Università degli Studi del Piemonte Orientale

Dipartimento di Scienze e Innovazione Tecnologica

Dottorato di Ricerca in Chemistry & Biology
Curriculum: Energy, Environmental and Food Sciences
XXIX ciclo a.a. 2015-2016
SSD: BIO/19

Exploring the microbiota of *Vitis vinifera* cv. Pinot Noir in two vineyards with different soil management: metagenomic and metaproteomic analysis

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Supervised by Prof.ssa Elisa Gamalero and Prof.ssa Graziella Berta

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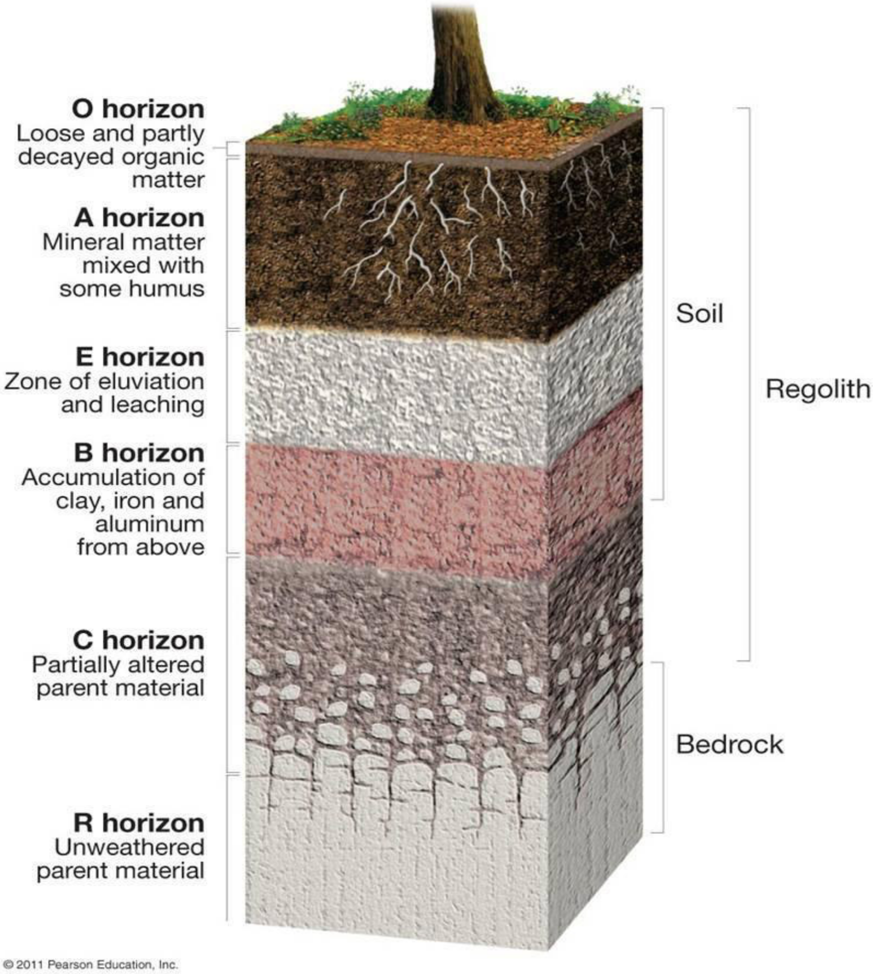
Introduction

1.1 Definition of soil

As usually happens for many common words, there is not a general consensus on the definition of "soil". Based on a traditional point of view, "**soil is the natural medium for the growth of plants"**. However, according to FAO, soil has also been defined as a "*natural body consisting of layers (soil horizons, Figure 1.1) that are composed of weathered mineral materials, organic material, air and water. Soil is the end product of the combined influence of climate, topography, organisms (flora, fauna and human) on parent materials (original rocks and minerals) over time. As a result soil differs from its parent material in texture, structure, consistency, colour, chemical, biological and physical characteristics*" (FAO, http://www.fao.org/soils-portal/about/all-definitions/en/).

The current definition of soil provided by the Soil Survey Staff (*Soil Taxonomy)* is: "*Soil is a natural body comprised of solids (mineral and organic matter), liquid, and gases that occurs on the land surface, occupies space, and is characterized by one or both of the following: horizons (Figure 1.1), or layers, that are distinguishable from the initial material as a result of additions, losses, transfers, and transformations of energy and matter or the ability to support rooted plants in a natural environment.*" (1).



<u>Figure 1.1</u> Subdivision of soil into soil horizons having typical chemical, physical and biological properties (Source:http://www.ctahr.hawaii.edu/mauisoil/a_profile.aspx)

In a very recent review Hartemink (2) analysed the definitions of soil given in the past 200 years and, in addition, attempted to give a brief scientific definition of soil: "*The soil is a living, four-dimensional natural entity containing solids, water (or ice) and air. Most soils are outside and are open systems, but soils also occur in shallow lakes and underneath pavement. A soil* *can have any colour, any age, be very shallow or deep, and consists mostly of a structured mixture of sand, silt and clay (inorganics), rocks and organic material (dead and alive). The soil has one or more genetic horizons, is an intrinsic part of the landscape, and changes over time. Soil are distributed across the earth mostly in a systematic manner. Soils store and transform energy and matter. The soil often supports vegetation, carries all terrestrial life, and produces most of our food. It is an integral part of the natural world interacting with the climate, lithosphere and hydrosphere. Soils are often studied in combination with land-use, climate, geomorphology or the hydrology of an area*".

Overall, soil is a heterogeneous, structured and discontinuous system, generally poor in energy sources and nutrients, resulting from the interactions among mineral, biological and chemical components present in soil (3-5).

Under a physical point of view soil is a highly complex environment of aggregated particles originating an intricate 3D network of pores that are filled with water and/or air (6). The formation of aggregates plays a key role in defining soil structure. Clay particles and humus form micro-aggregates characterized by a diameter lower than 250 μm, negative charge, and resistance to mechanical action (7); macro-aggregates are larger (size higher than 250 μm), less stable and can be destroyed by soil management (8-9).

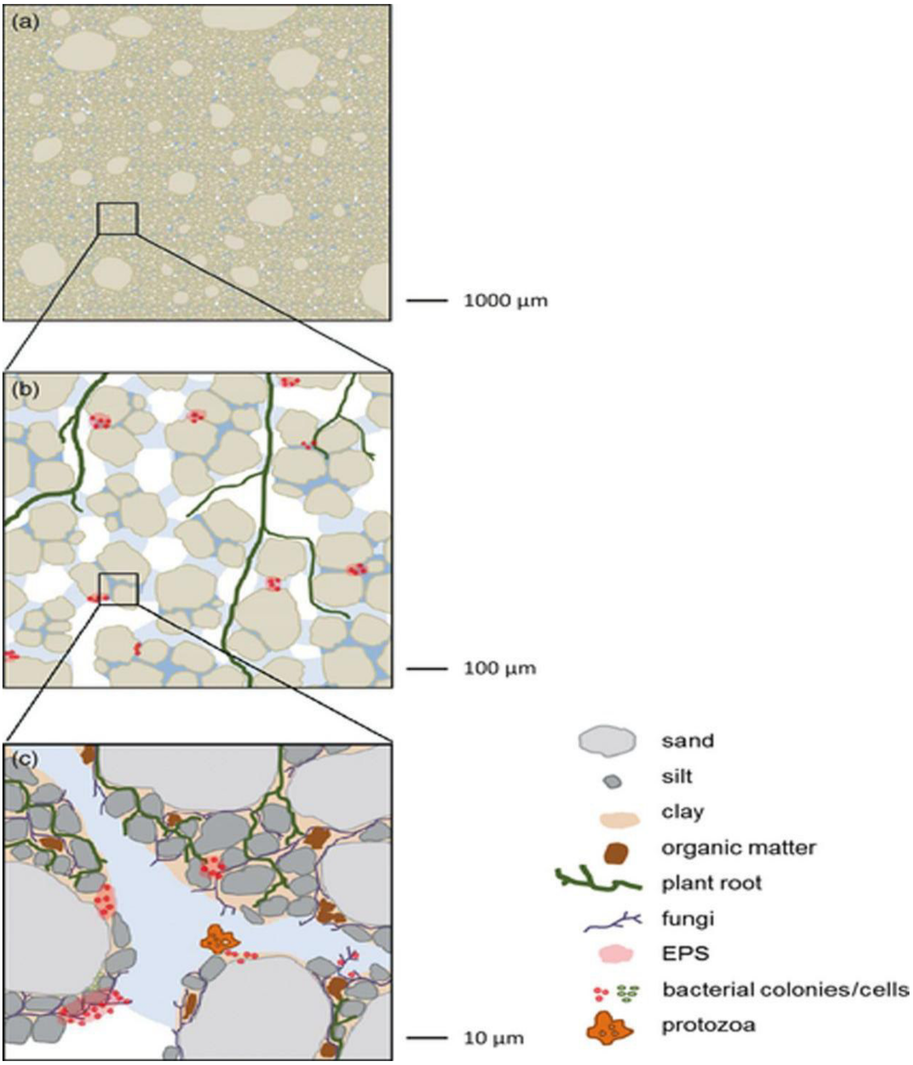
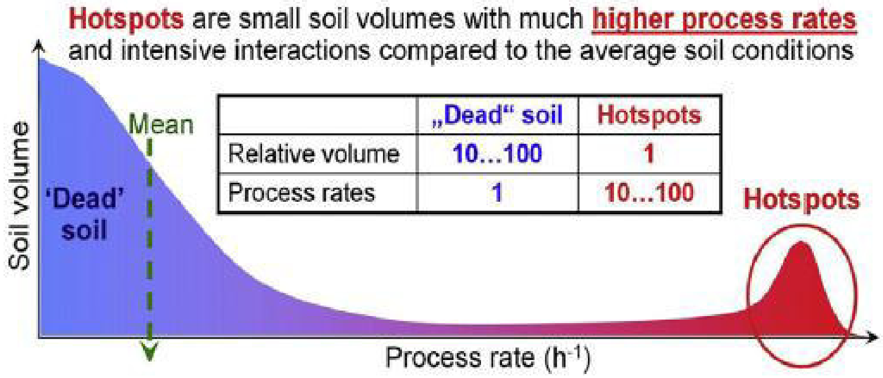


Figure 1.2 Soil representation at microscale level. While soil seems to be very homogeneous when looking at macroscale (a) it appears very heterogeneous at the microscale (b and c). b) Microaggregates carrying water filled (dark blu) micropores organize in clusters and originate macroaggregate. Pores having larger size (light blue and white) are localized between aggregates and are water or air filled, depending on the soil moisture status. c) The aggregates results from the combination of primary components, plant roots, fungal hyphae, and exopolysaccharides. Bacterial cells (red and green dots) mainly live in micropores, where they found protection against predators and dehydration. (Source: 10).

Bacteria relies on water availability for growth: as a consequence, in soil they live mainly associated to those districts of the pore network that are filled with water or covered by a water film. Typically, macro-pores largely drain out water and nutrients, while micro-pores hold back both water and nutrients. Both the size and the morphology of the pores affect water flow, thus influencing other parameters such as nutrient diffusion, aeration, redox potential and pH. Therefore, soil can be considered as a dynamic and complex habitat that includes a variety of microhabitats where microorganisms regulate plant growth and the maintenance of biogeochemical cycles (4,11).

Under a nutritional point of view, soil is an oligotrophic environment; as microorganisms are mainly heterotrophic, microbial growth in soil is limited by the scarce amount of readily available carbon and microbial cells are frequently in a “dormant” state that could be reversed if the limitations are removed (12).

Both the density and the biodiversity of bacteria in soil are affected by the soil conditions and by its management (13). Although the estimated microbial density is quite high and can reach 10^7-10^{12} cells per gram of soil (14), bacterial cells are grouped in clusters or covered by biofilm (15-16) occurring in very small microhabitats that occupy less than 1% of total soil volume (17) and 10^{-6} % of the soil surface area (18). These microhabitats, characterized by high microbial density, are known as microbial hotspots. According to Kuzyakov and Blagodatskaya (19) **hotspots** are “small portions of soil in which microbiological processes and interaction take place at higher rate compared to the average soil conditions” (Figura 1.3).



	„Dead" soil	Hotspots
Relative volume	10...100	1
Process rates	1	10...100

Figure 1.3 Graphical description of microbial hotspots in soil. Hotspots are small soil volumes characterized by a very intense biological activity and intensive interactions compared to the average soil conditions. The Table inset reports the measurement of the relative volume and process rates in both the hotspots and bulk soil. (Source: 19)

More in detail, hotspots harbour a high bacterial density which is typically 2-3 times greater of that occurring in bulk soil (20), so, although the bacterial cells are mainly in a dormant state, those microorganisms that maintain their activity drive most biochemical processes. Compared to bulk soil the number of active bacterial cells in the soil hotspots (i.e. the rhizosphere) has been reported to be 2 times greater (12).

Four hotspot groups are recognized on the basis of the sources and the localization of high input of labile organics (Figure 1.4):

1. Rhizosphere: input of labile organic carbon comes from the release of exudates by the roots occurring at various soil depths (21-22). A more exhaustive description of the rhizosphere is provided in the next chapter.

2. Detritusphere: input of organic carbon is represented mainly by recalcitrant molecules such as litter, located on the soil surface, and dead roots at various depths.

3. Biopores: they receive both labile and recalcitrant organic carbon by soil invertebrates and animal feces at various depths as well as by deep- growing roots and are maintained by roots themselves (23-24);

4. Aggregate surfaces: input of organic carbon is received by leaching of the detritusphere and by the rhizosphere (25).

While rhizosphere, detritusphere and biopores are originated by biological activity, the aggregate surface has abiotic origin.

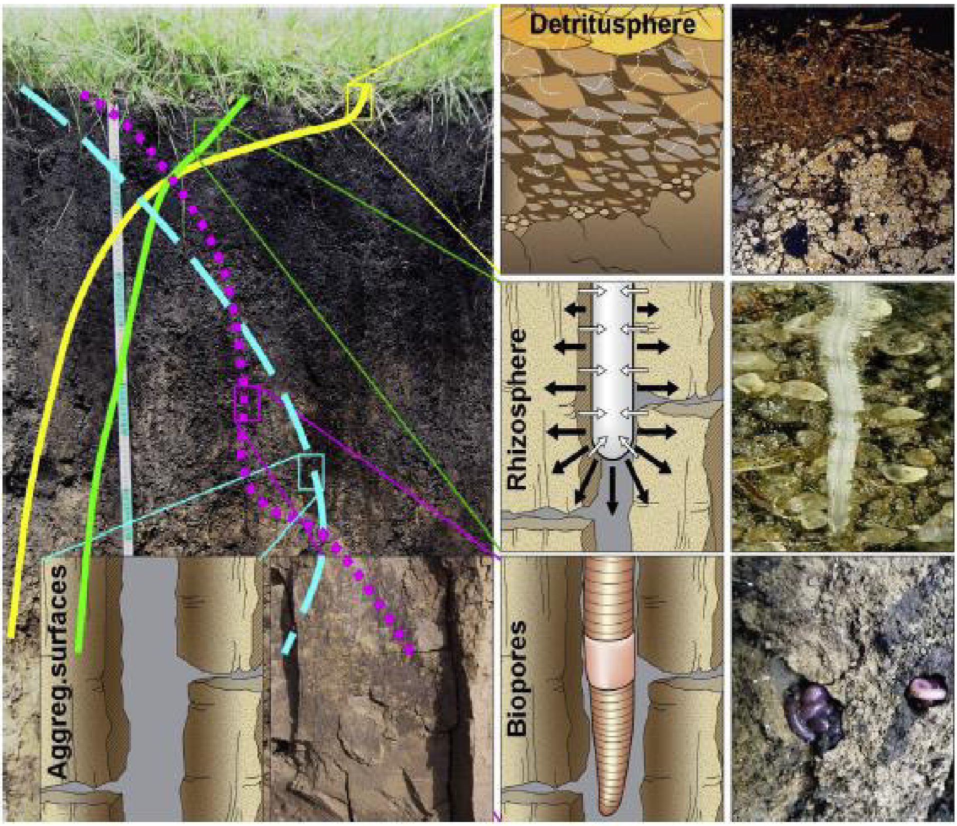


Figure 1.4. Representation of the four types of soil hotspot. Detritusphere (top right), Rhizosphere (middle right), Biopores (bottom right) and Aggregate surfaces (bottom left). The relative importance of these four soil hotspots is referred along 1 m depth and can vary according to soil parent material, plant coverage and climatic conditions. (Source:19)

Changes of soil physicochemical parameters (i.e temperature, pH, moisture and nutrient availability) at the level of microhabitats an induce a further differentiation of niches available for microbial colonization.

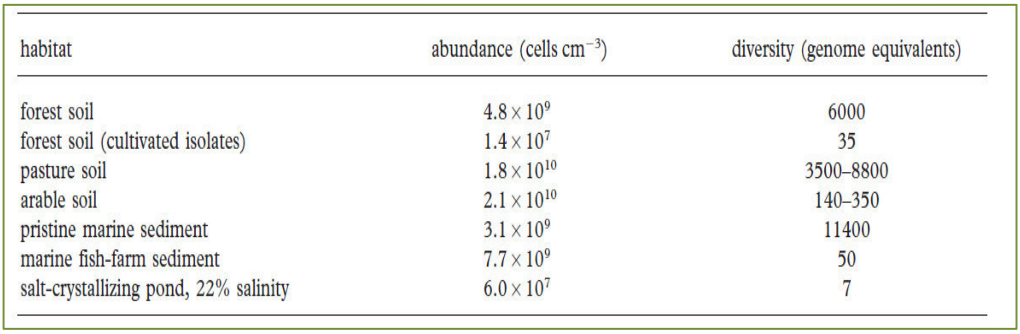
Soil harbours the most diverse populations of bacteria of any environment on earth and attention is moving toward a census of the bacterial species living in soil.

In this context, the paper published by Torsvik was a pioneer work: based on a DNA:DNA hybridization (26) it was estimated that 1 g of boreal forest soil contains about 7000 species or genetically distinct units.

In 2005, this approach was re-evaluated and nearly 10^7 microbial species per gram of soil were predicted by Gans and colleagues (27). Another attempt of quantification of the soil microbial biodiversity was provided by Schloss and Handelsman (28) who found 2000-5000 operational taxonomic units (OTUs) in one gram of soil.

In the Table 1.1 are reported both the bacterial abundance and the diversity in different soils.

Table 1.1 Abundance and diversity of soil bacteria. The number of cells /cm^3 was measured by fluorescence microscopy while biodiversity has been estimated by the DNA reassociation rate. (Source: 29)



habitat	abundance (cells cm^{-3})	diversity (genome equivalents)
forest soil	4.8×10^9	6000
forest soil (cultivated isolates)	1.4×10^7	35
pasture soil	1.8×10^{10}	3500–8800
arable soil	2.1×10^{10}	140–350
pristine marine sediment	3.1×10^9	11400
marine fish-farm sediment	7.7×10^9	50
salt-crystallizing pond, 22% salinity	6.0×10^7	7

According to the terminology used for microorganisms colonizing the human body (30-31), the overall communities of soil microorganisms are referred to as the soil microbiota, while the genes and genomes of the microbiota, as well as the products of the microbiota and the host environment, are defined as microbiome.

In this context, soil can be viewed as superorganisms relying on its microbiome for specific functions and traits.

Both the density and the biodiversity of the soil microbiome can be affected by a number of factors including soil type, plant cover (32), animal activity (33), wetness (34), fertilizer application (35), pH (36), and salinity (37).

As microbial density, diversity and activity can be affected by environmental factors; the interactions among different bacterial species can results in a wide range of relationships (competition, mutualism, predation or parasitism), whose intensity largely depend on the spatial distribution of the species. This cell-to- cell interactions may, in turn, have an impact on the soil environment.

Due to its sensitivity to small environmental changes, the soil microbiome can be considered as an indicator of soil quality (38). The concept of soil quality emerged in the ’90s and has been defined as “the capacity of a reference soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation” (39).

In this context, it should be considered that high species biodiversity may reflect redundancy within the soil microbiome, leading to fast recover after a stress occurrence (4) and to efficient protection against soil-borne pathogens (4, 40-41). In fact, when the biodiversity is high there is a low availability of free niche spaces for the development of plant pathogens (42). On the other side, a lack of evenness in the microbial community has been related to reduced plant productivity, probably due to the presence of an empty niche effect leaving some ecosystem services unful-filled.

Overall, high biodiversity of microbial communities leads to healthy soil, high rate of nutrient cycling and to efficient suppression of plant diseases and allow to achieve healthy and productive plants.

1.2 The Rhizosphere

The term rhizosphere, composed from the Greek words *rhiza* (root) and *sphaere* (surrounding), was coined for the first time by Lorenz Hiltner in 1904 (Figure 1.5) in order to describe the interactions between legume roots and microorganisms (43).

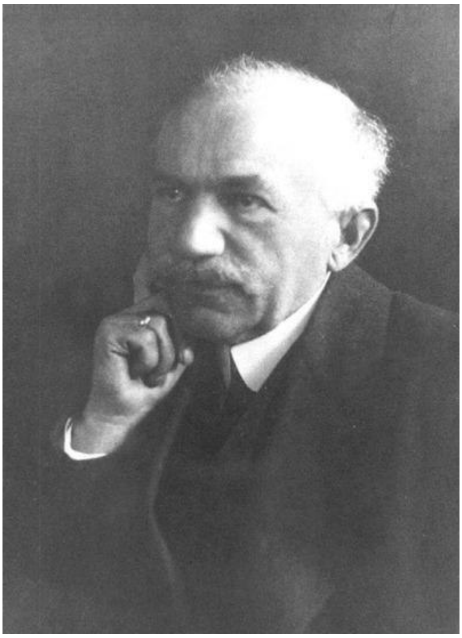


Figure 1.5 Picture of Lorenz Hiltner, President of the Bavarian Institute of Plant growth and Plant Protection of München and pioneer of the rhizosphere concept

In subsequent years, Lynch expanded and redefined the concept of the rhizosphere as “the volume of soil in close contact with the roots and directly influenced by the presence of these” (44).

The spatial extension of the rhizosphere is variable according to the soil structure, buffering capacity, water content and particle size (45).

The rhizosphere can be divided into three distinct zones (Figure 1.6): 1) ectorhizosphere (or rhizospheric soil, the soil immediately adjacent to the roots); 2) rhizoplane (root surface, where microorganisms and soil particles adhere); and 3) endorhizosphere (plant internal tissue, including cortex and endodermis) (46-48). Finally, the volume of soil that is not influenced by the presence of the root and is not part of the rhizosphere is called bulk soil (49).

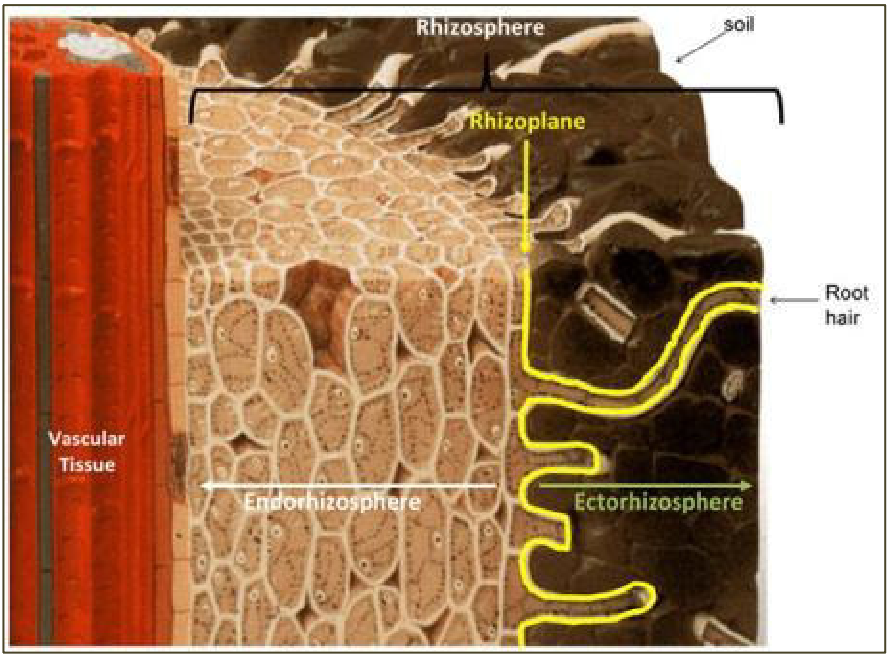


Figure 1.6. Representation of the different zones of the rhizosphere (Source:50)

The biochemical, biological, physical and chemical processes occurring in the rhizosphere are responsible for the different properties between bulk soils and rhizosphere that can be observed both in space and time (4).

The key difference between the bulk soil and the rhizosphere is the release of root exudates, a phenomenon known as rhizodeposition consisting in the transfer of carbon molecules (C) from roots into the surrounding soil (51-52). The rhizodeposition is involved in several rhizosphere processes including acquisition of nutrient, also influences soil ecology and plant growth (45) and plays a significant role in regulation of symbiotic association among microorganisms and plants (52).

The rhizodeposit composition comprises root exudates, mucilages and lysates from dead cells (52). Lysate compounds are important in maintaining the C/N ratio of soil organic matter (53). Instead, the root exudates constitute the major part of rhizodeposition and includes a wide variety of compounds (both low and high molecular weight molecules) such as organic acids, simple and complex sugars, amino acids, fatty acids, polypeptides and proteins, flavonoides, vitamins, phytosiderophores, growth factors, inorganic ions (e.g. $HCO3^-$, OH^-, H^+), gaseous molecules (CO_2, H_2), purines and nucleosides, hormones and enzymes (45; 54-58).

Depending on plant species, age and environmental conditions, rhizodeposits can account for up to 20 and 40% of net fixed carbon and about 17% of photosynthates is released by the root (13, 55). According to two different studies, exudates are divided into two classes: exudates which are released for a specific purpose (21) and exudates which are lost through basal exudation, and constitutes 3-5% of fixed C in photosynthesis (59).

Root exudates are released via two potential mechanisms: 1) plant products are transferred through the cellular membrane and transported into the rhizosphere via either active or passive mechanisms and 2) root compounds are released from root border cells (60-61). In particular, passive process trough ion channels, diffusion or vesicle transport (Figure 1.7) is considered the most used by plant (62).

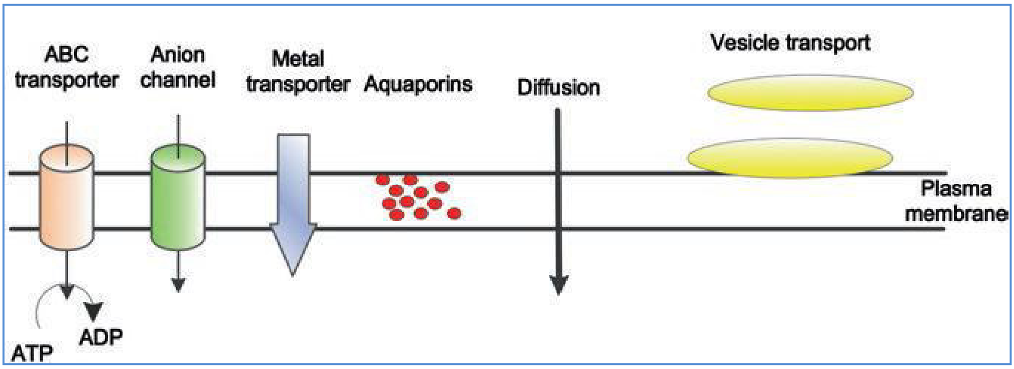


Figure 1.7 Overview of the mechanism which plant exude compounds (Source: 62)

In detail, ABC transporter are responsible for the exudation of flavonoids and lipids; metal transport for different metal; diffusion for a large number of compounds; aquaporins for water; vesicle transporter for protein and ion channel for sugar (62-63).

Several biotic and abiotic factors such as plant species, age, nutritional status, and developmental stage as well as various environmental factors including temperature, soil type and the presence of other microorganisms may affect the qualitative and quantitative composition of rhizodeposits (21, 32, 61, 64). Moreover, the release of root exudates varies in time and space (65). All these factors are summarized in Figure 1.8.

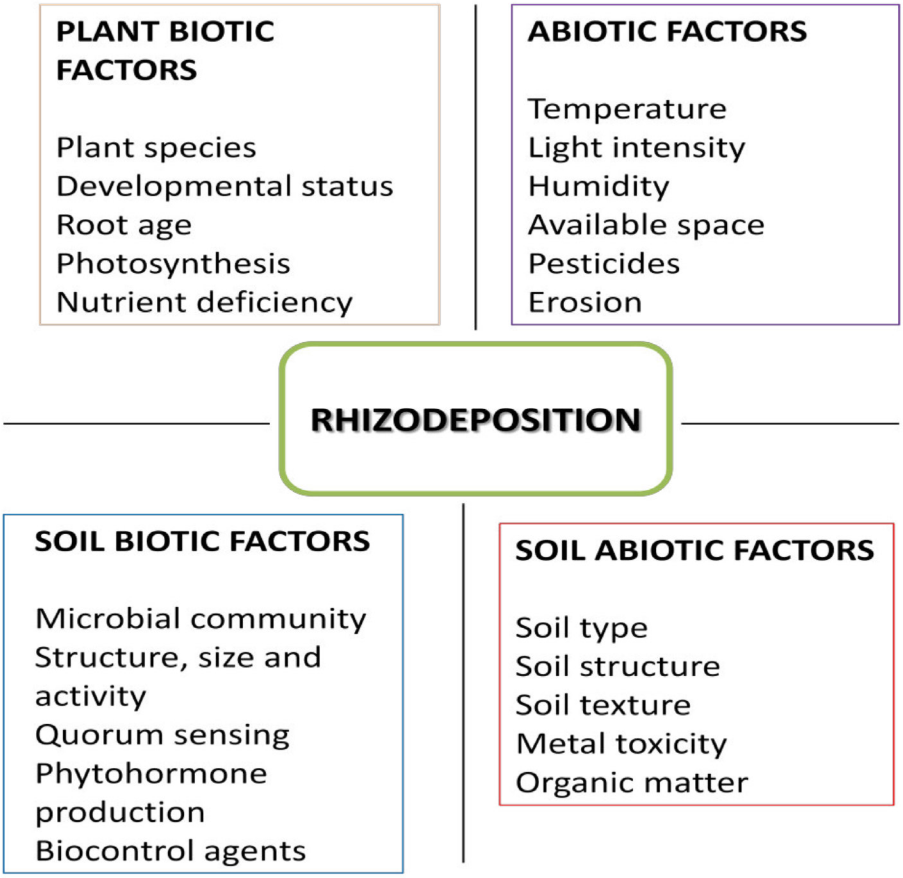
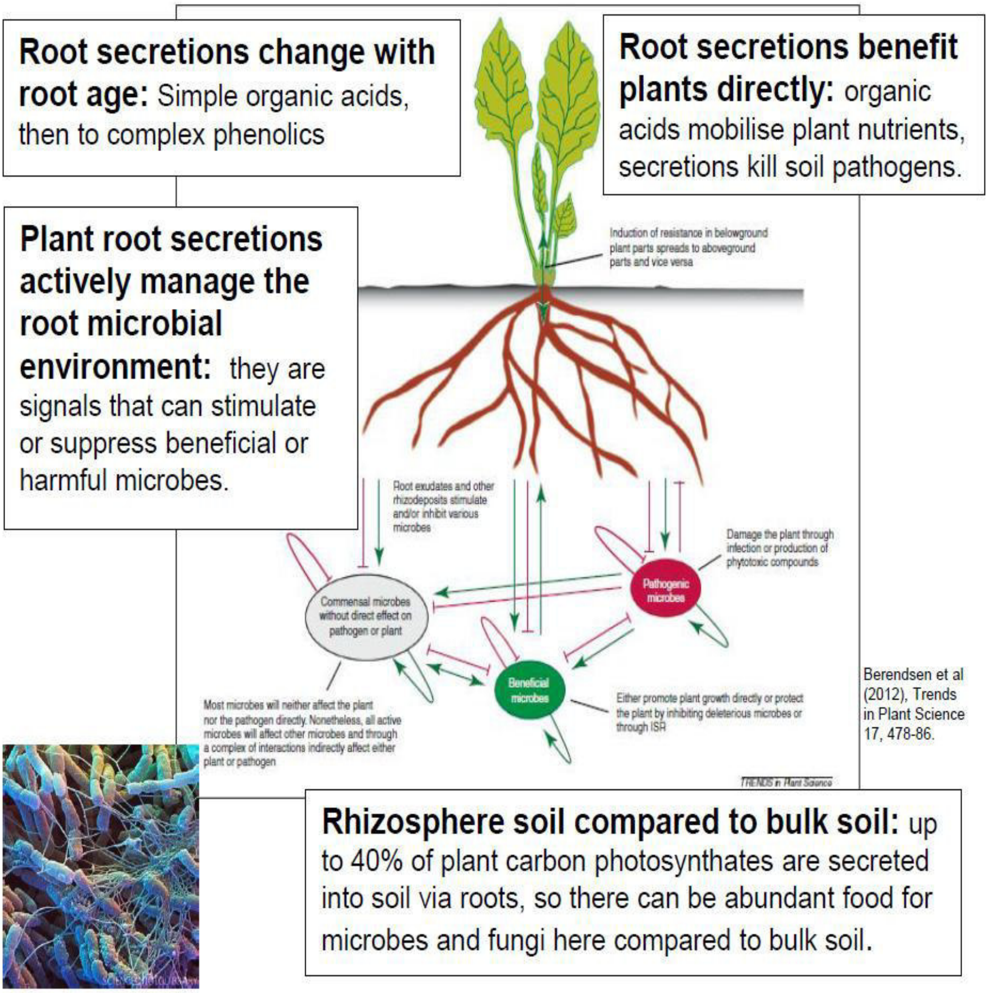


Figure 1.8. Representation of biotic and abiotic factors that influence the rhizodeposition (source: based on 21)

The release of root exudates can benefits the plant itself: organic acids can mobilize nutrients from soil and, at the same time, inhibit soil pathogens (66). Moreover, organic compounds released by the plant roots are important as source of energy and carbon for rhizospheric and soil microorganisms (67) (Figure 1.9). In fact, due to the root exudate release, bacterial density in the rhizosphere is higher than in bulk soil: this is the so called "rhizosphere effect" (21, 60, 68).



Root secretions change with root age: Simple organic acids, then to complex phenolics

Root secretions benefit plants directly: organic acids mobilise plant nutrients, secretions kill soil pathogens.

Plant root secretions actively manage the root microbial environment: they are signals that can stimulate or suppress beneficial or harmful microbes.

Rhizosphere soil compared to bulk soil: up to 40% of plant carbon photosynthates are secreted into soil via roots, so there can be abundant food for microbes and fungi here compared to bulk soil.

Berendsen et al (2012), Trends in Plant Science 17, 478-86.

<u>Figure 1.9</u> Plant-microbe and microbe-microbe interactions in the rhizosphere. Through exudate release, plants affect the composition and the activity of the associated bacterial communities. Rhizodeposition can result in a bacterial stimulation (green arrows) as well as in a inhibition (red blocked arrows). In turn, a wide variety of phytopathogenic/deleterious microorganisms competes with other microorganisms for colonization site and nutrients. Plant beneficial microorganisms can inhibit the phytopathogens development via the antibiotic synthesis, utilization of micronutrients and stimulating plant defences. Abbreviation: ISR, induced systemic resistance. (Source: 66)

The “rhizosphere effect” measures the population density by comparing the bacterial communities present in the rhizosphere (R) and bulk soil (S) and usually is expressed as the R/S ratio. The rhizosphere effect is higher for bacteria > fungi > actinomyces > protozoa (58, 69).

The effects of the rhizodeposition, in addition with other factors such as phenological stage of the plants, on the composition of soil microbial communities have been widely studied especially in annual crops, and grasslands (70-75).

According to different Authors, the rhizosphere is a niche that shape bacterial communities structure trough the interaction between plant species, root location and exudation, soil type and many others factors (41, 52, 76).

In fact, the rhizosphere is a dynamic environment, not only influenced by chemical and physical interaction, but shaped by the “rhizosphere trinity” represented by microorganisms, plants and soils (Figure 1.10) (44).

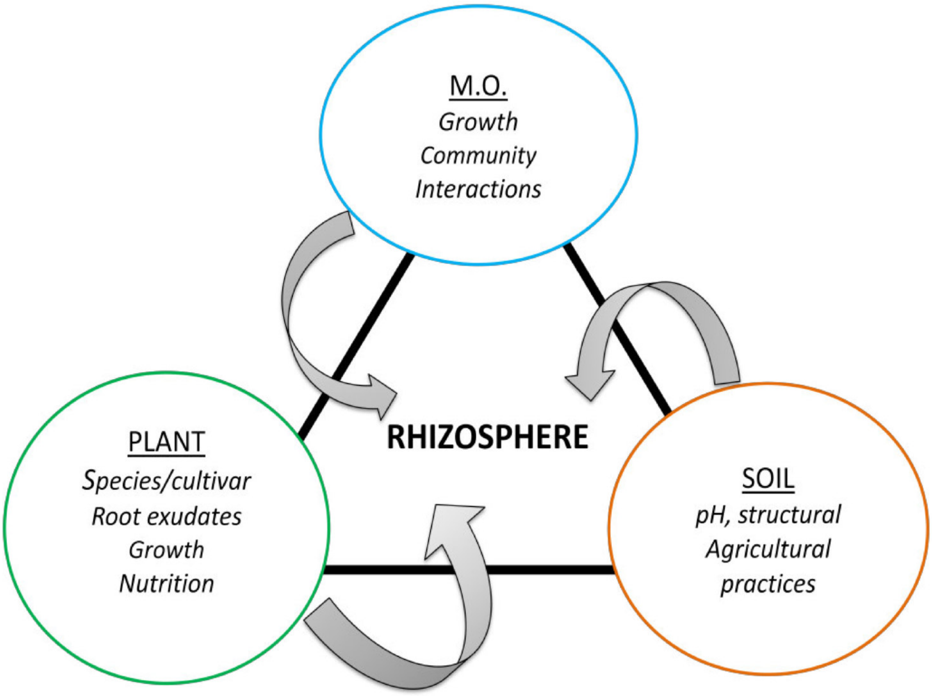


Figure 1.10 The rhizosphere trinity (Source: based on 44)

1.3 Microbial diversity in the rhizosphere

Bacterial diversity can be expressed in terms of genetic, taxonomic and functional diversity (77). In microbial terms, biodiversity describes the number and distribution of different species and their relative abundance within habitat and community (40). From the point of view of biological diversity, the microbial diversity refers at three levels: species number (species), within species (genetic) and community (ecological) diversity (74).

The rhizosphere is considered a “hot spot” for a wide range of microorganisms as well as bacteria, fungi (filamentous and mycorrhizal, AMF), algae, protozoa, viruses, oomycetes, and organisms like arthropods and nematodes (41, 79-80). Among these, bacteria and fungi are the most abundant, in particular density of bacteria are approximately 10^{10} cells per g of soil (14, 77).

While root exudates are important for the enrichment of microbial communities in the rhizosphere (32), the biodiversity of microbial community in the rhizosphere is usually lower than that observed in the bulk soil (81-83). Apparently, through rhizodeposition, plants actively shape the associated microbiome by selecting, from the bulk soil, those microorganisms that are able to use efficiently root exudates. Recently, the biodiversity of rhizospheric communities in crops and in medicinal and wild plants has been measured by metagenomics analysis: while the abundance of bacterial cells sum up to 10^{9}-10^{11} bacterial cells per gram of root, the number of bacterial species was found to overcome 1200 (Martina Köberl and Gabriele Berg, personal communication).

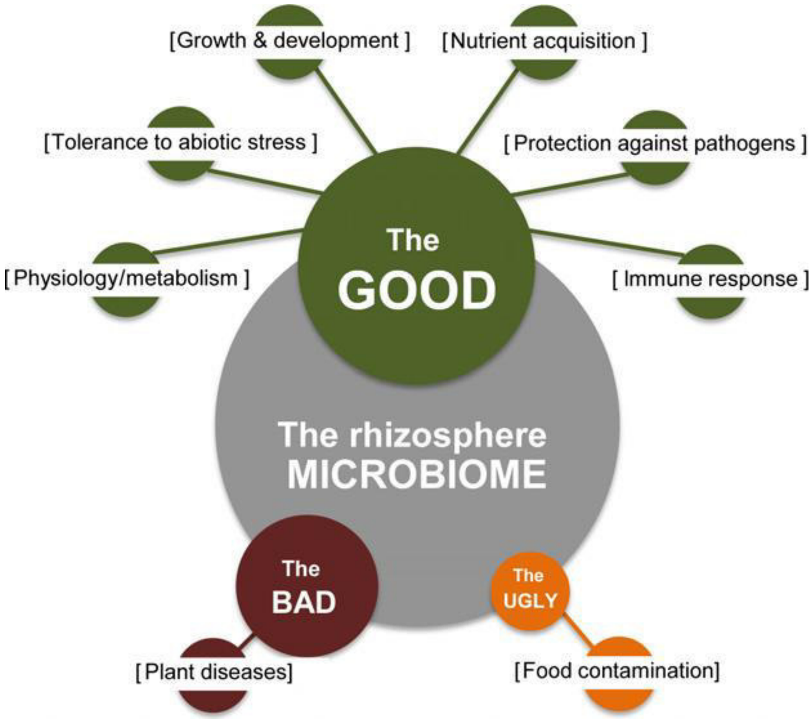
According to several Authors, the composition and structure of rhizospheric soil microorganisms communities vary in dependence on numerous factors such as plant species, plant phenological phase, age of the plant, root zone, soil texture and type, presence of plant pathogens and the chemicals exuded from roots (14, 22, 32, 57, 84-85). Together with soil type, the plant genotype plays a key role as driver of the microbial community composition in the rhizosphere (32). Thus, microbial communities associated to the roots of different plant species cultivated on the same soil frequently differ (64, 81). Moreover, the same plant species grown in different soil can originate similar microbial communities (86). Differences in the rhizosphere microbial communities have been observed in different genotypes of the same plant species (62, 72, 80).

Members of the phyla Proteobacteria, Acidobacteria, Actinobacteria and Bacteroidetes are prevalent and widespread, while members of Gemmatimonadetes, Firmicutes and Choloroflexi are less abundant (85, 87-88). According to the current genBank database from NCBI, among Proteobacteria the most abundant classes are considered alpha-Proteobacteria, beta-Proteobacteria and gamma-Poteobacteria (85). However, more than 10% of sequences found in soil sample are not assigned to known phyla (88-90).

In the last few years, some studies on the composition of microbial communities in the rhizosphere and bulk soil demonstrated that Acidobacteria, Actinobacteria, Protobacteria, Bacteroidetes and Gemmatimonadetes are the most abundant phyla (91-92).

In addition, some studies have shown that agricultural practices (crop management) and soil pH affect the composition of the bacterial communities (93-96). In turn, microflora can influence the health and the growth of the plants with positive, negative or deleterious effects (79). Recently, Mendes and co- workers defined the rhizosphere microbiome into three groups: “the good”, organisms with beneficial effects on plants, “the bad”, plant pathogenic microorganisms and “the ugly”, human pathogenic microorganisms (41).

Microorganisms that are beneficial for the plant growth, influence the nutrient status of plants and protect plants from pathogen attack by a wide mechanisms include plant growth-promoting bacteria (PGPB) (Figure 1.11) (41, 97).



<u>Figure 1.11</u> Schematic overview of the functions and impact of plant beneficial ('the good'), plant pathogenic ('the bad'), and human pathogenic microorganisms ('the ugly') on the host plant (Source: 41)

There are two types of bacteria beneficial for plants: one is represented by free-living bacteria and the other is represented by bacteria that "form a symbiotic relationship with the plant" (98-99).

1.4 Role of plant microbiome in plant health and productivity

Microorganisms living in soil are able to colonize the plant and affect (directly and indirectly) its yield, health and growth (100-101). Moreover, high bacterial biodiversity in soil seems to ensure plant health as well as high yield (101-102). Besides that, several plant beneficial bacteria are able to increase photosynthetic activity (102), confer stress tolerance (103-104) increase disease suppression (40-41), improve the performance of the plant iron acquisition (105) and affect the fruit and seed nutritional value (106-109).

1.4.1 PGPB and their mechanism of action

Plant growth-promoting bacteria (PGPB, 110) or rhizobacteria (PGPR, 111) are a heterogeneous groups of free-living bacteria isolated from diverse environments (112). The term PGPR was coined by Kloepper and Schroth (1978) to define “bacteria that are capable of promoting plant growth by colonizing the roots of plants under some conditions” (97, 113-116).

PGPB are associated with many plant species (117) and are able to colonize the roots of the plant in significant numbers, reaching 10^5-10^7 CFU per gram of fresh root (56). Generally, about 2-5% of the rhizosphere and soil bacteria are recognized as PGPB and exert a beneficial effect on plant growth (118).

On the basis of their activities, PGPB can be classified as phytostimulators (improving plant growth through phytohormones), biofertilizers (increasing the availability of nutrients), rhizomediators (degrading organic matter) and biocontrol agents or biopesticides (inhibiting phytopathogenic organisms diseases) (110, 119).

Moreover, PGPB can be divided into two groups according to their intimacy degree with the plant roots: the extracellular plant growth-promoting bacteria (e-PGPB) and intracellular plant growth-promoting (i-PGPB) (120). The i- PGPB are generally located inside of root cells or in the specialized nodular structure; on the other hand, e-PGPB live outside plant cells and not produce nodules (120).

Bacteria that can be classified as PGPB are characterized by three intrinsic characteristics: 1) they must be able to colonize the roots surface; 2) they promote plant growth and compete with other microorganisms; and 3) they need microhabitats associated to root surface to survive and multiply (119, 121).

It has been demonstrated that the PGPB positively influence the plants; in fact, after plant inoculation with PGPB one or more of the following traits can be observed: increased resistance to disease, increased tolerance to environmental stress, increased biomass, increased minerals content and enhance seed germination (62, 122).

As a consequence of the numerous studies on plant the number of bacterial species classified as PGPB has increased substantially. The main groups of PGPB belong to the phyla Actinobacteria, Proteobacteria, Firmicutes and Bacteroidetes and the range of bacteria that improve plant growth and inhibit plant pathogens include various species of *Pseudomonas*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Arthrobacter*, *Flavobacterium*, *Streptomyces*, *Klebsiella*, *Bradyrhizobium* (56, 67, 97, 123-124).

PGPB improve plant growth through a wide range of mechanisms, both direct and indirect. The stimulation of plant growth by PGPB occurs through: 1) the increase of the availability and acquisition of mineral nutrients, such as nitrogen, phosphorus and iron; 2) the synthesis of phytohormones such as auxin, cytokinin, abscisic acid and gibberellin; 3) the modulation of the ethylene levels in the plant tissues; and 4) the suppression of phytopathogens by induced systemic resistance and antibiosis (Figure 1.12) (115, 125-126).

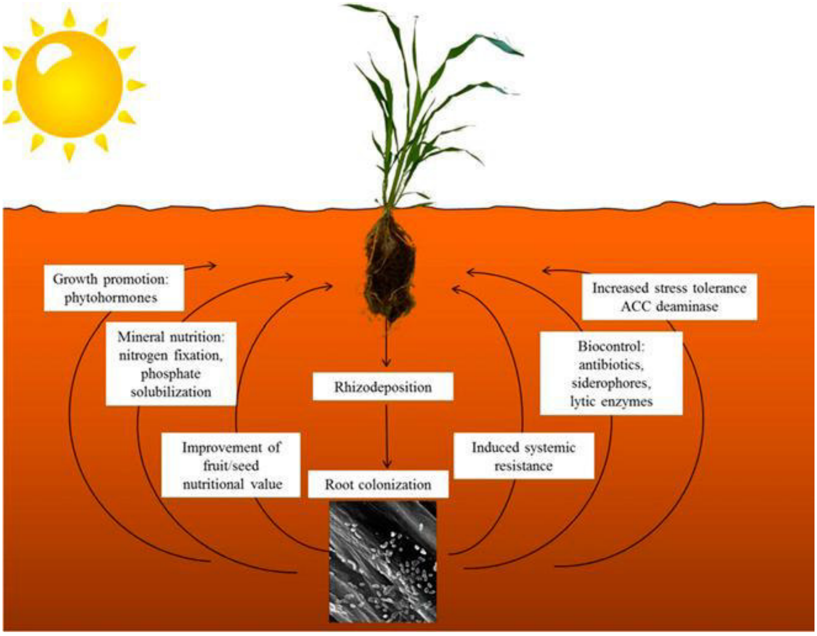


Figure 1.12. Overview of the main mechanisms used by PGPB (Source: 126)

1.4.1.1 Improvement of plant mineral nutrition

Nitrogen (N), together with phosphorous (P) is one of the most relevant nutrients for plant growth and productivity as they are integral part of the essential biomolecules, such as nucleic acids and proteins (126).

Although the high concentration of N in atmosphere and P in soil their bioavailability is very low (48,56, 114, 125, 126) and are considered the main plant limiting nutrients.

The conversion of atmospheric nitrogen (N_2) into utilizable forms for plants by a biological process is called biological N_2 fixation (BNF) (56).

The BNF is carried out both in rhizosphere and bulk soil, by nitrogen fixing bacteria having a complex enzyme system referred as nitrogenase (128).

The structural composition of nitrogenase was elucidated and consists of two conserved protein: 1) dinitrogenase reductase, or iron protein, a dimer of two identical subunits that contains the site for MgATP binding; and 2) dinitrogenase component which generally contain a prosthetic group with molybdenum (129-131) as a metal cofactor.

A wide range of bacteria can fix atmospheric nitrogen and generally this bacteria are classified as a) symbiotic nitrogen fixing bacteria (including members of the family Rhizobiaceae) and b) non-symbiotic nitrogen fixing bacteria (endophytes and free-living bacteria) (131).

Phosphorous is another essential macronutrients for plant growth (132); it is required for different metabolic processes such as respiration, energy transfer, macromolecular biosynthesis, signal transduction and photosynthesis (38,133).

In soils P occurs in both inorganic and organic forms. Inorganic P is represented by minerals such as apatite, hydroxyapatite and oxyapatite while, organic P is mainly represented by inositol phosphate (soil phytate) (125, 134).

The ability to convert insoluble phosphorus in an accessible form through mineralization and solubilization by phosphate-solubilising bacteria (PSB) is an important physiological trait of PGPB (114, 125, 131, 135).

Phosphate solubilisation by PGPB is carried out by 1) production of low molecular weight organic acids such as gluconic and citric acid (38, 114); 2) synthesis of extracellular enzymes like phosphatase enzymes (biochemical P mineralization) (84, 135); and 3) release of protons (H^+) to decrease soil pH (136-137).

In the rhizosphere and in soil there are a considerable population of PSB (97, 114).

Iron (Fe) is the fourth most abundant element on Earth (138) and its natural abundance in soil has been estimated to be about 20–40 g/kg (139). In aerobic soils, iron mainly occurs as hydroxides, oxyhydroxides, and oxides; in this way, the level of iron available for assimilation by living organisms is low, ranging from about 10^{-7} to 10^{-23} M at pH 3.5 and 8.5, respectively. Besides that, iron is an essential micronutrient for all organisms (67, 140). In fact, both microbes and plants have a high iron requirement (i.e., about 10^{-5}-$10^{-7}$and 10^{-4}-10^{-9} M, respectively); this specific condition is even more accentuated in the rhizosphere that is characterized by a strong competition for this element among plant, bacteria and fungi (141).

In plants, iron assimilation occurs by i) acidification of the rhizosphere mediated by release of H^+ that reduce the soil pH and ii) through the synthesis of phytosiderophores with high affinity against Fe (83, 131).

In order to increase iron availability, microorganisms and plants have evolved similar strategies; as happens in plants, when iron is lacking inside the bacterial cells, the synthesis of siderophores, having an extraordinary affinity for Fe^{3+} is induced (131, 141). The capability to acquire iron is one of the physiological traits that are considered fundamental for rhizospheric competence (142-143).

Bacterial siderophores play a dual role on the plant. If the plant is able to assimilate bacterial ferri-siderophores the interaction results in an improved mineral nutrition. On the other side, when bacteria outcompete with phytopathogens for this element the interaction may lead to the soil borne disease suppression. Siderophores production confers competitive advantages to PGPB and exclude other microorganisms from the rhizosphere (144).

1.4.1.2 Phytohormone production

The synthesis of phytohormones, such as auxins, gibberellins, and cytokinins is one of the direct mechanisms by which PGPB promote plant growth (97) (Figure 1.13). These molecules are involved in a variety of cellular process in plants (plant growth and development) and in the response of abiotic/biotic stresses (145).

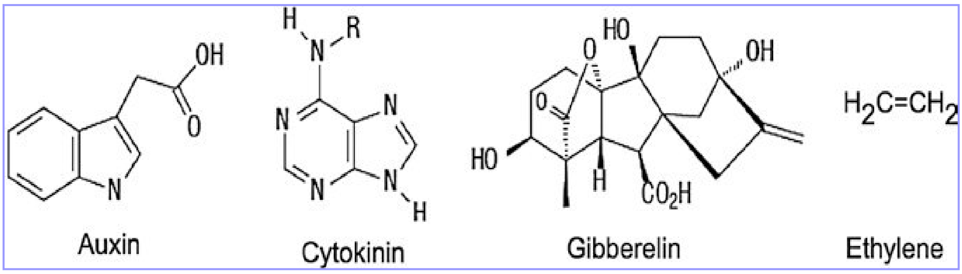


Figure 1.13 Structures of the major phytohormones (Source: 62)

Auxins are involved in a number of plant processes. This phytohormone influences the differentiation, the division, the extension of cells and plant tissues, stimulate seed germination, and control the vegetative processes (131, 146). As indole-3-acetic acid (IAA) is the most abundant member of the auxin family (56), the terms IAA and auxin are frequently used interchangeably (131).

Generally tryptophan, an amino acid commonly found in root exudates, is considered the main precursor of IAA: in fact, five out of the six auxin biosynthetic pathways in bacteria depend on this amino acid (146).

It has been shown that 80% of rhizosphere bacteria are capable to synthesize IAA (146-147).

Cytokinines are adenine derivates involved in different physiological processes such as cell division, elongation, seed germination, accumulation of chlorophyll and root development (148-150).

The cytokinines equilibrium is influenced by the levels of other growth phytohormones as auxin; this interaction between auxin and cytokinins has an important role in developmental processes of plants, in particular in apical dominance (48).

Gibberellins, instead, are a tetracyclic diterpenoid acids that demonstrated positive effects on flower and fruit development, seed germination, stem and leaf growth and elongation and also are implicated in promotion of root growth (151-152).

1.4.1.3 Lowering ethylene concentration

Ethylene is a gaseous plant hormone that regulates several physiological processes: it is involved in flowering initiation and senescence, root elongation, seed germination, fruit ripening and abscission, and production of organic compounds responsible for aroma in fruit (153). In addition, ethylene modulates the plant responses, to both biotic and abiotic stress (99). In the plant tissues ethylene is synthesized by methionine. In higher plants three enzymes are implicated in the biosynthesis of ethylene: 1) S-adenosyl-L-methionine (SAM) synthetase, which converts the methionine in SAM; 2) 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, which mediate the hydrolysis to ACC; and finally 3) ACC oxidase converting ACC to ethylene (131, 154).

Under stress condition (i.e exposure to high temperature, heavy metal, salinity, drought, or pathogen attack) the endogenous level of ethylene in plants raises (125, 155-156).

According to the model proposed by Glick (157) plants facing an environmental stress respond with a first small peak of ethylene (beneficial peak) inducing the synthesis of proteins related to plant defence's activation. In a second moment, if the stress become more intense a second deleterious peak of ethylene occurs (Figure 1.14). This second peak of ethylene is related to the activation of senescence phenomenon, chlorosis and abscission, and possibly death of the plant (125-126, 157).

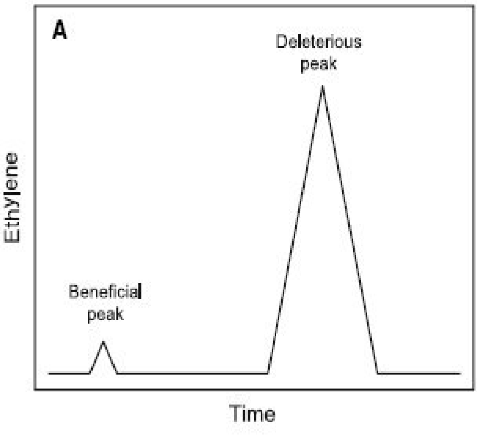


Figure 1.14. Production of ethylene in plants as a result of environmental stresses (Source:157)

Bacteria living on or in the root tissues, able to synthesize the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (157) increase plant tolerance to stress. In fact, ACC deaminase cleaves ACC into ammonia and α-ketobutyrate (Figure 1.15). Since ACC deaminase is an inducible enzyme it acts on the second peak of ethylene, decreasing the concentration of this phytohormone and improving the survival of the plant exposed to a wide range of stresses (123, 157-158).

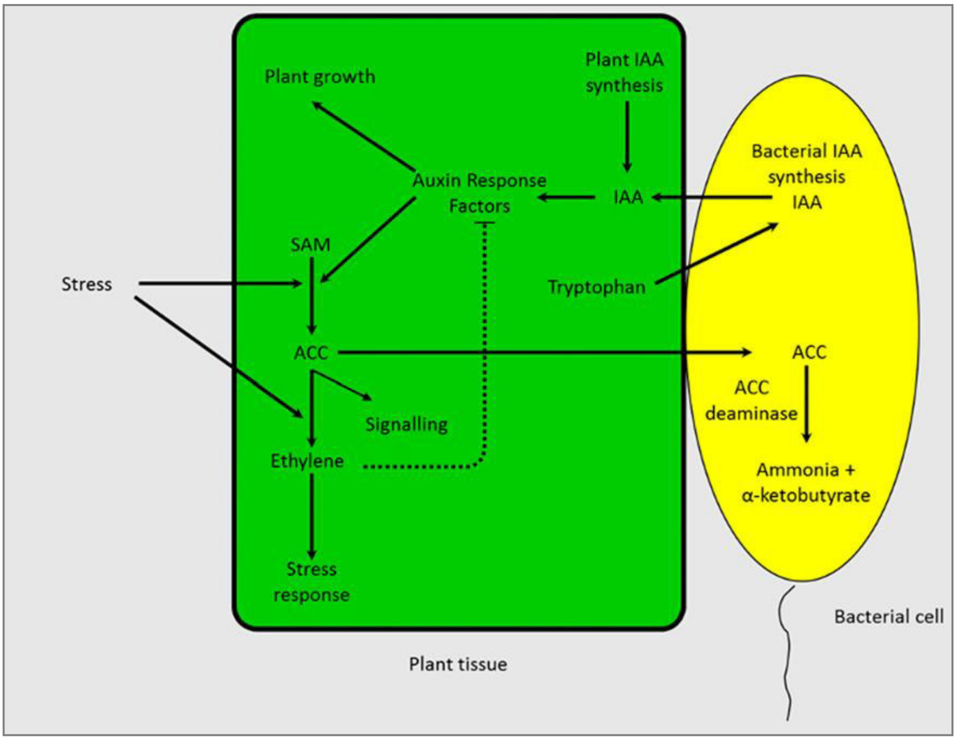


Figure 1.15 *Representation of how PGPB can increase plant tolerance to stresses through the action of the ACC deaminase, cleaving ACC, the immediate ethylene precursor, in ammonia and alpha-ketobutyrate (Source: 126)*

PGPB that are able to produce the enzyme ACC deaminase are involved in the protection of the plant in different situations such as growth in soil polluted by heavy metal (104, 125), in arid soils (159-160), in presence of excess of salt (161), anoxia (76). Plant inoculation with ACC deaminase producing bacteria also increases plant tolerance to soil borne diseases (162-163).

1.4.1.4 Soil borne disease suppression

The production and release of antibiotics, defined as molecules that kill or inhibit the development of various pathogens, is one of the most described mechanism for PGPB inhibition of plant pathogens (157, 164). Although a number of antibiotics are synthesized by different bacterial species, antibiotics produced by fluorescent pseudomonads are the most studied. The categories of antibiotics produced by fluorescent pseudomonads and involved in soil-borne disease suppression are phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, cyclic lipopeptides and hydrogen cyanide (165). It is well known that the main targets of these antibiotics are the electron transport chain (phenazines, pyrrolnitrin), metalloenzymes such as copper-containing cytochrome c oxidases (hydrogen cyanide), or cell membrane and zoospores (2,4- diacetylphloroglucinol, biosurfactants): however, information on their modes of action are scanty.

The first demonstration that PGPB can promote plant health by stimulating the plant's immune system was provided in 1991 by three works (166-168). Van Peer et al. (166) demonstrated that inoculating carnation (*Dianthus caryophyllus*) cuttings' roots with *Pseudomonas fluorescens* WCS417r an enhanced against the fungal pathogen *Fusarium oxysporum* in shoot was observed. In the same year Wei et al., (168) reported that inoculation of cucumber roots with strains of *Pseudomonas* spp. and *Serratia* spp. resulted in a reduced symptom expression of antrachnose due to leaf inoculation with *Colletotrichum orbiculare* (168). Finally, Almostrom (167) demonstrated that inoculation of bean seeds with a cell suspension of *P. fluorescens* S 97 led to protection against the halo blight caused by *Pseudomonas syringae* pv. *Phaseolicola* (167).

Overall, these three pioneer works clearly indicated that increased level of disease resistance was related to a plant-mediated immune response called induced systemic resistance (ISR). Typically, ISR is expressed not only in the zone of induction, but also in plant organs that are distant from the site of induction. A high amount of literature on this topic has been provided (for an excellent recent review see 169); at present, it has been proved that ISR is driven by a complex network of interconnected signalling pathways in which plant hormones, mainly jasmonate and ethylene, play a major regulatory role (169-170) (Figure 1.16). However, other bacterial molecules and components, such as the *O*-antigenic side chain of the bacterial outer membrane protein lipopolysaccharide, flagellar fractions, pyoverdine, the antibiotic DAPG, cyclic lipopeptide surfactants and, in some instances, salicylic acid, have been implicated as signals for the induction of systemic resistance (169).

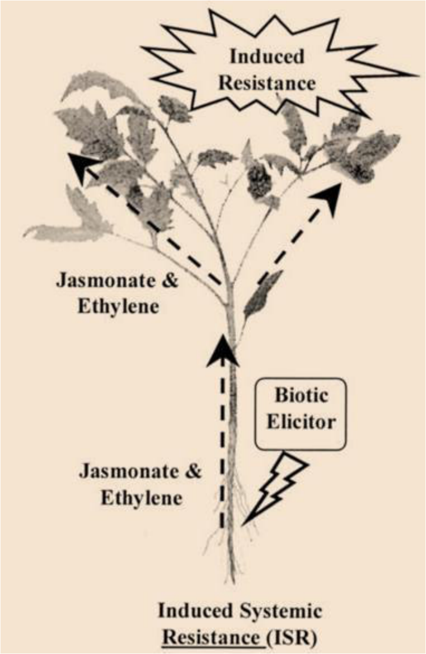


Figure 1.16. Induced systemic resistance (ISR) is typically activated after the root colonization by a biotic elicitor (plant beneficial microorganism). The activation of the systemic immunity in above-ground plant parts is given by a long-distance signal that travels starting from the root through the plant vascular system. Jasmonic acid (JA)- and ethylene (ET)-dependent signaling pathways are the molecules involved in the activation of plant gene defences.(Source: 171).

All of these signalling molecules activate the transcription and expression of defence-related plant genes that in turn lead to the construction of structural defence barriers.

1.5 Methods to characterize soil microbial communities

A high number of approaches have been used to study microbial diversity. Usually, they are classified in cultivation-dependent and cultivation-independent methods (172).

In the 1960s, microbiology studies were based on pure culture. In fact, traditional microbiological methods were based on isolation of the microorganisms in pure culture using non selective or selective or differential media designed in order to optimize the recovery of microorganisms (40, 173-174). However, this approach did not consider the interaction between microorganisms and between microorganisms and the surrounding environment. In the 1980s, one of the main progress was to characterize the microorganisms not only for the cell morphology but also for their density, diversity, and activity at the population level. Brock 1987 was the first to highlight that the behaviour of a microorganism under laboratory conditions can be very different when the microorganism lives in natural environments and is exposed to resource competition, predation, and stresses.

The major limitation of the pure culture approach lies on the fact that only a little fraction of the whole soil bacteria are culturable (48, 175). In fact, in 1985, Staley and Konopka coined the term “the great plate count anomaly” in order to describe the discrepancy between the numbers of cells extracted from natural environments able to form viable colonies on agar media and the number of cells observed under the microscope (Figure 1.17).

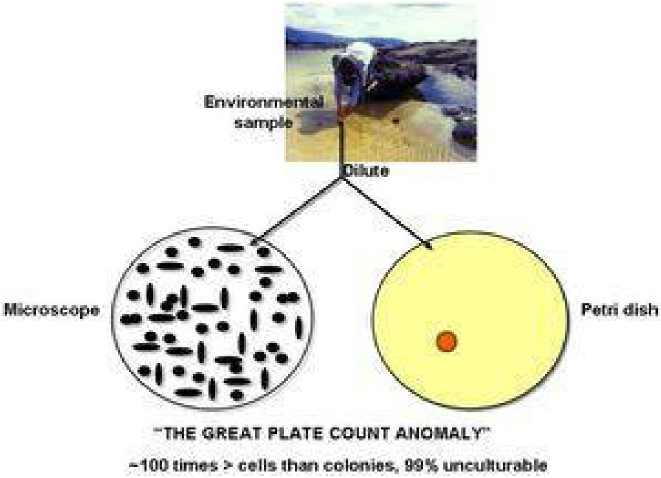


Figure 1.17 Representation of the great plate count anomaly: most of bacterial cells observed and counted with the microscope cannot currently be grown under laboratory conditions

The "great plate count anomaly" was related to i) those microbes that fail to grow in culture media because they have fastidious or unknown growth requirements ii) obligate symbiotic and parasitic bacteria and iii) viable but non culturable (VBNC) bacteria that are viable, active but resist to cultivation.

The VBNC concept was initially overlapped with that of dormant cells. However, further investigation allowed to define that while in VBNC the metabolic activity is measurable, in dormant cells, this activity is below the detection levels (176). Moreover, cells in VBNC status can resuscitate when environmental conditions become more favourable (177). While attention has been focused mainly on pathogenic VBNC bacteria (178), few data concerning the environmental functions of VBNC are available.

However, VBNC cells are active and can play an important but hidden role in environmental health.

In 1995, Amann and colleagues stated that the amount of microorganisms in the environment that resist to cultivation reaches 90% of the whole microbial community. Consequently, by using culture dependent approach most of the microbial diversity remained unexplored.

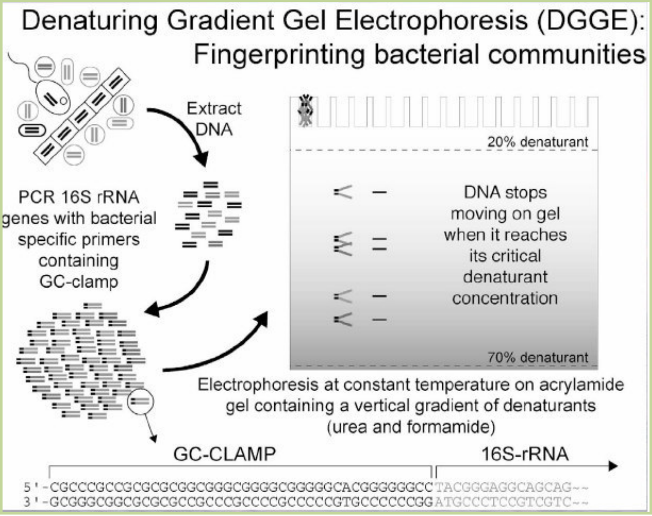
Taking into account all these historical steps, many efforts have been dedicated to the development of molecular methods that became essential in order to gain a broader knowledge on the diversity of the whole bacterial communities (173).

During the last few decades, a wide number of molecular tools have been developed to study microbial diversity in the rhizosphere (85, 173, 179). These techniques are based on the amplification of sequences derived from specific genes coding for the ribosomal RNA (rRNA) (180) or for the internal transcribed spacer (ITS), regions of DNA located between rRNA genes. These regions are present in bacteria (16S-23S) and fungi (18S-28S); on the basis of these genetic traits, it is possible to characterize and discriminate different bacterial species and fungi classes. Among these techniques the most used include PCR-based methods such as Denaturing Gradient Gel Electrophoresis (DGGE), Temperature Gradient Gel Electrophoresis (TGGE), Single Strand Conformation Polymorphism (SSCP), Ribosomal Intergenic Spacer Analysis (RISA) and Automated Ribosomal Intergenic Spacer Analysis (A-RISA) (174).

Generally, molecular analysis of environmental bacterial community are based on PCR performed on DNA extracted directly from soil (179, 181). DNA extraction from soil requires particular attention due the presence of PCR inhibitors such as humic acids (179).

Studies based on PCR methods are carried out using the ribosomal RNA operon, particularly the 16S rRNA gene. PCR amplification of rRNA genes from environmental sample associated with other fingerprint (microbial patterns) methods provides a high amount of detailed information about the species composition of the whole bacterial community (11).

PCR-DGGE (Figure 1.18) has been one of the earliest and most widely used methods proposed for the characterization of microbial communities in environmental samples (40).



<u>Figure 1.18</u> Image illustrating the PCR-DGGE technique

While different studies have utilized DGGE method for characterize rhizosphere and soil microbial communities (155, 182)the DGGE technique has several drawbacks such as: 1) low reproducibility of the results; 2) limited length of the obtained sequences (<500 bp); 3) generation of multiple bands for a single species (174, 183).

In recent years (2004), in order to overcome the bias of the molecular approach described before, more advanced techniques based on sequencing of DNA, known as “Next-Generation Sequencing” (NGS) technologies provided a new powerful tool to study bacterial communities.

The NGS is one of the more spread methods, in particular for studying large communities at the phylogenesys and/or the function level (85). The most commonly used NGS platforms are 454 pyrosequencing (Roche/454 Life Sciences) and Illumina HiSeq (Solexa Sequencing) (62, 183). This platforms differ in the procedure employed, but their basic strategy is similar.

In particular, Roche 454 uses the technique of pyrosequencing. The basis of pyrosequencing is the detection of the pyrophosphate release following nucleotide incorporation and is based on a series of enzymatic reactions, the last one being the generation of light by luciferase. The amount of light generated is approximately proportional to the number of nucleotides that are incorporated into the growing strand (62).

The pyrosequencing technology was used in several works for the characterization of microbial communities in rhizosphere soils samples such as apple (184) potato (185), grapevine (89, 186), maize (187), reygrass (188), rice (189) and oak (190).

The next generation sequencing have revolutionized the research on environmental microorganisms and allowed the development of metagenomics.

The term "metagenomics" was first used by Jo Handelsman and others, and first appeared in publication in 1998 (191) in order to describe the analysis of the collective microbial genomes occurring in an environmental sample (192-193). If metagenomics can provide new insights into microbial diversity and evolution, it is also true that information on the genetic potential does not correspond to the functionality of microbial communities in ecosystems. As stated by Chaparro et al., (2012) “it is not who is present but what they are doing that is more informative and revealing” (194).

Based on this idea, the progress made in technology have shed light on the need in determining the functional diversity together with the molecular diversity in the rhizosphere (195).

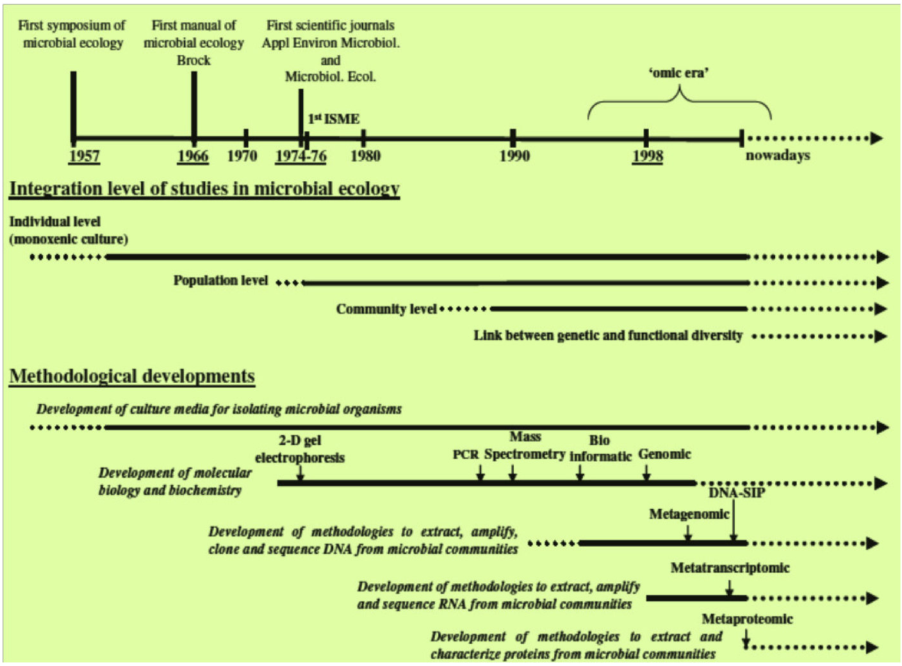
Several experimental procedures have been developed in order to recognize active and quiescent populations in natural environment (i.e. incorporation of labeled markers in microbial biomass). However, relying on these approaches, it’s possible to obtain information on the populations associated with a specific process, but not a full description of their functional role inside the community.

The analysis of the total content of gene transcripts (RNA copies of the genes) in a community (metatranscriptomics) in a specific moment of time has been applied to complex environment such as soil (196). The main objective of metatranscriptomics are to taxonomically classify transcripts, understand their functions and measure their abundances, and to relate these to environmental data in order to reveal how environmental conditions can have an influence on microbial communities. However, metatranscriptomic suffers of some drawbacks: i) the mRNA extraction from soil is a quite complex procedure; ii) difficulty in eliminating humic acids during the extraction process iii) the short half-life of RNA requires experience in manipulation; iv) there is a low correlation between the amount of RNA and the synthesis of the corresponding proteins.

Interest in metaproteome characterization is born by the awareness of these limitations, and in 2004 Wilmes and Bond defined metaproteomics as ‘‘*the large-scale characterization of the entire protein complement of environmental microbiota at a given point in time*’’ (197). Since proteins are the driving force of the biotransformation processes, metaproteome analysis offers a and “holistic” overview of the dynamics of microbial function (198) and allow to better understand possible interaction such as competition and cooperation for nutrients and distribution of metabolic activities within soil microorganism communities.

In the last years, metaproteomics studies have demonstrated that different protein profiles result from the interaction between microbial community and plants (199-200).

In recent years, it has been demonstrated that the development of techniques such as metagenomics and metaproteomics compared to the classical molecular techniques provide more information on the composition and activity of soil microbial communities (201-202). Also it has been showed that this two molecular approaches are essential to determine and characterize the phylogenetic and functional microbial diversities and to understand the interactions with different environmental factors, both biotic and abiotic (183).



<u>igure 1.18.</u> Chronological and step-by-step progresses in microbial ecology. (Source: 203)

1.6 Grapevine (*Vitis vinifera)*

Vitis vinifera (common name grapevine) is a species of *Vitis* belonging to the Rhamnales order and the Vitaceae family. The cultivated varieties (vine) are classified in *sativa* subspecie, while the wild form are classified in *silvestris* subspecie (Table 1.2).

Table 1.2 Systematic classification of Vitis vinifera

Kingdom	Plantae
Underkingdom	Tracheobionta (vascular plant)
Superdivision	Spermatophyta (seed plant)
Division	Magnoliophyta (flowering plant)
Class	Magnoliopsida (dicotyledons)
Order	Rhamnales
Family	Vitaceae
Genera	*Vitis* L.
Species	*Vitis vinifera* L.
Subspecies	*Vitis vinifera* L. ssp. *sativa* (cultivated grape) *Vitis vinifera* L. ssp. *silvestris* (wild form)

Grapevine is a woody perennial and climbing plant that is divided into a root system (underground part) and cauline system (aboveground part); this last one is formed by a skeleton (woody structure) and by a crown (herbaceous structure).

The roots of grapevine are typically multi-branching, producing lateral roots that further branch into smaller lateral roots. Roots, in addition to anchoring the plant, absorb nutrient and water from the soil, store carbohydrates and other compounds, for the winter season, and produce the substances that regulate vine growth.

The skeleton structure of the plant is articulated into a main axis, the so called trunk, from which originate secondary branches, know as shoots. The shoots are the vegetative organs that grow annually and supporting buds, leaves, inflorescence and tendrils. Branches and trunk look consist in typical external bark morphology, called rhytidome (204).

The trunk is permanent and supports the aboveground vegetative (leaves and stems) and reproductive (flowers and fruits) structures of the vine. The trunk of a mature vine has short branches, so called arms, which are located in different positions depending on the system. Some training system use semi- permanent branches of the trunk, know as cordons.

The grape leaves, provided with a long stalk, are simple, large and webbed, usually divided into 3-5 lobes with entire or dentate margin with more or less intense green colour, according to the variety. The leaves are positioned in the proximity of the nodes on each bud, in an alternate manner.

The plant climb with the help of tendrils, or cirrus, that are the characteristic elements of *V. vinifera*. The tendrils are branched and grow opposite the leaves at the node; this element automatically begin to coil when they contact another object (Figure 1.19) (204).

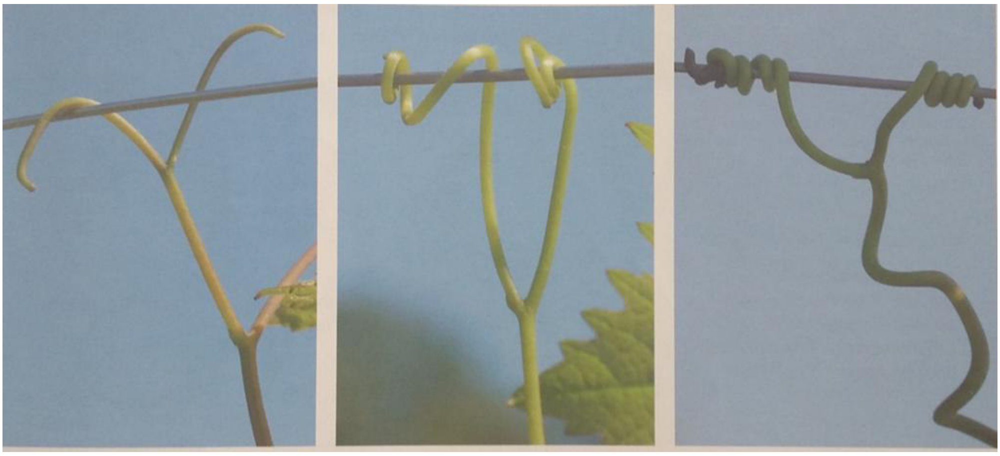


Figure 1.19 Rolling-up sequence of the tendril to a support (Source: 204)

After the fruit is harvested, the tendrils will harden and become wooden in nature.

Grape flowers are small (4-5 mm) and in cultivated varieties are typically hermaphrodites and pentamerous. Grape flowers are grouped in inflorescence or flower cluster, which are erect at first and pendulous then. The cluster is formed by a main axis, called rachis, and the single flowers are attached to the rachis through the pedicel. The flower cluster born on new shoots at the node opposite the foliage leaves in the same position as the tendrils.

The fruit is a berry, known as grape. The berries are placed on pedicels that, together with the ramification of the cluster, forms the stalk. From a structural point of view, the grape berry (Figure 1.20) may be divided into three types of tissue: 1) skin or exocarp, rich in colouring matter and aromatic compounds; 2) pulp or mesocarp; and 3) endocarp, formed by seed lodges (the perfect or normal number of seeds in the grape berry is four).

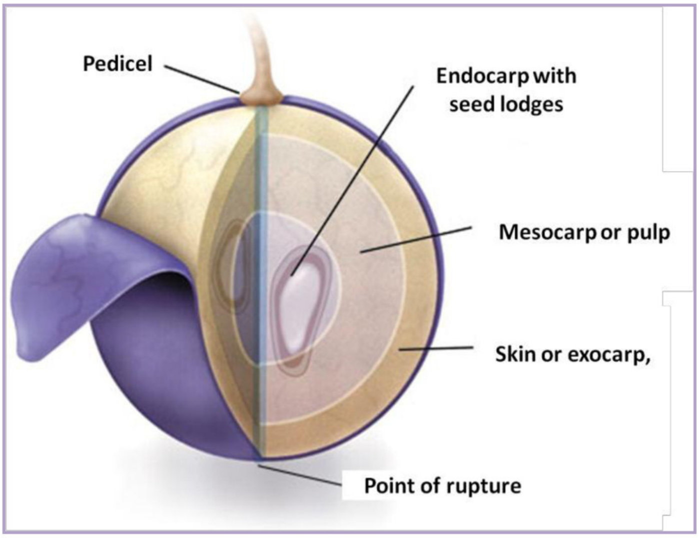


Figure 1.20 Structure of grapevine berry (Source: http://enoviti-hanumangirl.blogspot.it/2012/03/chemistry-of-grape-berry.html)

The colour (from green to yellow and from pink to purplish-red), the shape, the weight and the size of ripe berry depend on several factors such as the grape varieties (cultivar), the age of the plant, the climate and the type of cultivation (204).

1.6.1 Annual growth cycle of grapevine

The life cycle of the vine is a process occurring every year in the vineyard. The annual growth cycle of grapevine starts with the budburst in spring, following by inflorescence development/flowering, fruit development (fruit set and veraison), harvest and ends in winter with the dormancy (Figure 1.21).

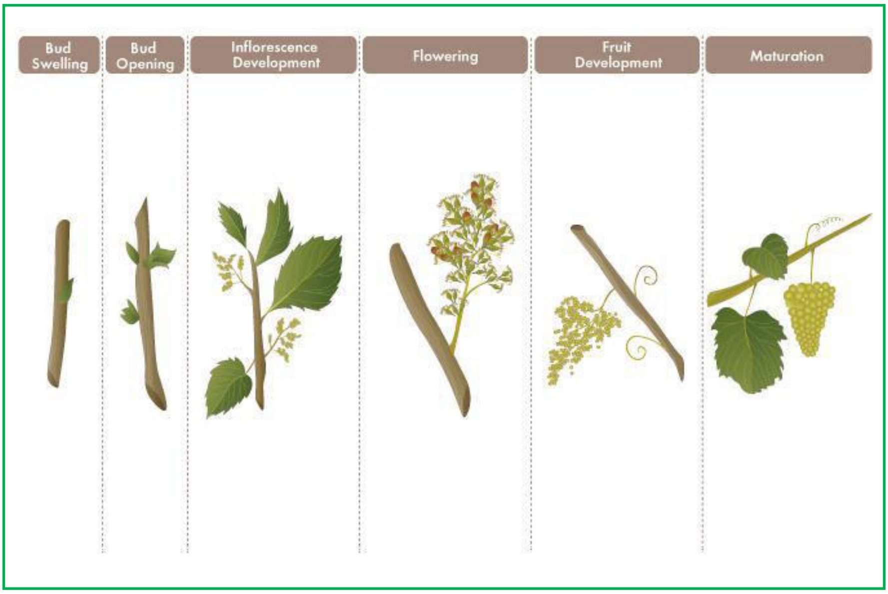


Figure 1.21 Phenological stages of V.vinifera (Source:http://www.sqm.com/enus/productos/nutricionvegetaldeespecialidad/cultivos/uvademesa.aspx#tabs-4)

The duration and the time between these stages varies depending on the grape cultivar, geographic location, environmental conditions, climate and seasonal weather, but the sequence of events remains constant (205).

The event commonly referred to as budburst, or bud break, represent the beginning of a new biological cycle and vegetative growth of the plant. This event starts in spring (with temperature around 10° C) when the dormant buds begin to grow with the production of shoots. Furthermore, this phase is conditioned by the time of pruning, the climate and soil (204).

After budburst the process of flowering (Figure 1.22 a) begins with small flower clusters; in this stage, grape flowers form, grow and open. Flower formation is complex and is greatly influenced by the viticultural practices and the environmental condition.

The process of flowering is immediately followed by fruit set (Figure 1.22 b), that is defined as the stage when the fertilized flower develop into berries with seeds; only a portion of those flowers will develop into berries. Many factors, including temperature, health of the plant, humidity and water stress, play an important role in the quality and quantity of flowers which are fertilized. Also, this stage is very important and crucial for the wine production because it determines the crop yield.

The stage of veraison, however, represent the transition from berry growth to berry ripening. Berry growth occurs, generally, in three phases: a rapid initial growth followed by a lag phase and finishing with the veraison phase. The first phase (Figure 1.c) is characterized by strong growth of the seed and berry; during this stage berries are dark green in colour and may double size in diameter. The next stage, know as lag phase, is a shorter period of slow growth. Finally, the final phase (Figure 1.d) of berry growth coincides with the beginning of fruit maturation, when acid level decrease, berries are soften, sugars are accumulated and varietal flavour and aromas develop.

The stage of harvest, that is the penultimate stage, describe the event in which the grape berry are removed from the plant. The ripeness and the measures of sugar, acid and tannin level determined the time of harvest. This stage is one of the most crucial steps in the process of wine-making.

Finally, in fall–winter, the grapevine enters in dormancy. In this phase, the vine create reserves in the vine’s trunk and roots and continues the process of photosynthesis. At that point the chlorophyll in the leaves begin to break down and the leaves change colour from green to yellow.

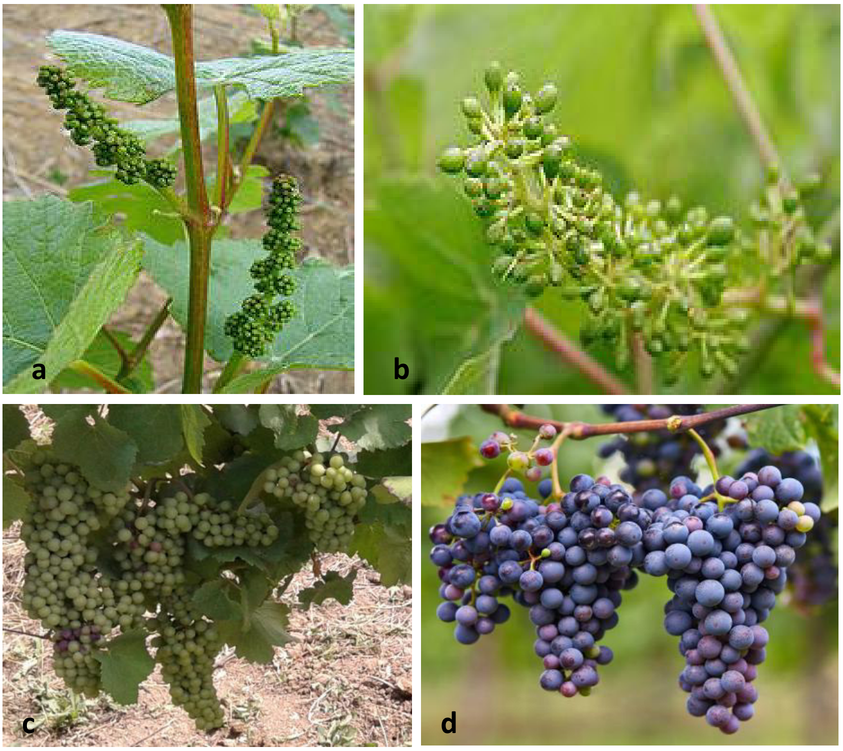


Figure 1.22 Images of phenological stage of V. vinifera: a) Flowering (inflorescence development); b) Fruit set (formation of young grapes); c) Fruit development; and d) Veraison (maturation of grapes)

1.6.2 Economic and cultural importance of grapevine

Vitis vinifera is one of the most important and widely cultivated fruit crops in the world due to its different uses such as production of wine, food product and grape juice. Furthermore, is a typical Mediterranean crop with a very relevant impact on the Italian economy, culture and landscape.

In the last few years has been estimated that the total area of vineyards in the world is 7.16 million hectares with a total production of wine amounted to 27million of tonnes (FAOSTAT 2013). Moreover, in Europe the country with highest area of vineyard is Spain, followed by France, Italy and Portugal (EUROSTAT 2014) (206).

In Italy, according to ISTAT, in 2011 over 750.000 hectares of territory were planted with vines, producing more than 72.000 tons of fruits; in the same year, wine production reached 43.000 tons with a net gain of more than 10 Billion Euro. In Piedmont, in particular, grapevine cultivation occurs in 54.000 hectares with a yield of 2.700 tons of high quality wine (about 6% of the total national production).

In terms of quantity and quality, the production of wine grapes is most influenced by three key factors: climate, soil and viticulture practices, which together are know as "terroir". In fact, variations in the location, soil, topography, and climate of single vineyards contribute to the diversity of wines. In other words, a wine produced in a given region is unique and this typical feature depends on the fruit composition which is due, in turn, to the growing in a specific geographical region, characterized by chemical and physical soil parameters, climate and by the specific interactions between the plant and the biotic and abiotic components of the surrounding environment (207). Therefore, the terroir may be also influenced by the local soil microbiota; grapevine microorganisms (including bacteria living both inside or outside plant tissues) and the plant-microbial interactions may influence the wine production and may condition the plant growth and the plant health status (89

Besides the economic importance, grapevine culture has an historical value in Piedmont; in fact, "vineyard landscape of Langhe-Roero and Monferrato" (Figure 1.23) has been added, in June 2014, to the list of UNESCO World Heritage Sites (http://whc.unesco.org/en/list/1390).

Figure 1.23 *Vineyard landscape of Langhe-Roero and Monferrato (Photo from UNESCO web site, http://whc.unesco.org)*

References:

(1) Soil Survey Staff (2010). Soil Taxonomy, eleventh ed. USDA National Resources Conservation Services, Washington DC

(2) Hartemink A.E. (2016). The Definition of Soil Since the Early 1800s. *Advancesin Agronomy.* Volume 137 Elsevier Inc. http://dx.doi.org/10.1016/bs.agron.2015.12.001

(3) Kennedy A.C. and Smith K.L. (1995). Soil microbial diversity and the sustainability of agricultural soils. *Plant and Soil*, 170: 75-86

(4) Nannipieri P., Ascher J., Ceccherini M.T., Landi L. Pietramellara G. and Renella G. (2003). Microbial diversity and soil functions. *European Journal of Soil Science*, 54: 655-670

(5) Rolf D. (2005). The metagenomics of soil. *Nature*, 3: 470-478

(6) Oades J.M. (1983). Soil orgnic matter and structural stability: mechanisms and implications for management. *Plant and Soil*, 76: 319-337

(7) Tisdall J.M. and Oades J.M. (1982). Organic matter and water-stable aggregates in soils. *J Soil Science,* 33: 141-163

(8) Six J., Bossuyt H., Degryze S. and Denef K. (2004). A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. *Soil & Tillage Research,* 79: 7-31

(9) Chenu C. and Cosentino D. (2011). Microbial Regulation of Soil Structural Dynamics. In: Architecture and Biology of Soils: Life in Inner Space. Ritz K. and Young I. (Eds.) Chap. 3, pp. 37-70

(10) Vos M., Wolf A.B., Jennings S.J. and Kowalchuk G.A. (2013). Micro-scale determinants of bacterial diversity in soil. *FEMS Microbiol Rev,* 1-19

(11) Torsvik V. and Øvreås L. (2002). Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion in Microbiology,* 5: 240-245

(12) Blagodatskaya E. and Kuzyakov Y. (2013). Active microorganisms in soil: critical review of estimation criteria and approaches. *Soil Biology & Biochemestry*, 67: 192-211

(13) Lemanceau P., Offre P., Mougel C., Gamalero E., Dessaux Y., Moënne-Loccoz Y. And Berta G. (2005). Microbial ecology of the rhizosphere. In: Microbiological methods for assessing soil quality. Bloem J., Hopkins D.W. and Benedetti A. (Eds) Chap 9.1, pp 228-230

(14) Watt M., Hugenholtz P., White R. and Vinall K. (2006). Numbers and locations of native bacteria on field-grown wheat roots quantified by fluorescence in situ hybridization (FISH). *Environ Microbiology*, 8: 871-884

(15) Hodge A., Paterson E., Grayston S.J., Campbell C.D., Ord B.G. and Killham K. (1998). Characterisation and microbial utilisation of exudate material from the rhizosphere of Lolium perenne grown under CO2 enrichment. *Soil Biology & Biochemestry,* 30: 1033-1043

(16) Ekschmitt K., Liu M.Q., Vetter S., Fox O. and Wolters V. (2005). Strategies used by soil biota to overcome soil organic matter stability - why is dead organic matter left over in the soil?. *Geoderma*, 128: 167-176

(17) Young I.M., Crawford J.W., Nunan N., Otten W. and Spiers A. (2008). Microbial distribution in soils: physics and scaling. In: Advances in

Agronomy. Sparks D.L. (Eds.) Elsevier Academic Press Inc, San Diego, pp. 81-121

(18) Young I.M. and Crawford J.W. (2004). Interactions and self-organization in the soil-microbe complex. *Science*, 304: 1634-1637

(19) Kuzyakov Y. and Blagodatskaya E. (2015). Microbial hotspots and hot moments in soil: concept & review. *Soil Biology and Biochemestry.,* 83: 184-189

(20) Marschner P., Marhan S. and Kandeler E. (2012). Microscale distribution and function of soil microorganisms in the interface between rhizosphere and detritusphere. *Soil Biology & Biochemestry*, 49: 174-183

(21) Jones D.L., Hodge A. and Kuzyakov Y. (2004). Plant and mycorrhizal regulation of rhizodeposition. *New Phytologist,* 163: 459-480

(22) Hinsinger P., Bengough A.G., Vetterlein D. and Young I.M. (2009). Rhizosphere: biophysics, biogeochemistry and ecological relevance. *Plant and Soil*, 321: 117-152

(23) Brown G.G., Barois I. and Lavelle P. (2000). Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains. *European Journal of Soil Biology*, 36: 177-198

(24) Tiunov A.V. and Scheu S. (2004). Carbon availability controls the growth of detritivores (Lumbricidae) and their effect on nitrogen mineralization. *Oecologia*, 138: 83-90

(25) Kaiser K. and Kalbitz K. (2012). Cycling downwards - dissolved organic matter in soils *Soil Biology & Biochemistry*, 52 : 9-32

(26) Torsvik V., Goksøyr J. and Daae F.L. (1990). High diversity in DNA of soil bacteria. *Appl Environ Microbiol*, 56(3): 782-787

(27) Gans J., Woilinsky M. and Dunbar J. (2005). Computational improvements reveal great bacterial diversity and high metal toxicity in soil. *Science*, 309: 1387-1390

(28) Schloss P.D. and Handelsman J. (2006). Toward a census of bacteria in soil. *PLOS Computational Biology*, 2: 786-793

(29) Horner-Devine M.C., Carney K.M. and Bohannan B.J.M. (2004). An ecological perspective on bacterial biodiversity. *Proc R Soc Lond,* 271: 113-122

(30) Zhao L. and Shen J. (2010). Whole-body systems approaches for gut microbiota-targeted, preventive healthcare. *J Biotechnol,* 149: 183-190

(31) Gevers D., Knight R., Petrosino J.F., Huang K., McGuire A.L., Birren B.W., Nelson K.E., White O., Methé B.A. and Huttenhower C. (2012). The human microbiome project: a community resource for the healthy human microbiome. *PLoS Biol,* 10(8): e1001377

(32) Berg G. and Smalla K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiol Ecol,* 68: 1-13

(33) Singh B.K., Nunan N. and Millard P. (2009). Response of fungal, bacterial and ureolytic communities to synthetic sheep urine deposition in a grassland soil. *FEMS Microbiol Ecol,* 70: 109-117

(34) Or D., Smets B.F., Wraith J.M., Dechesne A. and Friedman S.P. (2007b). Physical constraints affecting bacterial habitats and activity in unsaturated porous media – a review. *Adv Water Resour,* 30: 1505-1527

(35) Fierer N., Lauber C.L., Ramirez K.S., Zaneveld J., Bradford M.A. and Knight R. (2012). Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME J,* 6: 1007-1017

(36) Rousk J., Baath E., Brookes P.C., Lauber C.L., Lozupone C., Caporaso J.G., Knight R. and Fierer N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. I*SME J,* 4: 1340-1351

(37) Rajaniemi T.K. and Allison V.J. (2009). Abiotic conditions and plant cover differentially affect microbial biomass and community composition on dune gradients. *Soil Biol Biochem,* 41: 102-109

(38) Sharma S.B., Sayyed R.Z. Trivedi M.H. and Gobi T.A. (2013). Phosphate solubilising microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springerplus,* 2: 587.

(39) Karlen D.L., Mausbach M.J., Doran J.W., Cline R.G., Harris R.F. and Schuman G.E. (1997). Soil quality: A concept, definition, and framework for evaluation. *Soil Sci Soc AmJ,* 61:4-10

(40) Garbeva P., van Veen J.A. and van Elsas J.D. (2004). Microbial diversity in soil: Selection of Microbial populations by Plant and soil type and implications for disease suppressiveness. *Annu Rev Phytopathol*, 42:243-270

(41) Mendes R., Garbeva P. and Raaijmakers J.M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic ì, and human pathogenic microorganisms. *FEMS Microbiol Rev,* 37: 634-663

(42) Hillebrand H., Bennett D.M. and Cadotte M.W. (2008). Consequences of dominance: a review of evenness effects on local and regional ecosystem processes. *Ecology,* 89(6): 1510-1520

(43) Hartmann A., Rothballer M. and Schmid M. (2008). Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant Soil*, 312: 7-14

(44) Lynch J.M. (1990) Some consequences of microbial rhizosphere competence for plant and soil. In: The rhizosphere. Lynch J.M. (Eds). Wiley Interscience, New York, pp 1-10

(45) Bertin C., Yang X. and & Leslie A. Weston L.A. (2003). The role of root exudates and allelochemicals in the rhizosphere. *Plant and Soil,* 256: 67-83

(46) Morgan J.A.W., Bending G.D. and White P.J. (2005). Biological costs and benefits to plant–microbe interactions in the rhizosphere. *J Exp Bot,* 56: 1729-1739

(47) Prashar P., Kapoor N. and Sachdeva S. (2014). Rhizosphere: its structure, bacterial diversity and significance. *Rev Environ Sci Biotechnol*, 13: 63-77

(48) Velivelli S.L.S, Sessitsch A. and Prestwich B.D. (2015). The Role of Microbial Inoculants in Integrated Crop Management Systems. *Potato research,* DOI 10.1007/s11540-014-9278-9

(49) Gobat J.M., Aragno M. and Matthey W. (2004). The living soil: fundamentals of soil science and soil biology. Science Publishers, USA

(50) McNear Jr. D.H. (2013). The rhizosphere- roots, soil and everything in Between. *Nature Edu Know* 4(3):1

(51) Grayston S.J., Vaughan D. and Jones D. (1996). Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. *Appl Soil Ecol*, 5: 29-56

(52) Singh B.K., Millard P., Whiteley A.S., Murrell J.C. (2004). Unravelling rhizosphere–microbial interactions: opportunities and limitations. *Trends Microbiol,* 12: 386-393

(53) Jones D.L., Nguyen C. and Finlay R.D. (2009). Carbon flow in the rhizosphere: carbon trading at the soil–root interface. *Plant Soil*, 321: 5-33

(54) Farrar J., Hawes M., Jones D.L. and Lindow S. (2003)**.** How roots control the flux of carbon to the rhizosphere. *Ecology,* 84: 827-833.

(55) Nguyen C. (2003). Rhizodeposition of organic C by plants: mechanisms and controls. *Agronomie,* 23: 375-396

(56) Bulgarelli D., Schlaeppi K., Spaepen S., Ver Loren van Themaat E. and Schulze-Lefert P. (2013). Structure and functions of the bacterial microbiota of plants. *Annu Rev Plant Biol*, 64: 807-838.

(57) Turner T.R., James E.K. and Poole P.S.(2013). The plant microbiome. *Genome biology*, 14: 209

(58) Dotaniya M.L. and Meena V.D. (2015). Rhizosphere Effect on Nutrient Availability in Soil and Its Uptake by Plants: A Review. *Proc. Natl. Acad. Sci., India, Sect B Biol Sci*, 85(1): 1-12

(59) Pinton R., Varanini Z and Nannipieri P. (2001). The rhizosphere. New York USA: Marcel Dekker Inc.

(60) Bais H.P., Weir T.L., Perry L.G., Gilroy S. and Vivanco J.M. (2006). The reole of Root Exudates in rhizosphere interactions with Plants and Other organisms. *Annu Rev Plant Biol*, 57: 233-266

(61) Huang X-F., Chaparro J.M., Reardon K.F., Zhang R., Shen Q. and Vivanco J.M. (2014). Rhizosphere interctions: root exudates, micobes and microbial communities. *Botany*, 92: 267-275

(62) Glick B.R. (2016). Beneficial Plant-Bacteriaal Interactions. Springer International Publishing Switzerland. DOI 10.1007/978-3-319-13921-0_1

(63) Walker T.S., Bais H.P., Grotewold E. and Vivanco J.M. (2003). Root exudation and rhizosphere biology. *Plant physiol*, 132: 44-51

(64) Garbeva P., van Elsas J.D. and van Veen J.A. (2008). Rhizosphere microbial communities and its response to plant species and soil history. *Plan Soil*, 302: 19-32

(65) Dennis P.G., Miller A.J. and Hirsch P.R. (2010). Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities?. *FEMS Microbiol Ecol*, 72: 313-327

(66) Berendesen R.L., Pieterse C.M.J. and Bakker P.A.H.M. (2012).The rhizosphere microbiome and plant health. *Trends in Plant Science,* 17(8): 478-486

(67) Compant S., Duffy B., Nowak J., Clément C. and Barka E.A. (2005). Use of Plant Growth-Promoting Bacteria for biocontrol of plant Diseases: Principles, Mechanisms of ction and future prospects. *Applied and Environmental Microbiology,* 71: 4951-4959

(68) Lugtenberg B. and Kamilova F. (2009). Plant Growth-Promoting Rhizobacteria. *Annu Rev Microbiol,* 63: 541-556

(69) Atlas R. and Bartha R. (1997). Microbial ecology. Addison Wesley Longman, New York

(70) Mougel C., Offre P., Ranjard L., Corberand T., Gamalero E., Robin C. and Lemanceau P. (2006). Dynamic of the genetic structure of bacterial and fungal communities at different developmental stages of Medicago truncatula Gaertn. cv. Jemalong line J5. *New Phytologist,* 170(1): 165-175

(71) Houlden A., Timms-Wilson T.M., Day M.J. and Bailey M.J. (2008). Influence of plant developmental stage on microbial community structure and activity in the rhizosphere of three field crops. *FEMS Microbiol Ecology*, 65(2): 193-201

(72) Micallef S.A., Channer S., Shiaris M.P. and Colón-Carmona A. (2009). Plant age and genotype impact the progression of bacterial community succession in the Arabidopsis rhizosphere. *Plant Signal Behav*, 4(8): 777-780

(73) Xu Y., Wang G., Jin J., Liu J., Zhang Q. and Liu X. (2009). Bacterial communities in soybean rhizosphere in response to soil type, soybean genotype, and their growth stage. *Soil Biol Biochem*, 41(5): 919-925

(74) Chaparro J.M., Badri D.V., Bakker M.G., Sugiyama A., Manter D.K. and Vivanco J.M. (2013). Exudation of Phytochemicals in *Arabidopsis* follows specific patterns that are developmentally programmed and correlate with soil microbial functions. *PLoS One,* 8 (2): 1-10

(75) Sugiyama A., Ueda Y., Zushi T., Takase H. and Yazaki K. (2014). Changes in the bacterial community of soybean rhizospheres during growth in the field. *PLoS One*, 9(6): e100709

(76) Li X., Rui J., Maoa Y., Yannarell A. and Mackie R. (2014). Dynamics of the bacterial community structure in the rhizosphere of a maize cultivar. *Soil Biology & Biochemistry*, 68: 392-401

(77) Barriuso J., Ramos Solano B., Lucas J.A., Probanza Lobo A., Garcìa-Villaraco A. and Gutiérrez Mañero F.J. (2008). Ecology, Genetic diversity and screening strategies of Plant Growth-promoting Rhizobacteria (PGPR). In: Plant-Bacteria Interactions, strategies and Techniques to promote plant growth. Ahmad I., Pitchel J. and Hayat S. (Eds). Chap 1

(78) Fakruddin M.D. and Mannan K.S.B. (2013). Methods for Analyzing diversity of Microbial Communities in Natural Environments. *Ceylon Journal of Science* (*Bio Sci*) 42(1): 19-33

(79) Raaijmakers J.M., Paulitz T.C., Steinberg C., Alabouvette C. and Moënne-Loccoz Y. (2009). The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant Soil*, 321: 341-361

(80) Philippot L., Raaijmakers J.M., Lemanceau P. and van der Putten W.H. (2013). Going back to the roots: the microbial ecology of the rhizosphere. *NATURE Reviews Microbiol,* 11: 789-799

(81) Berg G., Opelt K., Zachow C., Lottmann J., Götz M., Costa R. and Smalla K. (2006). The rhizosphere effect on bacteria antagonistic towards the pathogenic fungus *Verticillium* differs depending on plant species and site. *FEMS Microbiol Ecol,* 56: 250-261

(82) Delmotte N., Knief C., Chaffron S., Innerebner G., Roschitzki B., Schlapbach R., Mering C. and von Vorholt J.A. (2009). Community proteogenomics reveales insights into the physiology of phyllosphere bacteria. *Proc Natl Acad Sci*, 106: 16428-16433

(83) Bulgarelli D., Garrido-Oter R., Münch P.C., Weiman A., Dröge J., McHardy A.C. and Schulze-Lefert P. (2015). Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host & Microbe,* 17: 392-403

(84) Marschner P., Crowley D and Rengel Z. (2011). Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis – model and research methods. *Soil Biology & Biochemistry*, 43: 883-894

(85) Lagos M.L., Maruyama F., Nannipieri P., Mora M.L., Ogram A. and Jorquera M.A. (2015). Current overview on the study of bacteria in the rhizosphere by modern molecular techniques: a mini-review. *Journal of Soil Science and Plant Nutrition,* 15: 504-523

(86) Miethling R., Wieland G., Backhaus H. and Tebbe C.C. (2000). Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with *Sinorhizobium meliloti* L 33. *Microb Ecol,* 40: 43-56

(87) Yang C., Hamel C., Gan Y. and Vujanovic V. (2012). Pyrosequencing reveals how pulses influence rhizobacterial communities with feedback on wheat growth in the semiarid Prairie. *Plant Soil,* 367: 493-505

(88) Aislabie J. and Deslippe J.R. (2013). Soil Microbes and their contribution to soil services. In: Ecosystem services in New Zealand – conditions and trends. Dymond J.R. (Eds). Chap 1.12, pp 143-161

(89) Janssen P.H. (2006). Identifying the Dominant Soil Bacterial Taxa in Libraries of 16S rRNA and 16S rRNA Genes MINIREVIEWS Identifying the Dominant Soil Bacterial Taxa in Libraries of 16S rRNA and 16S rRNA Genes. *Appl Environ Microbiol*, 72(3): 1719-1728

(90) Nacke H., Thürmer A., Wollherr A., Will C., Hodac L., Herold N., Schöning I., Schrumpf M. and Daniel R. (2011). Pyrosequencing-Based assessment of bacterial community structure along different management types in German forest and grassland soils. *PLoS One*, 6(2): e17000

(91) Opsi F., Landa B., Zecca O., Biddoccu M., Barmaz A. and Cavallo E. (2014). Diversity in soil bacterial communities structure in four high-altitude vineyards cultivated using different soil management techniques. *Geophys Res Abstr EGU Gen Assem*, 16: 14297.

(92) Zarraonaindia I,. Owens S.M., Weisenhorn P., West K., Hampton-Marcell J., Lax S., Bokulich N.A., Mills D.A., Martin G., Taghavi S., van der Lelie D. And Gilbert J.A. (2015). The Soil Microbiome Influences Grapevine-Associated Microbiota. *mBio*, 6(2): e02527-14

(93) Cheng Z., McConkey B.J. and Glick B.R. (2010). Proteomic studies of plant-bacterial interactions. *Soil Biol Biochem,* 42: 1673-1684

(94) Nguyen T.T., Fuentes S. and Marschner P. (2013). Effect of incorporated or mulched compost on leaf nutrient concentrations and performance of *Vitis vinifera* cv. Merlot. *J Soil Sci Plant Nutr,* 13: 485-497

(95) Newman M.M., Hoilett N., Lorenz N., Dick R.P., Liles M.R., Ramsier C. and Kloepper J.W. (2016). Glyphosate effects on soil rhizosphere-associated bacterial communities. *Science of the total Environment*, 543: 155-160

(96) Pinto C., Pinho D., Sousa S., Pinheiro M., Egas C. and Gomes A.C. (2014). Unravelling the Diversity of grapevine Microbiome. *PLoS One*, 9(1): e85622

(97) Glick B.R. (1995). The enhancement of plant growth by free living bacteria. *Canadian Journal of Microbiology*, 41 (2):109-114.

(98) Zahir Z.A., Arshad M. And Frankenberger Jr., W.T. (2004). Plant growth promoting rhizobacteria: applications and perspectives in agriculture. *Adv Agron,* 81, 97-168.

(99) Glick B.R. (2005). Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. *FEMS Microbiology Letters,* 251: 1-7

(100) Compant S., Clément C. and Sessitsch A. (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization,

mechanisms involved and prospects for utilization. *Soil Biol Biochem* 42: 669-678

(101) Lau J.A. and Lennon J.T (2011). Evolutionary ecology of plnt-microbe interactions: soil m.icrobial structure alters selection on plant traits. *New Phytologist,* 192(1): 215-224

(102) De Souza R., Ambrosini A. and Passaglia L.M.P. (2015). Plant growth-promoting bacteria as inoculants in agricultural soils. *Genet Mol Biol,* 38(4): 401-419

(103) Bashan Y., Puente M.E., de-Bashan L.E. and Hernandez J-P. (2008). Environmental uses of plant growth-promoting bacteria. In: Plant-Microbe Interactions, Barka E.A. and Clément C. (Eds). Chap 4, pp 69-93

(104) Gamalero E., Lingua G., Berta G. and Glick B.R. (2009). Beneficial role of plant growth bacteria and arbuscular mychorrhizal fungi on plant responses to heavy metal stress. *Can J Microbiol,* 55: 501-51

(105) Zhang H., Xue Y., Wang Z., Yang J. and Zhang J. (2009a). Morphological and physiological traits of roots and their relationships with shoot growth in super rice. *Field Crops Res,* 113: 31-40

(106) Lingua G., Bona E., Manassero P., Marsano F., Todeschini V., Cantamessa S., Copetta A., D'Agostino G., Gamalero E. and Berta G. (2013). Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads increases anthocyanin concentration in strawberry fruits (*Fragaria x ananassa* var. Selva) in conditions of reduced fertilization. *Int J Mol Sci,*14(8): 16207-16225.

(107) Berta G., Copetta A., Gamalero E., Bona E., Cesaro P., Scarafoni A. and D'Agostino G. (2014). Maize development and grain quality are differentially affected by mycorrhizal fungi and a growth-promoting

pseudomonad in the field. *Mycorrhiza*, 24: 161-170.

(108) Bona E., Lingua G., Manassero P., Cantamessa S., Marsano F., Todeschini V., Copetta A., D'Agostino G., Massa N., Avidano L., Gamalero E. and Berta G. (2015). AM fungi and PGP pseudomonads increase flowering, fruit production, and vitamin content in strawberry grown at low nitrogen and phosphorus levels. *Mycorrhiza*, 25(3): 181-193.

(109) Bona E, Lingua G, Todeschini V. Effect of bioinoculants on the quality of crops. In: Arora N, Mehnaz S, Balestrini R, (eds) Biformulations: for sustainable agriculture. Springer India. 2016. doi: 10.1007/978-81-322-2779-3_5.

(110) Bashan Y. and Holguin G. (1998). Proposalfor the division of plant growth promoting Rhizobacteria in two classidications: biocontrol-PGPB (Plant Growth-Prromoting Bacteria) and PGPB. *Soil Biol Biochem*, 30: 1225-1228

(111) Kloepper J.W., Leong J., Teintze M. and Schroth M.N. (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature,* 286: 885-886

(112) de-Bashan L.E., Hernandeza J-P. and Bashan Y. (2012). The potential contribution of plant growth-promoting bacteria to reduce environmental degradation – A comprehensive evaluation. *Applied Soil Ecology,* 61: 171-189

(113) Kloepper J.W. and Schroth M.N. (1978). Plant growth-promoting rhizobacteria on radishes. In: Proceedings 4th international conference on plant pathogenic bacteria. Gilbert C. (Eds.), pp 879-882

(114) Rodriguez H., Fraga R., Gonzalez T. and Bashan Y. (2006). Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant and Soil*, 287: 15-21

(115) Hayat R., Ali S., Amara U., Khalid R. and Ahmed I. (2010). Soil beneficial bacteria and their role in plant growth promotion: a review. *Ann Microbiol*, 60: 579-598

(116) Vejan P., Abdullah R., Khadiran T., Ismail S. and Boyce A.N. (2016). Role of Plant Growth Promoting Rhizobacteria in Agricultural Sustainability - A Review. *Molecules,* 21: 573

(117) Islam Md.R., Madhaiyan M., Deka Boruah H.P., Yim W., Lee G., Saravanan V.S., Fu Q., Hu H. and Sa T. (2009). Characterization of Plant Growth-Promoting Traits of Free-Living Diazotrophic Bacteria and Their Inoculation Effects on Growth and Nitrogen Uptake of Crop Plants. *J Microbiol Biotechnol*, 19(10): 1213-1222

(118) Antoun H. and Prevost D. (2005). Ecology of plant growth promoting rhizobacteria. In: PGPR: Biocontrol and Biofertilization, Siddiqui Z.A. (Eds.) Chap 1, pp 1-38

(119) Barea J.M., Pozo M.J., Azcón R. and Azcón-Aguilar C. (2005). Microbial co-operation in the rhizosphere. *Journal of Experimental Botany*, 56: 1761-1778

(120) Gray E.J. and Smith D.L. (2005). Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signalling processes. *Soil Biology & Biochemistry,* 37: 395-412

(121) Gamalero E., Lingua G., Caprì F.G., Fusconi A., Berta G. and Lemanceau P. (2004). Colonization pattern of primary tomato roots by *Pseudomonas fluorescens* A6RI characterized by diluition plating, flow cytometry,

fluorescence, confocal and scanning electron microscopy. *FEMS Microbiology Ecology,* 48: 79-87

(122) Wu C.H., Bernard S.M., Andersen G.L. and Chen W. (2009). Developing microbe–plant interactions for applications in plant-growth promotion and disease control, production of useful compounds, remediation and carbon sequestration. *Microb Biotechnol*, 2: 428-440

(123) Sessitsch A., Coenye T., Sturz A.V., Vandamme P., Barka E.A., Salles J.F., van Elsas D., Faure J.D., Reiter B., Glick B.R., Pruski G.W. and Nowak J. (2005*). Burkholderia phytofirmans* sp. nov., a novel plant-associated bacterium with plant-beneficial properties. *Int J Sys Evol Microbiol*, 55:1187-1192

(124) Soltani A.A., Khavazi K., Rahmani H.A., Omidvari M., Dahaji P.A. and Mirhoseyni H. (2010). Plant growth promoting characteristics in some *Flavobacterium spp*. isolated from soils of Iran. *J Agri Sci,* 2: 106-115

(125) Glick B.R. (2012). Plant growth promoting bacteria: mechanisms and applications. *Scientifica*

(126) Gamalero E. and Glick B. R. (2015). Bacterial modulation of plant ethylene levels. *Plant Physiology,* 169: 13-22

(127) Bøckman O.C. (1997). Fertilizers and biological nitrogen fixation as sources of plant nutrients: perspectives for future agriculture. *Plant and Soil*, 194: 11-14.

(128) Ahemad M. and Kibret M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *Journal of King Saud University – Science*, 26: 1-20

(129) Dean D.R. and Jacobson R. (1992). Biochemical genetics of nitrogenase. In: Nitrogen fixation, Stacey G., Burris R.H., Evans H.J. (Eds.), pp 763-817

(130) Franche C., Lindström K. and Elmerich C. (2009). Nitrogen-fixing bacteria associated with leguminous and non-legumious plants. *Plant Soil*, 321: 35-59

(131) Gamalero E. and Glick B. R. (2011). Mechanisms used by Plant Growth-Promoting Bacteria. In: Bacteria in Agrobiology: Plant Nutrient Management, Maheshwari D.K. (Eds.), Chap 2, pp 17-46

(132) Ehrlich H.L. (1990). Mikrobiologische und biochemische Verfahren stechnik. In: Einsele, A., Finn, R.K., Samhaber, W . (Eds.), Geomicrobiology, second ed. VCH Verlagsgesellschaft, Weinheim

(133) Khan M.S., Zaidi A., Ahemad M., Oves M. and Wani P.A. (2010). Plant growth promotion by phosphate solubilizing fungi – current perspective. *Arch Agron Soil Sci,* 56: 73-98

(134) Rodriguez H. and Fraga R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv,* 17: 319-339

(135) Richardson A.E., Barea J.M., McNeill A.M. and Prigent-Combaret C. (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil,* 321: 305-339

(136) Bashan Y., Kamnev A.A. and de Bashan L.E. (2013a). A proposal for isolating and testing phosphate-solubilizing bacteria that enhance plant growth. *Biol Fertil Soils,* 49: 1-2

(137) Gupta G., Parihar S.S., Ahirwar N.K., Snehi S.K. and Singh V. (2015). Plant Growth Promoting Rhizobacteria (PGPR): Current and future

prospects for development of sustainable agriculture. J *Microb Biochem Technol,* 7: 2

(138) Ma J.F. (2005). Plant root responses to three abundant soil minerals: silicon, aluminum and iron. *Crit Rev Plant Sci*, 24: 267-281

(139) Cornell R.M. and Schwertmann U. (2003). The Iron Oxides, 2nd edn. Wiley-VCH, Weinheim, New York

(140) Kobayashi T. and Nishizawa N.K. (2012). Iron uptake, translocation, and regulation in higher plants. *Annu Rev Plant Biol,* 63: 131– 152

(141) Mimmo T., Del buono D., Terzano R., Tomasi N., Vigani G., Crecchio C., Pinton R., Zocchi G. and Cesco S. (2014). Rhizospheric organic compounds in the soil–microorganism–plant system: their role in iron availability. *European Journal of Soil Science,* 65: 629-642

(142) Crowley D.E. (2006). Microbial siderophores in the plant rhizosphere. In: Iron nutrition in plants and rhizospheric microorganisms. Barton L.L. and Abadía J. (Eds). pp 169-198

(143) Shilev S. (2013). Soil Rhizobacteria Regulating the Uptake of Nutrients and Undesirable Elements by Plants. In: Plant Microbe Symbiosis: Fundamentals and Advances. Arora N.K. (Eds.) Chap 5, pp. 147-167

(144) Beneduzi A., Ambrosini A. and Passaglia L.M.P. (2012). Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology*, 35: 1044-1051

(145) Wani S.H., Kumar V., Shriram V. and Sah S.K. (2016). Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *The Crop Journal,* 4: 162-176

(146) Spaepen S. and Vanderleyden J. (2011). Auxin and Plant-Microbe Interactions. Cold Spring Harbor Laboratory Press

(147) Patten C. and Glick B.R. (1996). Bacterial biosynthesis of indole-3-acetic acid. *Can J Microbiol*, 42: 207-220

(148) Muller B. and Sheen J. (2007). Advances in cytokinin signaling. *Science,* 318: 68-69

(149) Hussain A. and Hasnain S. (2009). Cytokinin production by some bacteria: its impact on cell division in cucumber cotyledons. *Afr J Microbiol Res,* 3: 704-712

(150) Kundan R., Pant G., Jadon N. and Agrawal P.K. (2015). Plant Growth Promoting Rhizobacteria: Mechanism and Current Prospective. *Fertil Pestic*, 6: 2

(151) MacMillan J. (2001). Occurrence of Gibberellins in Vascular Plants, Fungi, and Bacteria. *J Plant Growth Regul*, 20(4): 387-442

(152) Bottini R., Cassán F. and Piccoli P. (2004). Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. *Appl Microbiol Biotechnol,* 65: 497-503

(153) Glick B.R. (2014). Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiological Research,* 169**:** 30-39

(154) Contesto C., Desbrosses G., Lefoulon C., Béna G., Borel F., Galland M., Gamet L., Varoquaux F. and Touraine B. (2008). Effects of rhizobacterial ACC deaminase activity on Arabidopsis indicate that ethylene mediates local root responses to plant growth-promoting rhizobacteria. *Plant Science*, 175: 178-189

(155) Gamalero E., Cesaro P., Cicatelli A., Todeschini V., Musso C., Castiglione S., Fabiani A. and Lingua G. (2012). Poplar clones of different sizes, grown on a heavy metal polluted site, are associated with microbial populations of varying composition. *Science of the Total Environment*, 425: 262-270

(156) Singh R.P., Shelke G.M., Kumar A. and Jha P.N. (2015). Biochemistry and genetics of ACC deaminase: a weapon to "stress ethylene" produced in plants. *Front Microbiol*, 6: 937.

(157) Glick B.R. (2007). Promotion of plant growth by bacterial ACC deaminase. *Crit Rev Plant Sci*, 26: 227-242.

(158) Penrose D.M., Moffatt B.A. and Glick B.R. (2001). Determination of 1-aminocycopropane-1-carboxylic acid (ACC) to assess the effects of ACC deaminase-containing bacteria on roots of canola seedlings. *Can J Microbiol,* 47: 77-80

(159) Mayak S., Tirosh T. and Glick B.R. (2004). Plant growth-promoting bacteria that confer resistance to water stress in tomato and pepper. *Plant Sci*, 166: 525-530

(160) Belimov A.A., Dodd I.C., Hontzeas N., Theobald J.C., Safronova V.I. and Davies W.J. (2009). Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase yield of plants grown in drying soil via both local and systemic hormone signalling. *New Phytol*, 181: 413-423

(161) Gamalero E., Berta G., Massa N., Glick B.R. and Lingua G. (2010). Interactions between *Pseudomonas putida* UW4 and *Gigaspora rosea* BEG9 and their consequences on the growth of cucumber under salt stress conditions. *J Appl Microbiol*, 108: 236-245

(162) Hao Y., Charles T.C. and Glick B.R. (2011) An ACC deaminase containing *A. tumefaciens* strain D3 shows biocontrol activity to crown gall disease. *Can J Microbiol*, 57: 278-286

(163) Gamalero E., Marzachì C., Galetto L., Veratti F., Massa N., Bona E., Novello G., Glick B. R., Ali S., Cantamessa S., D'Agostino G. and Berta G. (2016). An 1-Aminocyclopropane-1-carboxylate (ACC) deaminase-expressinf endophyte invreases plant resistance to flavescence dorée phytoplasma infection. *Plant Biosystems*, DOI: 10.1080/11263504.2016.1174172

(164) Whipps J.M. (2001). Microbial interactions and biocontrol in the rhizosphere. *Exp. Bot.,* 52(1): 487-511

(165) Haas D. and Défago G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews in Microbiology,* 3: 307-319

(166) Van Peer R., Niemann G.J. and Schippers B. (1991). Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas* sp. strain WCS417r. *Phytopathology,* 81: 728-734

(167) Alström S. (1991). Induction of disease resistance in common bean susceptible to halo blight bacterial pathogen after seed bacterization with rhizosphere pseudomonads. *Journal of General and Applied Microbiology,* 37: 495-501

(168) Wei G., Kloepper, J.W. and Tuzun S. (1991). Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant-growth promoting rhizobacteria. *Phytopathology* 81: 1508-15

(169) Pieterse C.M., Zamioudis C., Berendsen R.L., Weller D.M., Van Wees S.C. and. Bakker P.A. (2014). Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology,* 52: 347-375

(170) Conrath U. (2011). Molecular aspects of defence priming. *Trends Plant Science,* 16: 524-531.

(171) Vallad and Goodmaan (2004). Systemic Acquired resistance and induced systemic resistanc in conventional Agriculture. *Crop Sci,* 44: 1920-1934

(172) Ahmad F., Ahmad I. and Khan M.S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research*, 163: 173-181

(173) Hill G.T., Mitkowskia N.A., Aldrich-Wolfe L., Emelea L.R., Jurkonie D.D., Ficke A., Maldonado-Ramireza S., Lyncha S.T., and Nelsona E.B. (2000). Methods for assessing the composition and diversity of soil microbial communities . *Applied Soil Ecology,* 15: 25-36

(174) Kirk J.L., Beaudettea L.A., Hartb M., Moutoglisc P., Klironomosb J.N., Leea H. and Trevorsa J.T. (2004). Methods of studying soil microbial diversity. *Journal of Microbiological Methods*, 58: 169-188

(175) Hugenholtz P. and Pace N.R. (1996). Identifying microbial diversity in the natural environment: a molecular phylogenetic approach. *Trends Biotechnol.*, 14(6): 190-197

(176) Pinto D., Santos M.A. and Chambel L. Thirty years of viable but nonculturable state research: unsolved molecular mechanisms. *Crit Rev Microbiol (in press)*, doi:10.3109/1040841X.2013.79412

(177) Mukamolova G.V., Kaprelyants A.S., Young D.I., Young M. and Kell D.B. (1998). A bacterial cytokine. *P Natl Acad Sci*, 95: 8916-8921

(178) Signoretto C. and Canepari P. (2008). Towards more accurate detection of pathogenic Gram-positive bacteria in waters. *Curr Opin Biotech.,* 19: 248-253

(179) Kent A.D. and. Triplett E.W. (2002). Microbial communities and their interactions in soil and rhizosphere ecosystems. *Annu. Rev. Microbiol.*, 56: 211-236

(180) Ward D.M., Bateson M.M., Weller R. and Ruff-Roberts A.L. (1992). Ribosomal RNA analysis of microorganisms as they occur in nature, p.219-286. In K. C. Marshall (ed.), Advances in Microbial Ecology, vol. 12. Plenum Press, New York, U.S.A

(181) van Elsas J.D. and Boersma F.G.H. (2011). A review of molecular methods to study the microbiota of soil and the mycosphere. *European Journal of Soil Biology,* 47: 77-87

(182) Wakelin S., Mander C., Gerard E., Jansa J., Erb A., Young S., Condron L. and O'Callaghan M. (2012). Response of soil microbial communities to contrasted histories of phosphorus fertilisation in pastures. *Appl Soil Ecol,* 61: 40-48.

(183) Rincon-Florez V.A., Carvalhais L.C. and Schenk P.M. (2013). Culture-Independent Molecular Tools for Soil and Rhizosphere Microbiology**.** *Diversity*, 5: 581-612

(184) Sun J., Zhang Q., Zhou J. and Wei Q. (2014). Illumina amplicon sequencing of 16S rRNA tag reveals bacterial community development in the rhizosphere of apple nurseries at a replant disease site and a new planting site. *PLoS ONE*, 9: e111744

(185) Inceoğlu Ő., Waleed Abu Al-Soud, Joana Falcão Salles1, Alexander V. Semenov1, and van Elsas J.D. (Comparative Analysis of Bacterial

Communities in a Potato Field as Determined by Pyrosequencing. *PLoS ONE*, 6(8): e23321. doi:10.1371/journal.pone.0023321

(186) Castañeda L.E. and Barbosa O. (2016). Metagenomic analysis exploring taxonomic and functional diversity of soil microbial communities in Chilean vineyards and surrounding native forests. *PeerJ PrePrints*, 4: e16661v1.

(187) Correa-Galeote D., Bedmar E.J., Fernández-González A.J., Fernández-López M. and Arone G.J. (2016). Bacterial Communities in the Rhizosphere of Amilaceous Maize (*Zea mays* L.) as Assessed by Pyrosequencing. *Frontiers in Plant Science*, 7

(188) Lagos L., Navarrete O., Maruyama F., Crowley D.E., Cid F., Mora M.L. and Jorquera M.A. (2014). Bacterial community structures in rhizosphere microsites of ryegrass (*Lolium perenne* var. Nui) as revealed by pyrosequencing. *Biol Fert Soils*, 50: 1253-1266

(189) Breidenbach B., Pump J. and Dumont M.G. (2016). Microbial Community Structure in the Rhizosphere of Rice Plants. *Frontiers in Microbiology*, 6

(190) Uroz S., Buée M., Murat C., Frey–Klett P. and Martin F. (2010). Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. *Environ Microbiol,*2: 281-288

(191) Handelsman J. (2004). Metagenomics: Application of Genomics to Uncultured Microorganisms. *Microbiology and Molecular Biology Reviews,* 68: 669-685

(192) Riesenfeld C.S., Schloss P.D. and Handelsman J. (2004). Metagenomics: Genomic Analysis of Microbial Communities. *Annu Rev Genet,* 38: 525-52

(193) Leveau J.H.J. (2007). The magic and menace of metagenomics: prospects for the study of plant growth-promoting rhizobacteria. *Eur J Plant Pathol*, 119: 279-300

(194) Chaparro J.M., Badri D.V., Bakker M.G., Sugiyama A., Manter D.K. and VivancoJ.M. (2013). Exudation of Phytochemicals in *Arabidopsis* follows specific patterns that are developmentally programmed and correlate with soil microbial functions. *PLoS One,* 8(2): 1-10

(195) Hu J., Wei Z., Friman V-P., Gu S., Wang W.F., Eisenhauer N., Yang T-J., Ma J., Shen Q., Xu Y. and Jousset A. (2016). Probiotic diversity enhances rhizosphere microbiome function and Plant disease suppression. *mBio,* 7(6): e01790-16

(196) Franzosa E.A., Hsu T., Sirota-Madi A., Shafquat A., Abu-Ali G., Morgan X.C. and Huttenhower C. (2015). Sequencing and beyond: integrating molecular ‘omics for microbial community profiling. *Nat Rev Microbiol,* 13(6): 360-372

(197) Wilmes P. and L. Bond P.L. (2004). Metaproteomics: studying functional gene expression in microbial Ecosystems. *TRENDS in Microbiology*, 14

(198) Bastida F., Moreno J.L., Nicolás C., Hernández T. and Garcìa C. (2009). Soil metaproteomics: a review of an emerging environmental science. Significance, methodology and Perspectives. *European Journal of Soil Science*, 60: 845-859

(199) Moretti M., Minerdi D., Gehrig P., Garibaldi A., Gullino M.L. and Riedel K. (2012). A bacterial−fungal metaproteomic analysis enlightens an intriguing multicomponent interaction in the rhizosphere of *Lactuca sativa. Proteome Res.*, 11: 2061-2077.

(200) Lin W., Wu L., Lin S., Zhang A., Zhou M., Lin R., Wang H., Chen J., Zhang Z. and Lin R. (2013). Metaproteomic analysis of ratoon sugarcane rhizospheric soil. *BMC Microbiol,*13: 135-148.

(201) Sørensen J., Haubjerg Nicolaisen M., Ron E. and Simonet P. (2009). Molecular tools in rhizosphere Microbiology from single–cell to whole–community analysis. *Plant Soil*, 321: 483-512.

(202) Hirsch P.R., Mauchline T.H. and Clark I.M. (2010). Culture–independent molecular techniques for soil microbial ecology. *Soil Biology & Biochemestry,* 42: 878-887

(203) Maron P.A., Ranjard L., Mougel C. and Lemanceau P. (2007). Metaproteomics: A New Approach for Studying Functional Microbial Ecology. *Microbial Ecology,* 53: 486-493

(204) Eynard I. e Dalmasso G. (1990). Viticoltura moderna, IX edizione, Hoepli, Milano, 53-79.

(205) Jones G. and Davis R.E. (2000). Climate Influences on Grapevine Phenology, Grape Composition, and Wine Production and Quality for Bordeaux, France. *Am. J. Enol. Vitic.,* 51

(206) Pinto C. and Gomes A.C. (2016). *Vitis vinifera* microbiome: from basic research to technological development. *BioControl* DOI 10.1007/s10526-016-9725-4

(207) Van Leeuwen C. and Seguin G. (2006). The Concept of Terroir in Viticulture. *Journal of Wine Research*, 2006, Vol. 17: 1-10

Chapter II

Outline of the thesis

This PhD work has been realized as a part of the project “Marcandis3”, financially supported by Regione Piemonte PSR FEASR 2007/2013 BANDO 2012 Misura 124-Azione 1 “Cooperazione per lo sviluppo di nuovi prodotti, processi e tecnologie nel settore agro-alimentare”. This project was carried out in collaboration with different partners: University of Piemonte Orientale (DISIT and spin-off “SmartSeq s.r.l.), Vignaioli Piemontesi and Agrion Foundation for Research (Tennuta Cannona).

One of the goals of the project was to study the effects of chemical treatments and fertilization on the composition of the microbial community in two vineyards subjected to different soil managements, such as conventional and integrated pest management (IPM).

Thanks to the development of culture-independent methods and especially to the recent advances in next-generation sequencing strategies, the complexity of the soil/rhizosphere microbial communities has been extensively explored. Following this basic idea, the aim of this work was to describe the microbial communities living on the roots of *Vitis vinifera* cv. Pinot noir, by a metagenomic approach under a taxonomic perspective, paying special attention to the shifts induced by the phenological stage of the plant (comparison between flowering and early fruiting stages, variable: time) and by the rhizosphere effects itself (comparison between bulk soil and rhizosphere, variable: space).

Since the main limitation of the metagenomic analysis based on DNA is that it does not provide information about the activity of the identified bacteria, we decided to make a step further and characterize the bacterial communities in the integrated pest management vineyard under a functional point of view through a metaproteomic approach.

In fact, proteomic tools can be a better way to gain information about microbial community activity understanding the real interaction pathways and identifying the set of proteins that microorganisms use to compete and cooperate in such complex environmental scenario as roots and soil.

This study represents an attempt to provide new informations on the whole and active bacterial communities associated to grapevine roots related to the phenological stages and the rhizosphere effect, respectively. We trust that our results will be useful in order to unravel the microbial biodiversity and activity in this specific environment and identify possible biomarkers of the typical features of *Vitis vinifera* cv. Pinot Noir.

Chapter III

Materials and Methods to analyse the structure and activity of microbial communities

3.1 Soil sampling

Soils samples (both rhizosphere, Rhiz, and bulk soil, BS) were collected in two vineyards subjected to different agriculture practices: conventional and integrated pest management.

The integrated pest management vineyard (Tenuta Cannona, TC) is located close to Carpeneto (AL), Latitude: 44.683706 °N, Longitude: 8.6258889 °E and Altitude: 268.22 m above sea level. The vineyard following the conventional methods (Cantina Mantovana, CM) is located near Mantovana (AL), Latitude: 44.730294°N, Longitude: 8.6226556 °E and Altitude: 215.35 m above sea level.

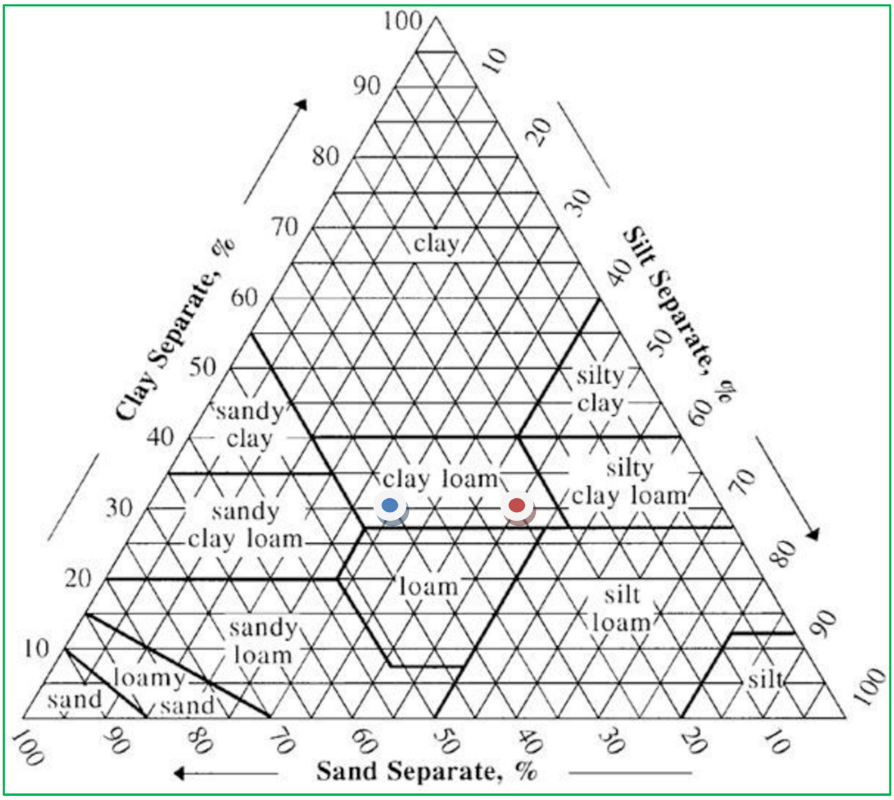
In addition, sampling were performed in two different phenological stages of plant of *Vitis vinifera* cv. Pinot noir. The first sampling was carried out in May 2014, corresponding to the inflorescence development/flowering time and the second sampling in July 2014 during the early fruit development (Figure 3.1).

<u>Figure3.1</u> Images of V. vinifera in two phenological stages: A) Inflorescence development/flowering (first sampling), B) Fruit development (second sampling)

The bulk soil (BS1 and BS2, for each sampling date) and the soil associated with the roots of *Vitis vinifera* cv. Pinot noir (Rhiz 1 and Rhiz 2, for each sampling date), five per each kind, were sampled at a depth of 30 cm, corresponding to the topsoil, after removing the surface layer (3.0–5.0 cm). Three soil cores were taken in the proximity of the stem (3 cm), therefore a total of fifteen cores were taken for each kind of soil. The roots entrapped in the soil cores collected close to the stem were considered for the sampling of rhizosphere soil. The soil adhering to these roots was removed using sterile gloves. The 5 bulk soil samples (three subsamples for each) were taken in a non cultivated area close to the vineyard (see Figure 4.1 and 5.1 in the result section). As recommended by the Italian law (GU 179/2002) for soil characterization analysis, the three subsamples of rhizosphere and bulk soil were then pooled in order to obtain a homogeneous sample. For biological purpose, in particular for DNA extraction, an aliquot of soil samples were maintained at -20 °C; furthermore, for proteomic analysis, an aliquot were frozen in liquid nitrogen and stored at -80° C.

The physical-chemical analyses of the soil (performed according to D.M. 13/09/99) sampled in Tenuta Cannona show that the soil is clay loam according to the USDA (United State Department of Agriculture) classification (Sand 29.8%, Silt 41.3%, Clay 28.9%) (figure 3.2, red spot), slightly alkaline (pH 7.89), with a total Nitrogen 0.69 g/Kg, a total organic carbon 4.3 g/Kg, C/N ratio 6.3 and cation exchange capacity (CEC) 15.9 meq/Kg. The soil sampled in Cantina Mantovana was clay loam (Sand 45.0%, Silt 26.8%, Clay 28.2%) (Figure 3.2, blue spot), acid (pH 5.99), with a total organic carbon 6.4 g/Kg, total Nitrogen 0.70 g/Kg, C/N ratio 9.06 and CEC 15.8 meq/Kg.

Complete physical-chemical analyses of the soil were reported in Annex I.



<u>Figure 3.2</u> Representation of soil texture; red spot soil sampled in TC (Tenuta Cannona) and blue spot soil sampled in CM (Cantina Mantovana)

3.2 DNA extraction from soil samples

The three sub-samples of the 5 bulk soil and 5 rhizosphere samples were pooled and homogenized before extraction of DNA.

DNA was extracted with the "PowerSoil®DNA Isolation" (Figure 3.3) kit (MoBio Laboratories, Inc) following the manufacturer's instructions.

Figure 3.3 "PowerSoil®DNA Isolation" kit (MoBio Lab)

This kit allows to extract genomic DNA directly from soil trough mechanical and chemical disruption of the cell associated with environmental matrix.

Briefly, 250 mg of samples were introduced into tubes containing marbles and added with SDS, an anionic detergent, and other lytic agents.

Subsequently, tubes were subjected to vigorous shaking in Tissue Lyser (QIAGEN) (Figure 3.4) for three cycles at 30 Hz for 1 minute and after were centrifuged at room temperature for 1 minute at 10.000 x g.

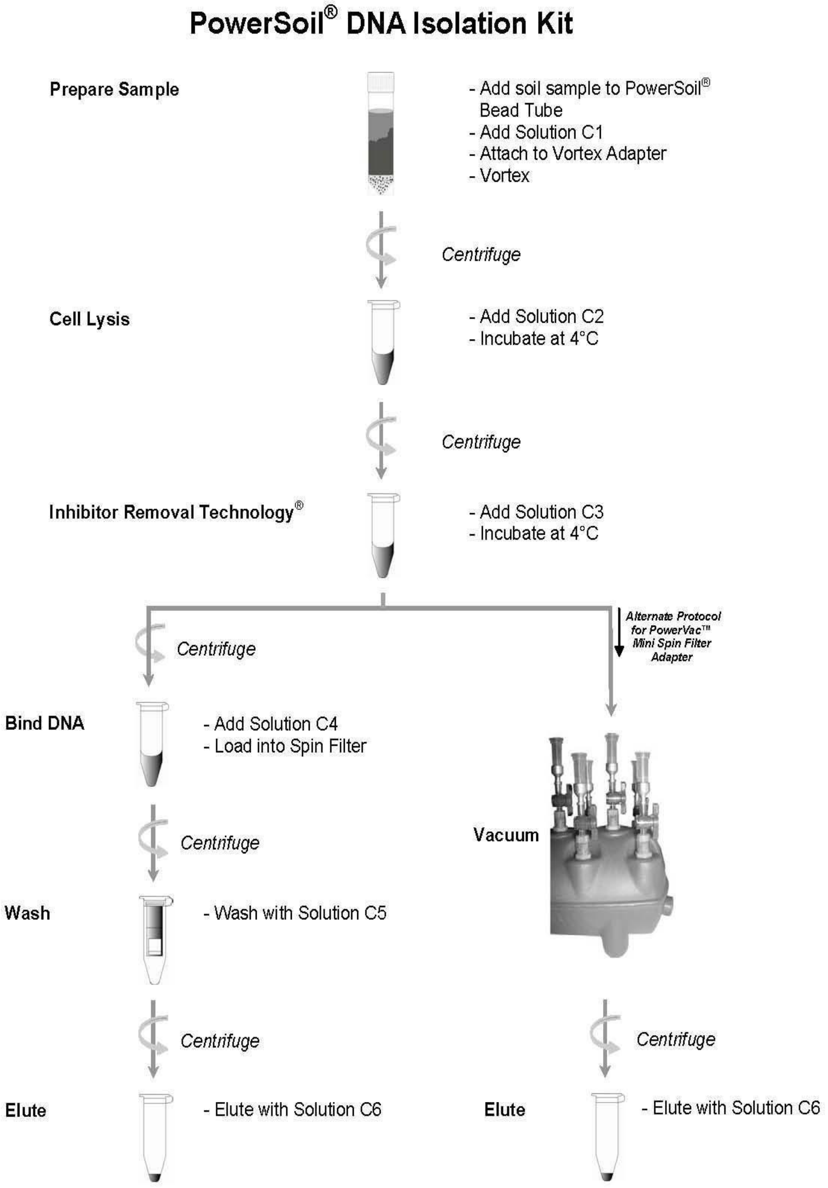
Figure 3.4 *Image of Tissue Lyser (QIAGEN)*

The supernatant obtained was transferred in a new tube and added, in two steps, with 250 µl of C2 solution and 300 µl of C3 solution allowing the precipitation of non-genomic material; each addition was followed by an incubation for 5 minutes at 4° C and by a centrifugation at 10.000 x g for 1 minute at room temperature.

In the next step, the supernatant was transferred in tube with a silica filter and centrifuged in three subsequently steps; in order to facilitate the binding of the DNA to the filter to a high salt concentration solution was added.

The DNA bind to the membrane was then washed with an ethanol solution to remove the possible contaminants (salt, humic acids) and was then eluted in TE buffer solution (provided in the kit).

The schematic representation of the “PowerSoil®DNA Isolation” kit protocol is reported in Figure 3.5.



<u>Figure 3.5</u> Schematic manufacturer's instruction for the use of the PowerSoil DNA Isolation kit for DNA extraction

The efficiency of the genomic DNA extraction was evaluated by electrophoresis on a 0.8% agarose gel in 1x TAE Buffer (Biorad) and the gel was visualized under UV light after ethidium bromure staining (1).

DNA extracts were stored at -20° C.

3.3 Quantification of nucleic acids

The assessment of the yield and pureness of the genomic DNA extracted as previously described, has been performed using spectrophotometric measurements. The optical density of the samples were measured in a range between 260 nm and 320 nm. The amount of genomic DNA extracted has been calculated according to the formula: $(A_{260} - A_{320}) \times 50$ and expressed in ng of nucleic acids.

Since the DNA absorbs light at 260 nm and protein (in particular aromatic amino acids) at 280 nm, the ratio 260/280 nm was used to asses DNA contamination of protein reagents. Pure preparations of DNA and RNA have a 260/280 OD ratio values of 1.8 and 2.0, respectively.

Finally, the ratio 260/230 was used as a secondary measure of nucleic acid purity and values was usually in the range of 2.0-2.2. Lower values of this ratio indicate contamination with reagent that absorb at 230 nm, such as a phenol and guanidine HCl.

3.4 Ethanol precipitation of sample

Ethanol precipitation is a commonly used technique for purifying and/or concentrating nucleic acids (DNA o RNA).The eluted DNA, obtained after the extraction, was added with 1/10 volume of sodium acetate (3M, pH 5.2) and 2.5 volume (calculated after addition of sodium acetate) of 100% ethanol. The addition of reagents was followed by an incubation at -20° C for 4 hours; then the suspension was incubated for 20 minutes at -80° C. Following these two steps, the DNA precipitated and was collected by centrifugation at 15.000 x g for 15 minutes at 4° C; afterwards, 70% of ethanol wash solution was added to pellet; the suspension was centrifuged again and the supernatant was removed.

Finally, the pellet was dried in SpeedVac and the DNA was re-suspended in TE buffer (elution buffer, provided with the DNA extraction kit).

After ethanol precipitation each samples was quantified by spectrophotometer following the protocol described in section 3.3

3.5 PCR amplification of soil bacterial communities

For the characterization of microbial communities, each DNA was amplified with a primers pair complementary to the variable V1 and V4 regions of the 16S rDNA (Figure 3.6).

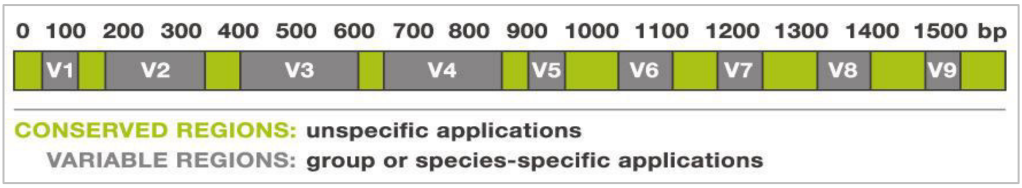


Figure 3.6 16S rDNA illustrating the conserve (green) and variable (grey) regions (Source: http://www.alimetrics.net/en/index.php/dna-sequence-analysis)

16S rDNA genes were amplified by PCR using the forward primer (5'-AGAGTTTGATCCTGGCTCAG-3') (2) and the reverse primer (5'-CTACCAGGGTATCTAATC-3') (3).

The DNA amplification reaction was carried out in a thermocycler TC-512 Techne (Figure 3.6); the protocol included an initial step of denaturation at 94° C for 5 minutes, followed by 34 cycles of denaturation at 94° C for 1 minute, annealing at 60° C for 1 minute and elongation at 72° C for 5 minutes, and a final elongation for 10 minutes t 72° C.

Each PCR reaction were performed in a final volume of 20 µl containing: 5 ng of extract DNA, 250 nM of each primer, 1x optimized buffer containing $MgCl_2$ (Thermo Scientific), 100 µM of each dNTPs (dATP, dCTP, dGTP, dTTP), 0.08 U Taq DNA polymerase (Thermo Scientific) and 5 % DMSO (Dimethyl Sulfoxide, SIGMA).

Figure 3.6 Thermocycler TC-512 Techne and particular of display

Amplification products were separated by electrophoresis on 1.4 % agarose gel at 65 Volts for 2 hours in TAE buffer (pH 8.5) and gel was visualized after staining with ethidium bromide under UV light (1).

3.6 Pyrosequencing

PCR products from bacteria were used for Pyrosequencing with 454 (Roche) technology following the Roche manufacturer's.

More in detail, amplicons were sequenced in droplet water in oil emulsion; DNA-carrying beads were loaded into individual wells on a PicoTiter plate and surrounded by enzyme (sulfurylase luciferase) beads. Nucleotides flowed one by one over the plate and template-dependent incorporation releases pyrosphosphate, which was converted to light through luciferin/luciferase enzymatic reaction. The light signals, which are proportional to the number of incorporated nucleotides in a given flow, are represented in flowgrams that are analysed and nucleotide sequence is determined for each reading with the GS Amplicon Variant Analyzer software.

The schematic workflow of the 454 Roche Junior GS+ is reported in Figure 3.7.

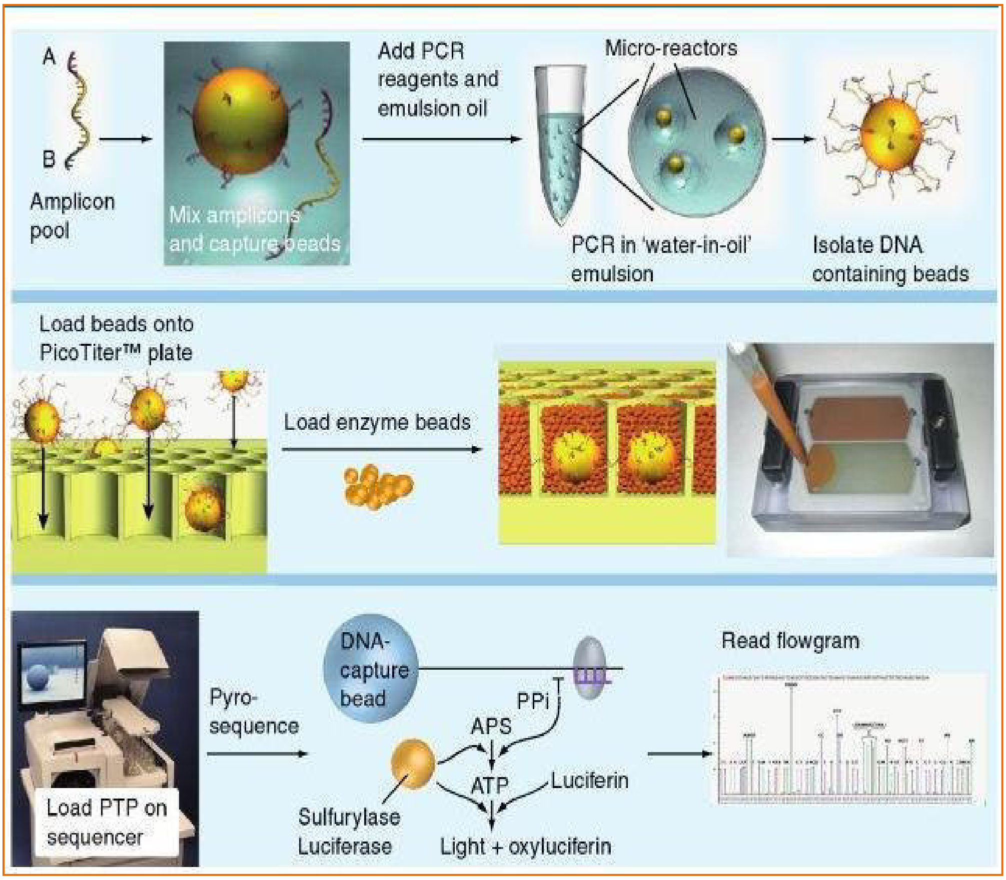


Figure 3.7 Workflow of the 454 Roche Junior GS+ pyrosequencer

3.7 Bioinformatic and statistical analysis

Data were analyzed using a custom bioinformatics pipeline. Raw sequence reads were demultiplexed to obtain a single file for each sample. During this process reads that meet following criteria were discarded: 1) read length shorter than 200 nt, 2) average Phred quality score (4) lesser than 25, and 3) read containing at least one ambiguous base.

For each sample, the taxonomy assignment up to genus level was performed using RDP (https://rdp.cme.msu.edu) classifier (5) and species-level resolution was attained by blasting reads against a core set of the RDP database.

Sequences were clustered according to similarity thresholds and the representative sequence of each cluster was identified with the name of the corresponding RDP hit for all taxonomic levels.

Finally, a table with absolute abundance for all samples was used as input for the analysis with RAM package of R statistical software to obtain: 1) alpha diversity graph, 2) PCoA ordination, and 3) biodiversity index (Shannon index, Simpson Index, Observed species).

Statistical analysis was performed with R statistical software. Data were analyzed by non-parametric Mann-Whitney test with cut-off significance at $p \leq 0.05$ to asses differences between treatments.

3.8 Protein extraction and digestion

Soil proteins were extracted using “NoviPure™Soil Protein Extraction” (Figure 3.8) kit (MoBio Laboratories, Inc) according to manufacturer’s instructions.

Figure 3.8 *“NoviPure™Soil Protein Extraction” Kit (MoBio Lab)*

This kit has been designed to extract intracellular and extracellular microbial proteins without co-extraction of interfering compounds such as humic substances.

The first step of the procedure was the addition of 5 g of soil into a bead tube (provided by the kit). Soil samples were then suspended intwo solution: the first one is a buffer that contain a detergent that will not co-extract humic substance, while the second one is represented by 1M Dithiothreitol (DTT) for a final concentration of 10 mM. Subsequently, the tubes were subjected to vortexing for completely mix and incubated on ice for 10 minutes. Samples were then centrifuged at 4.500 x g for 5 minutes at 4° C.

Tubes were placed on ice and added with a second solution (provided by the kit), vortexed to completely mix and incubated at 4° C for 30 minutes; the incubation of the samples with this solution allows to complete the lysis of microorganism and improves intracellular and extracellular protein recovery. The tubes were centrifuged at 4° C for 10 minutes at 4.500 x g.

The obatined supernatant was then transferred into a new tube and added with 100% Trichloroacetic Acid (TCA), vortexed briefly to mix and incubated at -20° C overnight.

After this step the tubes were centrifuged at 4.500 x g for 20 minutes at 4° C and the pellet obtained was washed, for three times with 100% ice cold HPLC-acetone and centrifuged at 20.000 x g for 5 minutes at 4° C.

Finally, the pellet was resuspended in 50 mM ammonium bicarbonate and quantified by Bradford method (6).

The schematic representation of the NoviPure™ Soil Protein Extraction kit is reported in Figure 3.9.

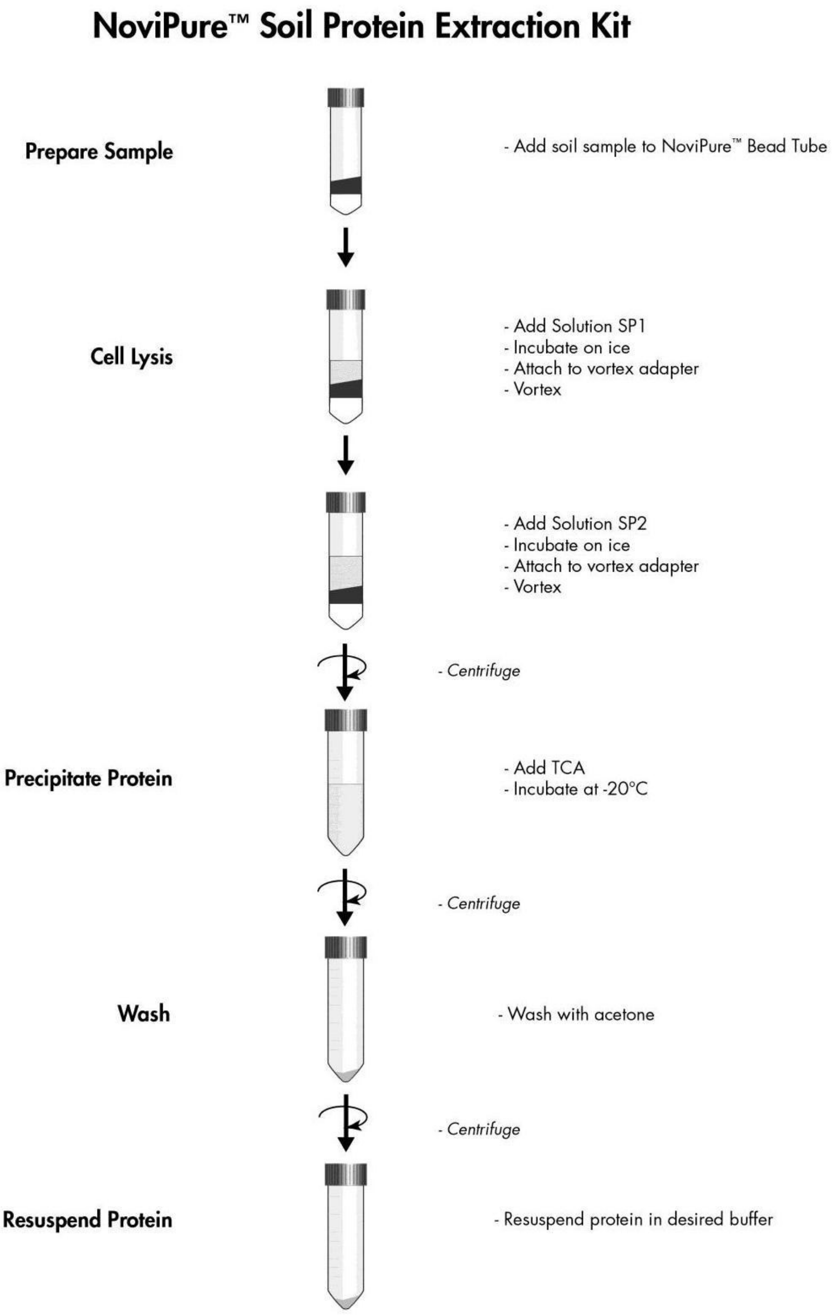


Figure 3.8 Schematic manufacturer's instruction for the use of the Protein extraction kit

After quantification, the extracted proteins were digested with trypsin (Roche, Segrate, Milano, Italy) and resuspended in 50 mM ammonium bicarbonate (37 °C overnight) after a reduction step (DTT to final concentration of 10 mM, 30 min at 60 °C) and an alkylation step (iodoacetamide to final concentration 20 mM, 30 min, room temperature in the dark).

3.9 MS/MS analysis of protein

The mass spectrometry analyses were performed using a micro-LC Eksigent Technologies (Dublin, USA) system with as stationary phase a Halo Fused C18 column (0.5 x 100 mm, 2.7 µm; Eksigent Technologies Dublin, USA). The injection volume was 4.0 µL and the oven temperature was set at 40 °C. The mobile phase was a mixture of 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in acetonitrile (B), eluting at a flow-rate of 15.0 mL min-1 at an increasing concentration of solvent B from 2% to 40 % in 30 minutes. The LC system was interfaced with a 5600+ TripleTOF system (AB Sciex) equipped with a DuoSpray Ion Source. The samples were subjected to the traditional data-dependent acquisition (DDA): the mass spectrometer analysis was performed using a mass range of 100–1500 Da (TOF scan with an accumulation time of 0.25 s), followed by a MS/MS product ion scan from 200 to 1250 Da (accumulation time of 5.0 ms) with the abundance threshold set at 30 cps (35 candidate ions can be monitored during every cycle). The ion source parameters in electrospray positive mode were set as follows: curtain gas (N2) at 25 psig, nebulizer gas GAS1 at 25 psig, and GAS2 at 20 psig, ionspray floating voltage (ISFV) at 5000 V, source temperature at 450 °C and declustering potential at 25 V. The MS data were acquired with Analyst TF 1.7 (AB SCIEX).

3.10 Protein database search and Blast2GO data analysis

For each taxonomic unit, all protein sequences present in NCBInr were downloaded and used to create an in-house protein database useful to perform analysis. The mass spectrometry files were searched using Mascot (Matrix Science Inc., Boston, USA). The Mascot search was performed on Mascot v. 2.3.0, the digestion enzyme selected was trypsin, with 3 maximum missed cleavages, a search tolerance of 0.4 Da was specified for the peptide mass tolerance, and 0.6 Da for the MS/MS tolerance. The charges of the peptides to search for, were set to 2+, 3+ and 4+, and the search was set on monoisotopic mass. The instrument was set to ESI-QUAD-TOF and the following modifications were specified for the search: oxidized methionine and deamidation (NQ) as variable modifications.

To perform the Blast2GO analysis (http://www.blast2go.com/b2ghome) we downloaded the protein FASTA sequences from http://www.ncbi.nlm.nih.gov using the GI code ID. Data analysis was performed with Blast2Go standard parameters.

The EC annotations, obtained by mapping from equivalent GO annotations, were visualized reconstructing the structure of the Gene Ontology relationships and ECs on KEGG maps (http://www.genome.jp/kegg). Data of biological process and molecular function were recorded.

References

(1) Sambrook J., Fritsch E.F. and Maniatis T. (1989). Molecular cloning: a laboratory manual, II edizione. Cold Spring Harbor Laboratory Press, NY

(2) Weisburg W.G., Barns S.M., Pelletie D.A., Lane D.J., Pelletier D.A. and Lane D.J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol*, 173(2): 697-703

(3) Wang Y. and Qian P.Y. (2009). Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. *PLoS One*, 4(10): e7401

(4) Ewing B., Hillier L., Wendl M. and Green P. (1998). Base-calling of automated sequencer traces usingPhred. I. Accuracy assessment. *Genome Res*, 175-185

(5) Wang Q., Garrity G.M., Tiedje J.M. and Cole J.R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol*, 73(16): 5261-5267

(6) Bradford M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem*, 72: 248-254

Chapter IV

Exploring the microbiota of *Vitis vinifera* cv. Pinot noir in a conventional pest management vineyard

In this section I’m going to describe the characterization of the microbial communities in a vineyard subjected to conventional pest management performed by a metagenomic approach, paying attention to the shifts induced by the phenological stage of the plant (flowering and early fruiting stage; variable time) and by the rhizosphere effects (rhizosphere and bulk soil; variable space).

Conventional pest management is an agricultural production system characterized by use of pesticides and insecticides and fertilizer relative to land area. In conventional practices, the inorganic fertilizers and the chemical pesticides are routinely applied.

The vineyard following the conventional methods analysed in this work is located in “Cantina Mantovana” near to Mantovana (AL), Latitude: 44.730294°N, Longitude: 8.6226556 °E and Altitude: 215.35 m above sea level. In figure 4.1 is reported the GIS map of the vineyard and the two sampling site (BS, bulk soil and Rhiz, rhizosphere).

The conventional management of the “Cantina Mantovana” vineyard was based on treatment with different chemicals: the herbicide glyphosate between the vineyard lines (in June), fungicide against *Oidium* spp. (Trifloxistrobin), fungicide against *Peronospora* spp. (Fosetyl-Al + copper) in June and July, one insecticide (Thiamethoxan) in July and, finally two sulphur treatments.

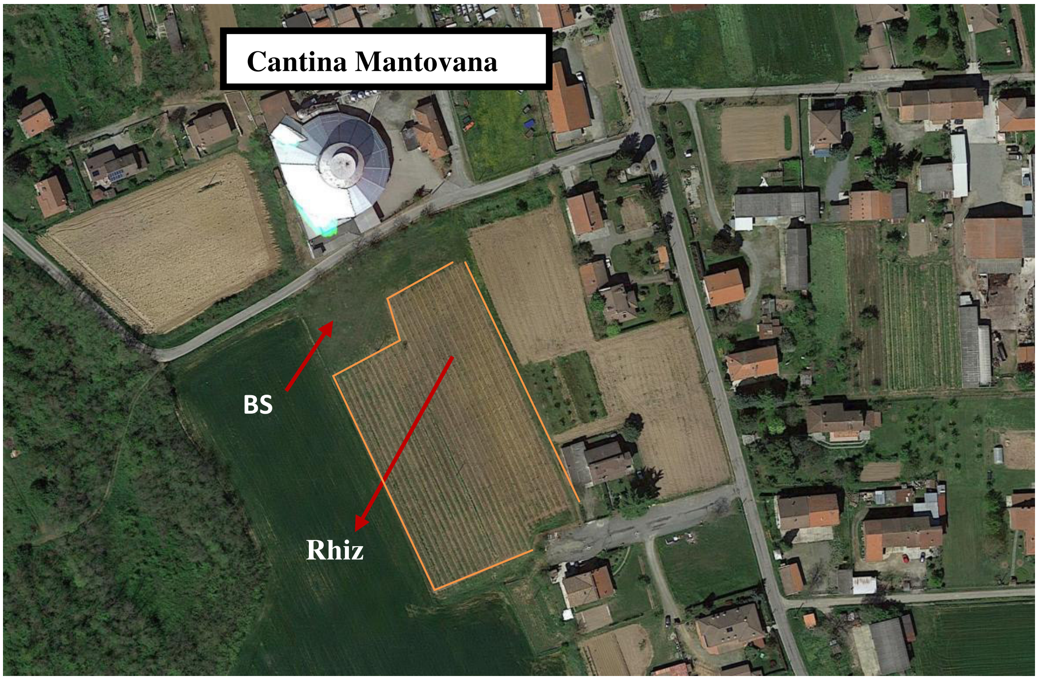
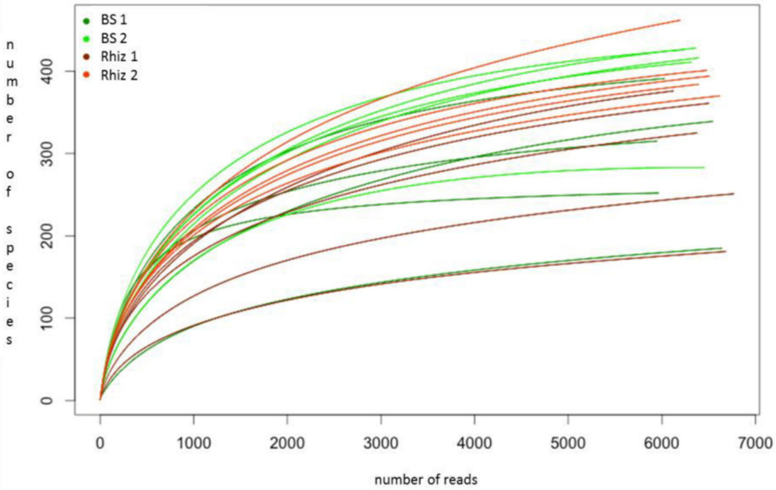


Figure 4.1 GIS map of the conventional pest management vineyard (Cantina Mantovana). The sampling points of the rhizosphere (Rhiz) and bulk soil (BS) are indicated by red arrow.

4.1 Biodiversity

The biodiversity of bacteria population between the soil samples (rhizosphere and bulk soil) at two sampling times was compared by the rarefaction curves (Figure 4.2), that is based on the observation that the curve of rarefied counts of any feature should plateau if the sample is close to saturation. According to Figure 4.2 a good coverage of the entire community was achieved in all the samples.



<u>Figure 4.2</u> Rarefaction curves for each soil samples (BS1, BS1, Rhiz1, Rhiz2, five sub-samples each)

Biodiversity has been expressed as number of bacterial species, Simpson's Index and Shannon-Wiener's Index. While the median number of bacterial species was comparable in the two sampling times for bulk soil and rhizosphere, it was higher during the early fruiting stage (Bulk soil, 416; Rhizosphere, 394) than the flowering stage (Bulk soil, 315; Rhizosphere, 325) both in bulk soil and rhizosphere (Figure 4.3 a). The median value of the Shannon-Wiener's Index was higher in the bulk soil at the two sampling times and in the rhizosphere during the early fruiting stage (Figure 4.3 b). The Simpson's Index did not differ among the samples (Figure 4.3 c).

a)

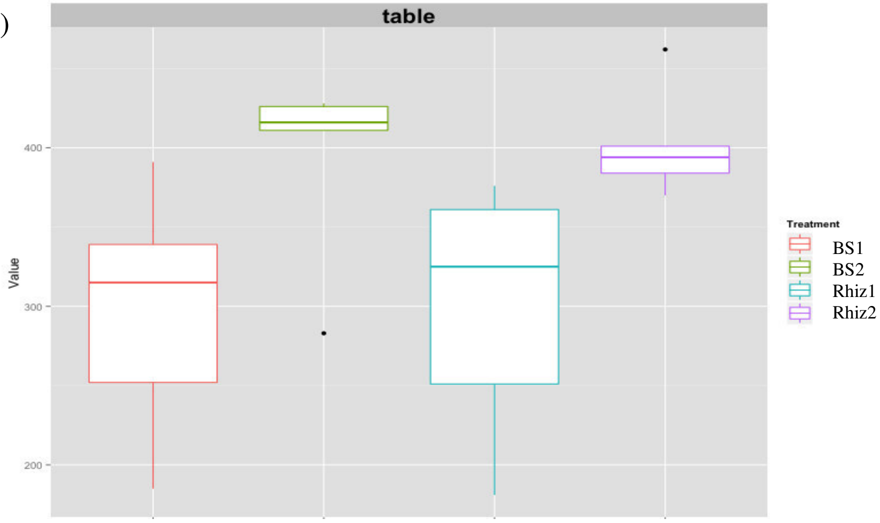


table
400
300
200
Value
Treatment
BS1
BS2
Rhiz1
Rhiz2

b)

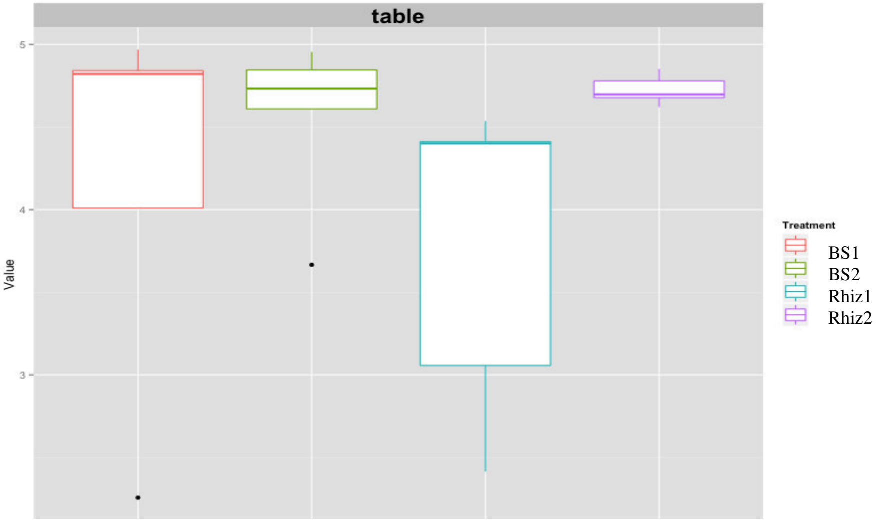


table
5
4
3
Value
Treatment
BS1
BS2
Rhiz1
Rhiz2

c)

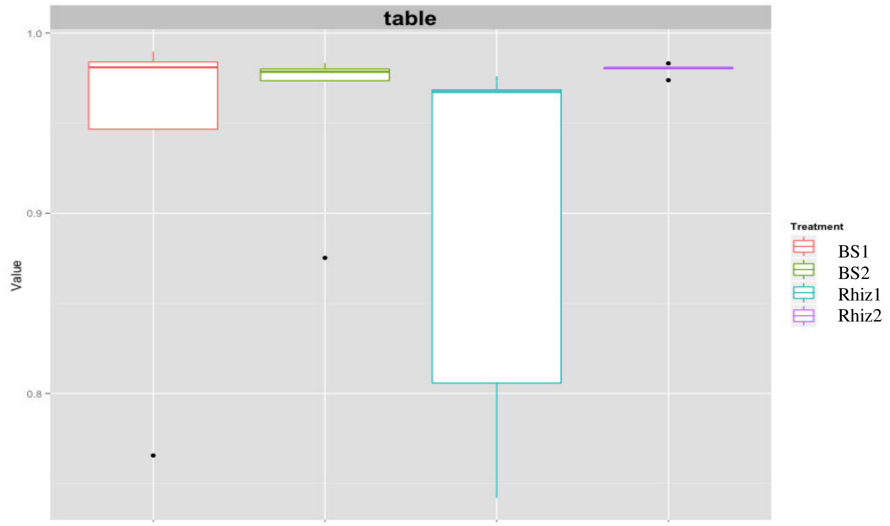


Figure 4.3 *a) Number of bacterial species, b) Shannon-Wiener's Index and c) Simpson's diversity index calculated in bulk soil and rhizosphere of V. vinifera cv. Pinot noir at two sampling times*

4. 1 Description of microbial communities recorded in the vineyard

The analysis of the microbial structure performed with the 454 Roche methods revealed, after demultiplexing steps, a total of 139.991 reads. These data were used for phyla description.

The phyla Actinobacteria, followed by Proteobacteria and Firmicutes were dominant in the bacterial communities of the conventional pest management vineyard (both in bulk soil and in rhizosphere) (Figure 4.4) (Annex II). In particular, the abundance of Firmicutes was higher in bulk soil at first sampling compared to all the other samples.

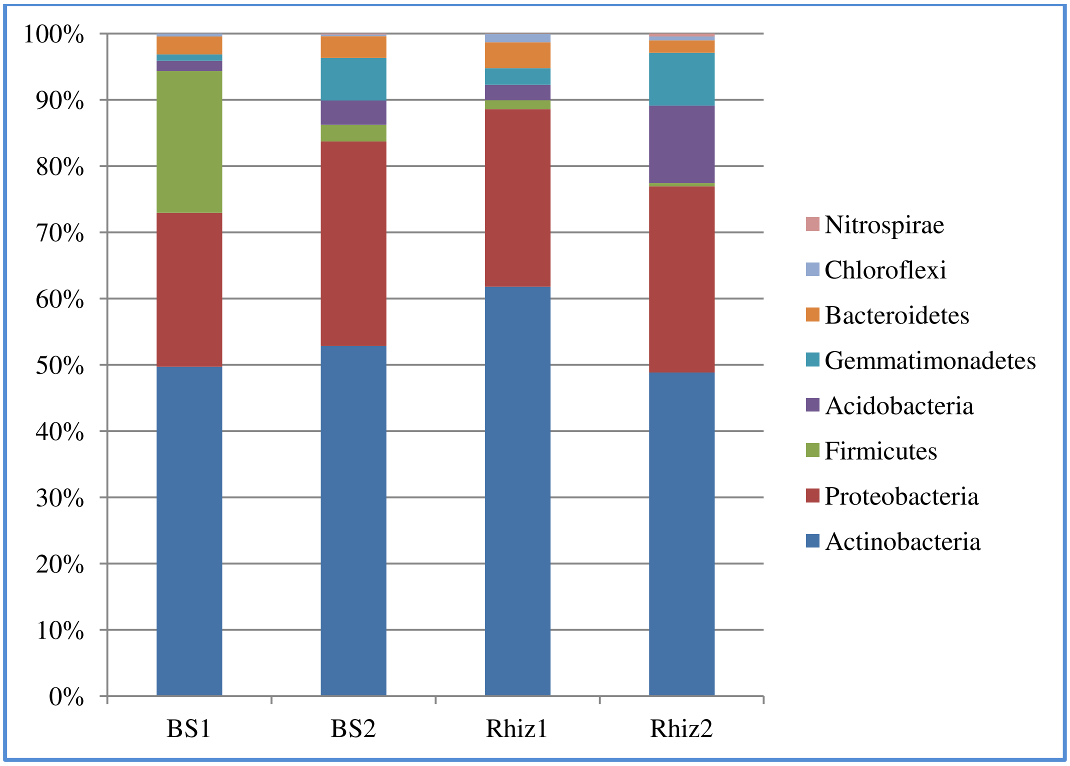


Figure 4.4 Phyla distribution in a conventional pest management vineyard in all soil samples (top 8 taxa).

Actinobacteria frequency did not change significantly between the soil samples (BS1 49.61%, BS2 52.80%, Rhiz1 61.71% and Rhiz2 48.76%). On the contrary, in bulk soil the frequency of readings ascribed to Proteobacteria and Firmicutes differed according to the time: while the frequency of Proteobacteria and Firmicutes during the flowering stage was 23.15% and 21.40%, respectively, during the early fruit development stage was 30.84% and 2.49%, ($p = 0.032$ and $p = 0.008$, respectively) (Figure 4.5 a and b).

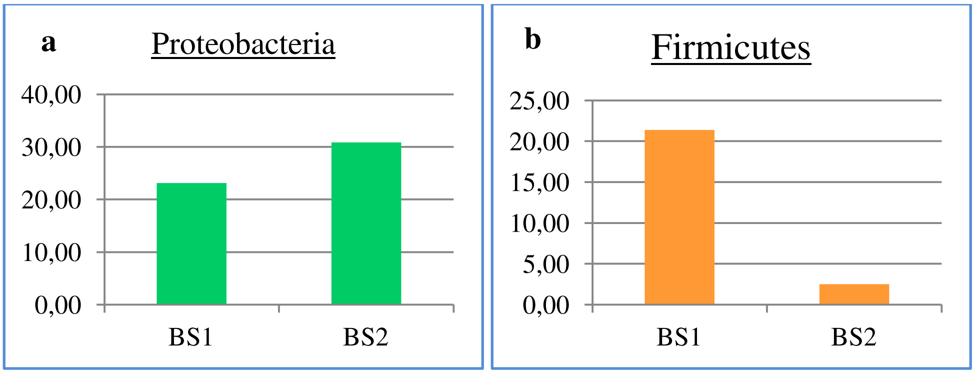


Figure 4.5 Frequency of a) Proteobacteria and b) Firmicutes phyla in bulk soil according to time variable

Similarly, the abundance of Acidobacteria and Gemmatimonadetes in the rhizosphere recorded at first sampling (2.31% and 2.49%, respectively) and at second sampling (11.63% and 7.99%, respectively) changed significantly (p=0.032 and p= 0.008, respectively) (Figure 4.6 a and b).

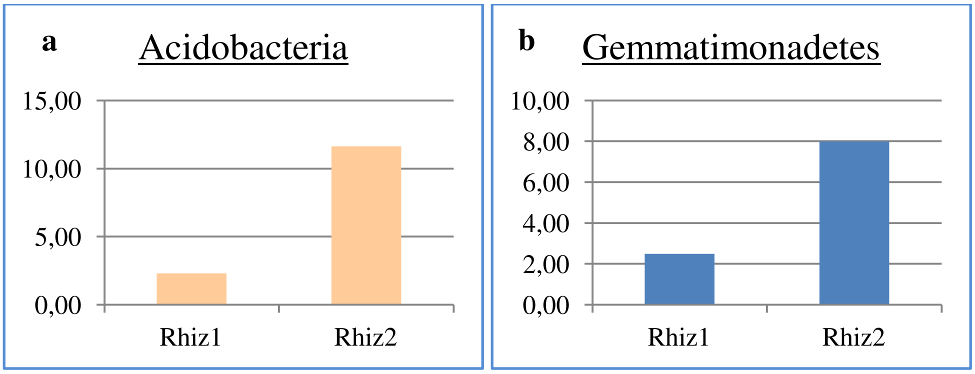


Figure 4.6 Frequency of a) Acidobacteria and b) Gemmatimonadetes phyla according to time variable in the rhizosphere

In addition, the amount of reads corresponding to Firmicutes was higher in bulk soil than in the rhizosphere during the flowering (BS1 21.40% and Rhiz1 1.36%, p= 0.032) (Figure 4.7 a); moreover, variations in the frequency of Nitrospirae occurred during early fruit development stage according to the variable space (BS2 0.14% and Rhiz2 0.47%, p= 0.047) (Figure 4.7 b).

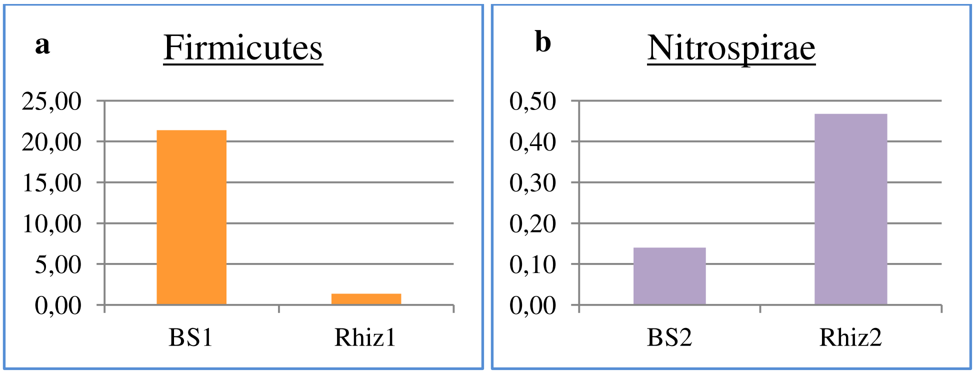
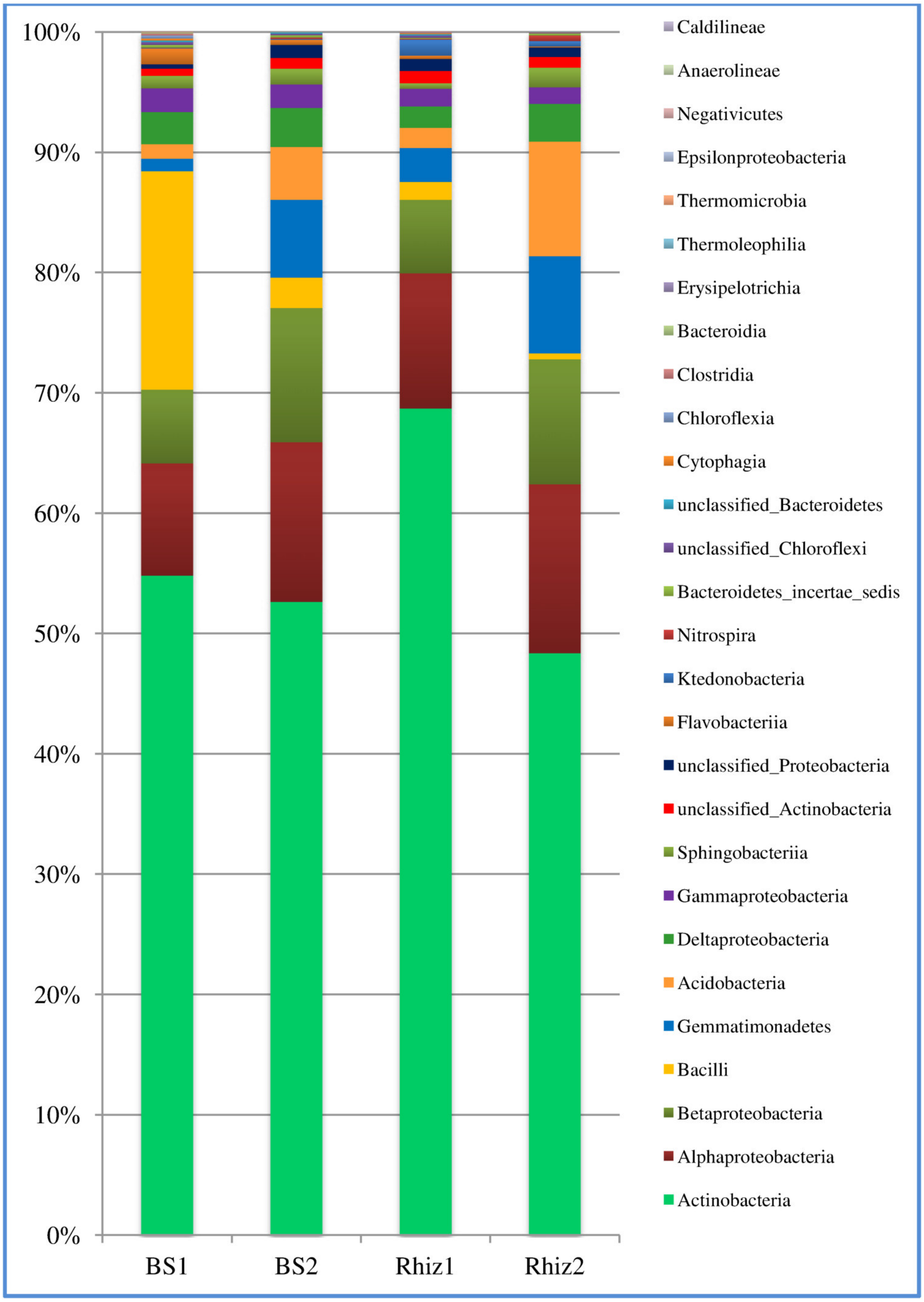


Figure 4.7 Frequency of a) Firmicutes and b) Nitrospirae phyla in soil and rhizosphere during the two sampling dates

At the classes level, Actinobacteria was the dominant one followed by α- and β-Proteobacteria (Figure 4.8) (Annex III).



<u>Figure 4.8</u> Microbial community composition in the bulk soil and rhizosphere of V. vinifera cv. Pinot Noir at the two sampling times (flowering and early fruiting) at the class level.

Actinobacteria, followed by unclassified_Actinobacteria, were the most represented classes belonging to the phylum Actinobacteria with high frequencies in all soil samples.

Inside Actinobacteria, both in bulk soil and in the rhizosphere at the two sampling times the dominant genera found were *Gaiella*, *Arthrobacter*, *Blastococcus, Streptomyces* and *Nocardioides* (Figure 4.9).

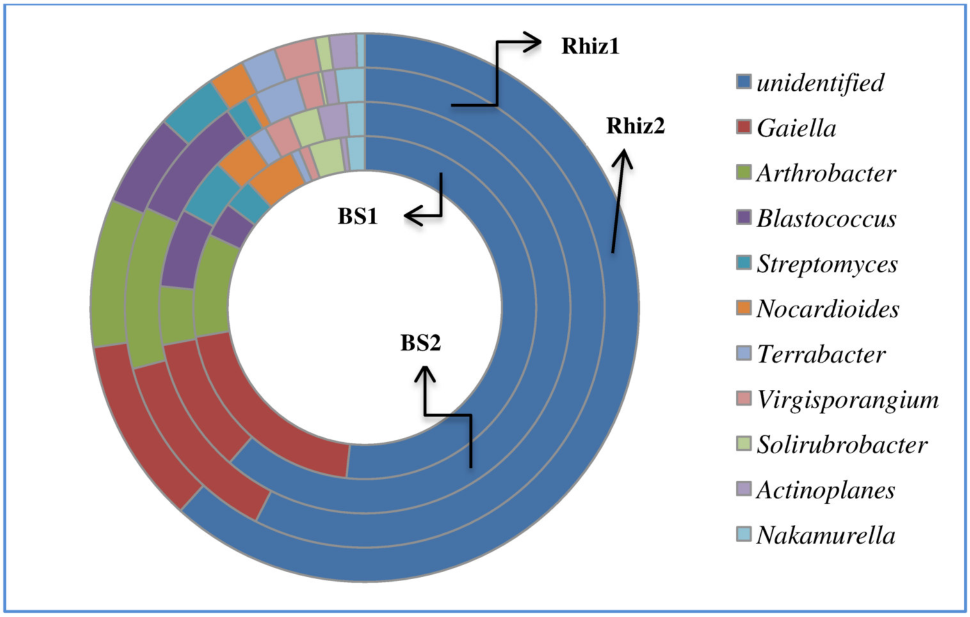
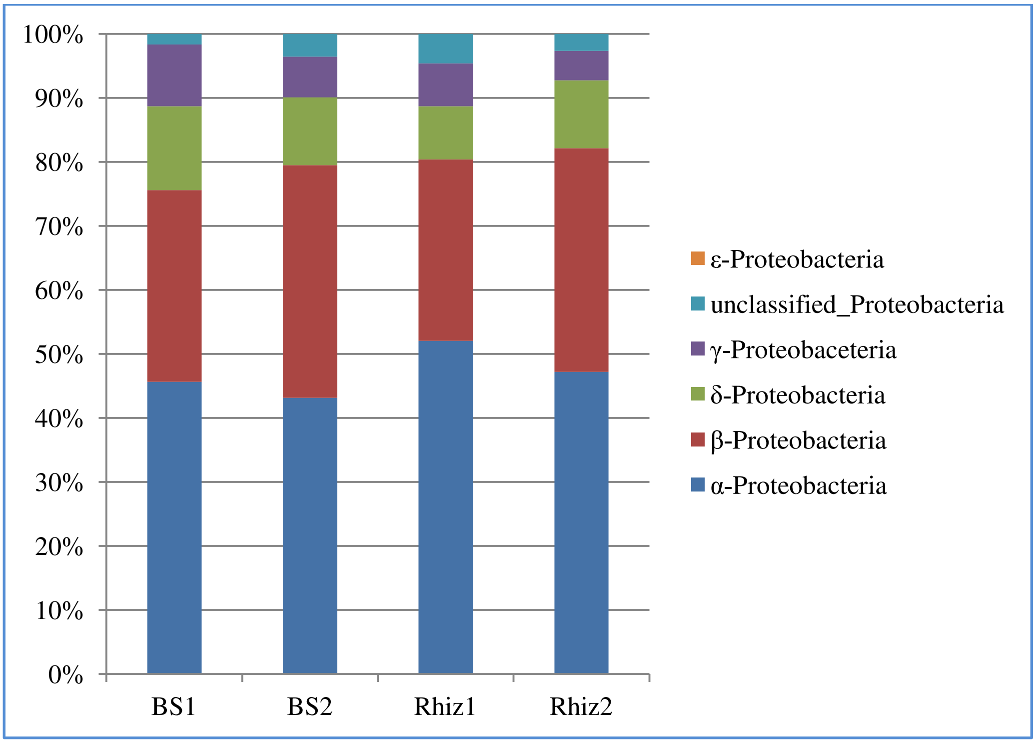


Figure 4.9 Distribution of genera belonging to Actinobacteria found in the rhizosphere and in bulk soil at two sampling times

The abundance of *Gaiella*, *Arthrobacter*, *Blastococcus* and *Streptomyces* did not change significantly and was similar in all soil samples. On the contrary, during the flowering stage (first sampling) the frequency of *Nocardioides* was higher in bulk soil than in rhizosphere (BS1 4.83% and Rhiz1 0.70%, p= 0.032).

The distribution of the different Proteobacteria classes is reported in figure 4.10. The results obtained by pyrosequencing indicated that α- followed by β- and δ-Proteobacteria were dominant in all the soil samples.



<u>Figure 4.10</u> Distribution of the Protebacteria classes in all soil samples

The frequency of α-Proteobacteria in the bulk soil decreased during time (BS1 45.62% and BS2 43.16%, p= 0.008).

Similarly, the amount of readings ascribed to δ-Proteobacteria changed significantly according to the time variable both in rhizosphere (Rhiz1 8.30% and Rhiz2 10.60%, p= 0.016) and in bulk soil (BS1 13.11% and BS2 10.59%, p= 0.028).

Among the α-Proteobacteria, the most dominant identified genus was *Bradyrhizobium* followed by *Phenylobacterium*, *Skermanella* and *Microvirga* (Figure 4.11).

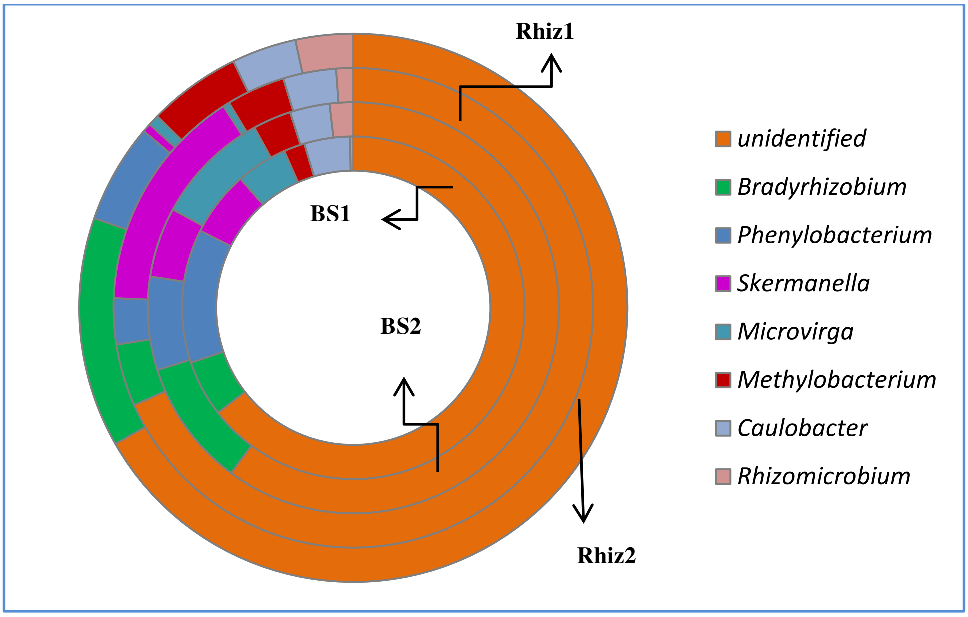


Figure 4.11 Distribution of the genera belonging to α-Proteobacteria in bulk soil and rhizosphere during the flowering (first sampling) and early fruiting stage (second sampling)

According to the phenological stage of the plant, the frequency of *Bradyrhizobium* differed in the rhizosphere (Rhiz1 4.19% and Rhiz2 13.57%, p= 0.016) and in bulk soil (BS1 5.31% and BS2 9.96%, p= 0.046).

Significant frequency variations during time were observed in the rhizosphere also for *Phenylobacterium* (Rhiz1 3.10% and Rhiz2 5.85%, p= 0.015). Moreover, the amount of readings corresponding to the genus *Microvirga* in bulk soil at the second sampling point was significantly higher than that recorded in the rhizosphere (BS2 9.07% and Rhiz2 0.65%, p= 0.008). Instead, the frequency of *Skermanella* did not differ among the soil samples.

The predominant identified genera belonging to the β-Proteobacteria was *Burkholderia,* followed by *Noviherbaspirillum* and *Massilia* (Figure 4.12).

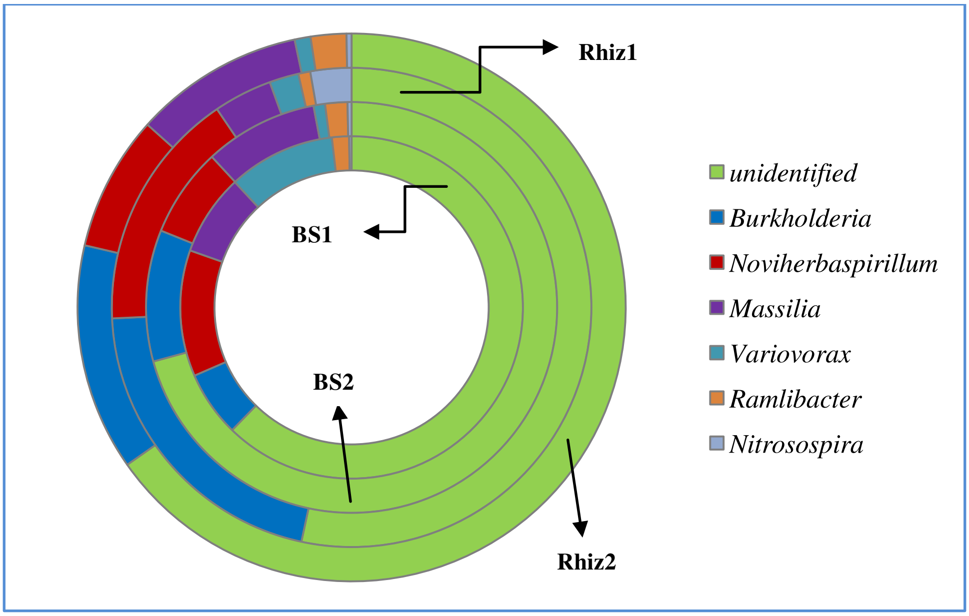


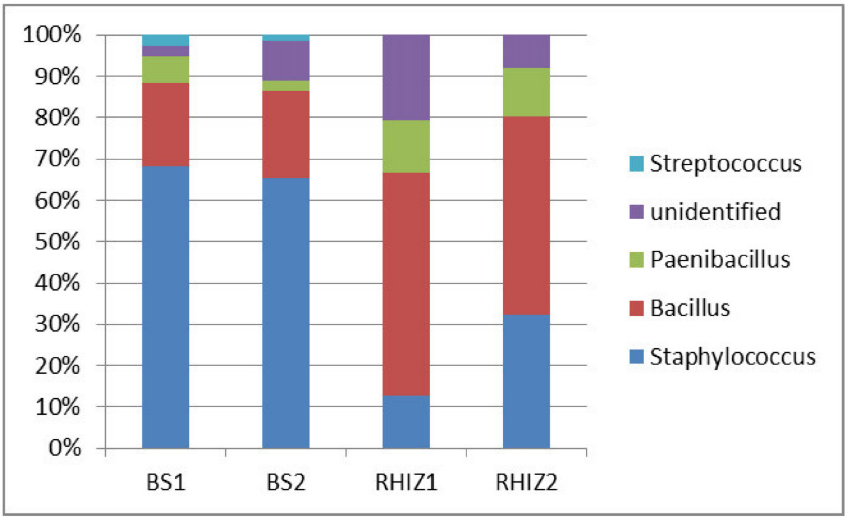
Figure 4.12 Abundance of the most represented genera belonging to β-Proteobacteria in all soil samples

The amount of readings ascribed to *Burkholderia* and *Noviherbaspirillum* did not change both in rhizosphere and in bulk soil at the two sampling points. In contrast, abundance of *Massilia* in the rhizosphere changed significantly during time, being higher at early fruit development than at the flowering time (Rhiz1 3.95% and Rhiz2 10.04%, p= 0.012).

Besides the unidentified genera, *Cystobacter* was the dominant identified genus belonging to δ-Proteobacteria. While it was more abundant in bulk soil than in rhizosphere at second sampling (early fruit development) (BS2 2.16% and Rhiz2 0.35%, p= 0.032), variations occurred in bulk soil too according to the sampling times (BS1 0.36% and BS2 2.16%, p= 0.036).

Inside the phylum Firmicutes the amount of sequences corresponding to the class Bacilli in bulk soil changed according to the time (BS1 75.97% and BS2 13.76%, p= 0.008); in addition, their frequency during the first sampling times was higher in bulk soil than in rhizosphere (BS1 75.97% and Rhiz1 6.06%, p= 0.032).

Among the class Bacilli, the genus *Staphylococcus* followed by *Bacillus* and *Paenibacillus* were dominant in all the soil samples (Figure 4.13).



<u>Figure 4.13</u> Abundance of the most represented genera belonging to the class Bacilli in all soil samples

While the frequency of *Staphylococcus* and *Bacillus* genera did not changed significantly among the samples, the amount of readings ascribed to *Paenibacillus* in bulk soil was higher during the first sampling than in the second one (BS1 6.24% and BS2 2.54%, p= 0.047).

The phylum Nitrospirae was represented only by the Nitrospira class and by the genus, *Nitrospira*. The abundance of this genus during the second sampling was higher in rhizosphere than in bulk soil (BS2 19.37% and Rhiz2 71.41%, p= 0.047) (Figure 4.14 a).

Similarly, the phylum Gemmatimonadetes was represent by the Gemmatimonadetes class including a unique the genus *Gemmatimonas*. The number of sequences corresponding to *Gemmatimonas* in the rhizosphere differed according to the phenological stage of the plant (Rhiz1 11.62% and Rhiz2 48.81%, p= 0.008) (Figure 4.14 b).

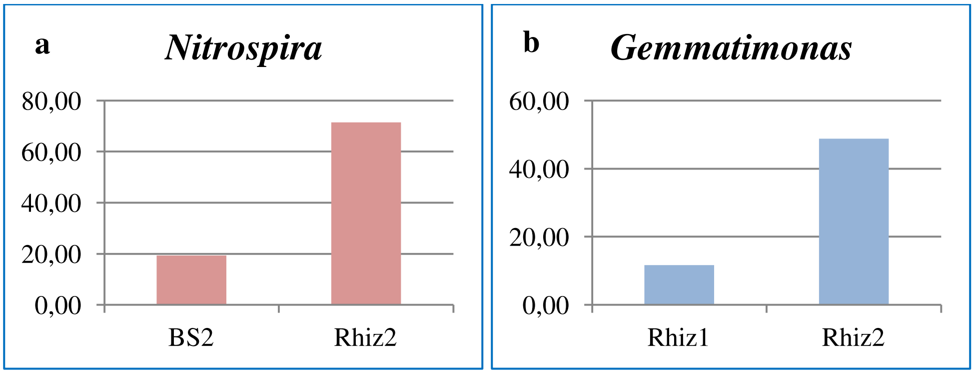


Figure 4.14 Relative abundance of (a)Nitrospira in bulk soil and rhizosphere during the second sampling and (b)Gemmatimonas in the rhizosphere according to the plant's phenological stage

Finally, the obtained data for the phylum Bacteroidetes showed that the Sphingobacteriia followed by Flavobacteria and Bacteroidetes_incertae_sedis were the most abundant classes both in rhizosphere and in bulk soil independently by the sampling time (Figure 4.15).

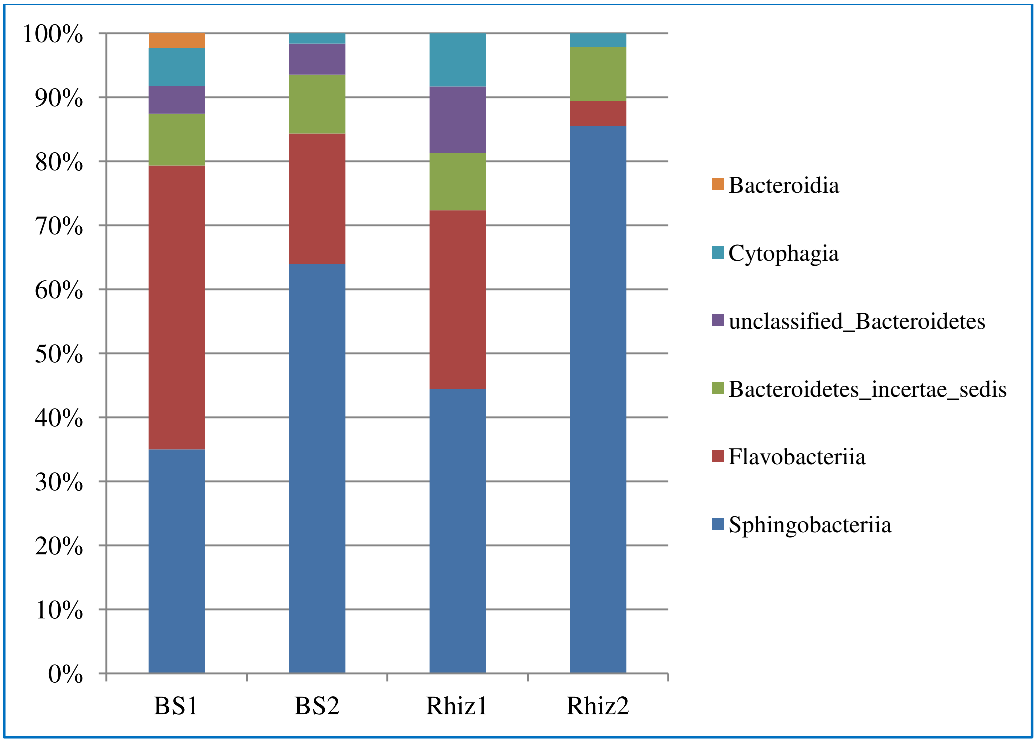


Figure 4.15 Distribution of the Bacteroidetes classes at the two sampling date both in rhizosphere and in bulk soil

In general, among the Bacteroidetes, the dominant genera was *Niastella* followed by *Flavisolibacter*, and *Segetibacter* belonging to Sphingobacteria class. Instead, the most frequent genera belonging to Flavobacteria class were *Chryseobacterium* followed by *Flavobacterium.* In addition, *Ohtaekwangia* was the dominant genus belonging to Bacteroidetes_incertea_sedis.

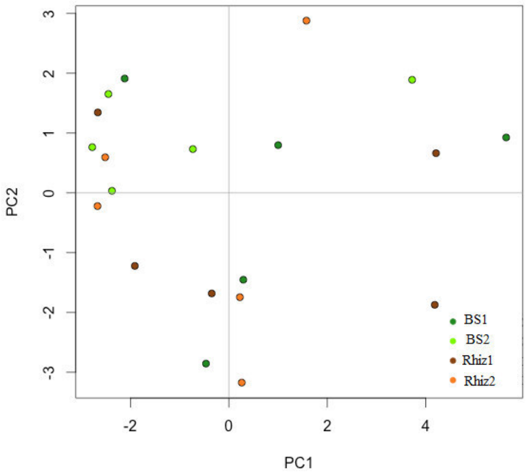
The frequency of all genera belonging to Bacteroidetes phyla did not change both in bulk soil and in rhizosphere during the two phenological stage of the plant.

The list of the bacterial genera described with their frequencies and significances are reported in Annex IV.

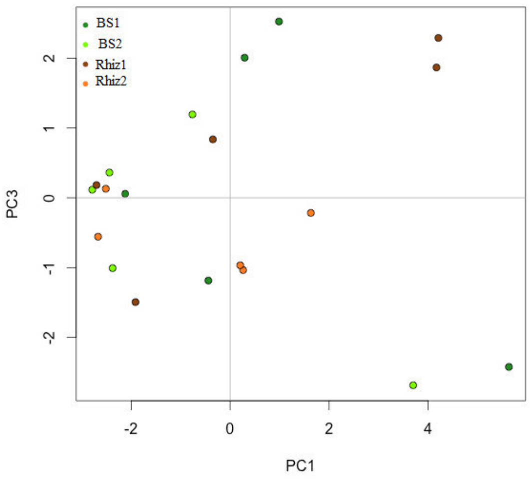
Overall, the dominant bacterial species were unclassified members of Actinomycetales and Solirubrobacterales whose frequency did not change among the soil samples. In contrast, the occurrence of unclassified Micromonosporaceae changed significantly according to sampling times (p= 0.032), especially in bulk soil. In addition, besides the unclassified species, *Staphylococcus epidermidis* was the predominant species whose their frequency did not differ in the soil samples. The full list of the bacterial species revealed in the conventional pest management vineyard with their frequencies are reported in Annex V.

Finally, PCA analysis revealed different structure for the soil samples: in general, rhizosphere and bulk soil were separated on the first axis in accordance with the sampling times (flowering and early fruit development stage). This clustering was represented by the axis1 (35.1%) that explained the diversity among the soil samples. The axis 2 explained about the 14.4% of biodiversity, while the axis 3 explained the 11% of diversity (Figure 4.16 a and b).

a)



b)



<u>Figure 4.16</u> Comparison of the genetic structure of bacterial communities by principal component analysis of microbiome profiles from the different sampling time (flowering and early fruit development)) and from the different compartments (rhizopheric soil and bulk soil) of Vitis vinifera cv Pinot noir: a) component 1 vs. component 2; b) component 1 vs. component 3.

4.3 Discussion

The effect of the plant species, cultivar and age on the microbial communities structure have been described in different studies (1-4); moreover, several studies characterized the impact on these communities of cropping practices, such as use of herbicide, and soil type (5-6). Finally, several works evaluated the impact of pesticides and herbicides on the rhizosphere bacterial communities (7-9).

It has been reported, in addition, that the amount and the quality of root exudates change between the growth stage of the plant and these changes can exert an effect on the composition of bacterial associated with plants (10).

Overall our results demonstrated that shifts in bacterial communities in a vineyard subjected to conventional management, occurred mainly according to sampling time (phenological stage of the plant). This suggested that the impact of the plant's phenological stage (variable time) on the microbial community, in this specific condition, is stronger than that exerted by the space variable (rhizosphere effect).

The number of species observed at early fruit development stage was higher than flowering stage both in bulk soil and in rhizosphere. Moreover, microbial biodiversity, measured as Shannon's Index was higher during early fruiting stage (second sampling) than the flowering stage (first sampling) independently by space variable. Furthermore, principal components analysis (PCA) based on the relative abundance confirm that the genetic structure of microbial communities differed significantly according to the time variable (flowering stage vs. early fruiting stage). Our results showed difference in the microbial communities due to the phenological stage of the plant and not to the rhizosphere effect.

Regarding phyla distribution, our data indicated that the predominant phyla, in all the soil samples, were Actinobacteria (with high frequencies, $\geq 50\%$), Proteobacteria, Firmicutes and Acidobacteria. These results are only in partial agreement with other recent works focused on the structure of microbial communities in the vineyard ecosystem (11-14).

According to a recent work, in a conventional pest management vineyard, Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes and Acidobacteria were the most represented phyla found through DGGE methods (14).

In addition, in a work based on culture-dependent a dominance of strains belonging to Proteobacteria, Firmicutes and Actinobacteria with an abundance comprises about 42.1, 33.7 and 9.5% respectively were reported in the rhizosphere of grapevine (11).

In general, all of these phyla were commonly found in both soil and rhizosphere and are well known for their effect on plant growth (15-16). It should also be considered that these microbial groups by P solubilization and N_2 fixation can play an essential role in nutrient cycling (17). In addition, these phyla may partially enhance the soil fertility and crop productivity (18). Notably, Actinobacteria and Proteobacteria, known as dominant phyla in soil, are supposed to be involved in the degradation of organic matter (19) as well as in the production of secondary metabolites (20).

Among the Proteobacteria, the most represented classes found in the vineyard (in both sampling time) were α, β and δ-Proteobacteria. In general, the amount of sequences ascribed to all Proteobacteria classes was higher at the second sampling (early fruiting stage) than in the first sampling (flowering stage) (data not show). According to Gregory (21), the increase of γ-Proteobacteria in the rhizosphere during different times can be ascribed to a higher or more favourable organic matter release during plant growth. Moreover, in recent years, the effect of herbicides and pesticides on bacterial communities in the rhizosphere of corn and soybean demonstrated that all Proteobacteria classes and especially γ-Proteobacteria increased following herbicides treatment (glyphosate) (22).

Regarding α-Proteobacteria the most represented identified genus was *Bradyrhizobium* that is known for its ability to promote plant growth and fix nitrogen (23).

Burkholderia was the predominant genus of the β-Proteobacteria. It is a gram-negative bacteria commonly found in several environment such as soil, plant rhizosphere, water and humans (24).

Some species of this bacterial genus are considered as plant growth-promoting bacteria, play an important role in bioremediation (25) and are able to suppress plant pathogens (26). At the same time, species belonging to *Burkholderia,* especially *B. cepacia* and *B. pseudomallei* has emerged as opportunistic human pathogen (27). Some species of *B. cepacia* were detected in the rhizosphere of different crop plants (28): this bacterial species is common in agricultural soils (29) and has been found as present in the rhizosphere of maize and other crop plants (30). Consistently, in the conventional pest management vineyard subjected to analysis we found microorganisms belonging to *Burkholderia sp.*

Moreover, sequences corresponding to the genus *Staphylococcus* have been found both in bulk soil and in rhizosphere. In particular, *Staphylococcus epidermidis* was the dominant species in the conventional pest management vineyard; moreover, the species *Staphylococcus haemolyticus* was present in soil samples although with low frequence.

Both these bacterial species are classified as human opportunistic pathogen (31-32). Also, the presence and the isolation of *S. haemolyticus* from internal tissue of plants has been documented (33).

Some opportunistic human bacterial pathogens are even able to colonize plant tissue (34) and the occurrence of these bacteria in the rhizosphere and soils received many attention in the last years. In fact, different works reported the presence of possible opportunistic human pathogenic bacteria associated with plant roots of several species such as potato, strawberry and rice (28, 35-38).

Recently, Yousaf and collaborators (39), by pyrosequencing approach found opportunistic human pathogens in the grapevine endosphere; in this work four bacteria genera recognized as opportunistic human pathogens were detected (*Burkholderia, Propionibacterium*, *Staphylococcus* and *Clostridium*). In the same year, Campisano and colleagues reported the presence of opportunistic human pathogens in grapevine (40).

References:

(1) Chiarini L., Bevivino A., Dalmastri C., Nacamulli C. and Tabacchioni S. (1998). Influence of plant development, cultivar and soil type on microbial colonization of maize roots Influence of plant development, cultivar and soil type on microbial colonization of maize roots. *Appl Soil Ecol,* 8: 11-18

(2) Smalla K., Wieland G., Buchner A., Zock A., Parzy J., Kaiser S., Roskot N., Heuer H. and Berg G. (2001). Bulk and rhizosphere soil bacterial communities studied by denaturating gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. *Appl Environ Microbiol*, 67: 4742-4751

(3) Baudoin E., Benizri E. and Guckert A. (2002). Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. *Appl Soil Ecol,* 19: 135-145

(4) Chaparro J.M., Badri D.V., Bakker M.G., Sugiyama A., Manter D.K. and Vivanco J.M. (2013). Exudation of Phytochemicals in *Arabidopsis* follows specific patterns that are developmentally programmed and correlate with soil microbial functions. *PLoS One,* 8 (2): 1-10

(5) Hardoim P.R., Andreote F.D., Reinhold-Hurek B., Sessitsch A., van Overbeek L.S. and van Elsas J.D. (2011). Rice root-associated bacteria: insights into community structures across 10 cultivars. *FEMS Microbiol Ecol,* 77: 154-164

(6) Dias A.C.F., Dini-Andreote F., Hannula S.E., Andreote F.D., Pereira e Silva M.dC., Salles J.F., deBoer W., van Veen J. and van Elsas J.D. (2013). Different selective effects on Rhizosphere Bacteria exerted by Genetically modified versus Conventional Potato Lines. *PLoS One,* 8(7): e67948

(7) Imfeld G. and Vuilleumier S. (2012). Measuring the effects of pesticides on bacterial communities in soil: a critical review. *Europ J of soil Biol*, 49: 22-30

(8) Jacobsen C.S. and Hjelmsø M.H. (2014). Agricultural soils, pesticides and microbial activity. *Current opinion in Biotech*, 27: 15-20

(9) Nettles R., Watkins J., Ricks K., Boyer M., Licht M., Atwood L.W., Peoples M., Smith R.G., Mortensen D.A. and Koide R.T. (2016). Influence of pesticide seed treatments on rhizosphere fungal and bacterial communities and leaf fungal endophyte communities in maize and soybean. *Appl Soil Ecol*, 102: 61-69

(10) Dini-Andreote F., Nunes da Rocha U., Araùjo W.L., Azevedo J.L. and van Overbeek L.S. (2010). Effect of bacterial inoculation, plant genotype and developmental stage on root-associated and endophytic bacterial communities in potato (*Solanum tuberosum*). *Antonie van Leeuwenhoek*, 97: 389-399

(11) Karagöz K., Ates F., Karagöz H., Kotan R. and Çakmakçi R. (2012). Characterization of plant growth-promoting traits of bacteria isolated from the rhizosphere of grapevine grown in alkaline and acidic soils. *European Journal of Soil Biology,* 50: 144-150

(12) Campisano A., Antonielli L., Pancher M., Yousaf S., Pindo M. and Pertot I. (2014a). Bacterial endophytic communities in the grapevine depend on pest management. *PLoS One*, 9(1): e02527-14

(13) Bulgari D., Casati P., Quaglino F. and Bianco P.A. (2014). Endophytic bacterial community of grapevine leaves influenced by sampling date and phytoplasma infection process. *BMC Microbiol,* 14: 198

(14) Vega-Avila A.D., Gumiere T., Andrade P.A.M., Lima-Perim J.E., Durrer A., Baigori M., Vazquez F. and Andreote F.D. (2015). Bacterial

communities in the rhizosphere of *Vitis vinifera* L. cultivated under distinct agricultural practices in Argenitina. *Antonie Van Leeuwenhoek,* 107(2): 575-588

(15) Berendesen R.L., Pieterse C.M.J. and Bakker P.A.H.M. (2012).The rhizosphere microbiome and plant health. *Trends in Plant Science,* 17(8): 478-486

(16) Philippot L., Raaijmakers J., Lemanceau P. and van der Putten W.H. (2013). Going backe to the roots: the microbial ecology of the rhizosphere. *Nature reviews Microbiology*, 11: 789-799

(17) Daquiado A.R., Kuppusamy S., Kim S.Y., Kim J.H., Yoon Y-E., Kim P.J., Oh S-H., Kwak Y-S. and Lee Y.B. (2016). Pyrosequencing analysis of bacterial community diversity in long-term fertilized paddy field soil. *Applied Soil Ecology*, 108: 84-91

(18) Lesaulnier C., Papamichail D., McCorkle S., Ollivier B., Skiena S., Taghavi S., Zak D. and van der Lelie D. (2008). Elevated atmospheric CO_2 affects soil microbial diversity associated with trembling aspen. *Environ Microbiol,* 10: 926-941

(19) Ahn J.H., Song J., Kim B.Y., Kim M.S., Joa J.H. and Weon H.Y. (2012). Charcaterization of the bacterial and archaeal communities in rice field soils subjected to long-term fertilization practices. *J Microbiol,* 50: 754-765

(20) Jenkins S.N., Waite I.S., Blackburn A., Husband R., Rushton S.P., Manning D.C. and O'Donnell A.G. (2009). Actinobacterial community dynamics in long term managed grasslands. *Antonie val Leeuwenhoek Int J Gen Mol Microbiol*, 95(4):319-334

(21) Gregory, P.J. (2006). Roots, rhizosphere and soil: The route to a better understanding of soil science? *Eur J Soil Sci*, 57: 2-12

(22) Newman M.M., Hoilett N., Lorenz N., Dick R.P., Liles M.R., Ramsier C. and Kloepper J.W. (2016). Glyphosate effects on soil rhizosphere-associated bacterial communities. *Science of the total Environment*, 543: 155-160

(23) Barea J.M., Pozo M.J., Azcón R. and Azcón-Aguilar C. (2005). Microbial co-operation in the rhizosphere. *J of Experiment Bot,* 56(417): 1761-1778

(24) Parke J.L. and Gurian-Sherman D. (2001). Diversity of the *Burkholderia cepacia* complex and implications for risk assessment of biological control strains. *Annu Rev Phytopathol,* 39: 225-258

(25) Chiarini L., Bevivino A., Dalmastri C., Tabacchioni S. and Visca P. (2006). *Burkholderia cepacia* complex species: health hazards and biotechnological potential. *Trends Microbiol,* 14: 277-286

(26) el-Banna, N., and Winkelmann G. (1998). Pyrrolnitrin from *Burkholderia cepacia*: antibiotic activity against fungi and novel activities against *Streptomyces*. *J Appl Microbiol,* 85:69-78.

(27) Coenye T. and Vandamme P. (2003). Diversity and significance of *Burkholderia* species occupying diverse ecological niches. *Environmental Microbiology,* 5(9): 719-729

(28) Berg G., Eberl L. and Hartmann A. (2005). The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environ Microbiol*, 7(11): 1673-1685

(29) Miller S.C., LiPuma J.J. and Parke J.L. (2002). Culture-based and non-growth-dependent detection of the *Burkholderia cepacia* complex in soil environments. *Appl Environ Microbiol,* 68: 3750-3758

(30) Dalmastri C., Baldwin A., Tabacchioni S., Bevivino A., Mahenthiralingam E., Chiarini L. and Dowson C. (2007). Investigating *Burkholderia cepacia*

complex populations recovered from Italian maize rhizosphere by multilocus sequence typing. *Envrin Microbiol,* 9(7): 1632-1639

(31) Voung C. and Otto M. (2002). *Staphylococcus epidermidis* infections. *Microbes infect,* 4(4): 481-489

(32) Takeuchi F., Watanabe S., Baba T., Yuzawa H., Ito T., Morimoto Y., Kuroda M., Cui L., Takahashi M., Ankai A., Baba S.I., Fukui S., Lee J.C. and Hiramatsul K. (2005). Whole-genome sequencing of *Stphylococcus haemolyrticus* uncovers the extreme plasticity of its genome and the evolution of human-colonizing staphylococcal species. *J Bacteriol*, 187(21): 7292-7308

(33) Krechel A., Faupel A., Hallmann J., Ulrich A. and Berg G. (2002). Potato-associated bacteria and their antagonistic potential towards plant-pathogenic fungi and the plant-parasitic nematode Meloidogyne incognita (Kofoid & White) Chitwwod. *Can J Microbiol,* 48(9): 772-786

(34) Tyler H.L. and Triplett E.W. (2008). Plants as a Habitat for beneficial and/or Human Pathogenic Bacteria. *Annu Rev Phytopathol*, 46(1): 53-73

(35) Gupta C.P., Sharma A., Dubey R.C. and Maheshwari D.K. (2001). Effect of metal ions on growth of *Pseudomonas aeruginosa* and siderophores and protein function. *J Exp Biol,* 39: 1318-1321

(36) Mehnaz S., Mirza M.S., Haurat J., Bally R., Normand P., Bano A. and Malik K.A. (2001). Isolation and 16S rRNA sequence analysis of the beneficial bacteria from the rhizosphere of rice. *Can J Microbiol,* 47: 110-117

(37) Sessitsch A., Reiter B. and Berg G. (2004). Endophytic bacterial communities of field-grown potato lines and their plant growth-promoting abilities. *Can J Microbiol,* 50: 239-249

(38) Berg G., Roskot N., Steidle A., Eberl L., Zock A. and Smalla K. (2002). Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different *Verticillium* host plants. *Appl Environ Microbio.,* 68: 3328-3338

(39) Yousaf S., Bulgari D., Bergna A., Pancher M., Quaglino F., Casati P. and Campisano A. (2014). Pyrosequencing detects human and animal pathogenic taxa in the grapevine endosphere. *Front Microbiol,* 5: 237

(40) Campisano A., Ometto L., Compant S., Pancher M., Antonielli L., Varotto C., Anfora G., Pertot I., Sessitsch A. and Rota-Stabelli O. (2014b). Interkingdom transfer of the acne-causing agent, propionibacterium acnes, from human to grapevine. *Mol Biol Evol.*, 31(5): 1059-1065

Chapter V

Exploring the microbiota of *Vitis vinifera* cv. Pinot noir in an integrated pest management vineyard

In this section I'm going to describe the rhizosphere microbiota of grapevine in an integrated pest management vineyard at two phenological stages corresponding to flowering and early fruit development (variable: time).

The composition of the rhizosphere bacterial community has been compared to that of bulk soil (variable: space).

According to the FAO definition (2012), integrated pest management is *"an ecosystem approach to crop production and protection combining different management strategies and practices to grow healthy crops and minimize the use of pesticides"*. In Europe, IPM is not yet regulated; however, its general principles are listed in the Annex III of Directive 2009/128/EC.

The integrated pest management vineyard is located in "Tenuta Cannona", Agrion Foundation for research, close to Carpeneto (AL): Altitude: 286 m a.s.l., Latitude: 44,683706 °N and Longitude: 8,6258889 °E. In figure 5.1 a is reported a GIS map of the vineyard and the two sampling site (BS, bulk soil and Rhiz, rhizosphere).

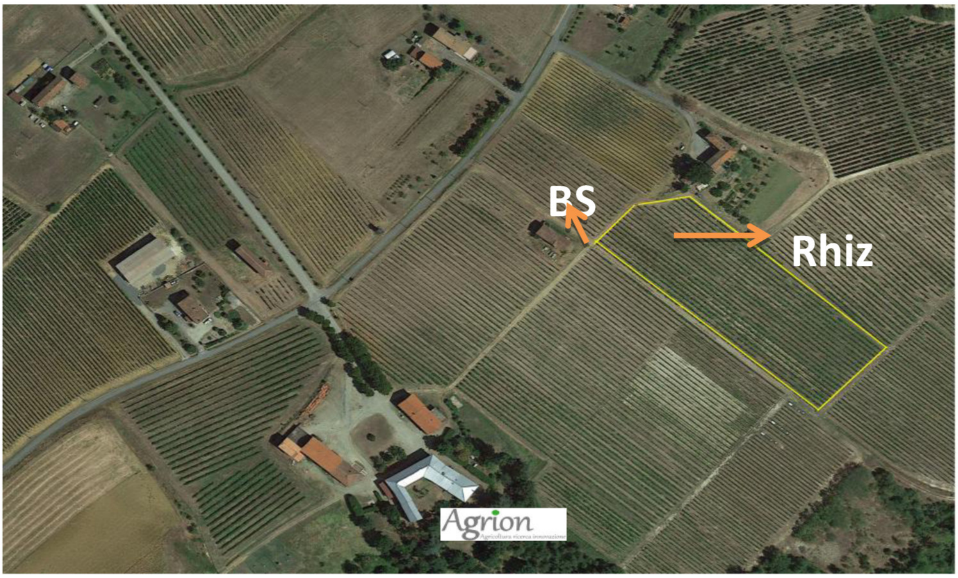


Figure 5.1 GIS map of the integrated pest management vineyard. The sampling points of the rhizosphere (Rhiz) and bulk soil (BS) are indicated by an orange arrow.

Chemical treatments performed during vine growth were weeding with glyphosate (in April) among the plants, but not between the lines (Figure 5.2), fungicide treatment against *Peronospora* spp. (Metalaxil-m + mancozeb), against *Oidium* spp. (Ciflufenamid) each month and against *Botrytis cinerea* (Cyprodinil + Fludioxonil) in July; finally, two insecticide treatments (Thiamethoxam and Clorpirifos-metile) in July.

Figure 5.2 Imagines of the line without the treatments

5.1 Biodiversity

The estimate of the coverage of the metagenomics dataset was provided by the rarefaction curves (Figure 5.3), that are based on the observation that the curve of rarefied counts of any feature should plateau if the sample is close to saturation (1). This kind of analysis allowed to measure the depth of our experiments.

The curves showed in Figure 5.3 show that a good coverage of the entire community was achieved, although the number of reads coming from one sample of bulk soil harvested during the first sampling was ten order of magnitude lower than the other soil samples.

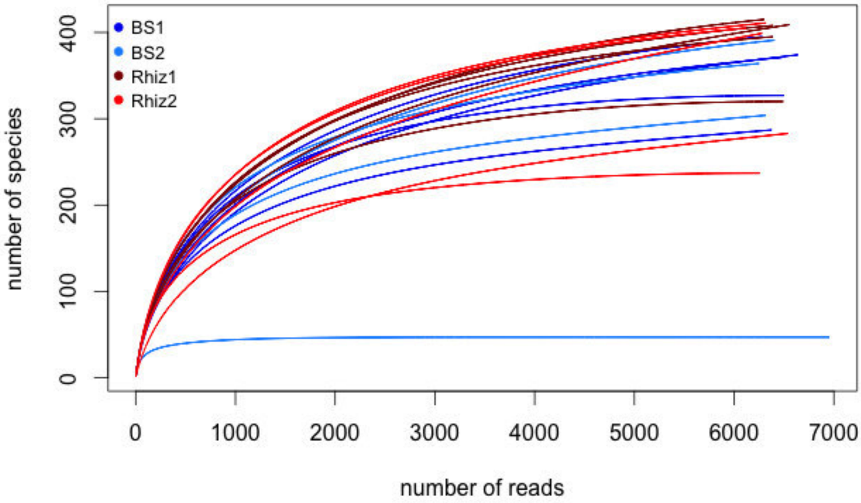


Figure 5.3 Rarefaction curves for each samples (BS1, BS1, Rhiz1, Rhiz2, five sub-samples each)

Biodiversity has been expressed as number of bacterial species, Shannon-Wiener's and Simpson's indices. The median number of bacterial species was similar in the two samplings; however, this parameter was higher in the rhizosphere (first sampling, 894; second sampling, 915) than in the bulk soil (first sampling 685; second sampling 639.5) in both the two samplings (Figure 5.4 a). The median value of the Shannon-Wiener's index for the rhizosphere was higher at the first sampling (flowering) than in all the other cases (Figure 5.4 b). The Simpson's index did not change in the different samples (Figure 5.4 c).

a)

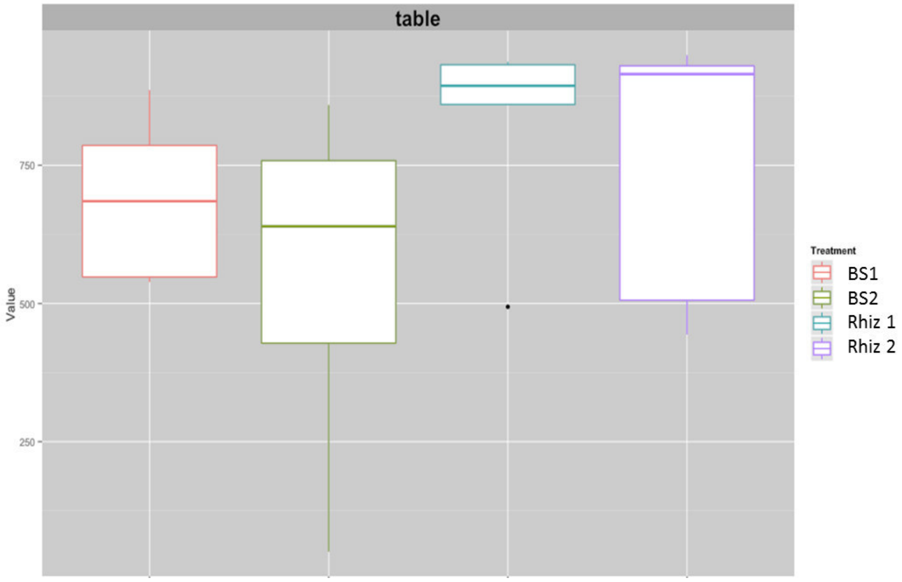


table
Value
750
500
250
Treatment
BS1
BS2
Rhiz 1
Rhiz 2

b)

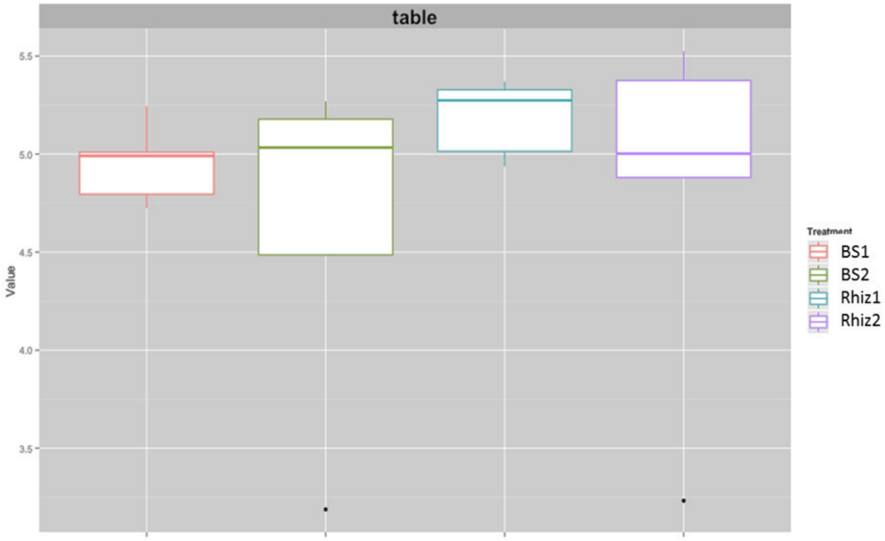


table
Value
5.5
5.0
4.5
4.0
3.5
Treatment
BS1
BS2
Rhiz1
Rhiz2

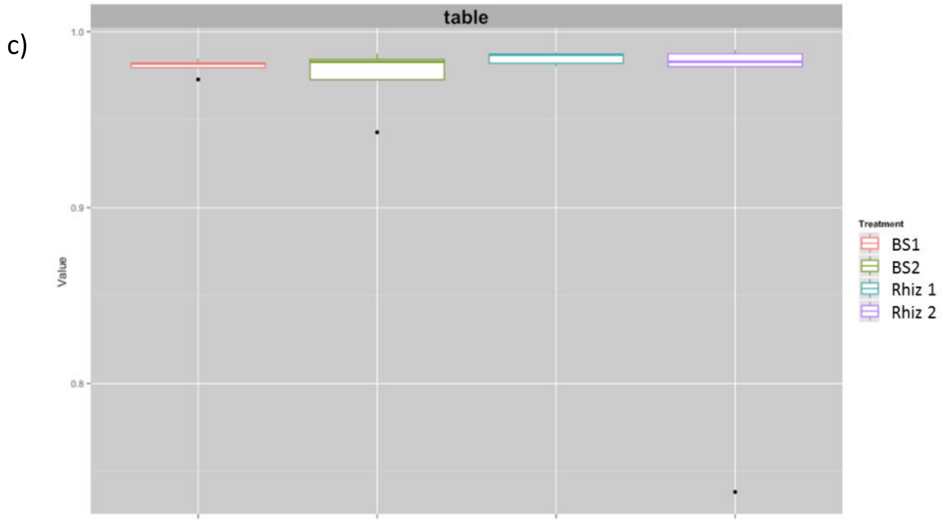


Figure 5.4 *a) Number of bacterial species, b) Shannon-Wiener's Index and c) Simpson's diversity index detected in bulk soil and rhizosphere of V. vinifera cv. Pinot noir at two sampling times*

5.2 Description of microbial communities observed in the vineyard

The analysis of the microbial structure performed with the pyrosequencing approach provided a total of 142908 reads with a mean value of 7500 reads per sample. Moreover, after demultiplexing steps a total of 128296 reads were obtained (with a mean value of 6800 reads) and used for further analysis and for phyla description.

The dominant phyla found in the integrated pest management vineyard were Actinobacteria followed by Proteobacteria and Gemmatimonadetes both in the rhizosphere and bulk soil (space) and both at the flowering and at the early fruit development (time) (Figure 5.5) (Annex VI).

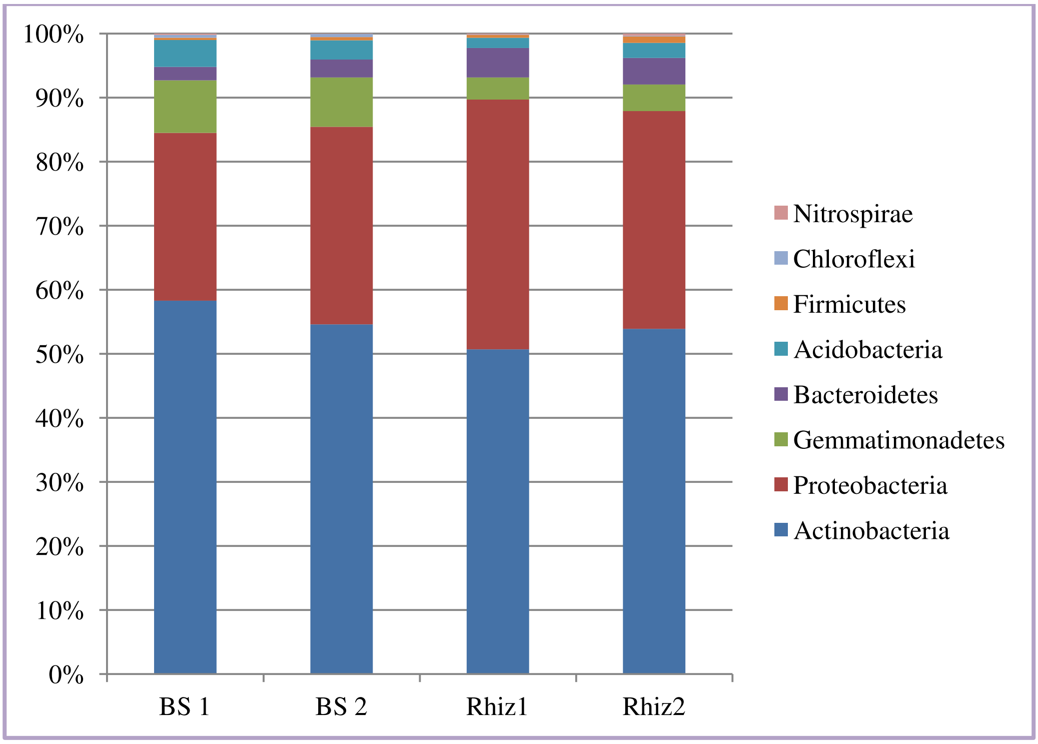


Figure 5.5 Microbial community composition in the rhizosphere and bulk soil at the two sampling time at the phylum level (top 8 taxa)

Actinobacteria frequency did not change significantly between the soil samples (BS1 58.24%; BS2 54.60%; Rhiz1 50.65%; Rhiz2 53.85%). Similarly, the amount of readings ascribed to Proteobacteria did not change considering sampling time or site (BS1 26.20%; BS2 30.81%; Rhiz1 39.00%; Rhiz2 33.95%).

In contrast, abundance of Gemmatimonadetes and Chloroflexi differed between bulk soil (8.15% and 0.45%, respectively) and rhizosphere (3.45% and 0.03%, respectively) during the flowering (first sampling) ($p=0.032$ and $p=0.012$, respectively) (Figure 5.6 a and b).

Furthermore, the amount of Nitrospirae recorded in bulk soil changed significantly according to the sampling time (BS1 0.06% and BS2 0.01%, p= 0.018); variations occurred also in space during the fruit development (second sampling) (BS2 0.01% and Rhiz2 0.09%, p= 0.016) (Figure 5.6 c and d).

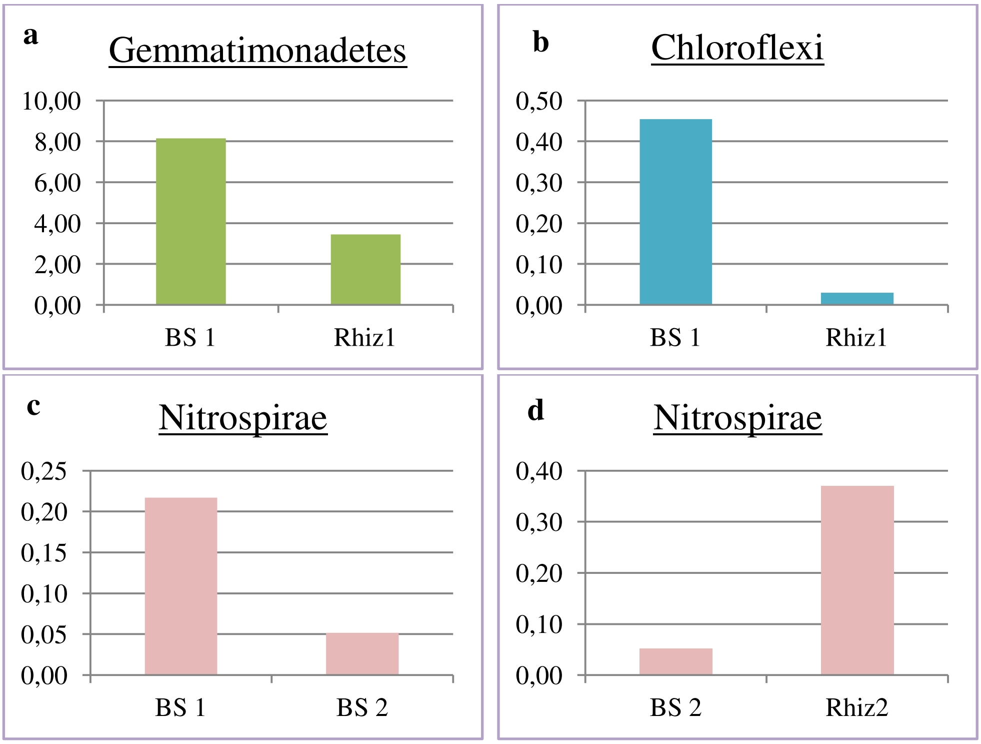


Figure 5.6 Frequency of a) Gemmatimonadetes, b) Chloroflexi and c,d) Nitrospirae phyla in soil and rhizosphere during the two sampling dates

No significant differences were observed when considering the frequency of Bacteroidetes, Firmicutes and Acidobacteria phyla.

At the classes level, Actinobacteria was the dominant one followed by α-Proteobacteria and β-Proteobacteria as shown in Figure 5.7 (Annex VII).

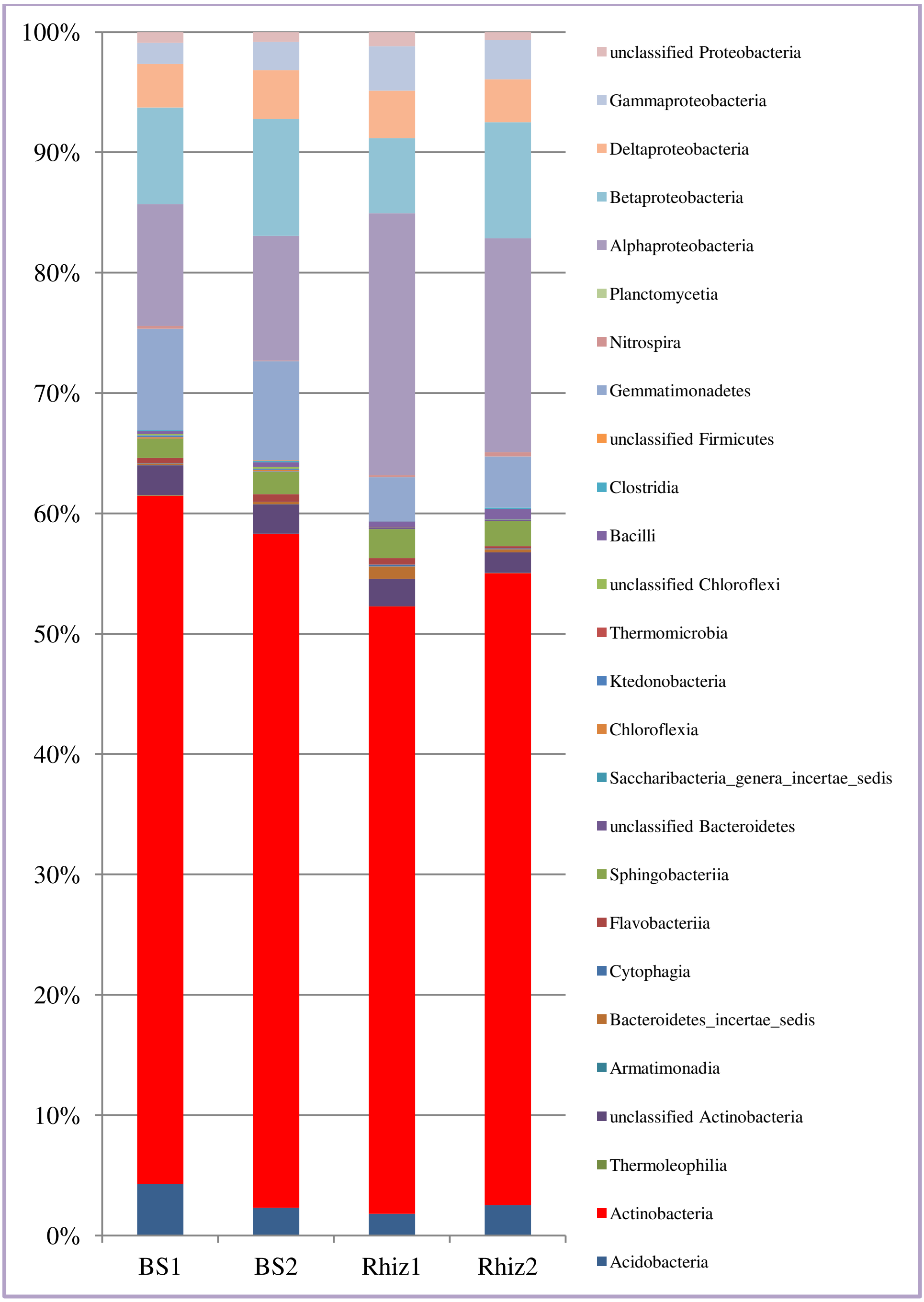


Figure 5.7 Microbial community composition in the bulk soil and rhizosphere of V. vinifera cv. Pinot Noir at the two sampling times (flowering and early fruiting) at the class level.

Actinobacteria was the most represented class belonging to Actinobacteria phyla with frequencies overcoming 50% in all the soil samples followed by unidentified_Actinobacteria (unculturable bacteria).

Considering all the soil samples, *Gaiella*, *Arthrobacter*, *Solirubrobacter*, *Blastococcus*, *Nocardioides* and *Streptomyces* were found to predominant genera belonging to Actinobacteria (Figure 5.8).

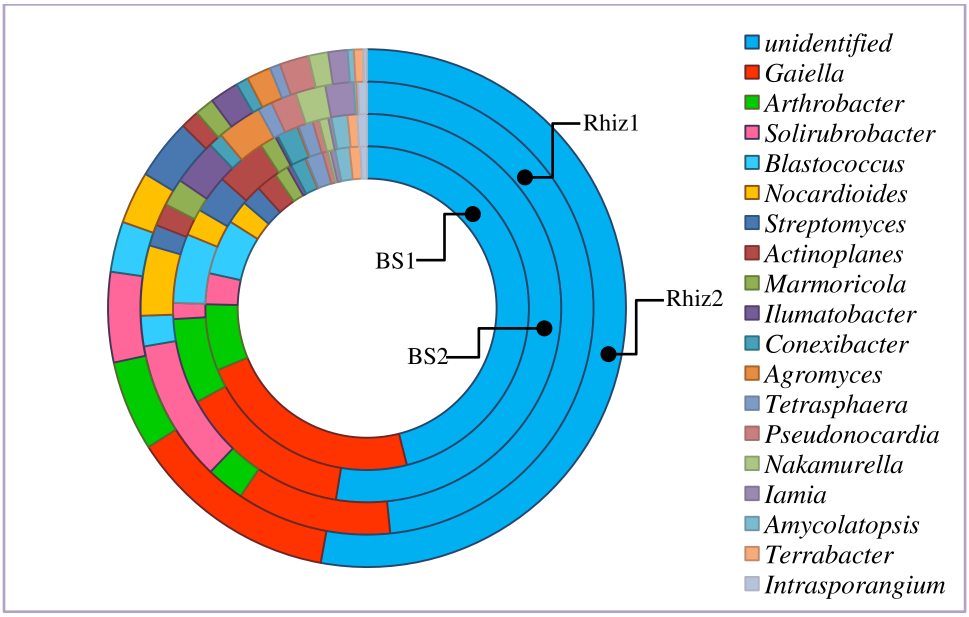


Figure 5.8 Distribution of the genera belonging to Actinobacteria in all the soil samples

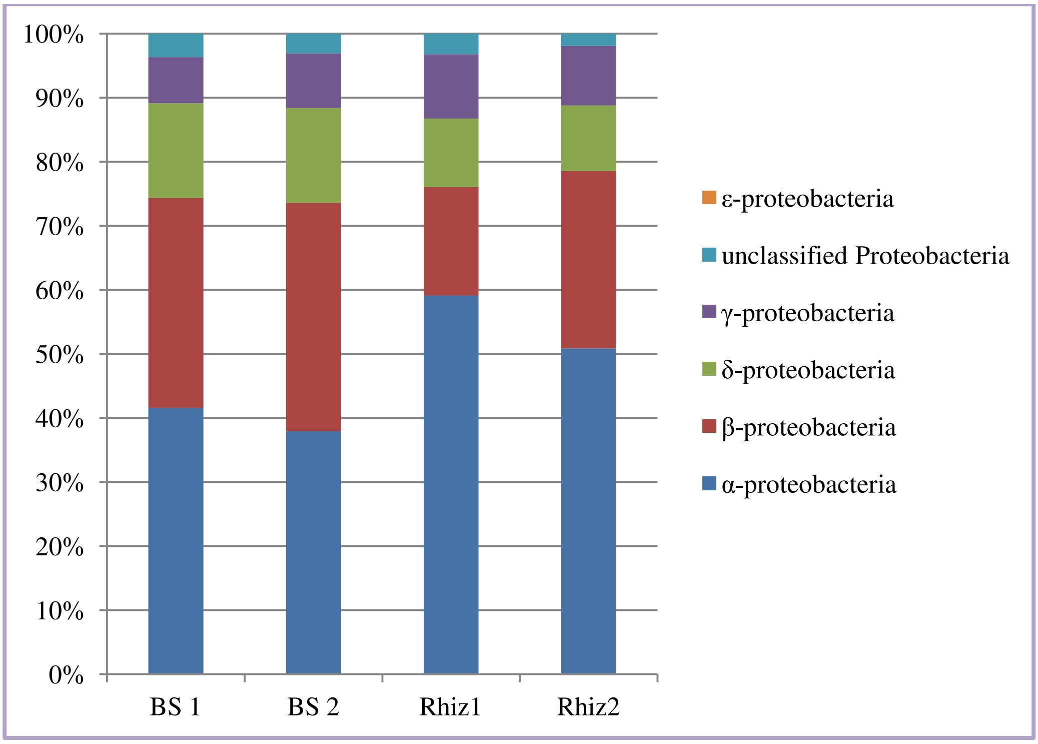
In particular, the most represented identified genus was *Gaiella* with frequency varying between 10 and 18%. Like *Gaiella* the frequency of *Blastococcus* and *Nocardioides* were similar in all samples.

On the contrary, *Solirubrobacter* and *Arthrobacter* genera differed between rhizosphere (9,5% and 2,5%, respectively) and bulk soil (3,0% and 6,4%, respectively) during the flowering stage ($p = 0.032$ and $p = 0.016$, respectively).

In addition, the frequency of these genera in the rhizosphere changed significantly during time (*Arthrobacter,* Rhiz1 2,5% and Rhiz2 5,2%, p=0.047 and *Solirubrobacter,* Rhiz1 9,5% and Rhiz2 5,2%, p= 0.032).

Moreover, the frequency of the genus *Streptomyces* in the rhizosphere differed in the two sampling times (Rhiz1 1,4% and Rhiz2 3,5%, p=0.028).

The distribution of different classes of Proteobacteria recorded in the vineyard was reported in Figure 5.9. The obtained data indicated that α-Proteobacteria, followed by β-Proteobacteria, were the most abundant in the all samples. In addition, no member belonging to class ε-Proteobacteria was found in the samples.



<u>Figure 5.9</u> Distribution of the classes Proteobacteria at two sampling times in bulk soil and rhizosphere

The frequency of α-Proteobacteria in the rhizosphere was significantly higher at flowering than at fruiting stage (Rhiz1 59.1% and Rhiz2 50.8%, p=0.032); moreover, γ-Proteobacteria frequency was significantly higher in the rhizosphere than in bulk soil at the first sampling date (BS1 7.2% and Rhiz1 10%, p=0.032). In contrast, abundance of β-Proteobacteria was similarly in all samples.

Among α-Proteobacteria, *Skermanella* was the most abundant identified genus (BS1 9.9%, BS2 8.8%, Rhiz1 24.0%, Rhiz2 18.9%) followed by *Bradyrhizobium* and *Microvirga* (Figure 5.10).

At the flowering stage the amount of readings corresponding to the genus *Bradyrhizobium* in the rhizosphere was significantly higher than that recorded in bulk soil (Rhiz1 1.16% and BS1 0.55%, p=0.028). Instead, the frequency of *Skermanella* and *Microvirga* genera did not differ significantly in all samples.

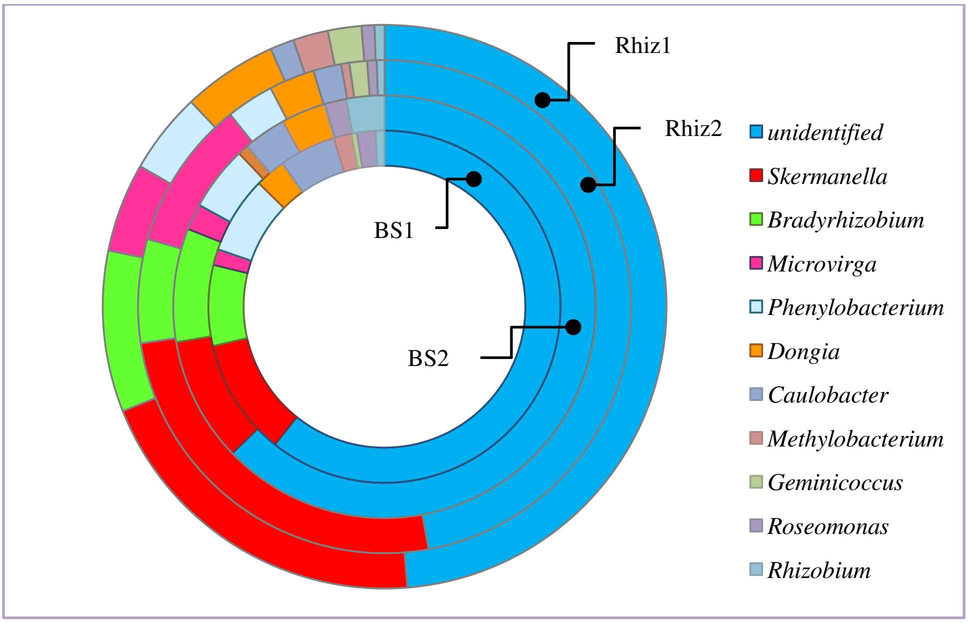


Figure 5.10 Abundance of the most represented genera belonging to α-Proteobacteria

Regarding β-Proteobacteria, besides the unidentified genera *Massilia* was the dominant identified genus (Figure 5.11) and was more abundant in bulk soil than in rhizosphere at flowering (BS1 6.53% and Rhiz1 1.01%, p=0.012). In addition, results obtained demonstrated significant variations in the rhizosphere according to with the phenological stage of the plant (Rhiz1 1.01% and Rhiz2 12.44%, p=0.012).

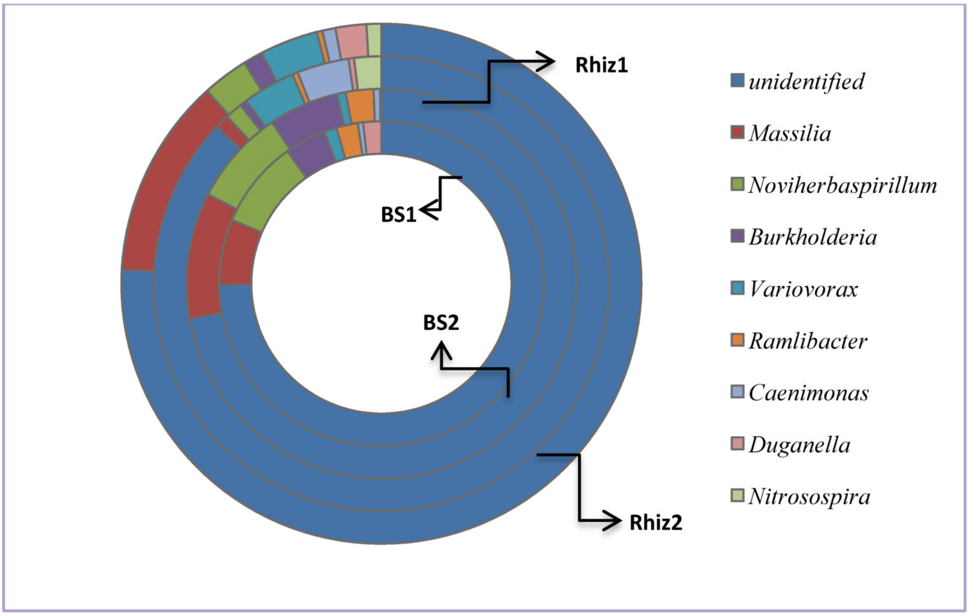


Figure 5.11 Distribution of the genera belonging to β-Proteobacteria in all soil samples

The most abundant identified genus belonging to the γ-Proteobacteria was *Steroidobacter* whose frequency changed according to the space variable during the first sampling (flowering) (BS1 16.1% and Rhiz1 42.4%, p=0.016).

The phylum Gemmatimonadetes was represented by the Gemmatimonadetes including a unique genus, *Gemmatimonas*. The abundance of this genus was the highest observed in the bulk soil compared to the rhizosphere during the early flowering phase of the plant (BS1 35.43% and Rhiz1 14.71%, p=0.032) (Figure 5.12 a).

Similarly to Gemmatimonadetes, Nitrospirae phylum was represented by only Nitrospira class and by a unique genus, *Nitrospira*. However, in contrast to *Gemmatimonas*, the frequency of this genus changed significantly during early fruit development stage (second sampling) and was higher in rhizosphere than in bulk soil (Rhiz 2 44.01% and BS2 6.44%, p=0.016) (Figure 5.12 b).

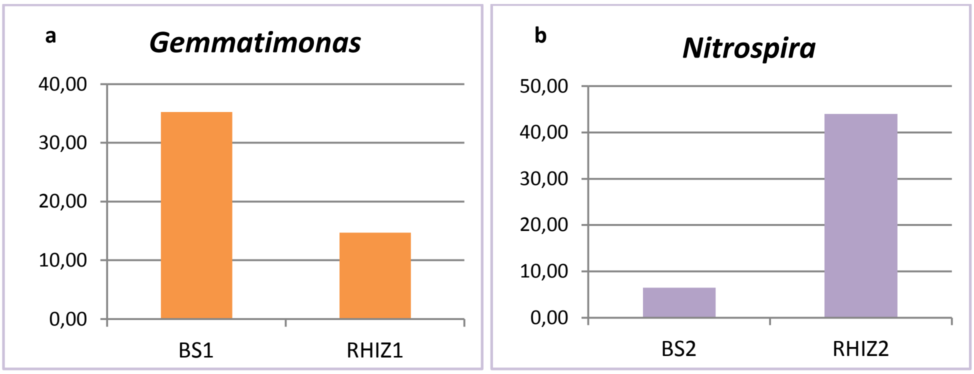


Figure 5.12 Relative abundance of Gemmatimonas (a) and Nitrospira (b) in bulk soil and rhizosphere at two sampling times

Regarding Bacteroidetes phyla, the most abundant classes found in all samples (both in bulk soil and rhizosphere, independently by time) were Sphingobacteria and Flavobacteria, followed by Bacteroidetes_incertea_sedis (Figure 5.13). No significant differences were reported for the Sphingobacteria and Flavobacteria classes; moreover, frequency of Bacteroidetes_incertea_sedis differed between bulk soil and rhizosphere during the first sampling (flowering) (BS1 6.17% and Rhiz1 23.81%, p= 0.032).

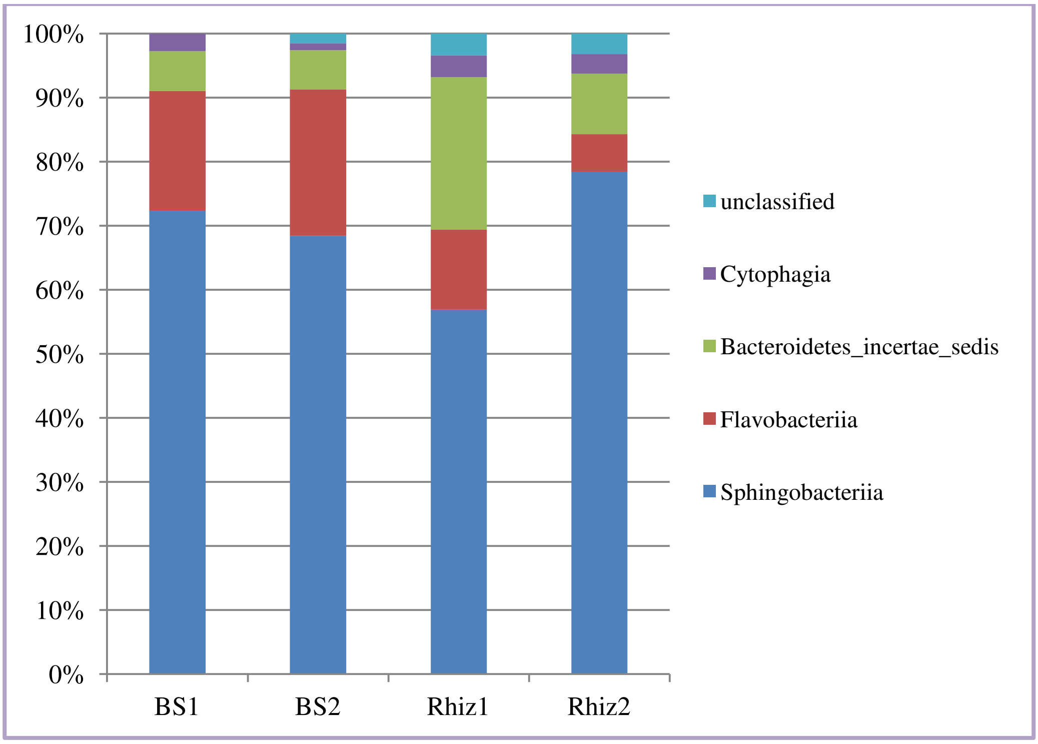
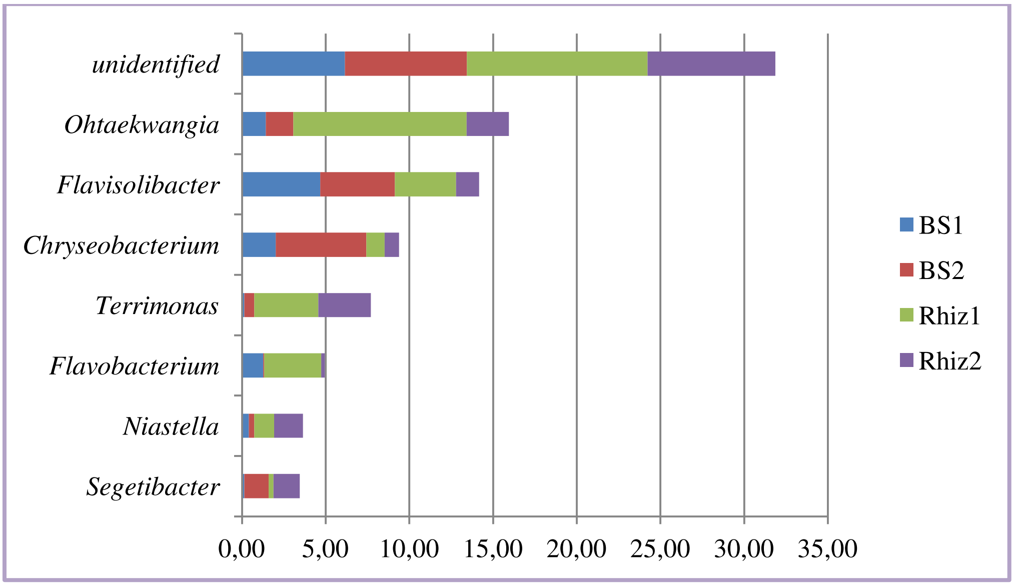


Figure 5.13 Abundance of Bacteroidetes at class level and their distribution in bulk soil and rhizosphere at two sampling times

In general, among the Bacteroidetes, the dominant genera were *Flavisolibacter*, *Terrimonas*, *Niastella* and *Segetibacter* belonging to Sphingobacteria class. The most frequent genera belonging to Flavobacteria class were *Chryseobacterium* and *Flavobacterium*.

In addition, *Ohtaekwangia* was the dominant genus belonging to Bacteroidetes_incertea_sedis (Figure 5.14).



<u>Figure 5.14</u> Representation of major genera belonging to different classes of Bacteroidetes_incertae_sedis recorded in all samples

The amount of sequences ascribed to Firmicutes was low in all samples compared to other phyla. At level class they were represented by Bacilli and Clostridia which did not differ between the soil samples.

Finally, results obtained for the phylum Acidobacteria were comparable both in the rhizosphere and in bulk soil during the two sampling times.

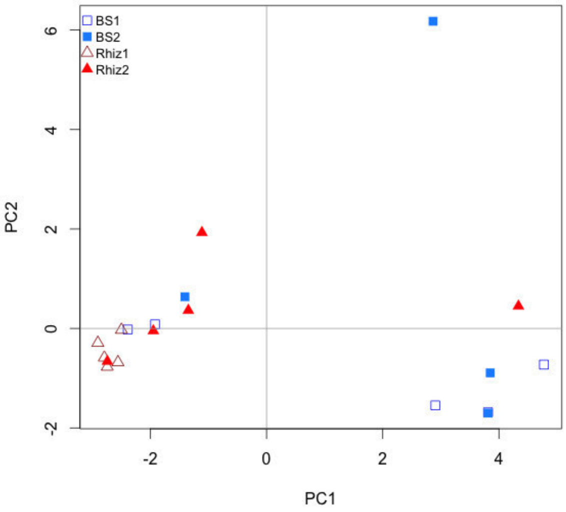
The list of the bacterial genera described with their frequencies and significances are reported in Annex VIII.

In general, the most dominant genera were unidentified-Actinobacteria, and their frequency did not change both in time and in space.

Dominant bacterial species were unclassified Actinomycetales, unclassified Solirubrobacterales and unclassified Micromonosporaceae whose frequency did not change among the samples. On the contrary, the occurrence of unclassified Acidimicrobiales, unclassified Nocardioidaceae and unclassified Bradyrhizobiaceae differed between the bulk soil and the rhizosphere (p=0.032, 0.016, 0.008; respectively), especially during the flowering. The full list of the bacterial species detected in the integrated pest management vineyard with their frequencies in different are reported in Annex IX.

Finally, PCA analysis revealed different structure for the soil samples: in general, rhizosphere and bulk soil were separated on the first axis, independently by the sampling date. The bacterial community associated to the rhizosphere harvested at the early flowering time (Rhiz1) were clearly separated by all the other samples. This clustering was represented by the axis1 (48.1%) that explained the highest diversity among the soil samples. The axis 2 explained about the 16% of biodiversity, while the axis 3 explained only the 6% of diversity (Figure 5.15 a and b).

a)



b)

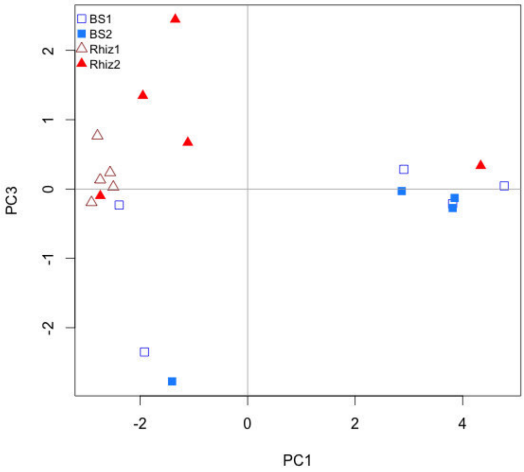


Figure 5.15 Comparison of the genetic structure of bacterial communities by principal component analysis of microbiome profiles from the different sampling time (flowering and early fruit development)and from the different compartments (rhizopheric soil and bulk soil) of Vitis vinifera cv Pinot noir: a) component 1 vs. component 2; b) component 1 vs. component 3.

5.3 Discussion

The possible effect of different plant phenological stages and of rhizodepositions on the composition of microbial communities has been extensively studied and described in annual crops (2-6). However, comparatively less information on bacterial communities in woody perennial agroecosystems are available (7-9).

Roots exudates can affect the structure of rhizosphere microbial communities (10). Moreover, in annual plants the amount of root exudates gradually increases until the flowering stage and decreases during plant maturity (11). Based on this observation it has been reported that during the seedling development root releases sugars that become substrates for a wide range of microorganisms, while when plant ages it release molecules able to select specific microbial species (12-13).

In contrast, our results showed differences of the microbial communities in accordance with space (rhizosphere effect), but not of the phenological stage. The number of species observed in the rhizosphere was higher than in bulk soil at both the sampling time. Consistently, microbial biodiversity measured as Shannon’s index was higher in the rhizosphere than in bulk soil during the first sampling. Moreover, the genetic structure of bacterial communities analysed by PCA was shown to change significantly according to the space factor (bulk soil vs. rhizosphere). Taken together these results suggest that the space factor has a dominant effect over the time factor in determining overall microbial community patterns in the rhizosphere. Moreover, the impact of the rhizosphere effect appears to be more pronounced during the flowering that during the fruit development.

Regarding phyla distribution, our results showed that, in all the samples, the predominant phyla were: Actinobacteria (with high frequencies, ≥ 50%), Proteobacteria, Gemmatimonadetes and Bacteroidetes. This is only in partial agreement with data recently reported in other studies on the bacterial communities in vineyards (14-15).

According to Opsi and co-workers (14), Proteobacteria (36%) followed by Actinobacteria (26%) and Acidobacteria (15%) have been described to be the prevalent phyla in vineyards. Similarly, Zarraonaindia and colleagues (15) analysing bulk soil and grapevine roots samples, observed a dominance of sequences ascribed to Proteobacteria (32% and 57%, respectively), *Acidobacteria* spp. (19% in soil; 10% in root), *Bacteroidetes* spp. (10% in soil;

13% in root) and *Verrumicrobia* spp. (8% in soil; 5% in root), with a higher relative abundance of *Planctomycetes* spp. in soils (7%) and of *Actinobacteria* spp. in roots (5.1%).

Moreover, Li and collaborators (16) demonstrated that the dominant phyla both in maize rhizosphere and in bulk soil were Proteobacteria, Acidobacteria, Actinobacteria, and Sugiyama and co-workers (6), studying changes in microbial structure of soybean rhizosphere, reported that Proteobacteria, Actinobacteria and Choroflexi were the dominant phyla. In general, several studies on the characterization of the soil microbial communities reported that Proteobacteria was the dominant phyla in both the rhizosphere of different plants and in bulk soil.

Actinobacteria and Proteobacteria are well known as dominant phyla in soil and widely distributed in nature. They are actively involved in production of secondary metabolites, in nutrient transformation and in carbon cycling (17).

Actinobacteria are considered as oligotrophic K-strategists (18) and are favoured over copiotrophic r-strategists especially where the availability of soil organic carbon is low and the inputs deriving from fertilizers and pesticides is reduced (19). This is consistent with the low amount of total organic carbon (0.43%) measured in this vineyard soil; moreover, it should be considered that the integrated pest management provides a lower amount of chemical inputs (i.e. pesticides and fertilizers) compared to conventional management that can, on the other side, stimulate the growth of copiotrophic microorganisms.

Among all the identified genera in all samples, our results showed that *Gaiella* was the most represented and was the dominant genus of Actinobacteria. Members of this genus grow as non-motile rod-shaped Gram-negative cells; they are strictly aerobic, oxidase and catalase positive, and the type species is *Gaiella occulta*, described for the firts time in 2011 by Albuquerque an colleagues (20).

Consequently, very little information is available for members of this genus and further studies on other genera phylogenetically close to *Gaiella* could be useful to improve our knowledge on the behaviour and the response of the genus.

Proteobacteria include organisms with a wide range of metabolisms; members of α, β, γ and δ-Proteobacteria, are commonly reported in soil. Members of the α, β and γ classes are considered to be copiotrophs (r-strategist), and they are prevalent where resource availability is high such as in rhizosphere soils (21). In our case this was true especially for α and γ-Proteobacteria, but not for the other classes.

The genus *Bradyrhizobium*, belonging to α-Proteobacteria, has been associated with the ability of this genus to fix N_2, promote plant growth and suppress soil-borne disease. In other studies of the composition of microbial communities in the grapevine rhizosphere, *Bradyrhizobium* was found as one of predominant genera (15). Among β-Proteobacteria, *Massilia* was the prevalent identified genus; cells of *Massilia* were first isolated from clinical samples and are described as flagellated, aerobic and non-spore forming (22). In recent years, however, *Massilia* was found and isolated from rhizosphere of different grapevine cultivar by using molecular approaches (23-24). This genus is spread in different environment and its presence has been reported in the rhizosphere of several plant species such as potato (25), poplar tree (7) and in very different environments such as phyllosphere (26), freshwater (27) and aerosols (28).

Interestingly, the occurrence of members belonging to the phylum Gemmatimonadetes in vineyard soils has never been reported before. However, the presence of sequences of Gemmatimonadetrs are often observed in environmental 16S rRNA gene libraries; it has been estimated that this phylum represents one of the top nine phyla commonly found in soils, representing about 2% of soil bacterial diversity (29). More recently, this information has been confirmed by DeBruyn et al. (30) by using high-throughput sequencing: according to this estimates, Gemmatimonadetes relative abundances in large libraries (>500 sequences) from soils range from 0.2% to 6.5%, with a mean of 2.2%. Our results showed that the abundance of sequences ascribed to Gemmatimonadetes (6937 in total) ranged from 4% in rhizosphere to 8% in bulk soil. While most of Gemmatimonadetes have been identified only at the genus level, about 32 sequences were ascribed to *Gemmatimonas aurantiaca* (24 coming from bulk soil) and 8 to the strain *G. aurantiaca* T27 (7 coming from rhizosphere). This species has been described by Zhang and collaborators (31) as a polyphosphate-accumulating strain isolated from wastewater; since the highest amount of Gemmatimonadetes were measured in arid soils with neutral pH it has been suggesting an adaption to neutral dry soil (30).

Our results showed the presence of lactic bacteria belonging to the family Lactobacillaceae such as *Lactobacillus iners* (data not shown). However, only 3 sequences corresponding to this species were detected in the rhizosphere of *V. vinifera* cv Pinot Noir, thus confirming that soil does not represent a favourable ecological niche or reservoir for microorganisms possibly involved in wine production (15, 32-33).

The occurrence of human opportunistic pathogens in rhizosphere and soils has been described many times (34-37) and attention has been paid especially to the pathogens able to colonize the plant internal tissues (38). Surprisingly, we did not detect any sequences corresponding to possible human or plant pathogens. On the other hand we did neither retrieve sequences belonging to typical plant growth promoting bacteria such as fluorescent pseudomonads.

References:

(1) Rodriguez-R L.M. and Konstantinidis K.T. (2014). Estimating coverage in metagenomic data sets and why it matters. *ISME J*, 8(8): 2349-2351

(2) Mougel C., Offre P., Ranjard L., Corberand T., Gamalero E., Robin C. and Lemanceau P. (2006). Dynamic of the genetic structure of bacterial and fungal communities at different developmental stages of *Medicago truncatula* Gaertn. cv. Jemalong line J5. *New Phytol,* 170(1): 165-175

(3) Houlden A., Timms-Wilson T.M., Day M.J. and Bailey M.J. (2008). Influence of plant developmental stage on microbial community structure and activity in the rhizosphere of three field crops. *FEMS Microb Ecol*, 65(2): 193-201

(4) Micallef S.A., Channer S., Shiaris M.P. and Colón-Carmona A. (2009). Plant age and genotype impact the progression of bacterial community succession in the Arabidopsis rhizosphere. *Plant Signal Behav*, 4(8): 777-780.

(5) Xu Y., Wang G., Jin J., Liu J., Zhang Q. and Liu X. (2009). Bacterial communities in soybean rhizosphere in response to soil type, soybean genotype, and their growth stage. *Soil Biol Biochem*, 41(5): 919-925

(6) Sugiyama A., Ueda Y., Zushi T., Takase H. and Yazaki K. (2014). Changes in the bacterial community of soybean rhizospheres during growth in the field. *PLoS One*, 9(6): e100709

(7) Ulrich K., Ulrich A. and Ewald D. (2008). Diversity of endophytic bacterial communities in poplar grown under field conditions. *FEMS Microb Ecol*, 63: 169-180

(8) Bulgari D., Casati P., Brusetti L., Quaglino F., Brasca M., Daffonchio D. and Bianco P.A. (2009). Endophytic bacterial diversity in Grapevine (*Vitis vinifera* L.) leaves described by 16S rRNA gene sequence analysis and Length Heterogeneity-PCR. *The J of Microb,* 47(4): 393-401

(9) Pinto C., Pinho D., Sousa S., Pinheiro M., Egas C. and Gomes A.C. (2014). Unravelling the diversity of Grapevine Microbiome. *PLoS One,* 9(1): e85622

(10) Jones D.L., Nguyen C. and Finlay R.D. (2009). Carbon flow in the rhizosphere: carbon trading at the soil-root interface. *Plant Soil*, 321: 5-33

(11) Aulakh M.S., Wassmann R., Bueno C., Kreuzwieser J. and Rennenberg H. (2001). Characterization of root exudates at different growth stages of ten rice (*Oryza sativa* L.) cultivars. *Plant Biol*, 3: 139-148

(12) Badri D.V., Chaparro J.M., Zhang R., Shen Q. and Vivanco J.M. (2013). Application of natural blends of phytochemicals derived from the root exudates of arabidopsis to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. *J Biol Chem*, 288(7): 4502-4512.

(13) Chaparro J.M., Badri D.V., Bakker M.G., Sugiyama A., Manter D.K. and Vivanco J.M. (2013). Root Exudation of Phytochemicals in *Arabidopsis* Follows Specific Patterns That Are Developmentally Programmed and Correlate with Soil Microbial Functions. *PLoS One*, 8(2): 1-10.

(14) Opsi F., Landa B., Zecca O., Biddoccu M., Barmaz A. and Cavallo E. (2014). Diversity in soil bacterial communities structure in four high-

altitude vineyards cultivated using different soil management techniques. *Geophys Res Abstr EGU Gen Assem*, 16: 14297.

(15) Zarraonaindia I., Owens S.M., Weisenhorn P., West K., Hampton-Marcell J., Lax S., Bokulich N.A., Mills D.A., Martin G., Taghavi S., van der Lelie D. And Gilbert J.A. (2015). The Soil Microbiome Influences Grapevine-Associated Microbiota. *mBio*, 6(2): e02527-14

(16) Li X., Rui J., Maoa Y., Yannarell A. and Mackie R. (2014). Dynamics of the bacterial community structure in the rhizosphere of a maize cultivar. *Soil Biol & Biochem*, 68: 392-401

(17) Jenkins S.N., Waite I.S., Blackburn A., Husband R., Rushton S.P., Manning D.C. and O'Donnell A.G. (2009). Actinobacterial community dynamics in long term managed grasslands. *Antonie van Leeuwenhoek, Int J Gen Mol Microbiol*, 95(4):319-334

(18) Fierer N., Schimel J.P. and Holden P.A. (2003). Variations in microbial community composition through two soil depth profiles. *Soil Biol Biochem*, 35(1): 167-176

(19) Panikov N.S. (1999). Understanding and prediction of soil microbial community dynamics under global change. *Appl Soil Ecol*, 11: 161-176

(20) Albuquerque L., Franca L., Rainey F.A., Schumann P., Nobre M.F. and Da Costa M.S. (2011). *Gaiella occulta* gen. nov., sp. nov., a novel representative of a deep branching phylogenetic lineage within the class Actinobacteria and proposal of Gaiellaceae fam. nov. and Gaiellales ord. nov. *Syst Appl Microbiol*, 34: 595-599

(21) Fierer N., Bradford M.A. and Jackson R.B. (2007). Toward an ecological classification of soil bacteria. *Ecology*, 88(6): 1354-1364

(22) La Scola B., Birtles R.J., Mallet M.N. and Raoult D. (1998) *Massilia timonae* gen. nov., sp. nov., isolated from blood of an immunocompromised patient with cerebellar lesions. *J Clin Microbiol,* 36: 2847-2852

(23) Leveau J.H.J and Tech J.J. (2010). Grapevine microbiomics: bacterial diversity on grape leaves and berries revealed by high-throughput sequence analysis of 16S rRNA amplicons. *Acta Horticul,* 905: 31-42

(24) Compant S., Mitter B., Colli Mull J.G., Gangl H. and Sesstisch A. (2011). Endophytes of grapevine flowers, berries and seeds: Identification of cultivable bacteria, comparison with other plant parts and visualization of niches of colonization. *Microb Ecol*, 62: 188-190

(25) Inceoğlu Ő., Falcão Salles J., van Overbeek L. and van Elsas J.D. (2010). Effects of plant genotype and growth stage on the betaproteobacterial communities associated with different potato cultivars in two fields. *Appl Environ Microbiol*, 76: 3675-3684.

(26) Zhou Z.X., Jiang H., Yang C., Yang M.Z. and Zhang H.B. (2010). Microbial community on healthy and diseased leaves of an invasive plant *Eupatorium adenophorum* in sowthwest China. *J Microbiol*, 48: 139-145.

(27) Gallego V., Sánchez-Porro C., Garcìa M.T. and Ventosa A. (2006). *Massilia aurea* sp. nov., isolated from drinking water. *Int J Syst Evol Microbiol,* 56: 2449-2453

(28) Ravva S.V., Sarreal C.Z. and Mandrell R.E. (2011). Bacterial Communities in Aerosols and Manure Samples from Two Different Dairies in Central and Sonoma Valleys of California. *PLoS ONE,* 6(2): e17281

(29) Janssen P.H. (2006). Identifying the Dominant Soil Bacterial Taxa in Libraries of 16S rRNA and 16S rRNA Genes MINIREVIEWS Identifying the Dominant Soil Bacterial Taxa in Libraries of 16S rRNA and 16S rRNA Genes. *Appl Environ Microbiol*, 72(3): 1719-1728

(30) DeBruyn J.M., Nixon L.T., Fawaz M.N., Johnson A.M. and Radosevich M. (2011). Global biogeography and quantitative seasonal dynamics of Gemmatimonadetes in soil. *Appl Environ Microbiol*, 77(17): 6295-6300

(31) Zhang H., Sekiguchi Y., Hanada S., Hugenholtz P., Kim H., Kamagata Y. and Nakamura K. (2003). *Gemmatimonas aurantiaca* gen. nov., sp. nov., a Gram-negative, aerobic, polyphosphate-accumulating micro-organism, the first cultured representative of the new bacterial phylum Gemmatimonadetes phyl. nov. *Int J Syst Evol Microbiol*, 53(4): 1155-1163

(32) Chen Y.S., Yanagida F. and Shinohara T. (2005). Isolation and identification of lactic acid bacteria from soil using an enrichment procedure. *Lett Appl Microbiol*, 40(3): 195-200

(33) Castañeda L.E. and Barbosa O. (2016). Metagenomic analysis exploring taxonomic and functional diversity of soil microbial communities in Chilean vineyards and surrounding native forests. *PeerJ PrePrints*, 4: e16661v1

(34) Berg G., Eberl L. and Hartmann A. (2005). The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environ Microbiol*, 7(11): 1673-1685

(35) Berg G., Erlacher A., Smalla K. and Krause R. (2014). Vegetable microbiomes: Is there a connection among opportunistic infections, human health and our “gut feeling”?. *Microb Biotechnol*, 7(6): 487-495

(36) Mendes R., Garbeva P. and Raaijmakers J.M. (2013). The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol Rev*, 37(5): 634-663

(37) Campisano A., Ometto L., Compant S., Pancher M., Antonielli L., Varotto C., Anfora G., Pertot I., Sessitsch A. and Rota-Stabelli O. (2014). Interkingdom transfer of the acne-causing agent, propionibacterium acnes, from human to grapevine. *Mol Biol Evol*, 31(5): 1059-1065

(38) Tyler H.L. and Triplett E.W. (2008). Plants as a Habitat for Beneficial and/or Human Pathogenic Bacteria. *Annu Rev Phytopathol*, 46(1): 53-73

Chapter VI

Functional and phylogenetic characterization in a IPM vineyard by metaproteomic approach

In this final section the active microbial community in the rhizosphere and soils sampled in the integrated pest management vineyard has been characterized by a metaproteome approach.

6.1 Protein identification by MS/MS analysis and phylogenetic classification of acquired proteins

Pyrosequencing of bacterial genomic DNA produced a total of 142908 reads obtained with a mean value of 7500 reads per sample. After the multiplexing step, a total of 128296 reads (with a mean value of 6800 reads per sample) were used for further analysis. A total of 27237 reads were obtained for phyla description and produced a protein database including a total 11788243 sequences and 3831487183 residues, useful for protein identification and for identification of bacterial genera.

Protein identification resulted in bulk soil (BS) and in rhizosphere (Rhiz) is reported in Annex X and XI.

Using MS/MS analysis, a total of 579 proteins were identified in the two soils. In particular, 259 proteins were identified only in bulk soil (BS) and 300 only in the rhizosphere soil (Rhiz), while 20 proteins were commonly expressed in both soils, as shown in the Venn diagram reported in figure 6.1

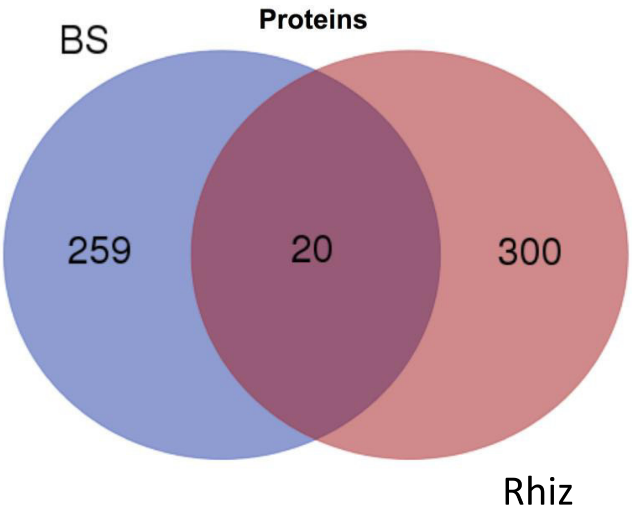


Figure 6.1 Venn diagram of the identified protein in bulk soil (BS) and in rhizosphere (Rhiz)

Identified proteins were expressed by a total of 150 genera and in particular by 49 genera occirring only in BS (about 33%), 42 (28%) only in Rhiz and 59 (40%) shared between the two soils (Figure 6.2, Annex X and XI).

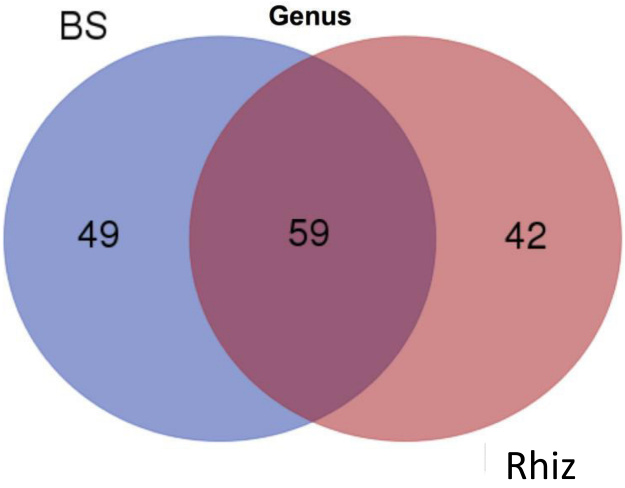
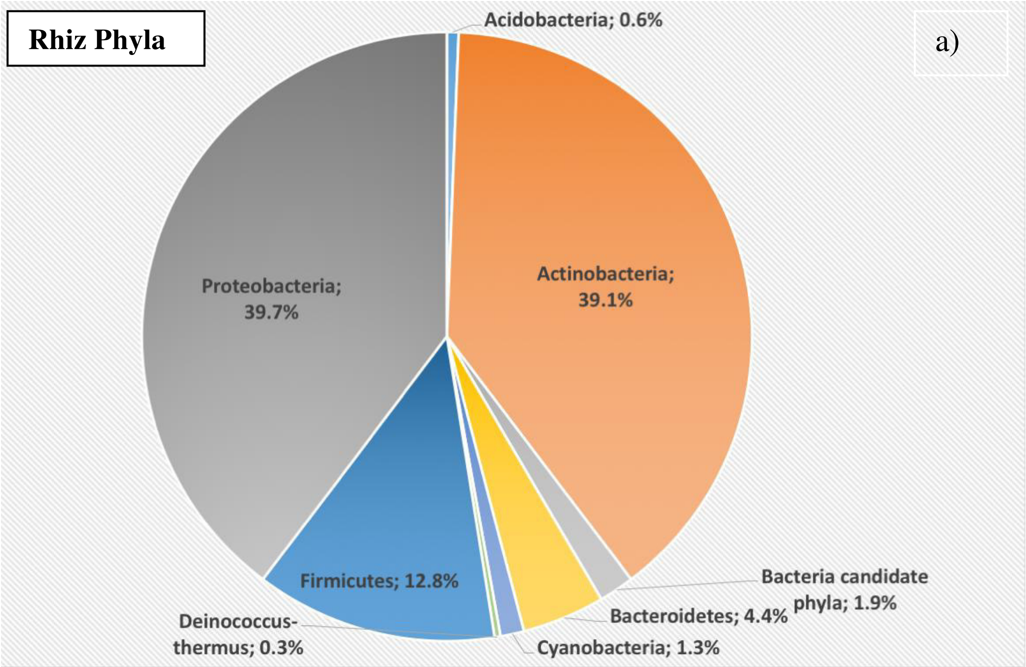


Figure 6.2 Venn diagram of the bacterial genus in the rhizosphere (Rhiz) of V. vinifera and in bulk soil (BS)

Genus identification following protein identification were used to calculate genus frequency in each samples and the frequencies were calculated as number of protein identified by the considered genus/ total of identified protein and expressed as frequency.

The identified genera belonged to 9 different phyla in BS and 8 phyla in Rhiz, as shown in figure 6.3 a and b. The most active phyla were Proteobacteria, followed by Actinobacteria and Firmicutes both in rhizosphere and in bulk soil (Figure 6.3 a and b). In accordance to figure 6.3, in rhizosphere, Actinobacteria expressed a higher number of proteins than in bulk soil (Rhiz 39.1% and BS 27.2%); on the contrary, the amount of proteins released by Proteobacteria was lower in Rhiz than in BS (Rhiz 39.7% and BS 50.9%).

Moreover, while proteins originated by the phylum Deinococcus-Thermus have been detected only in Rhiz, while those expressed by Chloroflexi and Gemmatimonadetes occurred only in BS (Figure 6.3 a and b).



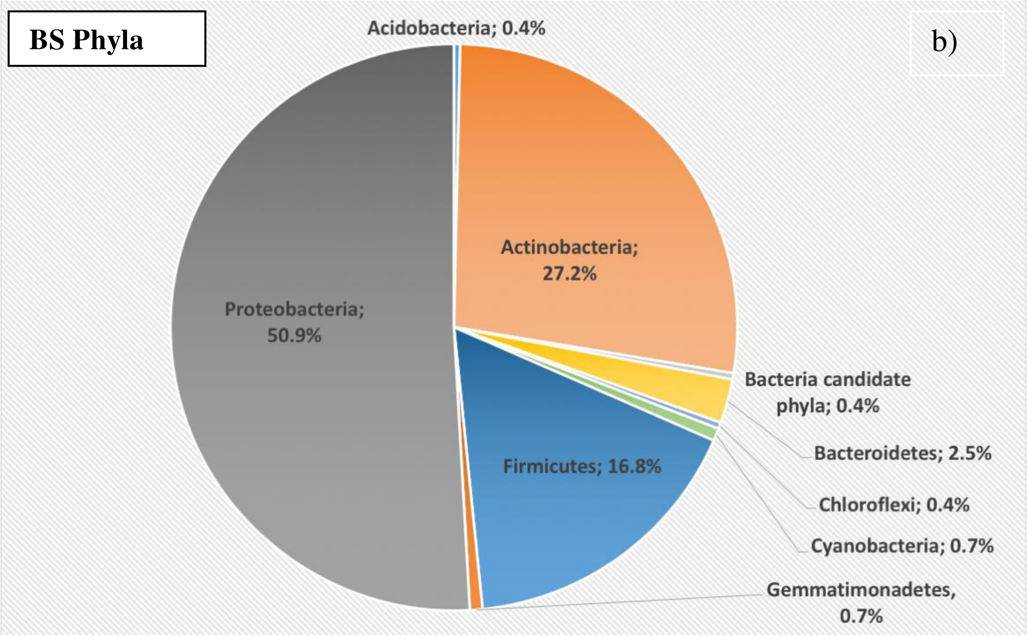
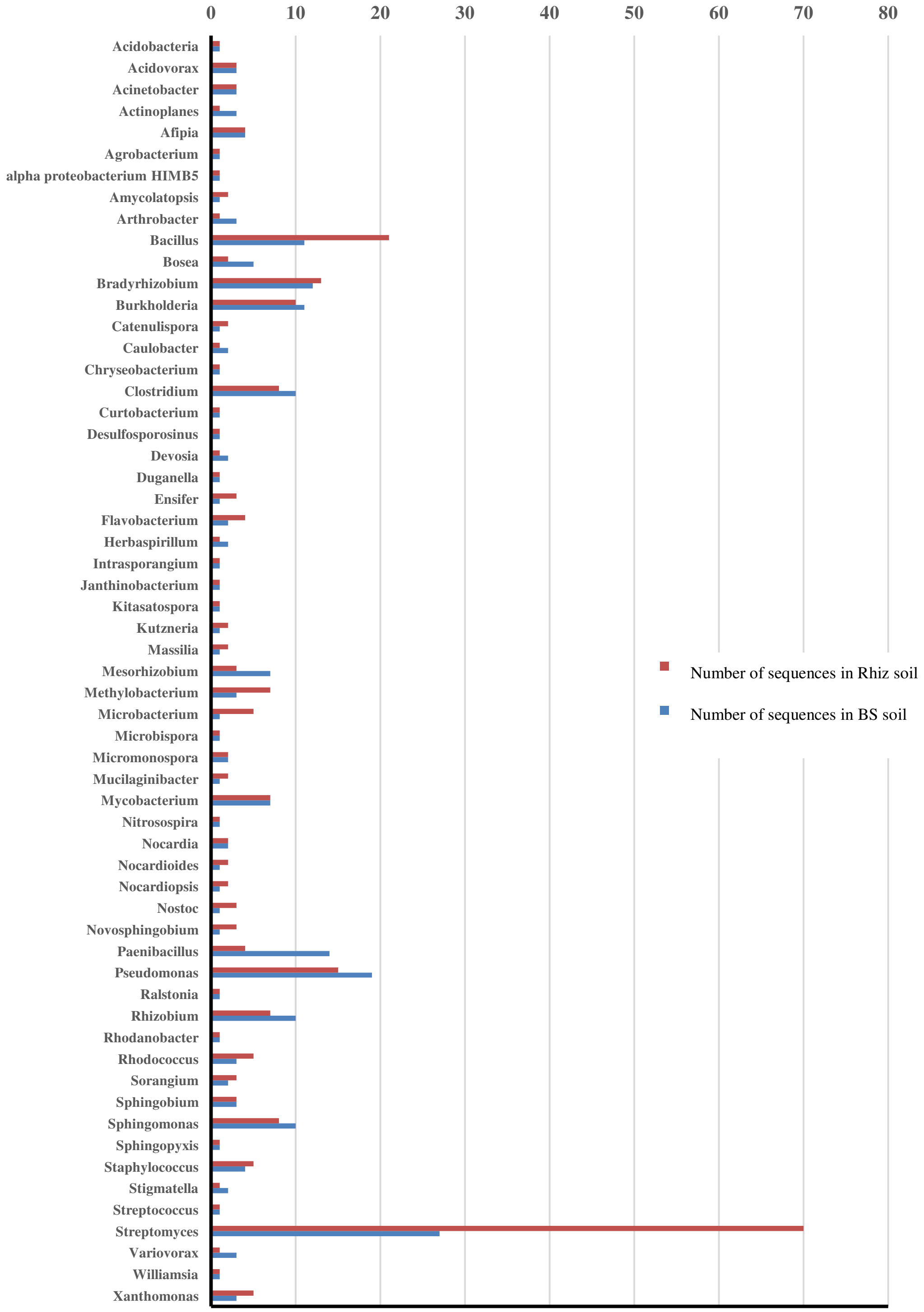


Figure 6.3 Microbial community composition in the rhizosphere of V. vinifera (a) and in bulk soil (b) at the phylum level

Sequence number of expressed proteins by each genus was summarized in figure 6.4. In both soils the most represented genera were *Streptomyces* (27 protein sequences in BS and 70 in Rhiz), *Bacillus* (11 protein sequences in BS and 21 in Rhiz), *Pseudomonas* (19 in BS and 15 in Rhiz) and *Bradyrhizobium* (12 protein sequences in BS and 13 in Rhiz). Other represented genera (expressing at least three proteins), both in BS and Rhiz, were: *Acidovorax, Acinetobacter, Afipia, Burkholderia, Clostridium, Ensifer, Flavobacterium, Mesorhizobium, Methylobacterium, Mycobacterium, Nostoc, Novosphingobium, Paenibacillus, Rhizobium, Rhodococcus, Sorangium, Sphingomonas, Staphylococcus* and *Xanthomonas*.

A total of 49 genera (Figure 6.2, Annex X -white rows) were detected as active in bulk soil (BS) while 42 bacterial genera occurred only in rhizosphere (Rhiz) (Figure 6.2, Annex XI -white rows).



<u>Figure 6.4</u> Sequence number of expressed protein in each genus.

6.2 Functional and biological classification of the identified proteins

The obtained results by Blast analysis and categorized as molecular function and biological processes were reported in Figure 6.5.

Molecular function category showed, in Rhiz a higher number of proteins involved in anion and cation binding activity, transpherase activity, transferring phosphorus-containing group, hydrolase activity and nucleoside binding, nucleic acid binding, nucleoside phosphate binding, nucleotide and ribonucleotide binding compared to BS. Finally, the passive transmembrane transporter activity was detected only in BS.

Biological processes involved in Rhiz are quite different from those occurring in BS. In particular, in spite of a substantial maintenance of the different biological processes involved in cell metabolism, in rhizosphere we observed a higher number of proteins working in the macromolecule and nucleobase-containing compound metabolic processes, as well as the appearance of phosphorus metabolic process (Annex XI, blue entries), regulation of biosynthetic, cellular, macromolecule, nitrogen compound (Annex XI, orange entries) and primary metabolic processes.

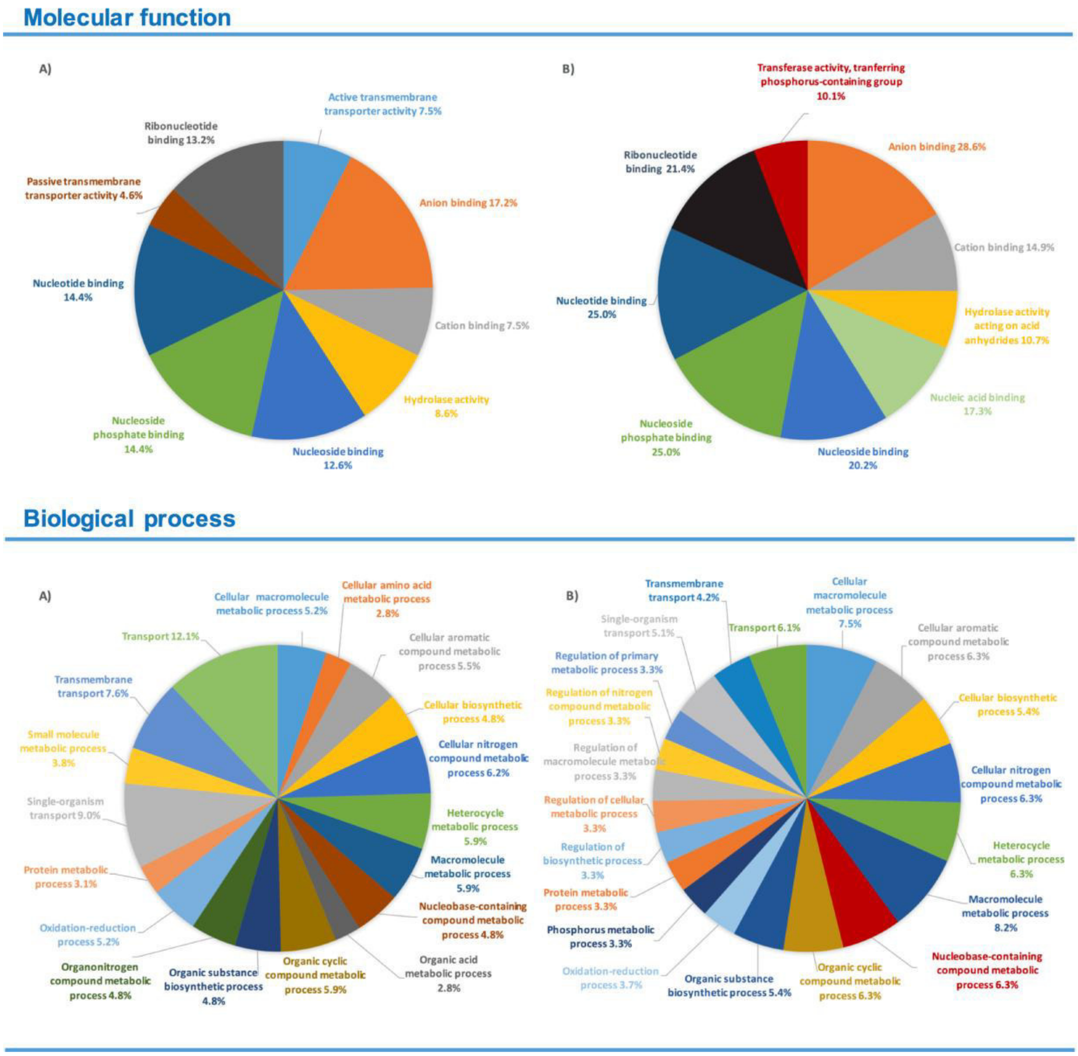


Figure 6. Pie charts with percentages of the identified proteins belonging to different functional categories and biological processes involved in the two samples. A: bulk soil (BS); B: soil associated with the roots of Vitis vinifera cv. Pinot noir (Rhiz)

6.3 Protein expression

Among the 59 genera active in the two soils, the three main representative genera in the rhizosphere of *V. vinifera* were *Streptomyces*, *Bacillus* and *Pseudomonas* (figure 6.4). The most active genus was *Streptomyces* expressing 70 different proteins, listed in Annex XI and showed in figure 6.4. The second important genus in protein expression was *Bacillus* leading to 21 expressed proteins (Annex XI), followed by *Pseudomonas* that expressed 15 proteins (Annex XI). These three genera were also represented in BS with 27 proteins from *Streptomyces* (listed in Annex X and showed in figure 6.4), 11 proteins from *Bacillus* and 19 proteins from *Pseudomonas*. In BS, *Paenibacillus* also was well represented with 19 proteins.

In the two soils (BS and Rhiz) 20 proteins were commonly expressed (independently identified) by the same genus (Annex X and Xi, green lines). Specific protein expression occurred in the two soils involving mutually exclusive genera: 56 proteins by 49 genera occurred only in BS (Annex X, white lines), while, in the rhizosphere, 54 proteins were expressed by 42 genera absent in BS (Annex XI, white lines).

6.4 Discussion

Rhizosphere is a complex microhabitat where interactions among plant root, bacteria, fungi and animals occur (1-2). This system was studied with different approaches in the past and in the present time and many papers characterizing bacteria selection near plant roots and the effects that these interactions could have on plant growth, production have been described (3-8).

Our work, for the first time, demonstrates, by exploring a metaproteomic approach, that in the vineyard rhizosphere the genus with higher protein expression are *Streptomyces* followed by *Bacillus* and *Pseudomonas*. These data are in agreement with the literature concerning soil culturable bacteria (3). Moreover, we identified different genera specifically involved in vineyard rhizosphere interaction as, for example, *Comamonas* and *Deinococcus*. The most active phyla were Proteobacteria, Actinobacteria and Firmicutes in both soils. Moreover, the phylum Deinococcus-Thermus was exclusive of Rhiz soil, while Chloroflexi and Gemmatimonadetes were exclusive of BS soil. These data are partially in agreement with those obtained in previous chapter by NGS. In fact, in the previous chapter our results demonstrated that the dominant phyla were Actinobacteria, Proteobacteria, Gemmatimonadetes and Bacteroidetes. Proteomic analysis showed that Gemmatimonadetes are active in BS soil but not in Rhiz, while Bacteroidetes are more active in rhizosphere than in BS. Our results demonstrated that in rhizosphere the most active phylum is Proteobacteria, followed by Actinobacteria; these results are in agreement with those obtained by Opsi and coworkers that reported Proteobacteria (36%), followed by Actinobacteria (26%) and Acidobacteria (15%) as the prevalent phyla in vineyard. These phyla are also described as main present in other kind of soils and rhizosphere (9). Moreover, the phylum Deinococcus-Thermus, detected as active only in grapevine rhizosphere, is also detected in two other proteomic works, by Knief and coworkers (10) and Lin et al. (11) in rhizosphere of rice and sugarcane.

These results underline the difference between the metagenomics and metaproteomic approach and the great potentiality of the proteomic techniques when describing the environmental bacterial community and their activity. In fact, while metagenome describe the whole bacterial community, the metaproteome clearly indicate really active species and allows kind to understand the function they exert.

Concerning the detailed protein expression occurring in the bulk soil and in rhizosphere we could summarized two main aspects: a common expression in term of protein identification and expressing genera that represent probably stable mechanisms that occurs in soil and a more specific protein expression occurring in the two different compartments. In particular, a set of twenty proteins were commonly expressed both in BS and Rhiz soils by the same genera. These proteins are involved in mechanisms of bacterial metabolism and response to environment stimuli not linked with plant root presence. Major facilitator superfamily (MFS) transporter (identified in our work in *Acidovorax*) together with beta-lactamase are included in the ancient and diverse antibiotic resistance genes (ARGs). These genes have previously been identified by Forsberg and coworkers from agricultural soil and included genes identical to those found in human pathogens. Despite the apparent overlap between soil and clinical resistomes, factors influencing ARG composition in soil and their movement between genomes and habitats remain largely unknown. MFS transporters were largely absent from Acidobacteria and were enriched among Actinobacteria and Proteobacteria (12), confirmed by our work. This protein is linked with antibiotic resistance and in particular resistance to amphenicol and tetracycline antibiotics occurred predominantly via the action of drug transporters, most of these belonging to the major facilitator superfamily (MFS transporter). MFS transporter was also identified in *Nitrosospira multiformis*, an ammonia oxidizing bacteria from soil environment (13), linked to N transport: for uptake of inorganic N, an ammonia permease, a NarK nitrate/nitrite transporter of the major facilitator superfamily (MFS), and a FNT-type nitrate/nitrite transporter were identified. MFS transporter are also involved in sulfate transporters, together with ABC transporter while phosphate transport in soil bacteria involves a complete ABC transporter, unlinked components of ABC transporters, and three phosphate-selective porins (13). Porins from *Bradyrhizobium* are detected in our soil samples confirming the role of this protein in soil. This protein also play a variety of roles depending on the bacterial species, including the maintenance of cellular structural integrity, bacterial conjugation and bacteriophage binding, antimicrobial resistance and pore formation to permit the penetration of small molecules (14).

Linked to phosphate metabolism in soil bacteria, two phosphate ABC transporter substrate-binding protein PstS were detected from *Afipia*. This expression could be linked with phosphate starvation in a poor phosphate soil such as vineyard soil, as demonstrated by Aguena and coworkers (15) in *Escherichia coli*. PstS is the substrate-binding component of the ABC-type transporter complex pstSACB, involved in phosphate import and the accumulation of this protein is enhanced under phosphate starvation (15). ABC transporters consist of two conserved regions: a highly conserved ATP binding cassette and a less conserved transmembrane domain. Most ABC transporters function as a dimer. ABC transporters are involved in the export or import of a wide variety of substrates ranging from small ions to macromolecules. The major function of ABC import systems is to provide essential nutrients to bacteria (11).

OmpA38, also detected as commonly protein in our sample, is the most abundant protein in the outer membranes of *A. baumannii*. Although OmpA38 primarily functions as a porin in the outer membranes, it seems to be an important virulence factor in the induction of apoptosis of epithelial cells (16). In addition, OmpA38 is highly homologous to AlnA. Some Acinetobacter species secrete AlnA and produce oil-in-water emulsions for efficient biodegradation of polyaromatic hydrocarbons (17). Bacteria of the genus Acinetobacter are ubiquitous microorganisms, which can be found in a variety of ecological niches including water and soil, and in clinical specimens of human and animal origins. Outer membrane proteins (Omps) of Gram-negative bacteria are known to be key players in bacterial adaptation and pathogenesis in host cells (18).

Secretion across the inner membrane in some Gram-negative bacteria occurs via the preprotein translocase pathway. Proteins are produced in the cytoplasm as precursors, and require a chaperone subunit to direct them to the translocase component. From there, the mature proteins are either targeted to the outer membrane, or remain as periplasmic proteins. The translocase itself comprises 7 proteins, including a chaperone protein (SecB), an ATPase (SecA), an integral membrane complex (SecCY, SecE and SecG), and two additional membrane proteins that promote the release of the mature peptide into the periplasm (SecD and SecF) (19). We detected both in BS and in Rhiz soil a protein-export membrane protein SecF. As reported by Tseng and coworkers, SecD and SecF homologs are found in nearly all prokaryotes, including archea, and sometimes they are fused as one polypeptide. SecD and SecF show some structural similarity to transport system of the RND-(resistance/nodulation/cell division) family (19).

The pyridine nucleotide-disulfide oxidoreductase is involved in electron transfer between pyridine nucleotides and disulphide compounds that is catalysed by three flavoproteins which are well characterized. The flavoproteins consist of two identical or near identical polypeptide chains, each with a functional cysteine residue, and a molecule of FAD which is non-covalent linked. Petrus and coworkers (20) identified the presence of a new mer gene, which they called merK, in *Xanthobacter autotrophycus*, a mercury resistant soil bacteria. These genes encode proteins with homology to members of the pyridine nucleotide disulfide oxidoreductase family, and are most similar to a glutathione reductase (20). They proposed that merK as novel addition to mer operons in Alpha- proteobacteria, and that this gene is particularly common in marine isolates. Bacteria expressing this protein are able to reduce glutathione (20).

23S rRNA (guanosine(2251)-2~-O)-methyltransferase RlmB, which we detected in soil from Intrasporangium, is a cytoplasmatic protein that belongs to the class IV-like SAM-binding methyltransferase superfamily, RNA methyltransferase TrmH family and RlmB subfamily. RlmB catalyzes the methylation of guanosine 2251, a modification conserved in the peptidyltransferase domain of 23S rRNA. The conserved residues in this novel family of 2'O-methyltransferases cluster in the knotted region, suggesting the location of the catalytic and AdoMet binding sites (21).

Proline-Proline-Glutamic acid (PPE) family protein from *Mycobacterium* was detected both in BS and in Rhiz soils. PPE family proteins were identified in *Mycobacterium* cells under different genotoxic stress conditions in Namouchi et al. and were considered a key-players in survival processes (22).

Glycosyl transferase family protein from *Pseudomonas* (detected in both soil) could be involved in response to osmotic stress in soils. In a transcriptomic study by Johnson and coworkers (23) this protein is upregulated in *Sphingomonas wittichii* under salinity stress that can occur in soil because the solute potential can dramatically decrease close to the surfaces of plant roots, where the uptake of water by plants can result in an up to 200-fold increase in the concentration of solutes (24).

TonB-dependent receptor was detected from *Sphingobium* could be linked with iron starvation. TonB-dependent receptors (TBDRs) are outer membrane proteins mainly known for the active transport of iron siderophore complexes in Gram-negative bacteria (25). In most cases, the expression of the genes encoding these receptors is under the control of the Fur (Ferric uptake regulator) repressor and activated under conditions of iron starvation (25).

Apolipoprotein N-acyltransferase are involved in lipoprotein lipidation in *Streptomyces*. In fact, following translocation, bacterial lipoproteins are lipidated by lipoprotein diacylglycerol transferase (Lgt) and cleaved of their signal peptides by lipoprotein signal peptidase (Lsp). Streptomyces are unusual among Gram-positive bacteria because they export large numbers of lipoproteins via the twin arginine protein transport (Tat) pathway. Furthermore, some *Streptomyces* species encode two Lgt homologues and all *Streptomyces* species encode two homologues of lipoprotein N-acyl transferase (Lnt) (26).

In soil proteome we detected both in BS and in Rhiz soil a peptidase from *Variovorax*. This protein is commonly detected in extracellular proteomes of the various Bacillus species, which contribute to the virulence and the supply of nutrients (27).

Regarding differentially expressed proteins and their roles in rhizospheric soils, the identified genus expressed protein involved mainly in macromolecule metabolic process and nucleobase-containing compound metabolic process, regulation of biosynthetic process, regulation of cellular metabolic process, regulation of macromolecule metabolic process and regulation of primary metabolic process. Very interesting, in our opinion, to better explain and clarify the role of microorganisms and the specific involved enzyme in "rhizosphere metabolism" is the appearance of phosphorus metabolic process and regulation of nitrogen compound metabolic process. In particular, protein involved in phosphorus metabolic process are enzyme with phosphate transfer and kinase activity. For example, *Bacillus* expresses a carbamate kinase, which expression is regulated in a manner that allows the enzyme to function as a provider of ammonia under aerobic conditions and of ATP under anaerobic conditions (28) and a thymidylate synthase that produce de novo thymidylate or dTMP, an essential DNA precursor; *Burkholderia* a sensor histidine kinase, a polyphosphate kinase 2 and a pantetheine-phosphate adenylyltransferase that catalyzes the fourth of five steps in the coenzyme A biosynthetic pathway, reversibly transferring an adenylyl group from ATP onto 4′-phosphopantetheine to yield dephospho-coenzyme A and pyrophosphate in *Burkholderia pseudomallei* as reported by Edwards et al. (29); *Comamonas*, *Myxococcus*, *Rhizobium* and *Stigmatella* that express different histidine kinase; *Methylobacterium* an ATPase; *Streptomyces* a two-component sensor histidine kinase, phosphoenolpyruvate synthase and putative molybdopterin biosynthesis protein. Finally, this part of metabolic process involved a glycosyl hydrolase family 15 from *Arthrobacter* linked to lignin degradation as reported by Jiménez et al (30) in a metatrascriptomic study of soil-derived microbial consortia that were trained to degrade once-used wheat straw, switchgrass and corn stover. Protein involved in the regulation of nitrogen compound metabolic process involved different transcriptional regulatory protein such as: yhcZ-like uncharacterized transcriptional regulatory protein from *Actinoplanes*, a transcriptional regulator from Bacillus, two transcriptional regulators from *Bradyrhizobium*, IclR family transcriptional regulator from *Caulobacter*, valine--tRNA ligase from *Mesorhizobium*, LysR family transcriptional regulator from *Pseudomonas*, AraC family transcriptional regulator from *Sorangium*, transcriptional regulator, two helix-turn-helix transcriptional regulator and Transcriptional regulator from *Streptomyces* and finally two-component system response regulator from *Xanthomonas*. The expression of different kind of transcriptional regulators could be linked with environmental response, in fact, most often, adaptive responses in bacteria are mediated by transcriptional regulators which, upon receiving the appropriate signal, trigger the specific transcriptional response. For example, a number of regulators belonging to the IclR family are involved in the control of catabolic pathways for the degradation of aromatic compounds (31). MerR family transcriptional regulator from Amycolatopsis, Micromonospora, Nocardia and Streptomyces. The MerR family is a group of transcriptional activators with similar N-terminal helix-turn-helix DNA binding regions and C-terminal effector binding regions that are specific to the effector recognized (32). Mer genes are linked with mercury resistance in bacteria.

References:

(1) Philippot L., Raaijmakers J.M., Lemanceau P., and van der Putten W.H. (2013). Going back to the roots : the microbial ecology of the rhizosphere. *Nat Publ Gr*, 11: 789-799

(2) Mendes R., Garbeva P. and Raaijmakers J.M. (2013). The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbio. Rev,* 37: 634-663

(3) Bevivino A., Paganin P., Bacci G., Florio A., Pellicer M.S., Papaleo M.C., Mengoni A., Ledda L., Fani R., Benedetti A. and Dalmastri, C. (2014). Soil bacterial community response to differences in agricultural management along with seasonal changes in a Mediterranean region. *PLoS One*, 9: doi:10.1371/journal.pone.0105515

(4) Nannipieri P., Ascher J., Ceccherini M.T., Lan D.L., Pietramellar G. and Renell G. (2003). Microbial diversity and soil functions. *Eur J Soil Sci*, 54: 655

(5) Bona E., Lingua G. and Todeschini V. (2016). Effect of Bioinoculants on the quality of crops. In: Bioformulations: For Sustainable Agriculture. Arora, Naveen Kumar, Mehnaz, Samina, Balestrini, R. (Eds.) pp. 93–124.

(6) Berta G., Copetta A., Gamalero E., Bona E., Cesaro P., Scarafoni A. and D’Agostino G. (2014). Maize development and grain quality are differentially affected by mycorrhizal fungi and a growth-promoting pseudomonad in the field. *Mycorrhiza,* doi:10.1007/s00572-013-0523-x

(7) Bona E., Lingua G., Manassero P., Cantamessa S., Marsano F., Todeschini V., Copetta A., D’Agostino G., Massa N., Avidano L., Gamalero E. and Berta G. (2015). AM fungi and PGP pseudomonads increase flowering, fruit production, and vitamin content in strawberry grown at low nitrogen

and phosphorus levels. *Mycorrhiza,* 25: 181-193

(8) Bona E., Lingua G., Manassero P., Marsano F., Todeschini V., Cantamessa, S., Copetta A., D'Agostino G., Massa N., Avidano L., Gamalero E. and Berta G. (2013). Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads improve strawberry fruit yield and quality in conditions of reduced fertilization. *J Agric Food Chem* (Submitted)

(9) Wang N.F., Zhang T., Zhang F., Wang E.T., He J.F. and Ding H. (2015). Diversity and structure of soil bacterial communities in the Fildes Region (maritime Antarctica) as revealed by 454 pyrosequencing. *Front in Microb,* 6: 1-11

(10) Knief C., Delmotte N., Chaffron S., Stark M., Innerebner G., Wassmann R., von Mering C. and Vorholt J.A. (2012). Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. *ISME J,* 6: 1378-1390

(11) Lin W., Wu L., Lin S., Zhang A., Zhou M., Lin R., Wang H., Chen J., Zhang Z. And Lin R. (2013). Metaproteomic analysis of ratoon sugarcane rhizospheric soil. *BMC Microbiol,* 13: 135-148

(12) Forsberg K.J., Patel S., Gibson M.K., Lauber C.L., Fierer N. Dantas, G. and Biology E. (2014). The tetracycline destructases: a novel family of tetracycline-inactivating enzymes. *HHS Public Access,* 509: 612-616

(13) Norton J.M., Klotz M.G., Stein L.Y., Arp D.J., Bottomley P.J., Chain P.S.G., Hauser L.J., Land M.L., Larimer F.W., Shin M.W. and Starkenburg S.R. (2008). Complete genome sequence of Nitrosospira multiformis, an ammonia-oxidizing bacterium from the soil environment. Appl Environ Microbiol 74: 3559-3572

(14) Poirel L. and Nordmann P. (2008). *Acinetobacter baumanii*: mechanisms of resistance, multiple beta-lactamase. In: Acinetobacter Biology and

Pathogenesis. Berezin E., Friedman H. and Bendinelli M. (Eds.) pp. 129-144.

(15) Aguena M., Ferreira G.M. and Spira B. (2009). Stability of the pstS transcript of Escherichia coli. Arch Microbiol, 191: 105–112.

(16) Choi C.H., Lee E.Y., Lee Y.C., Park T.I., Kim H.J., Hyun S.H., Kim S.A., Lee S.K. and Lee J.C. (2005). Outer membrane protein 38 of *Acinetobacter baumannii* localizes to the mitochondria and induces apoptosis of epithelial cells. *Cell Microbiol,* 7: 1127-1138

(17) Walzer G., Rosenberg E. and Ron E.Z. (2006). The *Acinetobacter* outer membrane protein A (OmpA) is a secreted emulsifier. *Environ Microbiol,* 8: 1026-1032

(18) Lin S. Z. and Qijing,J.H. (2002). Outer membrane proteins: key players for bacterial adaptation\rin host niches. *Microbes Infect* 4: 325-331.

(19) Tseng T., Gratwick K., Kollman J., Park D., Nies D., Goffean A. and Saier M. (1999). The RND permease superfamily: an ancient, ubiquitous and diverse family that includes human disease and development proteins. *J Mol Microb Biotecnol,* 1: 107-125.

(20) PetrusA.K., Rutner C., Liu S., Wang Y. and Wiatrowski H.A. (2015). Mercury reduction and methyl mercury degradation by the soil bacterium *Xanthobacter autotrophicus* Py2. *Appl Environ Microbiol.* 81: 7833-7838.

(21) Michel G., Sauvé V., Larocque R., Li Y., Matte A. and Cygler M. (2002). The structure of the RlmB 23S rRNA methyltransferase reveals a new methyltransferase fold with a unique knot. *Structure,* 10: 1303-1315

(22) Namouchi A., Gómez-Muñoz M., Frye S.A., Moen L.V., Rognes T., Tønjum T. and Balasingham S.V. (2016). The *Mycobacterium tuberculosis* transcriptional landscape under genotoxic stress. *BMC Genomics,* 17: 791

(23) Johnson D.R., Coronado E., Moreno-Forero S.K. and Heipieper H.J. (2011). Transcriptome and membrane fatty acid analyses reveal different strategies for responding to permeating and non-permeating solutes in the bacterium *Sphingomonas wittichii.*

(24) Csonka L. (1989). Physiological and genetic responses of bacteria to osmotic stress. *Microbiol Rev,* 53: 121-147.

(25) Blanvillain S., Meyer D., Boulanger A., Lautier M., Guynet C., Denancé N., Vasse J., Lauber E. and Arlat M. (2007). Plant carbohydrate scavenging through TonB-dependent receptors: A feature shared by phytopathogenic and aquatic bacteria. *PLoS One,* doi:10.1371/journal.pone.0000224

(26) Widdick D.A., Hicks M.G., Thompson B.J., Tschumi A., Chandra G., Sutcliffe I.C., Brülle J.K., Sander P., Palmer T. and Hutchings M.I. (2011). Dissecting the complete lipoprotein biogenesis pathway in *Streptomyces scabies*. *Mol Microbiol,* 80: 1395-1412

(27) Antelmann H., Williams R.C., Miethke M., Wipat A., Albrecht D., Harwood C.R. and Hecker M. (2005). The extracellular and cytoplasmic proteomes of the non-virulent *Bacillus anthracis* strain UM23C1-2. 3684–3695

(28) Abdel A., Bibb W. and Nainan O. (1982). Carbamate kinase from *Pseudomonas aeruginosa*: purification, characterization, physiological role and regulation. *J. Bacteriol,* 151: 1411-1419.

(29) Edwards T., Leibly D., Bhandari J., Statnekov J., Phan I., Dieterich S., Abendroth J., Staker B., Van Voorhis W., Myler P. and Stewart L. (2011). Structures of phosphopantetheine adenylyltransferase from *Burkholderia pseudomallei. Acta Crystallogr Sect F Struct Biol Cryst Commun,* 1032-1037

(30) Jiménez D.J., Julia M., Brossi D.L., Schückel J., Kre S., George W., Willats

T. and van Elsas J.D. (2016). Characterization of three plant biomass-degrading microbial consortia by metagenomics- and metasecretomics-based approaches. 10463-10477

(31) Molina-Henares A.J., Krell T., Eugenia Guazzaroni M., Segura A. and Ramos J.L. (2006). Members of the IclR family of bacterial transcriptional regulators function as activatorsand/or repressors. *FEMS Microbiol Rev,* 30: 157–186

(32) Brown N.L., Stoyanov J.V., Kidd S.P. and Hobman J.L. (2003). The MerR family of transcriptional regulators. *FEMS Microbiol Rev,* 27: 145-163

Chapter VII

Conclusion

Vitis vinifera is a naturally reservoir of microorganisms that interacting with the host plant may affect plant growth, health and accordingly wine quality and production (1-3).

The grapevine microbiome is very heterogeneous due to influence of environmental factors, grapevine cultivar and geographical location (1, 4).

The microbial communities living in bulk and rhizospheric soil associated to grapevine include bacteria with different physiological traits and activities (3) that play, on their turn, an important role in yield and crop quality (5).

In the last few years, the microbiome of *V. vinifera* has received much attention and in particular epiphyte and endophyte bacterial communities have been investigated in numerous studies; hovewer, few information are available concerning the structure of bacteria associated with the grapevine roots.

In fact, culturable bacteria living inside grapevine tissue were described by Baldan and collaborators (6) and similarly, by using culture-dependent methods, Karagoz and colleagues (7) isolated and identified epiphytic bacteria from roots and characterized their physiological activities.

Besides these culture dependent approach, different molecular procedures have been used to provide information on the microbial structural composition of *V. vinifera*. West and collaborators (8) characterized by DGGE (Denaturing Gradient Gel Electrophoresis) the bacterial endophytes of grapevine.

The analysis of epiphyte bacteria of fruits, leaves, bark and soil were performed by Length Heterogeneity-PCR (LH-PCR) on *V.* vinifera cv. Barbera (9); in the following years, Vega-Avila and colleagues (10) described trough DGGE the rhizosphere microbiome of grapevine cultivated under organic and conventional practices.

Recently, by using next generation sequencing (NGS) approach, the diversity of bacterial communities was assessed in the most common grape cultivars (Chardonnay and Cabernet Sauvignon) in California (4). Then the shifts of the microbial communities inside the plant tissue according to the infection by flavescence dorée phytoplasma (9) and to the management of the vineyard (11) were described. Finally, insight on the variability of bacterial structure of leaves, flowers, grapes, roots and soil during different phenological stage of the plant was provided by Zarraonaindia and co-workers (2). In addition, variability of Eukaryotic and Prokaryotic leaf epiphytic community was characterized, by 454 sequencing approach, according to the phenological stages of the plant (1).

Nevertheless, at our knowledge, only one paper reported information, obtained by PFLA analysis on the structural diversity of rhizospheric microbial community of grapevine belonging to the cultivar Pinot Noir (12).

Although genomic analyses provide a large number of information about bacterial community composition, they do not provide information regarding the real activity of the identified bacteria and the molecular interactions between the bacterial community and the plant roots. In this scenario, proteomic studies could be a good complementary tool to better understand the real interaction pathway with roots and soil, identifying the repertoire of proteins that microorganisms use to compete and cooperate in complex environmental communities.

However, there are scanty data available in literature using this approach regarding agricultural plants. In particular, Wang et al (13), Knief et al (14), Lin et al. (15) and Moretti et al. (16) characterized the rhizospheric proteome of sugar cane, rice, tobacco and lactuce, respectively. At our knowledge, no data are available in literature regarding rhizosphere proteome of *V. vinifera*.

In contrast to the literature, in the two vineyards considered in this study the predominant bacteria group both in rhizosphere and in bulk soil were represented by Actinobacteria phylum. This results is very interesting because Proteobacteria have been previously reported to be the dominant phylum in *V. vinifera* rhizosphere (1- 2, 7). This suggests that the possible effect of the plant cultivar, the phenological stage of the plant and the cropping practices can influence the composition of the microbial communities in the rhizosphere.

Moreover, the present study extends knowledge about the active component in the overall microbial communities. Interestingly, the data reported in the metagenomic analysis are different from those obtained with metaproteomic approach. In fact, the bacterial groups that were dominant when considering the whole bacterial community did not overlap with the groups identified as most active demonstrating that the bacterial species occurring in high number not always correspond to the most active, and vice versa.

In conclusion, the data presented in this work highlight the importance of studying the natural biodiversity of grapevine and the need of a more detailed characterization of the plant microbe interactions. Our results will contribute to the characterization both from a structural and functional point of view of the biodiversity of grapevines and particularly of *V. vinifera* cv. Pinot noir

References

(1) Pinto C., Pinho D., Sousa S., Pinheiro M., Egas C. and Gomes A.C. (2014). Unravelling the diversity of Grapevine Microbiome. *PLoS One,* 9(1): e85622

(2) Zarraonaindia I., Owens S.M., Weisenhorn P., West K., Hampton-Marcell J., Lax S., Bokulich N.A., Mills D.A., Martin G., Taghavi S., van der Lelie D. And Gilbert J.A. (2015). The Soil Microbiome Influences Grapevine-Associated Microbiota. *mBio*, 6(2): e02527-14

(3) Salvetti E., Campanro S., Campedelli I., Fracchetti F., Gobbi A., Tornielli G.B., Torriani S. and Felis G.E. (2016). Whole-Metagenome_sequencing_Based Community Profiles of *Vitis vinifera* L. cv. Corvina Berries Withered in two Post-harvest conditions. *Frontiers in Microbiol,* 7: 937

(4) Bokulich NA, Thorngate JH, Richardson PM, Mills DA (2014) Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proc Natl Acad Sci U S A,* 111(1): E139-148

(5) Martins G, Lauga B, Miot-Sertier C, Mercier A, Lonvaud A, Soulas ML, et al (2013) Characterization of Epiphytic Bacterial Communities from Grapes, Leaves, Bark and Soil of Grapevine Plants Grown, and Their Relations. *PLoS One,* 8(8): e73013

(6) Baldan E, Nigris S, Populin F, Zottini M, Squartini A, Baldan B (2014) Identification of culturable bacterial endophyte community isolated from tissues of *Vitis vinifera* " Glera ." *Plant Biosyst*, 148(3): 508-516

(7) Karagöz K, Ateş F, Karagöz H, Kotan R, Çakmakçi R (2012) Characterization of plant growth-promoting traits of bacteria isolated from

the rhizosphere of grapevine grown in alkaline and acidic soils. *Eur J Soil Biol*, 50: 144-150

(8) West ER, Cother EJ, Steel CC, Ash GJ (2010) The characterization and diversity of bacterial endophytes of grapevine. *Can J Microbiol*, 56(3): 209-216

(9) Bulgari D, Casati P, Quaglino F, Bianco PA (2014) Endophytic bacterial community of grapevine leaves influenced by sampling date and phytoplasma infection process. *BMC Microbiol,* 14: 198

(10) Vega-Avila AD, Gumiere T, Andrade PAM, Lima-Perim JE, Durrer A, Baigori M, et al (2015) Bacterial communities in the rhizosphere of *Vitis vinifera* L. cultivated under distinct agricultural practices in Argentina. *Antonie Van Leeuwenhoek,* 107(2): 575-588

(11) Campisano A, Antonielli L, Pancher M, Yousaf S, Pindo M, Pertot I (2014) Bacterial endophytic communities in the grapevine depend on pest management. *PLoS One*, (1): e112763

(12) Steenwerth KL, Drenovsky RE, Lambert JJ, Kluepfel DA, Scow KM, Smart DR (2008) Soil morphology, depth and grapevine root frequency influence microbial communities in a Pinot noir vineyard. *Soil Biol Biochem*, 40(6): 1330-1340

(13) Wang H.B., Zhang Z.X., Li H., He H.B., Fang C.X., Zhang A.J., Li Q.S., Chen R.S., Guo Z.K., Lin H.F., Wu L.K., Lin S., Chen T., Lin R.Y., Peng X.X. and Lin W.X. (2011). Charcaterization of Metaproteomics in Crop Rhizospheric Soil. *J of proteome res,* 10: 932-940

(14) Knief C., Delmotte N., Chaffron S., Stark M., Innerebner G., Wassmann R., von Mering C. and Vorholt J.A. (2012). Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. *ISME J,* 6: 1378-1390

(15) Lin W., Wu L., Lin S., Zhang A., Zhou M., Lin R., Wang H., Chen J., Zhang Z. And Lin R. (2013). Metaproteomic analysis of ratoon sugarcane rhizospheric soil. *BMC Microbiol,* 13: 135-148

(16) Moretti M., Minerdi D., Gehrig P., Garibaldi A., Gullino M.L. and Riedel K. (2012). A Bacterial-Fungal Metaproteomic analysis enlightens an intriguing multicomponent interaction in the rhizosphere of *Lactuca sativa. J of Proteome res,* 11: 2061-2077

Annex

Annex I: Physical-chemical analyses of the soil (performed by Enocontrol s.r.l.)

Enocontrol Scarl

CENTRO ANALISI E RICERCHE

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RAPPORTO DI PROVA N° 14TR00031

Campione: **TERRENO - TENUTA CANNONA**

Data rapporto di prova: 18/04/2014

RISULTATO DELL'ANALISI CHIMICO-FISICA DEL TERRENO

Prova:	**U.M.:**	**Ris.:**	**Giudizio**
SABBIA	%	**29,8**	franco-argilloso
LIMO	%	**41,3**	mediamente plastico.
ARGILLA	%	**28,9**	
Metodo: D.M. 13/09/99 Allegato II.5 (Andreasen)			
pH in ACQUA		**7,89**	Terreno moderatamente alcalino
Metodo: D.M. 13/09/99 Allegato III.1			
CALCARE attivo	g/kg	**29**	Dotazione media
Metodo: D.M. 13/09/99 Allegati V.1-V.2			
CARBONIO ORGANICO	g/kg	**4,3**	
SOSTANZA ORGANICA	g/kg	**7,4**	Dotazione bassa
Metodo: D.M. 13/09/99 Allegato VII.3			
AZOTO totale	g/kg	**0,69**	Dotazione bassa
Metodo: D.M. 13/09/99 Allegati XIV.2-XIV.3			
RAPPORTO C/N		**6,30**	Rapporto basso - mineralizzazione prevalente
CAPACITA' DI SCAMBIO CATIONICO	meq/100 g	**15,9**	Normale
CALCIO scambiabile	mg/kg	**3406**	Dotazione molto elevata
CALCIO scambiabile	meq/100 g	**16,99**	
MAGNESIO scambiabile	mg/kg	**500**	Dotazione molto elevata
MAGNESIO scambiabile	meq/100 g	**4,11**	
POTASSIO scambiabile	mg/kg	**205**	Dotazione elevata
POTASSIO scambiabile	meq/100 g	**0,52**	
Ca/Mg		**4,1**	Squilibrio Ca/Mg per eccesso relativo di magnesio
Ca/K		**32,5**	Rapporto equilibrato
Mg/K		**7,9**	Squilibrio Mg/K per eccesso relativo di magnesio
Metodo: D.M. 13/09/99 Allegati XIII.2-XIII.5			
FOSFORO assimilabile	mg/kg	**7**	Dotazione molto bassa
ANIDRIDE FOSFORICA assimilabile	mg/kg	**17**	
Metodo: D.M. 13/09/99 Allegato XV.3			

IL CHIMICO
Dr. Cerruti Marco

I risultati del presente rapporto di prova si riferiscono esclusivamente al campione sottoposto a prova.
I campioni sono conservati per un periodo di 5 giorni dalla Data Rapporto di prova.

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Enocontrol Scarl

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RAPPORTO DI PROVA N° 14TR00032

Campione: **TERRENO - CANTINA MANTOVANA**

Data rapporto di prova: 18/04/2014

RISULTATO DELL'ANALISI CHIMICO-FISICA DEL TERRENO

Prova:	U.M.:	Ris.:	Giudizio
SABBIA	%	45,0	franco-argilloso
LIMO	%	26,8	mediamente plastico.
ARGILLA	%	28,2	
Metodo: D.M. 13/09/99 Allegato II.5 (Andreasen)			
pH in ACQUA		5,99	Terreno moderatamente acido
Metodo: D.M. 13/09/99 Allegato III.1			
CALCARE attivo	g/kg	11	Dotazione scarsa
Metodo: D.M. 13/09/99 Allegati V.1-V.2			
CARBONIO ORGANICO	g/kg	6,4	
SOSTANZA ORGANICA	g/kg	11,0	Dotazione normale
Metodo: D.M. 13/09/99 Allegato VII.3			
AZOTO totale	g/kg	0,70	Dotazione bassa
Metodo: D.M. 13/09/99 Allegati XIV.2-XIV.3			
RAPPORTO C/N		9,06	Rapporto equilibrato - mineralizzazione normale
CAPACITA' DI SCAMBIO CATIONICO	meq/100 g	15,8	Normale
CALCIO scambiabile	mg/kg	1972	Dotazione normale
CALCIO scambiabile	meq/100 g	9,84	
MAGNESIO scambiabile	mg/kg	674	Dotazione molto elevata
MAGNESIO scambiabile	meq/100 g	5,54	
POTASSIO scambiabile	mg/kg	115	Dotazione bassa
POTASSIO scambiabile	meq/100 g	0,29	
Ca/Mg		1,8	Squilibrio Ca/Mg per eccesso relativo di magnesio
Ca/K		33,4	Rapporto equilibrato
Mg/K		18,8	Squilibrio Mg/K per eccesso relativo di magnesio
Metodo: D.M. 13/09/99 Allegati XIII.2-XIII.5			
FOSFORO assimilabile	mg/kg	10	Dotazione bassa
ANIDRIDE FOSFORICA assimilabile	mg/kg	23	
Metodo: D.M. 13/09/99 Allegato XV.3			

IL CHIMICO
Dr. Cerruti Marco

Annex II: Full list at Phylum level of bacterial communities in conventional pest management vineyard with their frequencies and significances

	Frequency				**Significancy**			
Phylum	**BS1**	**BS2**	**RHIZ1**	**RHIZ2**	**BS1 vs BS2**	**Bs1 vs Rhiz1**	**BS2 vs Rhiz2**	**Rhiz1 vs Rhiz2**
Actinobacteria	49,61	52,80	61,72	48,76	0,841	0,841	0,841	0,690
Proteobacteria	23,15	30,84	26,78	28,10	0,032	0,548	0,600	0,548
Firmicutes	21,40	2,49	1,36	0,50	0,008	0,032	0,151	0,463
Acidobacteria	1,53	3,70	2,31	11,63	0,222	0,841	0,095	0,032
Gemmatimonadetes	0,93	6,38	2,49	7,99	0,222	0,548	0,690	0,008
Bacteroidetes	2,73	3,31	3,90	1,87	0,548	0,841	0,310	0,690
Chloroflexi	0,41	0,24	1,27	0,56	0,310	0,690	0,346	0,249
Nitrospirae	0,02	0,14	0,06	0,47	0,172	0,292	0,047	0,151
Candidatus_Saccharibacteria	0,09	0,02	0,06	0,03	0,523	1,000	0,452	0,461
Armatimonadetes	0,03	0,06	0,02	0,05	1,000	0,832	0,829	0,673
Verrucomicrobia	0,00	0,02	0,02	0,04	1,000	1,000	0,591	1,000
Fusobacteria	0,05	0,00	0,00	0,00	0,025	0,057	NA	0,424
Cyanobacteria1Chloroplast	0,03	0,00	0,00	0,00	0,057	0,387	0,424	0,180
Spirochaetes	0,02	0,00	0,00	0,00	0,072	0,347	NA	0,424

Annex III: Full list at Class level of bacterial communities in conventional pest management vineyard with their frequencies and significances

		Frequencies				Significancies			
phylum	**class**	**BS1**	**BS2**	**RHIZ1**	**RHIZ2**	**BS1 vs BS2**	**Bs1 vs Rhiz1**	**BS2 vs Rhiz2**	**Rhiz1 vs Rhiz2**
Actinobacteria	Actinobacteria	98,91	98,31	98,54	98,16	0,841	0,841	0,841	0,690
Actinobacteria	unclassified_Actinobacteria	1,09	1,67	1,46	1,82	0,690	0,841	0,249	0,151
Proteobacteria	Alphaproteobacteria	45,6	43,2	52,1	47,2	0,008	0,548	0,421	0,151
Proteobacteria	Betaproteobacteria	29,9	36,3	28,3	35,0	0,151	1,000	0,690	0,095
Proteobacteria	Deltaproteobacteria	13,1	10,6	8,3	10,6	0,028	1,000	0,841	0,016
Proteobacteria	Gammaproteobacteria	9,6	6,4	6,7	4,6	0,310	0,690	1,000	0,222
Proteobacteria	unclassified _Proteobacteria	1,7	3,5	4,6	2,6	0,310	1,000	1,000	0,295
Gemmatimonadetes	Gemmatimonadetes	4,36	35,20	11,62	48,81	0,222	0,548	0,690	0,008
Firmicutes	Bacilli	98,83	100,00	96,16	97,30	0,008	0,032	0,222	0,690
Firmicutes	Clostridia	1,01	0,00	3,84	2,70	0,091	0,091	0,667	0,063
Bacteroidetes	Sphingobacteriia	34,97	63,97	44,43	85,48	0,917	0,222	0,548	0,222
Bacteroidetes	Flavobacteriia	44,34	20,37	27,92	3,96	1,000	1,000	0,599	0,295
Bacteroidetes	Bacteroidetes_incertae_sedis	8,12	9,20	8,92	8,41	0,834	0,548	0,916	0,675
Bacteroidetes	unclassified_Bacteroidetes	4,37	4,85	10,40	0,00	0,753	0,458	0,338	0,504
Bacteroidetes	Cytophagia	5,90	1,62	8,33	2,15	0,168	0,548	0,916	1,000
Bacteroidetes	Bacteroidia	2,30	0,00	0,00	0,00	0,025	0,094	NA	NA
Chloroflexi	Ktedonobacteria	7,95	30,00	85,72	89,04	0,746	0,046	0,036	0,402
Chloroflexi	denovo	42,55	70,00	11,90	10,96	0,295	0,402	0,463	0,141
Chloroflexi	Chloroflexia	46,22	0,00	2,38	0,00	0,067	0,249	0,519	0,094
Nitrospirae	Nitrospira	1,94	19,37	7,27	71,41	0,172	0,292	0,047	0,151

Annex IV: List of the bacterial genera described in the conventional pest management vineyard with their frequencies and significancies in all soil samples

Phylum	Class	Order	Family	Genus	BS1	BS2	Rhiz1	Rhiz2	BS1 vs BS2	Bs1 vs Rhiz1	BS2 vs Rhiz2	Rhiz1 vs Rhiz2
Actinobacteria	Actinobacteria	unidentified_Actinobacteria	unidentified_Actinobacteria	unidentified_Actinobacteria	51,71	61,41	57,51	61,73	1,000	0,841	0,421	0,249
Actinobacteria	Actinobacteria	Gaiellales	Gaiellaceae	Gaiella	20,56	10,67	13,38	10,95	0,310	1,000	0,421	1,000
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Arthrobacter	9,71	4,58	10,91	8,71	1,000	0,222	0,222	0,690
Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae	Blastococcus	3,30	6,19	8,49	5,50	0,548	0,841	0,841	0,917
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	2,79	4,45	1,47	3,59	0,095	0,917	0,548	0,095
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Nocardioides	4,83	3,18	0,70	2,14	1,000	0,032	0,310	0,056
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	Terrabacter	0,83	1,47	2,95	2,04	0,675	0,421	0,012	1,000
Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	Virgisporangium	0,95	2,05	1,46	2,44	0,090	1,000	0,045	0,734
Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter	3,05	2,31	0,27	0,81	1,000	0,056	0,095	0,222
Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	Actinoplanes	0,54	2,26	0,87	1,58	0,056	0,548	0,530	0,059
Actinobacteria	Actinobacteria	Actinomycetales	Nakamurellaceae	Nakamurella	1,72	1,43	1,99	0,50	0,548	0,841	0,032	0,310
Proteobacteria	α-Proteobacteria	unidentified	unidentified	unidentified _	64,50	60,14	68,35	66,73	0,310	1,000	0,530	0,059
Proteobacteria	α-Proteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	5,31	9,96	4,19	13,57	0,046	0,530	0,222	0,016
Proteobacteria	α-Proteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	12,62	7,34	3,10	5,85	0,222	0,402	0,834	0,015
Proteobacteria	α-Proteobacteria	Rhodospirillales	Rhodospirillaceae	Skermanella	6,13	5,50	15,25	0,52	0,141	0,672	0,094	0,690
Proteobacteria	α-Proteobacteria	Rhizobiales	Methylobacteriaceae	Microvirga	4,84	9,07	0,43	0,65	0,222	0,092	0,008	0,527
Proteobacteria	α-Proteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	1,98	3,01	3,96	5,47	0,421	0,421	0,222	0,249
Proteobacteria	α-Proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	4,31	3,13	3,57	3,82	0,421	0,841	0,463	0,463
Proteobacteria	α-Proteobacteria	Alphaproteobacteria_incertae_sedis	Rhizomicrobium	Rhizomicrobium	0,31	1,84	1,14	3,39	0,093	0,399	0,293	0,092
Proteobacteria	β-Proteobacteria	unidentified_β-Proteobacteria	unidentified_β-Proteobacteria	unidentified	62,29	70,76	53,36	65,26	0,056	0,841	0,841	0,008
Proteobacteria	β-Proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	6,19	10,34	20,91	13,38	0,222	0,173	0,222	0,151

Proteobacteria	β-Proteobacteria	Burkholderiales	Oxalobacteraceae	Noviherbaspirillum	11,92	7,00	16,24	7,98	0,402	0,548	0,675	0,690
Proteobacteria	β-Proteobacteria	Burkholderiales	Oxalobacteraceae	Massilia	7,67	8,93	3,95	10,04	0,056	1,000	0,690	0,012
Proteobacteria	β-Proteobacteria	Burkholderiales	Comamonadaceae	Variovorax	10,09	0,90	1,97	0,94	0,056	0,209	0,528	0,290
Proteobacteria	β-Proteobacteria	Burkholderiales	Comamonadaceae	Ramlibacter	1,61	1,75	0,81	2,10	0,027	0,672	0,599	0,012
Proteobacteria	β-Proteobacteria	Nitrosomonadales	Nitrosomonadaceae	Nitrosospira	0,23	0,32	2,76	0,30	0,821	0,332	1,000	0,527
Proteobacteria	δ-Proteobacteria	Myxococcales	Cystobacteraceae	Cystobacter	0,05	0,78	0,08	0,12	0,036	0,671	0,032	0,243
Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	68,05	65,35	12,84	32,34	0,222	0,095	0,222	0,600
Firmicutes	Bacilli	Bacillales	Bacillaceae_1	Bacillus	20,35	21,15	53,86	47,78	0,222	0,151	0,222	0,222
Firmicutes	Bacilli	Bacillales	Paenibacillaceae_1	Paenibacillus	6,24	2,54	12,73	11,79	0,047	0,059	0,916	1,000
Firmicutes	Bacilli	unidentified	unidentified	unidentified	2,66	9,59	20,57	8,09	0,651	0,345	0,519	1,000
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	2,70	1,37	0,00	0,00	0,059	0,052	0,158	1,000
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas	4,36	35,20	11,62	48,81	0,222	0,548	0,690	0,008
Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira	1,94	19,37	7,27	71,41	0,172	0,292	0,047	0,151
Bacteroidetes	unidentified_Bacteroidetes	unidentified_Bacteroidetes	unidentified_Bacteroidetes	unidentified_Bacteroidetes	28,80	19,89	25,78	39,36	0,916	0,917	0,462	0,421
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	Niastella	2,70	8,89	4,60	20,73	0,344	0,599	0,209	0,094
Bacteroidetes	Bacteroidetes_incertae_sedis	Ohtaekwangia	Ohtaekwangia	Ohtaekwangia	11,70	10,11	9,21	9,04	0,834	0,548	0,916	0,675
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	Flavisolibacter	4,14	14,22	5,73	10,03	0,548	0,600	0,674	0,421
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Chryseobacterium	0,94	18,77	8,16	1,54	0,344	1,000	0,398	1,000
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	17,10	1,33	13,81	1,06	0,075	0,530	0,670	0,281
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	Segetibacter	5,40	4,87	6,40	6,95	0,530	1,000	0,672	0,289

Annex V: Full list of the bacterial species identified in the conventional pest management vineyard and their frequencies and significancies.

phylum	class	order	genus	species	BS1	BS2	Rhiz1	Rhiz2	BS1 vs BS2	Bs1 vs Rhiz1	BS2 vs Rhiz2	Rhiz1 vs Rhiz2
Actinobacteria	Actinobacteria	Actinomycetales	denovo	denovo	8,49	7,18	16,9	8,75	0,222	0,056	0,151	0,841
Actinobacteria	Actinobacteria	Actinomycetales	denovo	denovo	4,95	7,92	7,57	5,56	0,032	0,151	0,548	0,690
Actinobacteria	Actinobacteria	Solirubrobacterales	denovo	denovo	4,46	3,66	2,98	3,82	0,690	0,841	0,421	0,095
Actinobacteria	Actinobacteria	denovo	denovo	denovo	3,88	2,02	2,89	2,01	1,000	0,841	0,421	0,249
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Gemmatimonadaceae bacterium LWQ133	0,44	3,36	0,83	2,47	0,094	0,753	0,690	0,008
Proteobacteria	Alphaproteobacteria	Rhizobiales	denovo	denovo	2,09	1,94	2,51	1,62	0,008	0,310	0,548	0,310
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	bacterium Ellin6526	2,41	1,33	2,81	1,77	0,841	0,690	0,095	1,000
Proteobacteria	Betaproteobacteria	denovo	denovo	denovo	0,64	2,42	1,08	2,56	0,056	0,841	0,841	0,008
Actinobacteria	Actinobacteria	Actinomycetales	denovo	denovo	0,61	2,36	1,53	2,23	0,690	0,421	0,310	0,421
Actinobacteria	Actinobacteria	Actinomycetales	denovo	denovo	0,78	0,99	4,48	1,52	0,142	0,008	0,021	0,095
Proteobacteria	Alphaproteobacteria	Rhizobiales	denovo	denovo	0,58	2,18	0,82	2,00	0,016	0,841	0,753	0,008
Proteobacteria	Alphaproteobacteria	Rhodospirillales	denovo	denovo	1,31	1,07	1,69	1,93	0,173	0,222	0,016	0,222
Proteobacteria	Deltaproteobacteria	Myxococcales	denovo	denovo	1,26	1,30	1,24	1,80	0,222	0,690	0,548	0,095
Proteobacteria	Betaproteobacteria	Burkholderiales	denovo	denovo	0,55	2,16	0,69	1,64	0,180	1,000	0,180	1,000
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	bacterium Ellin7530	0,61	1,13	2,25	1,52	0,095	0,462	0,295	0,059

Actinobacteria	Actinobacteria	Actinomycetales	Blastococcus	bacterium Ellin6023	0,88	1,14	2,12	1,36	0,690	0,690	0,310	0,841
Actinobacteria	Actinobacteria	Acidimicrobiales	denovo	denovo	2,81	1,11	1,13	0,81	0,310	1,000	0,530	0,059
Actinobacteria	Actinobacteria	Actinomycetales	denovo	denovo	2,52	1,07	0,87	1,12	0,548	0,600	0,310	0,548
Proteobacteria	Betaproteobacteria	Burkholderiales	denovo	denovo	1,48	1,90	0,43	1,09	0,690	0,841	0,249	0,151
Proteobacteria	Alphaproteobacteria	Rhodospirillales	denovo	denovo	1,27	1,22	1,06	1,24	0,095	0,753	0,222	0,463
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	agricultural soil bacterium SC-I-92	2,28	0,81	1,29	0,81	0,841	0,421	0,690	0,095
Firmicutes	Bacilli	Bacillales	Staphylococcus	Staphylococcus epidermidis	5,76	0,59	0,03	0,05	0,526	0,518	0,248	0,528
Actinobacteria	denovo	denovo	denovo	denovo	1,04	1,03	1,43	1,04	0,056	0,095	0,095	0,056
Proteobacteria	denovo	denovo	denovo	denovo	0,59	1,26	1,40	0,91	0,310	0,548	0,421	0,310
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Gemmatimonadetes bacterium KBS708	0,29	1,38	0,35	1,34	0,151	0,548	0,421	0,222
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	unidentified bacterium	0,44	0,95	0,89	1,30	0,421	0,841	0,421	0,095
Proteobacteria	Alphaproteobacteria	denovo	denovo	denovo	0,88	0,83	1,28	0,99	0,310	1,000	1,000	0,295
Actinobacteria	Actinobacteria	Actinomycetales	denovo	denovo	0,57	0,94	1,09	1,13	0,530	0,841	0,462	0,463
Actinobacteria	Actinobacteria	Actinomycetales	denovo	denovo	1,58	0,50	1,29	0,73	0,032	1,000	0,690	0,047
Acidobacteria	Acidobacteria_Gp1	denovo	denovo	denovo	0,16	0,55	0,56	1,69	0,690	0,310	0,548	0,690
Proteobacteria	Betaproteobacteria	Burkholderiales	denovo	denovo	0,64	1,01	0,63	0,99	0,340	0,012	0,151	0,841
Proteobacteria	Betaproteobacteria	Burkholderiales	denovo	denovo	1,26	0,99	0,64	0,57	0,032	0,528	0,421	0,008
Actinobacteria	Actinobacteria	Actinomycetales	Blastococcus	Blastococcus sp. OS1-29	0,34	0,61	1,42	0,76	0,916	0,917	0,462	0,421
Proteobacteria	Deltaproteobacteria	Myxococcales	denovo	denovo	0,80	0,79	0,69	0,70	1,000	1,000	0,346	0,151

Actinobacteria	Actinobacteria	Actinomycetales	denovo	denovo	0,03	0,21	1,62	1,03	0,293	0,421	0,209	0,834
Firmicutes	Bacilli	Bacillales	Staphylococcus	Staphylococcus epidermidis PM221	3,54	0,39	0,05	0,06	0,222	0,095	0,310	0,753
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	bacterium Ellin7545	0,83	0,60	0,76	0,69	0,402	0,295	0,753	0,834
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	denovo	denovo	1,03	0,43	0,37	0,81	0,690	0,690	0,421	0,222
Actinobacteria	Actinobacteria	Actinomycetales	Kineosporia	Kineosporia rhamnosa (T)	1,45	0,68	0,17	0,33	0,548	0,032	0,094	0,295
Acidobacteria	Acidobacteria_Gp3	Gp3	Gp3	unidentified bacterium	0,17	0,33	0,25	1,20	1,000	0,032	0,032	0,841
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. L13	0,13	0,05	1,37	0,82	1,000	0,090	0,054	0,402
Acidobacteria	Acidobacteria_Gp3	Gp3	Gp3	bacterium enrichment culture clone SC-2_22	0,00	0,71	0,21	0,81	0,012	0,753	0,674	0,095
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Microcystis sp. SAG 43.90	0,03	0,78	0,20	0,61	0,056	0,833	0,753	0,012
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. S88	0,38	0,34	0,90	0,38	0,295	0,829	1,000	0,021
Chloroflexi	Ktedonobacteria	Ktedonobacterales	denovo	denovo	0,07	0,00	1,52	0,46	0,034	0,139	0,310	0,095
Actinobacteria	Actinobacteria	Actinomycetales	denovo	denovo	0,35	0,51	0,38	0,39	0,046	0,112	0,047	0,008
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Gemmatimonadetes bacterium Ellin7146	0,32	0,40	0,17	0,62	0,139	0,139	0,421	0,056
Proteobacteria	Gammaproteobacteria	Xanthomonadales	denovo	denovo	0,21	0,63	0,15	0,47	0,095	0,753	1,000	0,151
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobium	Bradyrhizobium sp. WSM471	0,35	0,57	0,07	0,49	0,151	0,344	0,917	0,675
Acidobacteria	Acidobacteria_Gp1	Gp1	Gp1	bacterium Ellin6528	0,03	0,37	0,13	0,72	1,000	0,690	0,095	0,151
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	bacterium Ellin5290	0,02	0,49	0,33	0,49	0,834	0,115	0,295	0,172
Actinobacteria	Actinobacteria	Actinomycetales	denovo	denovo	0,44	0,63	0,26	0,20	0,172	0,656	0,094	0,746
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	bacterium enrichment culture clone auto9_4W	0,03	0,31	0,17	0,72	0,031	0,052	0,834	0,548

Actinobacteria	Actinobacteria	Actinomycetales	Nakamurella	Humicoccus sp. L1886	0,50	0,28	0,94	0,08	0,021	1,000	0,753	0,008
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	Gaiella occulta (T)	1,16	0,36	0,14	0,13	0,402	0,829	0,463	1,000
Firmicutes	Bacilli	Bacillales	Staphylococcus	Staphylococcus epidermidis RP62A	1,54	0,28	0,00	0,02	0,526	0,239	0,168	0,480
Proteobacteria	Betaproteobacteria	Burkholderiales	Noviherbaspirillum	Comamonadaceae bacterium MSCB-9	0,00	0,32	0,60	0,33	0,249	0,141	0,095	0,093
Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacter	bacterium Ellin504	0,22	0,18	0,46	0,39	0,548	0,075	0,690	0,095
Proteobacteria	Gammaproteobacteria	denovo	denovo	denovo	0,16	0,40	0,40	0,25	0,036	0,340	0,310	0,008
Acidobacteria	Acidobacteria_Gp3	denovo	denovo	denovo	0,00	0,59	0,01	0,32	0,690	0,346	0,222	0,421
Actinobacteria	Actinobacteria	Actinomycetales	Blastococcus	Blastococcus sp. L1961	0,76	0,20	0,13	0,17	0,249	0,462	0,834	0,666
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderia	Burkholderia sp. MS4t	0,00	0,41	0,23	0,27	1,000	0,670	0,141	0,525
Actinobacteria	Actinobacteria	Actinomycetales	Marmoricola	Marmoricola sp. Gsoil 818	0,50	0,27	0,20	0,17	0,016	0,116	0,600	0,841
Proteobacteria	Alphaproteobacteria	Rhizobiales	denovo	denovo	0,12	0,23	0,03	0,47	0,310	0,750	0,151	0,059
Actinobacteria	Actinobacteria	Actinomycetales	Terrabacter	Terrabacter lapilli (T)	0,02	0,11	0,69	0,21	0,753	0,548	0,248	0,458
Actinobacteria	Actinobacteria	Actinomycetales	Blastococcus	Blastococcus aggregatus	0,53	0,28	0,16	0,11	0,424	0,045	0,025	0,140
Proteobacteria	Alphaproteobacteria	Rhodospirillales	denovo	denovo	0,12	0,24	0,38	0,21	0,052	0,180	0,094	0,059
Proteobacteria	Betaproteobacteria	Burkholderiales	Noviherbaspirillum	Herbaspirillum sp. Sco-D20	0,10	0,26	0,36	0,19	0,310	0,458	0,841	0,115
Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacter	Rubrobacterineae bacterium BR7-21	0,13	0,31	0,19	0,21	0,195	0,084	0,047	0,690
Acidobacteria	Acidobacteria_Gp3	Gp3	Gp3	Acidobacteria bacterium SCGC AAA204-D14	0,00	0,09	0,08	0,51	0,027	0,829	0,012	0,012
Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacter	bacterium Ellin6048	0,41	0,19	0,09	0,22	1,000	0,518	0,753	0,344
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioides	bacterium Ellin6014	0,39	0,21	0,10	0,21	0,753	0,458	0,338	0,504

Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. SaCR11	0,03	0,09	0,49	0,25	0,396	0,057	0,012	0,346
Acidobacteria	Acidobacteria_Gp1	Gp1	Gp1	Actinomycetales	0,16	0,18	0,10	0,31	0,295	0,402	0,463	0,141
Actinobacteria	Actinobacteria	Actinomycetales	Blastococcus	Candidatus Blastococcus massiliensis AP3	0,26	0,06	0,29	0,26	0,090	0,289	0,690	0,249
Proteobacteria	Deltaproteobacteria	Myxococcales	denovo	denovo	0,00	0,23	0,17	0,27	0,205	0,834	0,057	0,209
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. HS4-1	0,45	0,06	0,33	0,11	0,014	0,054	0,036	0,074
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacterium	Methylobacterium sp. MG-2011-64-DP	0,00	0,22	0,13	0,27	0,528	0,596	0,248	0,461
Actinobacteria	Actinobacteria	Acidimicrobiales	Iamia	bacterium Ellin5273	0,51	0,17	0,08	0,10	0,441	0,906	0,424	0,180
Actinobacteria	Actinobacteria	Actinomycetales	Nakamurella	Nakamurella flavida	0,16	0,31	0,10	0,12	1,000	0,402	0,202	0,834
Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacter	bacterium Ellin5249	0,59	0,23	0,03	0,03	0,665	0,504	0,823	0,813
Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacter	Solirubrobacter ginsenosidimutans	0,63	0,23	0,02	0,02	0,346	1,000	0,458	0,136
Acidobacteria	Acidobacteria_Gp3	Gp3	Gp3	Actinomycetales	0,00	0,09	0,04	0,42	0,463	0,463	0,917	1,000
Chloroflexi	denovo	denovo	denovo	denovo	0,39	0,14	0,26	0,06	0,295	0,036	0,016	1,000
Acidobacteria	Acidobacteria_Gp1	Gp1	Gp1	bacterium Ellin6547	0,03	0,18	0,12	0,25	0,753	0,344	0,690	0,036
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Gemmatimonadetes bacterium LX87	0,09	0,26	0,02	0,21	0,829	0,914	0,074	0,142
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	bacterium Ellin7507	0,08	0,17	0,19	0,19	0,401	0,525	0,032	0,141
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangium	Intrasporangium calvum DSM 43043	0,38	0,26	0,05	0,06	NA	0,180	0,424	0,519
Acidobacteria	Acidobacteria_Gp1	Gp1	Gp1	bacterium Ellin7544	0,13	0,08	0,21	0,24	0,018	0,441	0,530	0,090
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Gemmatimonas aurantiaca	0,00	0,21	0,03	0,26	0,441	0,724	0,424	0,180
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	unidentified bacterium	0,48	0,06	0,25	0,05	0,753	0,046	0,139	0,284

Proteobacteria	Deltaproteobacteria	Myxococcales	Kofleria	Kofleria flava	0,01	0,17	0,05	0,27	0,448	0,750	0,345	0,234
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. M29	0,00	0,12	0,26	0,20	0,289	1,000	0,528	0,012
Actinobacteria	Actinobacteria	Actinomycetales	Marmoricola	Nocardioides iriomotensis	0,42	0,16	0,12	0,04	0,917	0,036	0,142	0,584
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter oryzae	0,48	0,15	0,02	0,08	0,434	0,724	0,194	0,441
Acidobacteria	Acidobacteria_Gp1	Candidatus_Koribacter	Candidatus_Koribacter	Candidatus Koribacter versatilis Ellin345	0,00	0,11	0,12	0,28	0,072	0,746	NA	0,072
Proteobacteria	Alphaproteobacteria	Sphingomonadales	denovo	denovo	0,18	0,24	0,11	0,08	1,000	0,674	0,115	0,753
Proteobacteria	Alphaproteobacteria	Caulobacterales	Phenylobacterium	Phenylobacterium sp. W2.09-62	0,24	0,13	0,09	0,16	0,396	1,000	0,916	1,000
Proteobacteria	Alphaproteobacteria	Rhizobiales	denovo	denovo	0,06	0,09	0,21	0,20	0,009	0,192	1,000	0,020
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. 7B-895	0,00	0,03	0,18	0,30	0,015	0,168	0,753	0,462
Nitrospirae	Nitrospira	Nitrospirales	Nitrospira	Candidatus Nitrospira bockiana	0,03	0,06	0,05	0,34	0,205	0,373	0,917	0,753
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Skermanella	Skermanella aerolata	0,28	0,30	0,00	0,02	0,012	0,525	0,690	0,012
Actinobacteria	Actinobacteria	Actinomycetales	Terrabacter	Terrabacter carboxydivorans (T)	0,03	0,03	0,28	0,20	0,138	1,000	0,517	0,401
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	bacterium enrichment culture clone F44x_D7_T3_E05	0,23	0,03	0,37	0,06	0,180	0,441	NA	0,424
Actinobacteria	Actinobacteria	Actinomycetales	Tetrasphaera	Tetrasphaera sp. Ellin115	0,44	0,20	0,03	0,00	0,834	0,600	0,141	0,675
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioides	Nocardioides sp. S147	0,45	0,19	0,00	0,02	0,071	0,434	0,528	1,000
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioides	Nocardioides islandensis (T)	0,16	0,19	0,04	0,13	0,530	0,222	0,292	0,752
Bacteroidetes	Flavobacteriia	Flavobacteriales	Chryseobacterium	Chryseobacterium sp. CI02	0,03	0,37	0,02	0,03	0,290	0,914	0,289	0,670
Acidobacteria	Acidobacteria_Gp1	Gp1	Gp1	bacterium Ellin7522	0,06	0,15	0,10	0,17	0,387	0,737	0,093	0,142
Actinobacteria	Actinobacteria	Actinomycetales	Streptomyces	Streptomyces viridochromogenes subsp. komabensis	0,02	0,25	0,00	0,16	0,441	0,724	0,424	0,180

Proteobacteria	Betaproteobacteria	Burkholderiales	Noviherbaspirillum	Oxalobacter sp. W1.09-142	0,19	0,08	0,12	0,16	0,115	0,113	0,402	0,195
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter globiformis	0,26	0,13	0,14	0,05	0,750	0,834	0,172	0,173
Proteobacteria	Alphaproteobacteria	Caulobacterales	denovo	denovo	0,18	0,17	0,08	0,08	0,203	0,830	1,000	0,015
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Skermanella	Azospirillum sp. enrichment culture clone VanCtr101	0,09	0,08	0,45	0,00	1,000	0,341	0,070	0,027
Proteobacteria	Alphaproteobacteria	Sphingomonadales	denovo	denovo	0,02	0,17	0,17	0,10	0,084	0,130	0,501	0,576
Acidobacteria	Acidobacteria_Gp3	Gp3	Gp3	bacterium Ellin7504	0,00	0,04	0,05	0,29	0,396	1,000	0,517	0,205
Acidobacteria	Acidobacteria_Gp10	Gp10	Gp10	Acidobacteria bacterium WY11	0,10	0,23	0,05	0,06	1,000	1,000	0,015	0,591
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobium	bacterium Ellin6538	0,00	0,09	0,00	0,25	0,158	0,519	1,000	0,011
Firmicutes	Bacilli	Bacillales	Bacillus	Bacillus sp. SKM136	0,47	0,04	0,07	0,04	0,242	0,290	0,043	0,130
Acidobacteria	Acidobacteria_Gp3	Gp3	Gp3	agricultural soil bacterium SC-I-77	0,06	0,09	0,03	0,20	0,674	1,000	0,093	1,000
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Segetibacter	Segetibacter aerophilus	0,16	0,09	0,08	0,11	0,527	1,000	0,016	0,239
Actinobacteria	Actinobacteria	Actinomycetales	Motilibacter	Actinomycetales bacterium RS-16	0,00	0,09	0,05	0,19	0,023	0,018	0,075	0,032
Proteobacteria	Betaproteobacteria	Burkholderiales	Ramlibacter	Ramlibacter tataouinensis TTB310	0,09	0,08	0,03	0,17	0,169	0,830	0,058	0,331
Actinobacteria	Actinobacteria	Actinomycetales	denovo	denovo	0,22	0,10	0,07	0,07	0,829	0,265	0,595	0,480
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. LM3(2011)	0,24	0,09	0,10	0,05	1,000	0,085	0,056	0,478
Actinobacteria	Actinobacteria	Acidimicrobiales	Aciditerrimonas	Acidimicrobidae bacterium Ellin7143	0,03	0,11	0,16	0,08	0,600	0,230	0,036	0,443
Acidobacteria	Acidobacteria_Gp3	Gp3	Gp3	Acidobacteria bacterium LWQ4	0,09	0,11	0,05	0,12	1,000	0,746	0,245	0,750
Bacteroidetes	denovo	denovo	denovo	denovo	0,23	0,11	0,15	0,00	0,527	1,000	0,599	0,116
Proteobacteria	Deltaproteobacteria	denovo	denovo	denovo	0,21	0,13	0,05	0,05	0,834	1,000	0,173	0,402

Actinobacteria	Actinobacteria	Actinomycetales	Amycolatopsis	Amycolatopsis lexingtonensis (T)	0,16	0,18	0,00	0,05	0,746	0,280	0,264	0,821
Actinobacteria	Actinobacteria	Actinomycetales	Terrabacter	Terrabacter terrae (T)	0,00	0,06	0,11	0,17	0,797	0,345	0,041	0,202
Actinobacteria	Actinobacteria	Acidimicrobiales	Ilumatobacter	unidentified bacterium	0,27	0,09	0,03	0,05	0,065	0,018	0,074	0,112
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Roseomonas	Acetobacteraceae bacterium GIMN 1.017	0,00	0,18	0,10	0,06	0,674	0,106	0,206	0,445
Acidobacteria	Acidobacteria_Gp1	Gp1	Gp1	bacterium Ellin7529	0,00	0,17	0,00	0,12	0,278	0,906	0,398	0,066
Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacter	Solirubrobacter sp. Gsoil 917	0,13	0,12	0,07	0,06	0,295	1,000	0,112	0,747
Firmicutes	Bacilli	Bacillales	denovo	denovo	0,38	0,03	0,12	0,00	0,401	0,280	1,000	0,089
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacter	Caulobacter vibrioides	0,00	0,10	0,09	0,12	0,093	0,480	0,462	0,085
Firmicutes	Bacilli	Bacillales	denovo	denovo	0,06	0,18	0,07	0,03	0,519	0,203	0,012	1,000
Actinobacteria	Actinobacteria	Actinomycetales	Actinoplanes	Actinoplanes sp. IR56-Co102	0,00	0,24	0,00	0,04	1,000	0,607	0,192	1,000
Actinobacteria	Actinobacteria	Actinomycetales	Mycobacterium	Mycobacterium sp. N1730C	0,32	0,11	0,01	0,00	1,000	0,525	0,015	0,338
Actinobacteria	Actinobacteria	Actinomycetales	Terrabacter	Terrabacter sp. A2-62	0,16	0,04	0,10	0,09	0,009	1,000	0,399	0,010
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. CFM 20	0,21	0,07	0,08	0,04	0,667	0,332	0,023	0,462
Actinobacteria	Actinobacteria	Actinomycetales	Streptomyces	Streptomyces sp. 1MR-8	0,00	0,17	0,03	0,08	0,829	0,462	0,289	0,916
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderia	Burkholderia sp. MG-2011-1-AC	0,00	0,04	0,09	0,16	0,449	0,666	0,243	0,833
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderia	bacterium enrichment culture clone SC-2_86	0,00	0,01	0,21	0,11	0,034	0,734	0,249	0,011
Proteobacteria	Alphaproteobacteria	Rhizobiales	Pseudolabrys	methanogenic prokaryote enrichment culture B19_111	0,03	0,06	0,07	0,14	0,396	0,723	0,009	0,600
Proteobacteria	Alphaproteobacteria	Rhizobiales	denovo	denovo	0,00	0,15	0,07	0,06	0,071	0,331	1,000	0,243
Proteobacteria	Alphaproteobacteria	Caulobacterales	Phenylobacterium	Phenylobacterium sp. P-28	0,02	0,17	0,03	0,06	0,752	0,829	0,172	0,115

Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_incertae_sedis	Rhizomicrobium	bacterium Ellin329	0,03	0,09	0,00	0,14	0,584	0,502	0,034	0,209
Actinobacteria	Actinobacteria	Actinomycetales	Cryptosporangium	Cryptosporangium minutisporangium (T)	0,12	0,17	0,00	0,02	0,066	0,501	0,203	0,012
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobium	Bradyrhizobium sp. IV-102	0,00	0,09	0,00	0,16	0,511	1,000	0,262	0,101
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Skermanella	Skermanella sp. JC5	0,03	0,18	0,04	0,03	0,236	0,034	0,463	0,675
Actinobacteria	Actinobacteria	Actinomycetales	Tetrasphaera	actinomycete S23403	0,45	0,02	0,01	0,00	0,007	0,029	0,424	0,600
Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_incertae_sedis	Rhizomicrobium	bacterium Ellin335	0,00	0,07	0,03	0,14	0,249	0,236	0,018	0,515
Proteobacteria	Deltaproteobacteria	Myxococcales	Kofleria	Kofleria sp. DSM 53797	0,03	0,07	0,03	0,13	0,044	0,911	0,116	0,091
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioides	Nocardioides sp. S23405	0,17	0,11	0,00	0,05	0,478	0,406	0,246	0,141
Proteobacteria	Betaproteobacteria	Burkholderiales	Massilia	Kartchner Caverns bacterium MI-10a	0,00	0,11	0,00	0,13	0,180	0,180	NA	NA
Actinobacteria	Actinobacteria	Actinomycetales	Actinoplanes	Actinoplanes sp. RI44-Va104	0,00	0,11	0,02	0,11	0,283	0,434	0,114	0,675
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	metal-contaminated soil clone K20-25	0,11	0,18	0,01	0,00	1,000	1,000	1,000	0,833
Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacter	Solirubrobacter soli (T)	0,15	0,14	0,01	0,02	1,000	0,195	0,289	0,371
Actinobacteria	Actinobacteria	Actinomycetales	Streptomyces	Streptomyces canu	0,29	0,08	0,01	0,02	0,009	0,232	0,598	0,059
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	Actinomycetales	0,09	0,01	0,23	0,03	1,000	0,284	0,675	0,168
Proteobacteria	Alphaproteobacteria	Caulobacterales	Phenylobacterium	Phenylobacterium sp. DLS-83	0,09	0,09	0,07	0,05	0,071	0,180	1,000	0,020
Actinobacteria	Actinobacteria	Acidimicrobiales	Aciditerrimonas	Aciditerrimonas ferrireducens (T)	0,03	0,07	0,03	0,11	0,025	0,118	NA	0,424
Actinobacteria	Actinobacteria	Actinomycetales	Amycolatopsis	Amycolatopsis rifamycinica (T)	0,06	0,11	0,05	0,06	0,670	0,478	0,134	0,797
Actinobacteria	Actinobacteria	Actinomycetales	Streptomyces	Streptomyces sp. NEAU-CF1	0,06	0,09	0,07	0,06	0,915	0,823	0,525	0,338
Acidobacteria	Acidobacteria_Gp1	Gp1	Gp1	Acidobacteriaceae bacterium A2-1c	0,00	0,09	0,03	0,11	0,239	0,023	0,027	0,753

Proteobacteria	Betaproteobacteria	Burkholderiales	Massilia	Massilia aerilata	0,06	0,09	0,00	0,09	0,737	0,737	0,737	0,504
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Pseudomonas moraviensis	0,03	0,00	0,08	0,14	0,180	0,441	NA	0,424
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter defluvii	0,05	0,03	0,07	0,11	0,072	0,239	NA	0,424
Acidobacteria	Acidobacteria_Gp1	Gp1	Gp1	bacterium K-5b2	0,00	0,03	0,05	0,14	0,130	0,813	0,346	0,055
Actinobacteria	Actinobacteria	Actinomycetales	Amycolatopsis	Amycolatopsis bullii	0,06	0,10	0,07	0,03	0,345	0,441	0,138	0,112
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacterium	Methylobacterium longum	0,08	0,04	0,07	0,08	0,138	0,827	0,824	0,202
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	soil bacterium 14V-14	0,00	0,04	0,10	0,09	0,171	0,914	0,110	0,434
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Gemmatimonadetes bacterium WWH24	0,00	0,09	0,00	0,11	0,329	0,140	0,337	0,596
Proteobacteria	Alphaproteobacteria	Caulobacterales	Phenylobacterium	bacterium Ellin5060	0,03	0,09	0,02	0,08	0,344	0,057	1,000	0,596
Actinobacteria	Actinobacteria	Acidimicrobiales	Ilumatobacter	unidentified marine bacterioplankton	0,25	0,04	0,03	0,01	0,398	0,441	0,007	0,424
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckia	Beijerinckia mobilis (T)	0,00	0,09	0,00	0,11	0,745	0,504	0,825	0,742
Acidobacteria	Acidobacteria_Gp1	Gp1	Gp1	bacterium enrichment culture clone PA_42	0,00	0,11	0,01	0,08	0,528	0,829	0,071	1,000
Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacter	bacterium Ellin5025	0,02	0,02	0,12	0,09	0,025	0,180	0,246	0,014
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobium	Bradyrhizobium group bacterium Ellin127	0,00	0,09	0,00	0,11	0,130	0,373	0,753	0,456
Proteobacteria	Betaproteobacteria	Burkholderiales	Ramlibacter	Variovorax sp. P-9	0,03	0,07	0,03	0,09	0,607	0,441	0,424	0,424
Actinobacteria	Actinobacteria	Actinomycetales	Terrabacter	Terrabacter sp. Ellin102	0,09	0,06	0,05	0,06	0,106	0,504	0,332	0,386
Firmicutes	Bacilli	Bacillales	Bacillus	Bacillus sp. SKM7	0,23	0,06	0,03	0,02	0,287	0,346	0,242	0,091
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Dongia	bacterium Ellin314	0,08	0,09	0,00	0,06	0,833	0,265	0,045	0,441
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	soil bacterium 14V-08	0,03	0,10	0,07	0,05	0,020	1,000	0,916	0,011

Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckia	Beijerinckia sp. p310-1	0,03	0,11	0,00	0,07	0,243	0,101	0,194	0,600
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Rhizobacter	Rhizobacter fulvus	0,06	0,06	0,03	0,08	0,057	0,119	1,000	1,000
Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilus	Geodermatophilus saharensis (T)	0,00	0,04	0,04	0,13	0,066	0,796	0,032	0,013
Firmicutes	Bacilli	Bacillales	Bacillus	Bacillus megaterium	0,24	0,04	0,07	0,00	0,206	0,749	0,401	0,027
Actinobacteria	Actinobacteria	Acidimicrobiales	Ilumatobacter	Iamia sp. T2-YC6790	0,06	0,07	0,09	0,03	0,671	0,278	0,070	0,651
Actinobacteria	Actinobacteria	Actinomycetales	Marmoricola	Kribbella sp. enrichment culture clone VanCtr42	0,06	0,09	0,05	0,04	0,172	0,915	0,169	0,525
Proteobacteria	Betaproteobacteria	Burkholderiales	Massilia	Massilia sp. BS-1	0,03	0,06	0,00	0,11	0,051	0,750	0,456	0,331
Actinobacteria	Actinobacteria	Actinomycetales	Krasilnikovia	Krasilnikovia cinnamomea (T)	0,05	0,03	0,13	0,05	0,090	0,366	0,456	0,029
Actinobacteria	Actinobacteria	Actinomycetales	Marmoricola	Marmoricola bigeumensis (T)	0,13	0,06	0,05	0,03	0,519	0,026	0,056	0,074
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. MWR-A01	0,02	0,00	0,25	0,03	0,180	0,180	0,424	0,424
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Gemmatimonas sp. WX54	0,00	0,07	0,00	0,11	0,107	1,000	0,585	0,101
Proteobacteria	Alphaproteobacteria	Sphingomonadales	denovo	denovo	0,13	0,04	0,00	0,08	0,007	0,180	0,525	0,011
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacter	Caulobacter vibrioides NA1000	0,00	0,08	0,03	0,09	0,180	0,180	NA	NA
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. EP_L_35	0,05	0,03	0,10	0,06	0,180	0,441	NA	0,424
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. Lc30-1	0,05	0,06	0,08	0,05	0,402	0,119	0,065	1,000
Actinobacteria	Actinobacteria	Actinomycetales	Streptomyces	Streptomyces sp. JL-22	0,00	0,00	0,10	0,11	0,668	0,737	1,000	0,334
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderia	Burkholderia sp. DPA61	0,00	0,06	0,03	0,10	0,072	0,288	NA	0,424

Annex VI: Full list at Phylum level of bacterial communities in integrated pest management vineyard with their frequencies and significances

	Frequencies				**Significancy**			
phylum	**BS1**	**BS2**	**RHIZ1**	**RHIZ2**	**BS1 vs BS2**	**Bs1 vs Rhiz1**	**BS2 vs Rhiz2**	**Rhiz1 vs Rhiz2**
Actinobacteria	58,24	54,60	50,65	53,85	0,286	0,421	0,111	0,548
Proteobacteria	26,20	30,81	39,00	33,95	0,556	0,095	1,000	0,421
Gemmatimonadetes	8,15	7,69	3,45	4,16	0,905	0,032	0,556	0,548
Bacteroidetes	2,11	2,80	4,57	4,15	0,413	0,095	0,190	1,000
Acidobacteria	4,19	3,00	1,59	2,35	0,623	0,151	0,905	0,548
Firmicutes	0,35	0,52	0,48	0,98	0,556	0,346	0,730	0,753
Chloroflexi	0,45	0,50	0,03	0,10	0,905	0,012	0,063	0,599
Nitrospirae	0,22	0,05	0,18	0,37	0,018	0,456	0,016	0,209
Verrucomicrobia	0,00	0,00	0,01	0,08	0,771	0,589	0,093	0,461
Armatimonadetes	0,05	0,01	0,01	0,00	0,084	0,020	0,662	0,262
Candidatus_Saccharibacteria	0,01	0,01	0,01	0,01	0,607	0,914	0,519	1,000

Annex VII: Full list at Class level of bacterial communities in integrated pest management vineyard with their frequencies and significances

		Frequencies				**Significancies**			
Phylum	**Class**	**BS1**	**BS2**	**RHIZ1**	**RHIZ2**	**BS1 vs BS2**	**Bs1 vs Rhiz1**	**BS2 vs Rhiz2**	**Rhiz1 vs Rhiz2**
Actinobacteria	Actinobacteria	95,8	95,8	95,6	96,8	0,190	0,548	0,286	0,548
Actinobacteria	unidentified_Actinobacteria	4,1	4,2	4,3	3,1	0,730	0,675	0,190	0,094
Proteobacteria	α-proteobacteria	41,5	38,0	59,1	50,8	0,905	0,095	0,730	0,032
Proteobacteria	β-proteobacteria	32,8	35,6	17,0	27,7	0,730	0,095	0,730	0,056
Proteobacteria	δ-proteobacteria	14,8	14,8	10,7	10,2	0,413	0,151	0,730	0,548
Proteobacteria	γ-proteobacteria	7,2	8,6	10,0	9,3	0,556	0,032	0,286	0,841
Proteobacteria	unclassified Proteobacteria	3,7	3,0	3,2	1,9	1,000	0,036	0,712	0,548
Gemmatimonadetes	Gemmatimonadetes	35,23	33,02	14,71	17,03	0,905	0,032	0,556	0,548
Bacteroidetes	Sphingobacteriia	72,36	68,38	56,87	78,43	0,730	0,151	0,905	0,841
Bacteroidetes	Flavobacteriia	18,69	22,92	12,52	5,87	1,000	0,675	0,901	0,463
Bacteroidetes	Bacteroidetes_incertae_sedis	6,17	6,10	23,81	9,41	0,902	0,032	0,268	0,056
Bacteroidetes	Cytophagia	2,78	1,08	3,40	3,08	0,171	0,914	0,711	0,690
Bacteroidetes	unclassified	0,00	1,53	3,40	3,20	0,787	0,071	0,383	0,421
Chloroflexi	Chloroflexia	33,7	32,8	50,0	31,0	0,905	0,020	0,383	0,745
Chloroflexi	unidentified_Chloroflexi	23,3	45,0	0,0	51,8	0,171	0,024	0,268	0,434
Chloroflexi	Ktedonobacteria	43,0	22,2	0,0	17,3	0,607	0,072	0,898	0,072
Nitrospirae	Nitrospira	27,29	6,44	22,26	44,01	0,018	0,456	0,016	0,209
Firmicutes	Bacilli	75,00	69,22	88,55	89,97	0,219	0,151	0,730	0,690
Firmicutes	Clostridia	25,00	25,68	11,45	10,03	0,803	0,753	1,000	0,753
Firmicutes	unidentified_Firmicutes	0,00	5,10	0,00	0,00	0,590	1,000	0,590	1,000

Annex VIII: List of the bacterial genera described in integrated pest management vineyard with their frequencies and significancies in all soil samples

Phylum	**Class**	**Order**	**Family**	**Genus**	**BS1**	**BS2**	**Rhiz1**	**Rhiz2**	**BS1 vs BS2**	**Bs1 vs Rhiz1**	**BS2 vs Rhiz2**	**Rhiz1 vs Rhiz2**
Actinobacteria	Actinobacteria	Gaiellales	Gaiellaceae	Gaiella	21,7	13,7	10,4	12,2	0,286	0,056	0,905	1,000
Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Arthrobacter	6,4	6,8	2,5	5,2	0,905	0,016	0,413	0,047
Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter	3,0	1,3	9,5	5,2	0,413	0,032	0,111	0,032
Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilaceae	Blastococcus	5,3	5,5	2,0	2,9	0,905	0,151	0,556	0,690
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Nocardioides	2,2	2,1	4,7	3,0	0,556	0,142	0,905	0,344
Actinobacteria	Actinobacteria	Actinomycetales	Streptomycetaceae	Streptomyces	1,9	3,0	1,4	3,5	0,413	0,151	0,905	0,028
Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	Actinoplanes	2,1	4,0	1,5	1,1	0,286	0,075	0,286	0,421
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioidaceae	Marmoricola	1,3	1,3	1,8	1,1	1,000	0,209	1,000	0,222
Actinobacteria	Actinobacteria	Acidimicrobiales	Acidimicrobiaceae	Ilumatobacter	0,4	0,2	3,1	1,7	0,171	0,059	0,286	0,016
Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacteraceae	Conexibacter	1,5	1,7	1,0	0,7	0,905	0,173	0,413	0,222
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Agromyces	0,1	0,1	3,0	1,4	0,902	0,016	0,459	0,151
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	Tetrasphaera	1,6	1,2	1,0	0,6	0,461	0,310	0,461	0,095
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Pseudonocardia	0,5	0,5	1,7	1,7	1,000	0,690	0,806	0,310
Actinobacteria	Actinobacteria	Actinomycetales	Nakamurellaceae	Nakamurella	0,3	0,7	2,0	1,1	0,902	0,059	0,413	0,249
Actinobacteria	Actinobacteria	Acidimicrobiales	Iamiaceae	Iamia	0,5	0,3	1,9	1,1	0,325	0,016	0,556	0,008
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	Amycolatopsis	1,4	1,3	0,2	0,4	0,730	0,116	0,556	0,401
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	Terrabacter	1,0	0,8	0,1	0,5	0,623	0,057	0,623	0,399
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	Intrasporangium	0,6	0,8	0,6	0,2	1,000	0,674	0,264	0,032
Proteobacteria	α-proteobacteria	Rhodospirillales	Rhodospirillaceae	Skermanella	9,87	8,76	24,04	18,93	0,905	0,421	0,623	0,421
Proteobacteria	α-proteobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	6,82	8,00	6,42	8,62	1,000	0,028	0,190	0,346
Proteobacteria	α-proteobacteria	Rhizobiales	Methylobacteriaceae	Microvirga	1,35	1,84	9,25	4,77	0,556	0,421	0,623	0,095

Proteobacteria	α-proteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	6,52	4,54	2,94	4,35	0,063	0,917	0,286	0,917
Proteobacteria	α-proteobacteria	Rhodospirillales	Rhodospirillaceae	Dongia	2,57	0,77	2,82	5,08	0,110	0,222	0,105	0,753
Proteobacteria	α-proteobacteria	Caulobacterales	Caulobacteraceae	Caulobacter	4,78	3,01	1,72	1,28	0,111	0,421	0,905	0,222
Proteobacteria	α-proteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	1,50	3,09	0,51	1,88	1,000	0,834	1,000	0,112
Proteobacteria	α-proteobacteria	Alphaproteobacteria_incertae_sedis	Geminicoccus	Geminicoccus	0,47	0,07	1,10	1,80	0,532	0,036	0,533	1,000
Proteobacteria	α-proteobacteria	Rhodospirillales	Acetobacteraceae	Roseomonas	1,56	1,52	0,58	0,69	1,000	0,690	0,709	0,399
Proteobacteria	α-proteobacteria	Rhizobiales	Rhizobiaceae	Rhizobium	0,75	2,67	0,48	0,54	0,286	0,205	0,539	0,671
Proteobacteria	β-proteobacteria	Burkholderiales	Oxalobacteraceae	Massilia		10,46	1,01	12,44	0,286	0,012	0,556	0,012
Proteobacteria	β-proteobacteria	Burkholderiales	Oxalobacteraceae	Noviherbaspirillum	8,75	7,94	1,23	2,87	0,730	0,008	0,286	0,075
Proteobacteria	β-proteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	4,13	5,81	0,50	1,21	0,901	0,521	1,000	0,458
Proteobacteria	β-proteobacteria	Burkholderiales	Comamonadaceae	Variovorax	1,22	0,74	3,74	3,62	0,533	0,057	0,110	0,462
Proteobacteria	β-proteobacteria	Burkholderiales	Comamonadaceae	Ramlibacter	2,14	2,29	0,39	0,34	0,905	0,012	0,262	0,915
Proteobacteria	β-proteobacteria	Burkholderiales	Comamonadaceae	Caenimonas	0,52	0,54	3,69	0,82	0,522	0,036	0,711	0,036
Proteobacteria	β-proteobacteria	Burkholderiales	Oxalobacteraceae	Duganella	1,81	0,11	0,39	1,91	0,064	0,012	0,211	0,172
Proteobacteria	β-proteobacteria	Nitrosomonadales	Nitrosomonadaceae	Nitrosospira	0,00	0,00	1,93	0,92	1,000	0,025	0,247	0,526
Proteobacteria	γ-proteobacteria	Xanthomonadales	Sinobacteraceae	Steroidobacter	16,1	17,0	42,4	36,6	0,902	0,016	0,111	0,600
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	Gemmatimonas	35,23	33,02	14,71	17,03	0,905	0,032	0,556	0,548
Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira	27,29	6,44	22,26	44,01	0,018	0,456	0,016	0,209
Bacteroidetes	Bacteroidetes_incertae_sedis	Ohtaekwangia	Ohtaekwangia	Ohtaekwangia	1,41	1,66	10,35	2,53	0,902	0,032	0,268	0,056
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	Flavisolibacter	4,68	4,45	3,65	1,39	0,905	0,222	0,539	0,599
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Chryseobacterium	2,02	5,41	1,10	0,86	0,901	0,463	0,901	0,917
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	Terrimonas	0,14	0,60	3,81	3,15	0,620	0,059	0,105	0,151
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	1,27	0,06	3,40	0,21	0,133	0,151	0,607	0,036
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	Niastella	0,41	0,33	1,18	1,73	1,000	0,034	0,325	0,675
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	Segetibacter	0,14	1,46	0,29	1,56	1,000	0,670	0,710	0,671

Annex IX: Full list of the bacterial species identified in the integrated pest management vineyard and their frequency in the different soil samples.

phylum	**class**	**order**	**genus**	**species**	BS1	BS2	Rhiz 1	Rhiz 2	BS1 vs Bs2	BS1 vs Rhiz1	BS2 vs Rhiz2	Rhiz1 vs Rhiz 2
Actinobacteria	Actinobacteria	Actinomycetales	unclassified	unclassified	7,75	8,87	4,61	7,73	0,905	0,095	0,325	0,690
Actinobacteria	Actinobacteria	Solirubrobacterales	unclassified	unclassified	5,36	5,42	6,07	4,22	0,712	1,000	0,286	0,056
Actinobacteria	Actinobacteria	Actinomycetales	unclassified	unclassified	5,36	7,20	3,56	5,17	0,413	0,249	0,063	0,841
Actinobacteria	Actinobacteria	unclassified	unclassified	unclassified	3,43	2,65	3,86	4,28	0,556	0,151	0,556	0,222
Proteobacteria	Betaproteobacteria	unclassified	unclassified	unclassified	2,90	4,46	2,48	2,97	0,556	1,000	0,413	0,548
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	bacterium Ellin6526	5,78	2,82	1,47	1,66	0,413	0,116	0,730	1,000
Proteobacteria	Alphaproteobacteria	Rhizobiales	unclassified	unclassified	2,08	1,99	4,18	3,12	0,806	0,222	0,325	0,548
Actinobacteria	unclassified	unclassified	unclassified	unclassified	3,03	3,17	2,49	2,40	0,730	0,675	0,190	0,094
Proteobacteria	Deltaproteobacteria	Myxococcales	unclassified	unclassified	1,68	2,23	2,14	2,65	1,000	0,209	0,730	0,421
Actinobacteria	Actinobacteria	Acidimicrobiales	unclassified	unclassified	1,07	1,80	2,48	2,64	0,556	0,032	1,000	0,463
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Gemmatimonadaceae bacterium LWQ133	2,14	3,37	1,07	0,97	0,730	0,056	0,730	0,548
Actinobacteria	Actinobacteria	Actinomycetales	unclassified	unclassified	1,73	1,97	1,92	1,63	1,000	0,016	0,730	0,600
Proteobacteria	Alphaproteobacteria	unclassified	unclassified	unclassified	0,94	1,30	1,86	2,16	0,556	0,016	0,730	0,310
Proteobacteria	Betaproteobacteria	Burkholderiales	unclassified	unclassified	1,48	1,40	1,56	1,79	0,905	0,222	1,000	0,548
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	unidentified bacterium	1,43	1,39	1,12	1,66	0,730	0,310	0,730	0,917
Actinobacteria	Actinobacteria	Actinomycetales	unclassified	unclassified	1,22	1,39	1,11	0,88	0,730	0,462	0,325	0,059

Actinobacteria	Actinobacteria	Gaiellales	Gaiella	Gaiella occulta (T)	0,61	0,32	1,73	1,79	0,461	0,421	0,413	0,548
Proteobacteria	unclassified	unclassified	unclassified	unclassified	1,10	1,07	1,29	0,95	1,000	0,036	0,712	0,548
Proteobacteria	Alphaproteobacteria	Rhizobiales	unclassified	unclassified	0,70	0,71	1,50	1,29	0,730	0,008	0,712	0,209
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Gemmatimonadetes bacterium KBS708	2,05	1,65	0,18	0,28	1,000	0,142	0,389	0,834
Actinobacteria	Actinobacteria	Actinomycetales	unclassified	unclassified	1,31	1,63	0,46	0,77	1,000	0,346	0,413	0,346
Proteobacteria	Deltaproteobacteria	Myxococcales	unclassified	unclassified	0,65	0,95	0,98	1,55	0,730	0,421	0,730	1,000
Proteobacteria	Alphaproteobacteria	Rhodospirillales	unclassified	unclassified	1,49	1,29	0,48	0,62	1,000	0,401	0,176	0,833
Proteobacteria	Betaproteobacteria	Burkholderiales	unclassified	unclassified	0,90	0,64	0,88	1,33	0,905	0,690	0,286	0,222
Actinobacteria	Actinobacteria	Actinomycetales	Blastococcus	bacterium Ellin6023	1,31	1,10	0,49	0,76	0,730	0,008	0,905	0,548
Proteobacteria	Gammaproteobacteria	unclassified	unclassified	unclassified	0,80	0,60	0,80	1,28	0,712	0,295	0,556	1,000
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	agricultural soil bacterium SC-I-92	2,00	0,92	0,18	0,29	0,413	0,548	0,806	0,917
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	unclassified	unclassified	0,69	0,81	0,93	0,92	0,539	0,094	1,000	1,000
Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacter	Solirubrobacter ginsenosidimutans	0,82	0,26	1,29	0,67	0,323	0,116	0,323	0,047
Proteobacteria	Betaproteobacteria	Burkholderiales	unclassified	unclassified	1,01	0,89	0,56	0,68	0,905	0,151	0,016	0,310
Proteobacteria	Betaproteobacteria	Burkholderiales	unclassified	unclassified	0,91	0,69	0,32	1,26	0,730	0,295	0,905	0,295
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	bacterium Ellin7530	1,73	0,92	0,12	0,24	0,413	0,095	0,623	0,346
Actinobacteria	Actinobacteria	Actinomycetales	unclassified	unclassified	0,87	1,09	0,29	0,56	1,000	0,020	0,556	0,590
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Skermanella	Skermanella aerolata	0,24	0,38	1,16	0,73	1,000	0,151	0,806	0,222
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Skermanella	Skermanella sp. JC5	0,38	0,19	1,10	0,79	0,537	0,095	0,711	0,249

Actinobacteria	Actinobacteria	Actinomycetales	Blastococcus	Blastococcus sp. OS1-29	1,06	0,99	0,16	0,21	0,905	0,295	0,268	0,671
Actinobacteria	Actinobacteria	Actinomycetales	unclassified	unclassified	0,51	0,70	0,66	0,51	0,730	0,548	0,905	0,463
Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacter	bacterium Ellin6048	0,16	0,07	1,09	0,96	0,387	0,032	0,140	0,222
Proteobacteria	Gammaproteobacteria	Xanthomonadales	unclassified	unclassified	0,23	0,75	0,63	0,74	0,111	0,047	0,556	0,690
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	bacterium Ellin7545	1,02	0,93	0,14	0,22	0,730	0,075	0,556	0,421
Proteobacteria	Alphaproteobacteria	Rhodospirillales	unclassified	unclassified	0,30	0,41	0,71	0,81	0,902	0,059	0,032	0,841
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Skermanella	Azospirillum sp. AP-500	0,19	0,23	0,95	0,70	1,000	0,249	0,806	0,249
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobium	Bradyrhizobium sp. WSM471	0,30	0,47	0,56	0,72	0,712	0,056	0,621	0,463
Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacter	Solirubrobacter sp. Gsoil 917	0,33	0,27	0,78	0,54	0,730	0,056	0,459	0,142
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Gemmatimonadetes bacterium Ellin7146	0,62	0,60	0,35	0,37	1,000	0,675	0,905	0,462
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Steroidobacter	agricultural soil bacterium SC-I-87	0,13	0,14	0,90	0,59	0,806	0,016	0,190	0,310
Actinobacteria	Actinobacteria	Actinomycetales	unclassified	unclassified	0,36	0,50	0,53	0,35	0,902	0,675	0,905	0,222
Acidobacteria	Acidobacteria_Gp1	unclassified	unclassified	unclassified	0,75	0,51	0,00	0,22	1,000	0,106	0,621	0,265
Actinobacteria	Actinobacteria	Acidimicrobiales	Iamia	bacterium Ellin5273	0,19	0,09	0,61	0,43	0,413	0,047	0,413	0,047
Actinobacteria	Actinobacteria	Acidimicrobiales	Ilumatobacter	unidentified marine bacterioplankton	0,02	0,00	0,73	0,56	0,199	0,016	0,125	0,008
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Gemmatimonadetes bacterium LX87	0,33	0,13	0,49	0,35	0,190	0,222	0,268	0,142
Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangium	Intrasporangium calvum DSM 43043	0,37	0,43	0,31	0,11	0,905	0,753	0,266	0,059
Actinobacteria	Actinobacteria	Actinomycetales	Tetrasphaera	Tetrasphaera sp. Ellin115	0,50	0,22	0,27	0,18	0,325	0,917	0,902	0,173
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Microcystis sp. SAG 43.90	0,37	0,69	0,03	0,13	1,000	0,008	0,389	0,074

Acidobacteria	Acidobacteria_Gp3	Gp3	Gp3	unidentified bacterium	0,47	0,23	0,14	0,35	0,325	0,310	1,000	0,600
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	bacterium Ellin7507	0,55	0,36	0,11	0,09	0,389	0,075	0,900	0,916
Bacteroidetes	Flavobacteriia	Flavobacteriales	Chryseobacterium	Chryseobacterium sp. CI02	0,24	0,69	0,12	0,10	0,901	0,600	0,901	0,834
Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacter	bacterium Ellin5249	0,08	0,09	0,50	0,39	0,533	0,095	0,211	0,222
Actinobacteria	Actinobacteria	Actinomycetales	Kineosporia	Kineosporia rhamnosa (T)	0,28	0,39	0,21	0,20	0,539	0,675	0,556	0,674
Actinobacteria	Actinobacteria	Actinomycetales	unclassified	unclassified	0,27	0,22	0,11	0,50	0,730	0,295	0,413	0,205
Actinobacteria	Actinobacteria	Actinomycetales	Marmoricola	Marmoricola sp. Gsoil 818	0,30	0,33	0,24	0,15	1,000	0,402	0,623	0,675
Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacter	bacterium Ellin504	0,28	0,39	0,17	0,20	0,268	0,248	0,286	0,528
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. R40S	0,03	0,01	0,27	0,77	0,453	0,116	0,133	0,548
Acidobacteria	Acidobacteria_Gp3	Gp3	Gp3	bacterium enrichment culture clone SC-2_22	0,45	0,21	0,14	0,20	0,556	0,222	1,000	1,000
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Dongia	Rhodospirillales bacterium WX36	0,03	0,00	0,43	0,53	0,502	0,142	0,073	1,000
Acidobacteria	Acidobacteria_Gp10	Gp10	Gp10	Acidobacteria bacterium WY11	0,10	0,05	0,37	0,45	0,621	0,012	0,190	0,421
Proteobacteria	Alphaproteobacteria	Rhodospirillales	unclassified	unclassified	0,14	0,12	0,39	0,29	0,905	0,095	0,905	0,016
Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacter	Rubrobacterineae bacterium BR7-21	0,46	0,33	0,07	0,11	0,905	0,010	0,539	0,239
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Skermanella	Azospirillum sp. enrichment culture clone VanCtr101	0,13	0,05	0,47	0,26	0,806	0,548	0,623	0,151
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	bacterium Ellin5290	0,35	0,26	0,14	0,21	0,806	0,527	1,000	0,916
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	actinobacterium WWH12	0,05	0,02	0,52	0,32	0,137	0,209	0,262	0,421
Proteobacteria	Deltaproteobacteria	unclassified	unclassified	unclassified	0,24	0,07	0,27	0,35	0,806	0,463	0,461	0,841
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Skermanella	Azospirillum sp. LH-CAB12	0,05	0,01	0,63	0,18	0,898	0,142	0,533	0,056

Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter globiformis	0,15	0,14	0,25	0,41	0,902	0,458	0,461	0,173
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	bacterium enrichment culture clone auto9_4W	0,40	0,35	0,07	0,12	0,806	0,046	0,730	0,462
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. S88	0,21	0,73	0,00	0,00	0,533	0,158	0,201	0,519
Proteobacteria	Deltaproteobacteria	Myxococcales	unclassified	unclassified	0,16	0,42	0,16	0,15	0,082	0,599	0,268	0,600
Proteobacteria	Alphaproteobacteria	Rhizobiales	Microvirga	Microvirga zambiensis (T)	0,00	0,00	0,55	0,26	0,561	0,034	0,073	0,032
Actinobacteria	Actinobacteria	Actinomycetales	Nakamurella	Humicoccus sp. L1886	0,05	0,05	0,50	0,15	0,806	0,095	0,266	0,116
Actinobacteria	Actinobacteria	Actinomycetales	Blastococcus	Blastococcus sp. L1961	0,28	0,22	0,14	0,15	0,461	0,249	0,905	0,598
Proteobacteria	Alphaproteobacteria	Rhizobiales	Microvirga	alpha proteobacterium TP1	0,00	0,01	0,44	0,29	0,893	0,070	0,171	0,344
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	bacterium enrichment culture clone F44x_D7_T3_E05	0,23	0,11	0,18	0,23	0,413	1,000	0,413	0,753
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardia	Pseudonocardia sp. CNS139 PL04	0,19	0,04	0,23	0,28	0,902	1,000	0,706	0,205
Acidobacteria	Acidobacteria_Gp3	unclassified	unclassified	unclassified	0,25	0,21	0,07	0,21	0,325	0,310	1,000	0,600
Actinobacteria	Actinobacteria	Actinomycetales	Blastococcus	Blastococcus aggregatus	0,21	0,14	0,14	0,24	0,323	0,141	0,806	0,675
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Skermanella	Skermanella sp. B-121-1-2	0,02	0,05	0,42	0,18	0,802	0,021	0,621	0,036
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioides	Nocardioides sp. S23405	0,21	0,21	0,14	0,12	0,802	0,021	0,621	0,036
Acidobacteria	Acidobacteria_Gp7	Gp7	Gp7	unidentified bacterium	0,03	0,02	0,26	0,38	0,321	0,142	0,105	1,000
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardia	Pseudonocardia sp. ACT-0146	0,10	0,03	0,25	0,29	0,902	0,421	0,623	0,310
Bacteroidetes	Bacteroidetes_incertae_sedis	Ohtaekwangia	Ohtaekwangia	Sphingobacteria bacterium RYG	0,03	0,07	0,34	0,21	0,802	0,046	0,105	0,462
Actinobacteria	Actinobacteria	Actinomycetales	Agromyces	Agromyces ramosus (T)	0,00	0,01	0,35	0,28	1,000	0,044	0,105	0,346
Acidobacteria	Acidobacteria_Gp3	Gp3	Gp3	Acidobacteria bacterium LWQ4	0,21	0,13	0,16	0,15	0,387	0,834	1,000	0,243

Proteobacteria	Alphaproteobacteria	Rhodospirillales	Skermanella	Skermanella sp. VTT E-073090	0,05	0,03	0,37	0,13	0,802	0,222	0,532	0,139
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Gemmatimonas aurantiaca	0,19	0,27	0,02	0,16	0,902	0,206	1,000	0,091
Proteobacteria	Deltaproteobacteria	Myxococcales	Kofleria	Kofleria flava	0,17	0,13	0,13	0,18	0,623	0,600	1,000	1,000
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	unidentified bacterium	0,32	0,10	0,06	0,12	0,140	0,071	1,000	0,340
Proteobacteria	Alphaproteobacteria	Caulobacterales	Phenylobacterium	Phenylobacterium sp. W2.09-62	0,11	0,14	0,17	0,17	0,902	0,346	0,711	0,530
Actinobacteria	Actinobacteria	Actinomycetales	Marmoricola	Nocardioides iriomotensis	0,13	0,19	0,13	0,15	0,900	0,599	0,902	0,548
Actinobacteria	Actinobacteria	Acidimicrobiales	Ilumatobacter	unidentified bacterium	0,07	0,02	0,32	0,15	0,217	0,095	0,133	0,151
Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_incertae_sedis	Geminicoccus	alpha proteobacterium EXPOSESPACE_OU21	0,05	0,01	0,20	0,34	0,617	0,075	0,533	1,000
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. M29	0,26	0,30	0,00	0,02	1,000	0,025	0,169	0,071
Actinobacteria	Actinobacteria	Actinomycetales	Streptomyces	Streptomyces canu	0,07	0,13	0,17	0,20	1,000	0,344	0,900	0,598
Actinobacteria	Actinobacteria	Actinomycetales	Agromyces	Agromyces sp. L2361	0,02	0,01	0,39	0,10	0,797	0,012	0,163	0,172
Proteobacteria	Betaproteobacteria	Burkholderiales	Noviherbaspirillum	Comamonadaceae bacterium MSCB-9	0,21	0,28	0,02	0,06	0,905	0,070	0,539	0,136
Actinobacteria	Actinobacteria	Actinomycetales	Nakamurella	Nakamurella flavida	0,05	0,16	0,23	0,10	0,804	0,093	0,804	0,293
Proteobacteria	Alphaproteobacteria	Sphingomonadales	unclassified	unclassified	0,05	0,24	0,15	0,12	0,176	0,141	0,084	0,059
Acidobacteria	Acidobacteria_Gp1	Gp1	Gp1	bacterium Ellin7522	0,35	0,18	0,00	0,02	0,260	0,101	1,000	0,366
Proteobacteria	Alphaproteobacteria	Sphingomonadales	unclassified	unclassified	0,16	0,10	0,13	0,14	0,712	1,000	0,537	1,000
Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacter	Solirubrobacter soli (T)	0,10	0,01	0,24	0,16	0,104	0,095	0,019	0,047
Actinobacteria	Actinobacteria	Actinomycetales	Virgisporangium	Virgisporangium ochraceum	0,02	0,01	0,34	0,13	0,797	0,141	0,453	0,059
Actinobacteria	Actinobacteria	Acidimicrobiales	Ilumatobacter	Ilumatobacter fluminis (T)	0,02	0,00	0,30	0,18	0,348	0,402	0,443	0,095

Actinobacteria	Actinobacteria	Actinomycetales	Catelliglobosispora	Catelliglobosispora koreensis (T)	0,05	0,00	0,30	0,14	0,500	0,092	0,348	0,093
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	Actinomycetales	0,16	0,09	0,11	0,15	0,413	1,000	0,711	0,673
Actinobacteria	Actinobacteria	Actinomycetales	Streptomyces	Streptomyces viridochromogenes subsp. komabensis	0,18	0,28	0,00	0,04	0,901	0,045	0,901	0,057
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderia	Burkholderia sp. MS4t	0,16	0,27	0,01	0,04	1,000	0,387	1,000	0,588
Actinobacteria	Actinobacteria	Actinomycetales	Marmoricola	Nocardioides sp. MTD22	0,05	0,04	0,20	0,15	0,711	0,310	0,387	0,310
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Roseomonas	Acetobacteraceae bacterium GIMN 1.017	0,13	0,14	0,08	0,10	0,806	1,000	0,533	0,674
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioides	Nocardioides islandensis (T)	0,13	0,10	0,14	0,06	0,806	0,600	1,000	0,310
Proteobacteria	Betaproteobacteria	Burkholderiales	Noviherbaspirillum	Oxalobacter sp. W1.09-142	0,22	0,12	0,04	0,05	0,533	0,521	0,623	0,829
Proteobacteria	Betaproteobacteria	Burkholderiales	Noviherbaspirillum	Herbaspirillum sp. Sco-D20	0,21	0,18	0,00	0,05	0,457	0,009	0,169	0,158
Proteobacteria	Alphaproteobacteria	Rhizobiales	Microvirga	Microvirga sp. SV2184P	0,00	0,06	0,25	0,09	0,893	0,671	0,901	0,526
Actinobacteria	Actinobacteria	Actinomycetales	Actinoplanes	Actinoplanes sp. RI44-Va104	0,11	0,32	0,00	0,00	0,701	0,072	0,467	0,424
Actinobacteria	Actinobacteria	Actinomycetales	Agromyces	glacial ice bacterium G500K-1	0,00	0,00	0,27	0,12	0,240	0,020	0,044	0,056
Actinobacteria	Actinobacteria	Acidimicrobiales	Iamia	Iamia majanohamensis (T)	0,10	0,04	0,16	0,10	0,381	0,093	0,901	0,074
Actinobacteria	Actinobacteria	Actinomycetales	Aeromicrobium	Aeromicrobium sp. ZS218-2	0,00	0,00	0,30	0,07	1,000	0,031	0,125	0,116
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacterium	Methylobacterium sp. MG-2011-64-DP	0,05	0,25	0,00	0,13	0,701	0,072	1,000	0,007
Acidobacteria	Acidobacteria_Gp1	Candidatus_Koribacter	Candidatus_Koribacter	Candidatus Koribacter versatilis Ellin345	0,16	0,21	0,01	0,02	0,901	0,243	1,000	1,000
Proteobacteria	Deltaproteobacteria	Myxococcales	Kofleria	Kofleria sp. DSM 53797	0,10	0,06	0,12	0,10	0,157	0,387	0,459	0,248
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioides	Nocardioides sp. MSL 18	0,02	0,01	0,20	0,15	0,607	0,293	0,262	0,530
Actinobacteria	Actinobacteria	Actinomycetales	Actinoplanes	Micromonosporaceae bacterium 231729	0,05	0,23	0,08	0,02	0,268	0,916	0,171	0,242

Actinobacteria	Actinobacteria	Actinomycetales	Marmoricola	Kribbella sp. enrichment culture clone VanCtr42	0,03	0,04	0,16	0,15	0,530	0,047	0,385	0,917
Actinobacteria	Actinobacteria	Actinomycetales	Nakamurella	Nakamurella sp. 1153-i1wt	0,00	0,04	0,20	0,13	0,602	0,071	0,381	0,462
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Gemmatimonadetes bacterium LP81	0,05	0,07	0,09	0,16	1,000	0,222	0,176	0,599
Actinobacteria	Actinobacteria	Actinomycetales	Actinophytocola	Actinophytocola oryzae (T)	0,00	0,00	0,05	0,35	1,000	0,022	0,125	0,451
Actinobacteria	Actinobacteria	Acidimicrobiales	Ilumatobacter	Iamia sp. T2-YC6790	0,12	0,05	0,11	0,07	0,323	0,458	0,804	0,020
Proteobacteria	Deltaproteobacteria	Myxococcales	Byssovorax	Byssovorax cruenta (T)	0,08	0,05	0,13	0,10	0,532	0,206	0,459	0,203
Chloroflexi	unclassified	unclassified	unclassified	unclassified	0,10	0,21	0,00	0,05	0,171	0,024	0,268	0,434
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Steroidobacter	bacterium D29	0,02	0,02	0,14	0,18	1,000	0,343	0,260	1,000
Actinobacteria	Actinobacteria	Actinomycetales	Tetrasphaera	Tetrasphaera japonica (T)	0,20	0,11	0,03	0,00	0,623	0,140	0,140	0,130
Proteobacteria	Alphaproteobacteria	Caulobacterales	unclassified	unclassified	0,10	0,10	0,08	0,06	0,711	0,399	0,453	0,334
Proteobacteria	Deltaproteobacteria	Myxococcales	Sorangium	Sorangium cellulosum	0,03	0,03	0,16	0,11	0,797	0,012	0,077	0,011
Acidobacteria	Acidobacteria_Gp3	Gp3	Gp3	Acidobacteria bacterium SCGC AAA204-D14	0,18	0,16	0,00	0,00	1,000	0,264	0,893	0,723
Actinobacteria	Actinobacteria	Actinomycetales	Amycolatopsis	Amycolatopsis mediterranei S699	0,17	0,17	0,00	0,00	0,902	0,106	0,247	1,000
Bacteroidetes	unclassified	unclassified	unclassified	unclassified	0,00	0,05	0,16	0,12	0,787	0,071	0,383	0,421
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Segetibacter	Segetibacter aerophilus	0,02	0,10	0,03	0,22	0,797	0,831	0,453	0,525
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Gemmatimonadetes bacterium enrichment culture clone phylotype P10	0,03	0,00	0,10	0,21	0,306	0,753	0,201	0,753
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacter	Caulobacter vibrioides	0,06	0,07	0,13	0,06	1,000	0,015	1,000	0,021
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Steroidobacter	Steroidobacter sp. WWH78	0,00	0,02	0,17	0,13	0,893	0,033	0,133	0,093
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobium	Bradyrhizobium valentinum	0,00	0,00	0,20	0,10	0,240	0,057	0,044	0,528

Actinobacteria	Actinobacteria	Actinomycetales	Nocardioides	actinobacterium GWS-BW-H259	0,02	0,00	0,17	0,12	0,687	0,401	0,687	0,171
Bacteroidetes	Bacteroidetes_incertae_sedis	Ohtaekwangia	Ohtaekwangia	unidentified bacterium	0,03	0,04	0,17	0,05	0,617	0,046	0,459	0,036
Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacter	Conexibacter sp. L1948	0,06	0,03	0,17	0,03	0,157	0,206	1,000	0,046
Acidobacteria	Acidobacteria_Gp3	Gp3	Gp3	Actinomycetales	0,13	0,07	0,00	0,12	0,617	0,160	0,617	0,160
Acidobacteria	Acidobacteria_Gp3	Gp3	Gp3	uncultivated soil bacterium clone C002	0,05	0,06	0,16	0,02	1,000	0,142	0,617	0,141
Proteobacteria	Alphaproteobacteria	Rhizobiales	Pseudolabrys	methanogenic prokaryote enrichment culture B19_111	0,12	0,06	0,06	0,06	0,286	0,401	1,000	0,396
Actinobacteria	Actinobacteria	Actinomycetales	Nakamurella	Nakamurella multipartita DSM 44233	0,02	0,00	0,13	0,15	0,245	0,015	0,073	0,753
Chloroflexi	Ktedonobacteria	Ktedonobacterales	unclassified	unclassified	0,19	0,10	0,00	0,00	0,701	0,072	0,687	0,180
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioides	Nocardioides sp. 02SU6	0,12	0,05	0,07	0,05	0,108	0,746	1,000	0,193
Actinobacteria	Actinobacteria	Acidimicrobiales	Aciditerrimonas	Aciditerrimonas ferrireducens (T)	0,08	0,05	0,05	0,12	0,459	0,914	0,266	0,665
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Skermanella	Azospirillum sp. I54	0,03	0,00	0,07	0,20	0,348	0,599	0,073	0,548
Firmicutes	Bacilli	Bacillales	unclassified	unclassified	0,05	0,08	0,07	0,10	0,805	0,338	0,902	1,000
Actinobacteria	Actinobacteria	Actinomycetales	Pimelobacter	Nocardioides aromaticivorans	0,10	0,05	0,10	0,02	0,321	1,000	0,900	0,287
Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetospora	bacterium Ellin5115	0,00	0,00	0,18	0,09	1,000	0,091	0,441	0,142
Actinobacteria	Actinobacteria	Actinomycetales	Marmoricola	Marmoricola bigeumensis (T)	0,07	0,08	0,08	0,05	1,000	0,525	0,701	0,092
Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacter	Solirubrobacter sp. L1977	0,02	0,02	0,13	0,12	0,418	0,074	0,526	0,462
Actinobacteria	Actinobacteria	Gaiellales	Gaiella	grassland soil clone saf2_202	0,02	0,00	0,17	0,08	0,348	0,094	0,125	0,249
Actinobacteria	Actinobacteria	Actinomycetales	Terrabacter	Terrabacter lapilli (T)	0,16	0,10	0,00	0,02	0,898	0,072	0,898	0,072
Proteobacteria	Alphaproteobacteria	Caulobacterales	Phenylobacterium	Phenylobacterium immobile	0,03	0,03	0,09	0,12	0,453	0,340	0,048	1,000

Proteobacteria	Alphaproteobacteria	Rhizobiales	unclassified	unclassified	0,00	0,07	0,14	0,06	0,131	0,025	1,000	0,242
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. L13	0,02	0,25	0,00	0,02	0,898	0,072	1,000	0,071
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobium	Bradyrhizobium sp. NBRC 101128	0,03	0,00	0,09	0,15	0,093	0,073	0,024	0,399
Actinobacteria	Actinobacteria	Actinomycetales	Virgisporangium	Virgisporangium aurantiacum (T)	0,03	0,00	0,12	0,12	0,687	0,035	0,443	0,401
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	bacterium enrichment culture clone R1492-14	0,01	0,00	0,14	0,10	0,338	0,199	0,073	0,463
Acidobacteria	Acidobacteria_Gp1	Gp1	Gp1	Acidobacteriaceae bacterium A2-1c	0,14	0,12	0,00	0,00	0,898	0,158	0,467	1,000
Actinobacteria	Actinobacteria	Actinomycetales	Amycolatopsis	Amycolatopsis sp. GDS	0,12	0,15	0,00	0,00	1,000	0,066	0,123	1,000
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Flavisolibacter	Flavisolibacter sp. Gsoil 636	0,08	0,04	0,08	0,06	0,321	1,000	1,000	0,344
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Flavisolibacter	bacterium enrichment culture clone SC-2_14	0,07	0,10	0,05	0,04	0,533	0,456	0,323	0,672
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobium	Bradyrhizobium sp. VUPME29	0,03	0,00	0,16	0,05	0,281	0,027	0,024	0,092
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Rhizobacter	Rhizobacter fulvus	0,06	0,02	0,03	0,15	0,797	0,916	0,063	0,165
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Flavisolibacter	Flavisolibacter sp. enrichment culture clone 02SUJ3	0,08	0,11	0,03	0,03	1,000	0,596	0,215	0,338
Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilus	Geodermatophilus	0,13	0,02	0,06	0,03	0,532	0,461	0,900	0,461
Actinobacteria	Actinobacteria	Actinomycetales	Agromyces	Agromyces ramosus	0,02	0,00	0,13	0,09	0,441	0,246	0,441	0,203
Actinobacteria	Actinobacteria	Solirubrobacterales	Conexibacter	bacterium Ellin5025	0,09	0,13	0,02	0,02	0,730	0,020	0,375	0,507
Nitrospirae	Nitrospira	Nitrospirales	Nitrospira	Nitrospira japonica	0,08	0,01	0,09	0,06	0,163	0,249	0,033	0,752
Actinobacteria	Actinobacteria	Actinomycetales	unclassified	unclassified	0,05	0,11	0,00	0,10	0,623	0,230	0,901	0,065
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobium	Bradyrhizobium group bacterium Ellin127	0,03	0,00	0,11	0,10	0,281	0,092	0,072	0,092
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardia	Pseudonocardiaceae	0,00	0,17	0,05	0,03	0,140	0,119	0,262	0,594

Proteobacteria	Gammaproteobacteria	Xanthomonadales	Steroidobacter	Pseudomonas sp. VT1B	0,03	0,00	0,08	0,15	0,687	0,055	0,441	0,597
Proteobacteria	Alphaproteobacteria	Rhodospirillales	Dongia	bacterium Ellin314	0,12	0,00	0,08	0,03	0,201	0,599	0,441	0,034
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. CFM 20	0,18	0,06	0,00	0,00	0,902	0,025	0,042	NA
Actinobacteria	Actinobacteria	Actinomycetales	Terrabacter	Terrabacter terrae (T)	0,14	0,09	0,00	0,00	0,709	0,158	0,042	0,424
Actinobacteria	Actinobacteria	Actinomycetales	Geodermatophilus	Geodermatophilus saharensis (T)	0,08	0,13	0,01	0,02	0,902	0,199	0,260	1,000
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. HS4-1	0,19	0,03	0,00	0,00	0,522	0,072	0,467	0,424
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rudaea	unidentified bacterium	0,15	0,07	0,00	0,00	0,901	0,072	0,609	0,180
Proteobacteria	Alphaproteobacteria	Caulobacterales	Phenylobacterium	Phenylobacterium sp. P-28	0,05	0,03	0,06	0,08	0,610	0,831	0,321	0,831
Actinobacteria	Actinobacteria	Solirubrobacterales	Solirubrobacter	bacterium Ellin404	0,04	0,00	0,11	0,05	0,156	0,036	0,116	0,056
Proteobacteria	Alphaproteobacteria	Rhizobiales	Pedomicrobium	Pedomicrobium manganicum (T)	0,00	0,00	0,07	0,17	0,240	0,014	0,107	0,673
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonas	Gemmatimonas sp. enrichment culture clone AOCRB-EC-6	0,03	0,08	0,05	0,06	0,900	0,916	1,000	0,828
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Terrimonas	unidentified bacterium	0,02	0,01	0,10	0,08	0,699	0,045	0,133	0,243
Actinobacteria	Actinobacteria	Actinomycetales	Nocardioides	bacterium Ellin6014	0,10	0,08	0,03	0,00	0,900	0,287	0,787	0,655
Bacteroidetes	Flavobacteriia	Flavobacteriales	unclassified	unclassified	0,03	0,08	0,07	0,04	0,700	0,338	1,000	0,671
Actinobacteria	Actinobacteria	Actinomycetales	Pimelobacter	Nocardioides sp. 2145C	0,05	0,04	0,08	0,05	0,900	0,160	0,797	0,246
Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobium	bacterium Ellin6538	0,03	0,12	0,03	0,03	0,701	0,830	0,900	1,000
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	soil bacterium 14V-08	0,17	0,04	0,00	0,00	0,709	0,025	0,500	0,177
Actinobacteria	Actinobacteria	Actinomycetales	Actinoplanes	Actinoplanes cyaneus (T)	0,03	0,16	0,02	0,00	0,264	0,395	0,123	0,822
Proteobacteria	Betaproteobacteria	Rhodocyclales	unclassified	unclassified	0,06	0,00	0,07	0,08	0,109	0,523	0,044	1,000

Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. JCM 1339	0,10	0,11	0,00	0,00	1,000	0,072	0,081	0,424
Actinobacteria	Actinobacteria	Actinomycetales	Arthrobacter	Arthrobacter sp. EP_L_35	0,15	0,05	0,00	0,00	0,537	0,067	0,606	0,724
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Flavisolibacter	bacterium enrichment culture clone SC-2_38	0,07	0,04	0,04	0,06	0,899	0,459	1,000	0,452
Chloroflexi	Chloroflexia	Chloroflexales	unclassified	unclassified	0,10	0,11	0,00	0,00	0,902	0,025	0,247	0,180
Proteobacteria	Alphaproteobacteria	Rhizobiales	Balneimonas	Microvirga flocculans (T)	0,02	0,01	0,16	0,00	0,607	0,115	0,893	0,057
Actinobacteri	Actinobacteria	Actinomycetales	Cryptosporangium	Cryptosporangium minutisporangium (T)	0.08	0.00	0.08	0.03	0.443	0.833	0.439	0.165
Actinobacetria	Actinobacteria	Actinomycetales	Terrabacter	Terrabacter sp. A2-62	0.10	0.11	0.00	0.00	0.701	0.158	0.308	1.000

Annex X: Full list of identified protein and relative genus in bulk soil in a vineyard subjected to integrated pest management. In green line were reported the protein commonly expressed in the two soil samples by the same genus, while in yellow line were reported commonly genus that expressed different proteins.

Protein accession number	**Protein description**	**Blast results**	**Reference organism**	**Genus**	**Phylum**	**protein score**	**protein mass**	**protein pi**
gi|493431452	ABC transporter		Achromobacter xylosoxidans	Achromobacter	Proteobacteria	73	129049	8,3
gi|310763793	TonB-dependent siderophore receptor family protein 23		Achromobacter xylosoxidans A8	Achromobacter	Proteobacteria	65	89981	6,73
gi|522203346	hypothetical protein	desaturase	Acidobacteriaceae bacterium KBS 83	Acidobacteria	Acidobacteria	69	51800	8,91
gi|947758754	MFS transporter		Acidovorax sp.	Acidovorax	Proteobacteria	47	41015	10,65
gi|394312134	cellobiose phosphorylase, partial		Acidovorax sp. CF316	Acidovorax	Proteobacteria	76	138100	5,74
gi|950156312	hypothetical protein, partial	NF	Acidovorax sp. Root217	Acidovorax	Proteobacteria	42	18385	9,58
gi|491317843	outer membrane protein omp38		Acinetobacter sp. CIP 53.82	Acinetobacter	Proteobacteria	155	37963	5,02
gi|491340570	hypothetical protein	NF	Acinetobacter sp. NIPH 298	Acinetobacter	Proteobacteria	57	59464	6,69
gi|926240301	hypothetical protein	outer membrane protein omp38	Acinetobacter sp. TTH0-4	Acinetobacter	Proteobacteria	193	37992	5,13
gi|973275263	hypothetical protein ADL15_09345	NF	Actinoplanes awajinensis subsp. mycoplanecinus	Actinoplanes	Actinobacteria	84	54968	5,68
gi|504253384	transcriptional regulator		Actinoplanes missouriensis	Actinoplanes	Actinobacteria	57	25048	5,23
gi|505431016	transcriptional regulator		Actinoplanes sp. N902-109	Actinoplanes	Actinobacteria	72	32642	5,61
gi|504503280	hypothetical protein	GGDEF-domain containing protein	Actinoplanes sp. SE50/110	Actinoplanes	Actinobacteria	88	77744	5,85
gi|488803129	hypothetical protein	porin	Afipia felis	Afipia	Proteobacteria	63	49977	8,19
gi|488805021	phosphate ABC transporter substrate-binding protein PstS		Afipia felis	Afipia	Proteobacteria	118	35802	8,7
gi|492876585	phosphate ABC transporter substrate-binding protein PstS		Afipia sp.	Afipia	Proteobacteria	201	35846	8,86
gi|639257240	membrane protein		Afipia sp. OHSU_II-C1	Afipia	Proteobacteria	84	25041	5,85
gi|333791974	hypothetical protein AGRO_3924	NF	Agrobacterium sp. ATCC 31749	Agrobacterium	Proteobacteria	38	5255	7,82
gi|946935796	isoleucine--tRNA ligase		Agromyces sp. Leaf222	Agromyces	Actinobacteria	67	121473	4,78

gi|947990172	tRNA-dihydrouridine synthase		Agromyces sp. Root81	Agromyces	Actinobacteria	58	41275	5,3
gi|504766130	protein-export membrane protein SecF		alpha proteobacterium HIMB5	alpha proteobacterium HIMB5	Proteobacteria	44	33177	6,64
gi|516068890	membrane assembly protein AsmA		alpha proteobacterium LLX12A	alpha proteobacterium LLX12A	Proteobacteria	78	65229	10,83
gi|521085880	hypothetical protein	NF	Amycolatopsis sp. ATCC 39116	Amycolatopsis	Actinobacteria	76	1892347	5,45
gi|517945848	ABC transporter permease		Anaerococcus sp. PH9	Anaerococcus	Firmicutes	59	64316	7,1
gi|517593071	hypothetical protein	NF	Arthrobacter sp. 135MFCol5.1	Arthrobacter	Actinobacteria	56	45545	5,12
gi|651503230	hypothetical protein	chromosome partitioning protein	Arthrobacter sp. 35W	Arthrobacter	Actinobacteria	49	46402	6,03
gi|769944021	ABC transporter		Arthrobacter sp. IHBB 11108	Arthrobacter	Actinobacteria	69	66625	8,76
gi|490538295	hypothetical protein	NF	Bacillus cereus group	Bacillus	Firmicutes	61	21696	9,38
gi|489307740	thiol reductant ABC exporter subunit CydD		Bacillus pumilus	Bacillus	Firmicutes	66	64756	7,85
gi|736216089	electron transfer flavoprotein		Bacillus sp.	Bacillus	Firmicutes	34	20781	7,37
gi|924342542	pyridine nucleotide-disulfide oxidoreductase		Bacillus sp. FJAT-21945	Bacillus	Firmicutes	60	19763	6,07
gi|922745140	hypothetical protein	phage tail tape measure protein	Bacillus sp. FJAT-26652	Bacillus	Firmicutes	63	280506	8,75
gi|922740140	hypothetical protein	NF	Bacillus sp. FJAT-26652	Bacillus	Firmicutes	35	17418	9,69
gi|929004779	alpha-glucan phosphorylase		Bacillus sp. FJAT-28004	Bacillus	Firmicutes	57	81059	5,77
gi|657212408	hypothetical protein	NF	Bacillus sp. m3-13	Bacillus	Firmicutes	71	60940	5,3
gi|148851746	cation diffusion facilitator family transporter		Bacillus sp. SG-1	Bacillus	Firmicutes	56	31569	6,87
gi|651571039	electron transfer flavoprotein		Bacillus sp. UNC438CL73TsuS30	Bacillus	Firmicutes	34	20742	6,77
gi|653070648	electron transfer flavoprotein		Bacillus sp. URHB0009	Bacillus	Firmicutes	34	20787	6,77
gi|544855683	penicillin amidase		Bacteriovorax sp. BSW11_IV	Bacteriovorax	Proteobacteria	64	84390	7,22
gi|544899768	ABC transporter ATP-binding protein		Bacteroidetes bacterium oral taxon 272	Bacteroidetes bacterium oral taxon 272	Bacteroidetes	58	65372	9,43
gi|425859941	chaperone protein dnaK		Bdellovibrio bacteriovorus str. Tiberius	Bdellovibrio	Proteobacteria	38	65674	5,09
gi|930051604	MSHA biogenesis protein MshE		beta proteobacterium AAP51	beta proteobacterium AAP51	Proteobacteria	56	63690	6,36
gi|996006396	transcriptional regulator		Bosea sp. PAMC 26642	Bosea	Proteobacteria	66	29052	6,19

gi|996003716	ABC transporter ATP-binding protein		Bosea sp. PAMC 26642	Bosea	Proteobacteria	62	35710	9,44
gi|1011012909	multidrug ABC transporter ATP-binding protein		Bosea sp. Root670	Bosea	Proteobacteria	75	67683	8,62
gi|1011009863	hypothetical protein	NF	Bosea sp. Root670	Bosea	Proteobacteria	57	22547	10,36
gi|973333027	pyridoxal 4-dehydrogenase		Bosea sp. WAO	Bosea	Proteobacteria	70	35436	6
gi|653555505	hypothetical protein	porin	Bradyrhizobium sp. Ai1a-2	Bradyrhizobium	Proteobacteria	87	56373	8,18
gi|639171817	polymerase		Bradyrhizobium sp. ARR65	Bradyrhizobium	Proteobacteria	89	54225	5,94
gi|983701343	cysteine desulfurase NifS		Bradyrhizobium sp. BR 10303	Bradyrhizobium	Proteobacteria	66	42114	6,94
gi|500989631	polymerase		Bradyrhizobium sp. BTAi1	Bradyrhizobium	Proteobacteria	117	52289	6,51
gi|992049334	MATE family efflux transporter		Bradyrhizobium sp. CCH5-F6	Bradyrhizobium	Proteobacteria	62	50271	9,53
gi|493663268	polymerase		Bradyrhizobium sp. ORS 285	Bradyrhizobium	Proteobacteria	117	54117	6,29
gi|493661417	hypothetical protein	decarboxylase	Bradyrhizobium sp. ORS 285	Bradyrhizobium	Proteobacteria	48	58134	6,86
gi|365288252	T-box transcription factor TBX1		Bradyrhizobium sp. ORS 375	Bradyrhizobium	Proteobacteria	38	4885	8,53
gi|496249382	polymerase		Bradyrhizobium sp. STM 3809	Bradyrhizobium	Proteobacteria	117	53144	6,41
gi|496254114	polymerase		Bradyrhizobium sp. STM 3843	Bradyrhizobium	Proteobacteria	83	53818	5,96
gi|494869824	polymerase		Bradyrhizobium sp. WSM1253	Bradyrhizobium	Proteobacteria	117	54890	5,95
gi|653526576	cysteine desulfurase NifS		Bradyrhizobium sp. WSM1743	Bradyrhizobium	Proteobacteria	73	41819	7,23
gi|1000875335	diguanylate cyclase		Burkholderia sp.	Burkholderia	Proteobacteria	73	114270	5,37
gi|1000852615	hypothetical protein	NF	Burkholderia sp.	Burkholderia	Proteobacteria	61	99005	6,11
gi|1000853879	ABC transporter ATP-binding protein		Burkholderia sp.	Burkholderia	Proteobacteria	64	71259	5,15
gi|640674108	hypothetical protein	NF	Burkholderia sp. A1	Burkholderia	Proteobacteria	56	13872	10,35
gi|740965005	glutathione ABC transporter ATP-binding protein		Burkholderia sp. ABCPW 111	Burkholderia	Proteobacteria	72	72901	8,49
gi|705472445	hypothetical protein X946_3487	NF	Burkholderia sp. ABCPW 111	Burkholderia	Proteobacteria	59	60107	11,77
gi|976473933	type VI secretion protein		Burkholderia sp. ABCPW 14	Burkholderia	Proteobacteria	62	23676	8,21
gi|976480342	glycosyl transferase		Burkholderia sp. ABCPW 14	Burkholderia	Proteobacteria	38	35510	5,99

gi|984155950	Zinc carboxypeptidase		Burkholderia sp. LMG 29314	Burkholderia	Proteobacteria	37	39425	8,5
gi|984266944	hypothetical protein AWB74_04550	NF	Burkholderia sp. LMG 29317	Burkholderia	Proteobacteria	65	22556	9,32
gi|992093337	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase		Burkholderia sp. PAMC 26561	Burkholderia	Proteobacteria	62	84274	5,9
gi|860488409	hypothetical protein	NF	Caenimonas sp. SL110	Caenimonas	Proteobacteria	82	40231	9,12
gi|818890707	HNH endonuclease		candidate division Kazan bacterium GW2011_GWC1_52_13	candidate division Kazan bacterium	Bacteria candidate phyla	35	25367	9,15
gi|506274285	SARP family transcriptional regulator		Catenulispora acidiphila	Catenulispora	Actinobacteria	58	105379	5,04
gi|494949943	peptidase C39		Caulobacter sp. AP07	Caulobacter	Proteobacteria	75	78686	7,92
gi|501244920	TonB-dependent receptor		Caulobacter sp. K31	Caulobacter	Proteobacteria	39	100966	5,27
gi|917075105	FAD-linked oxidase		Cellulomonas sp. HZM	Cellulomonas	Actinobacteria	76	49298	5,34
gi|947139704	hypothetical protein	NF	Cellulomonas sp. Root485	Cellulomonas	Actinobacteria	56	47448	5,73
gi|654578556	acyl-phosphate glycerol 3-phosphate acyltransferase		Cellulomonas sp. URHE0023	Cellulomonas	Actinobacteria	61	20791	10,05
gi|1000250602	molybdenum cofactor biosynthesis protein C		Chloroflexi bacterium OLB14	Chloroflexi bacterium	Chloroflexi	61	16989	6,06
gi|736721475	hypothetical protein	NF	Chryseobacterium sp. JM1	Chryseobacterium	Bacteroidetes	37	17449	9,67
gi|928931981	hypothetical protein	NF	Clostridiaceae bacterium mt12	Clostridiaceae bacterium	Firmicutes	34	127888	8,99
gi|496091499	thiamine ABC transporter permease		Clostridiales	Clostridiales	Firmicutes	76	67026	5,42
gi|639695004	3-phosphoglycerate dehydrogenase		Clostridiales bacterium VE202-01	Clostridiales bacterium	Firmicutes	56	42520	5,23
gi|490134689	hypothetical protein	phage tail protein	Clostridium sp. ASF356	Clostridium	Firmicutes	38	48725	4,7
gi|545419474	isoleucine--tRNA ligase		Clostridium sp. ATCC 29733	Clostridium	Firmicutes	74	118449	5,16
gi|973345504	Aldehyde-alcohol dehydrogenase		Clostridium sp. C105KSO15	Clostridium	Firmicutes	71	52861	8,09
gi|524721572	60 kDa chaperonin		Clostridium sp. CAG:307	Clostridium	Firmicutes	58	56761	4,96
gi|524463290	unknown		Clostridium sp. CAG:343	Clostridium	Firmicutes	66	28484	5,65
gi|524292528	aBC transporter ATP-binding protein		Clostridium sp. CAG:533	Clostridium	Firmicutes	75	65513	8,84
gi|755045374	DeoR family transcriptional regulator		Clostridium sp. JCC	Clostridium	Firmicutes	66	28250	6,47

gi|737305541	phenylalanine--tRNA ligase subunit beta		Clostridium sp. KNHs214	Clostridium	Firmicutes	67	89229	5,17
gi|924279196	ABC transporter		Clostridium sp. L74	Clostridium	Firmicutes	63	65198	9,29
gi|503744122	ribonuclease G and E		Clostridium sp. SY8519	Clostridium	Firmicutes	66	44972	9,01
gi|703597603	hypothetical protein NI26_03725	NF	Curtobacterium sp. MR_MD2014	Curtobacterium	Actinobacteria	58	34523	4,61
gi|504855600	glutamate synthase		Dehalobacter	Dehalobacter	Firmicutes	60	53594	9,53
gi|344330360	ABC transporter family protein		Desulfosporosinus sp. OT	Desulfosporosinus	Firmicutes	39	16487	9,43
gi|941028321	hypothetical protein	ABC transporter ATP-binding protein	Devosia sp. A16	Devosia	Proteobacteria	75	61669	9,19
gi|946955742	LacI family transcriptional regulator		Devosia sp. Root105	Devosia	Proteobacteria	56	36362	6,67
gi|947492451	translation elongation factor EF-1 subunit alpha		Duganella sp. Leaf126	Duganella	Proteobacteria	99	49258	9,14
gi|645057801	dihydroxyacetone kinase		Ensifer adhaerens	Ensifer	Proteobacteria	62	58545	5
gi|915473776	hypothetical protein	RNA-binding protein	Flavobacteriaceae bacterium HQM9	Flavobacteriaceae bacterium HQM9	Bacteroidetes	36	57966	5,16
gi|737698797	site-specific tyrosine recombinase XerD		Flavobacterium sp. 83	Flavobacterium	Bacteroidetes	56	34955	8,9
gi|495085431	ATP-dependent DNA helicase		Flavobacterium sp. CF136	Flavobacterium	Bacteroidetes	38	88729	7,63
gi|496314927	multidrug transporter AcrB		gamma proteobacterium NOR5-3	gamma proteobacterium	Proteobacteria	63	112996	4,98
gi|931416789	hypothetical protein AMS20_10310, partial	formate dehydrogenase	Gemmatimonas sp. SG8_28	Gemmatimonas	Gemmatimonadetes	42	57704	5,46
gi|931475717	DNA-directed RNA polymerase subunit beta, partial		Gemmatimonas sp. SG8_38_2	Gemmatimonas	Gemmatimonadetes	61	76307	6,13
gi|763020319	dehydrogenase		Geobacillus stearothermophilus	Geobacillus	Firmicutes	36	44555	6,04
gi|729034132	tRNA pseudouridine synthase C		Geobacter sp. OR-1	Geobacter	Proteobacteria	57	30890	9,35
gi|754581630	hypothetical protein	NF	Geobacter sp. OR-1	Geobacter	Proteobacteria	57	17621	9,61
gi|946837770	hypothetical protein	PilZ domain-containing protein	Geodermatophilus sp. Leaf369	Geodermatophilus	Actinobacteria	37	21692	9,13
gi|502591560	Glu/Leu/Phe/Val dehydrogenase		Haliangium ochraceum	Haliangium	Proteobacteria	68	40378	5,45
gi|495602037	membrane protein		Herbaspirillum sp. GW103	Herbaspirillum	Proteobacteria	88	57732	5,19
gi|386434508	hypothetical protein GWL_15740	NF	Herbaspirillum sp. GW103	Herbaspirillum	Proteobacteria	38	37146	5,94
gi|503259067	23S rRNA (guanosine(2251)-2~-O)-methyltransferase RlmB		Intrasporangium calvum	Intrasporangium	Actinobacteria	56	33582	10,07

gi|84383470	CTP synthetase		Janibacter sp. HTCC2649	Janibacter	Actinobacteria	38	62298	5,36
gi|930610919	hypothetical protein	NF	Janthinobacterium sp. CG23_2	Janthinobacterium	Proteobacteria	65	35879	9,09
gi|754831405	3-oxoacyl-ACP synthase		Kibdelosporangium sp. MJ126-NF4	Kibdelosporangium	Actinobacteria	74	33957	5,76
gi|727526101	hypothetical protein	peptidase C39	Kitasatospora sp. MBT63	Kitasatospora	Actinobacteria	74	114008	5,89
gi|516444434	tRNA threonylcarbamoyladenosine biosynthesis protein TsaE		Kocuria	Kocuria	Actinobacteria	80	18872	4,57
gi|502687446	CTP synthetase		Kribbella flavida	Kribbella	Actinobacteria	38	62917	5,46
gi|578010126	hypothetical protein KALB_5022	beta-ketoacyl synthase	Kutzneria albida DSM 43870	Kutzneria	Actinobacteria	58	74505	6,21
gi|917332071	non-ribosomal peptide synthetase		Luteibacter sp. 9135	Luteibacter	Proteobacteria	67	315054	5,47
gi|913424491	amino acid dehydrogenase		Massilia sp. NR 4-1	Massilia	Proteobacteria	66	45595	8,82
gi|947926669	2-octaprenyl-6-methoxyphenyl hydroxylase		Mesorhizobium	Mesorhizobium	Proteobacteria	93	45386	6,53
gi|503294226	2-octaprenyl-6-methoxyphenyl hydroxylase		Mesorhizobium ciceri	Mesorhizobium	Proteobacteria	93	44785	6,12
gi|563248696	hypothetical protein X726_29050, partial	NF	Mesorhizobium sp. L103C105A0	Mesorhizobium	Proteobacteria	63	10269	10,47
gi|564007455	AIPR family protein		Mesorhizobium sp. L103C105A0	Mesorhizobium	Proteobacteria	46	78895	6,51
gi|563076641	hypothetical protein X755_06985	NF	Mesorhizobium sp. LNJC405B00	Mesorhizobium	Proteobacteria	38	22028	6,75
gi|947435028	hypothetical protein	NF	Mesorhizobium sp. Root157	Mesorhizobium	Proteobacteria	62	16998	6,75
gi|947444115	sorbosone dehydrogenase		Mesorhizobium sp. Root157	Mesorhizobium	Proteobacteria	36	53116	5,79
gi|947811389	chromosome segregation protein SMC		Methylobacterium sp. Leaf104	Methylobacterium	Proteobacteria	63	121682	5,45
gi|947576436	organic solvent tolerance protein		Methylobacterium sp. Leaf456	Methylobacterium	Proteobacteria	68	95741	5,63
gi|947549306	hypothetical protein	NF	Methylobacterium sp. Leaf94	Methylobacterium	Proteobacteria	57	11931	5,1
gi|651603954	phosphoenolpyruvate carboxylase		Methylocystis sp. LW5	Methylocystis	Proteobacteria	68	101519	5,92
gi|519030662	hypothetical protein	RND transporter	Methylopila sp. 73B	Methylopila	Proteobacteria	62	51768	9,47
gi|835551887	ATP-dependent helicase		Methylotenera sp. G11	Methylotenera	Proteobacteria	59	154607	8,42
gi|518941281	cell division ATP-binding protein FtsE		Microbacterium sp. 11MF	Microbacterium	Actinobacteria	71	38146	5,25
gi|943653998	hypothetical protein	NF	Microbispora sp. GMKU363	Microbispora	Actinobacteria	69	102007	6,11

gi|765340761	DNA processing protein DprA		Micrococcus sp. MS-ASIII-49	Micrococcus	Actinobacteria	70	41077	6,52
gi|237882412	radical SAM domain-containing protein		Micromonospora sp. ATCC 39149	Micromonospora	Actinobacteria	82	71732	5,01
gi|494134540	hypothetical protein	NF	Micromonospora sp. ATCC 39149	Micromonospora	Actinobacteria	47	17936	10,25
gi|821555652	hypothetical protein	NF	Microvirga sp. JC119	Microvirga	Proteobacteria	59	31137	8,56
gi|495782669	integrase		Mucilaginibacter paludis	Mucilaginibacter	Bacteroidetes	70	51710	9,57
gi|953302910	cell division protein FtsH		Mycobacterium chelonae group	Mycobacterium	Actinobacteria	75	81398	5,85
gi|953303084	two-component system response regulator		Mycobacterium chelonae group	Mycobacterium	Actinobacteria	62	25258	5,98
gi|491283902	glycosyl transferase		Mycobacterium rhodesiae	Mycobacterium	Actinobacteria	37	72095	6,3
gi|521714005	PPE family protein		Mycobacterium sp. 012931	Mycobacterium	Actinobacteria	44	14251	5,26
gi|947899381	magnesium transporter		Mycobacterium sp. Root135	Mycobacterium	Actinobacteria	68	48098	4,82
gi|950020723	hypothetical protein	membrane protein	Mycobacterium sp. Soil538	Mycobacterium	Actinobacteria	58	49215	4,56
gi|759676893	ABC transporter		Mycobacterium sp. UNC280MFTsu5.1	Mycobacterium	Actinobacteria	70	62988	5,81
gi|931469740	hypothetical protein AMJ63_11955, partial	NF	Myxococcales bacterium SG8_38_1	Myxococcales	Proteobacteria	38	68303	7,48
gi|506227062	ABC transporter ATP-binding protein		Nakamurella multipartita	Nakamurella	Actinobacteria	74	56493	7,33
gi|817706300	lysophospholipase		Neorhizobium galegae	Neorhizobium	Proteobacteria	61	23746	6,6
gi|499830650	polymerase		Nitrobacter hamburgensis	Nitrobacter	Proteobacteria	63	52080	8,64
gi|752473708	elongation factor 1-alpha, partial		Nitrosospira sp. NpAV	Nitrosospira	Proteobacteria	63	8721	9,85
gi|926409255	hypothetical protein	NF	Nocardia sp. NRRL S-836	Nocardia	Actinobacteria	98	715274	5,24
gi|926408706	apolipoprotein N-acyltransferase		Nocardia sp. NRRL S-836	Nocardia	Actinobacteria	35	54601	9,87
gi|948267855	hypothetical protein	helix-turn-helix transcriptional regulator	Nocardioides sp. Soil805	Nocardioides	Actinobacteria	35	25397	6,17
gi|655237454	hydrolase		Nocardiopsis sp. CNT312	Nocardiopsis	Actinobacteria	71	40757	4,63
gi|427362835	WD-40 repeat-containing protein		Nostoc sp. PCC 7107	Nostoc	Cyanobacteria	38	67124	5,97
gi|975154923	ABC transporter		Novosphingobium sp. Fuku2-ISO-50	Novosphingobium	Proteobacteria	70	60624	10,02
gi|918973513	non-ribosomal peptide synthetase		Paenibacillus sp. E194	Paenibacillus	Firmicutes	96	317697	5,57

gi|754770466	alpha-galactosidase		Paenibacillus sp. FSL P4-0081	Paenibacillus	Firmicutes	64	82311	5,53
gi|754766940	spore gernimation protein		Paenibacillus sp. FSL R5-0345	Paenibacillus	Firmicutes	31	24526	5,71
gi|754797941	hypothetical protein	ABC transporter	Paenibacillus sp. G1	Paenibacillus	Firmicutes	67	136829	8,74
gi|939704073	transcriptional regulator		Paenibacillus sp. GD6	Paenibacillus	Firmicutes	42	40758	6,21
gi|497277159	PAS domain-containing sensor histidine kinase		Paenibacillus sp. HGF5	Paenibacillus	Firmicutes	40	114125	5,05
gi|733494499	hypothetical protein QW71_36385	NF	Paenibacillus sp. IHB B 3415	Paenibacillus	Firmicutes	76	10704	4,75
gi|746263136	photosystem I reaction center PsaK		Paenibacillus sp. IHB B 3415	Paenibacillus	Firmicutes	58	12969	10,9
gi|947374108	hypothetical protein	ABC transporter ATP-binding protein	Paenibacillus sp. Leaf72	Paenibacillus	Firmicutes	62	66322	6,29
gi|727082997	Lipoprotein LipO precursor		Paenibacillus sp. P1XP2	Paenibacillus	Firmicutes	77	27785	8,34
gi|917007502	ABC transporter		Paenibacillus sp. UNC217MF	Paenibacillus	Firmicutes	76	80259	8,45
gi|655100805	ABC transporter		Paenibacillus sp. UNC451MF	Paenibacillus	Firmicutes	56	80708	7,25
gi|908696628	non-ribosomal peptide synthetase		Paenibacillus sp. UNCCL52	Paenibacillus	Firmicutes	64	1582156	4,89
gi|655107421	multidrug ABC transporter ATP-binding protein		Paenibacillus sp. URHA0014	Paenibacillus	Firmicutes	68	64122	8,72
gi|655302471	NAD(P) transhydrogenase subunit alpha		Paracoccus sp. J55	Paracoccus	Proteobacteria	57	55436	5,8
gi|800954639	cobyrinic acid a,c-diamide synthase		Paracoccus sp. S4493	Paracoccus	Proteobacteria	60	46639	7,02
gi|947608170	ABC transporter ATP-binding protein		Pelomonas	Pelomonas	Proteobacteria	84	38702	6,07
gi|917008118	GntR family transcriptional regulator		Phyllobacterium sp. UNC302MFCol5.2	Phyllobacterium	Proteobacteria	65	24646	9,05
gi|739075781	helix-turn-helix transcriptional regulator		Promicromonosporaceae bacterium W15	Promicromonosporaceae bacterium W15	Actinobacteria	39	95299	5,73
gi|981367273	flavoprotein		pseudomallei group	Pseudomonas	Proteobacteria	73	48442	9,8
gi|115587027	putative ABC transport system, membrane protein		Pseudomonas aeruginosa UCBPP-PA14	Pseudomonas	Proteobacteria	56	44928	6,58
gi|771574828	hypothetical protein	NF	Pseudomonas sp. 2(2015)	Pseudomonas	Proteobacteria	104	10464	8,98
gi|696286713	MexH family multidrug efflux RND transporter periplasmic adaptor subunit		Pseudomonas sp. FH4	Pseudomonas	Proteobacteria	69	41914	7,08
gi|495206412	hypothetical protein	NF	Pseudomonas sp. GM17	Pseudomonas	Proteobacteria	73	34188	6,12
gi|495222977	N-methylproline demethylase		Pseudomonas sp. GM21	Pseudomonas	Proteobacteria	46	75527	5,87

gi|398140949	outer membrane autotransporter barrel domain-containing protein		Pseudomonas sp. GM25	Pseudomonas	Proteobacteria	38	34787	4,8
gi|495353628	HDOD domain-containing protein		Pseudomonas sp. GM80	Pseudomonas	Proteobacteria	62	57497	9,62
gi|495374519	hypothetical protein	isoleucyl-tRNA synthetase	Pseudomonas sp. GM84	Pseudomonas	Proteobacteria	67	69648	6,11
gi|495640909	hypothetical protein	NF	Pseudomonas sp. M47T1	Pseudomonas	Proteobacteria	73	34190	6,52
gi|495644437	membrane protein		Pseudomonas sp. M47T1	Pseudomonas	Proteobacteria	70	47814	5,74
gi|764705166	hypothetical protein	NF	Pseudomonas sp. MRSN12121	Pseudomonas	Proteobacteria	73	34044	6,63
gi|835644116	hypothetical protein	NF	Pseudomonas sp. RIT288	Pseudomonas	Proteobacteria	43	183288	5,74
gi|489471593	sigma-54-dependent Fis family transcriptional regulator		Pseudomonas syringae	Pseudomonas	Proteobacteria	64	64800	5,92
gi|929528107	DEAD/DEAH box helicase		Pseudomonas syringae group genomosp. 3	Pseudomonas	Proteobacteria	64	113324	5,64
gi|519305603	glycosyl transferase family protein		Pseudomonas syringae pv. actinidiae ICMP 19096	Pseudomonas	Proteobacteria	85	34069	5,9
gi|940059420	Unknown protein sequence		Pseudomonas syringae pv. coriandricola	Pseudomonas	Proteobacteria	44	7587	7,88
gi|330898218	major outer membrane lipoprotein		Pseudomonas syringae pv. japonica str. M301072	Pseudomonas	Proteobacteria	100	8839	6,23
gi|928338908	Uncharacterized protein AC506_4814		Pseudomonas syringae pv. maculicola str. M6	Pseudomonas	Proteobacteria	68	47872	5,37
gi|702549177	methylmalonate-semialdehyde dehydrogenase (acylating)		Pseudonocardia sp. P1	Pseudonocardia	Actinobacteria	37	53278	5,47
gi|746569490	type VI secretion protein		Ralstonia sp. A12	Ralstonia	Proteobacteria	72	72707	10,09
gi|946974378	methylmalonate-semialdehyde dehydrogenase (acylating)		Ramlibacter sp. Leaf400	Ramlibacter	Proteobacteria	59	54049	6,09
gi|946871710	hypothetical protein	NF	Rathayibacter sp. Leaf299	Rathayibacter	Actinobacteria	74	105428	5,22
gi|1011366580	multidrug ABC transporter ATP-binding protein		Rhizobium	Rhizobium	Proteobacteria	66	66823	8,96
gi|983323837	peptide ABC transporter ATP-binding protein		Rhizobium	Rhizobium	Proteobacteria	66	26996	9,13
gi|1011754231	thiamine ABC transporter ATP-binding protein		Rhizobium	Rhizobium	Proteobacteria	71	26404	6,4
gi|983326284	dipeptide ABC transporter ATP-binding protein		Rhizobium	Rhizobium	Proteobacteria	66	74070	8,93
gi|983320573	DNA primase		Rhizobium	Rhizobium	Proteobacteria	38	73784	6,37
gi|489636062	uroporphyrin-III C-methyltransferase		Rhizobium leguminosarum	Rhizobium	Proteobacteria	64	29488	7,88
gi|1011712608	hypothetical protein	type III secretion system protein	Rhizobium sp. Root149	Rhizobium	Proteobacteria	76	29241	9,24

gi|1011834312	excinuclease ABC subunit A		Rhizobium sp. Root651	Rhizobium	Proteobacteria	56	95517	5,87
gi|739344918	hypothetical protein	NF	Rhizobium sp. YR295	Rhizobium	Proteobacteria	38	80516	7,67
gi|739316628	Na+/H+ antiporter		Rhizobium sp. YR519	Rhizobium	Proteobacteria	56	58691	7,85
gi|489607508	methyl-accepting chemotaxis protein		Rhizobium/Agrobacterium group	Rhizobium/Agrobacterium group	Proteobacteria	34	83101	5,28
gi|653330202	malonyl-[acyl-carrier protein] O-methyltransferase BioC		Rhodanobacter sp. OR444	Rhodanobacter	Proteobacteria	39	32643	9,15
gi|443417259	ABC transporter ATP-binding protein		Rhodococcus sp. AW25M09	Rhodococcus	Actinobacteria	69	63942	5,42
gi|653335964	hypothetical protein	NF	Rhodococcus sp. JG-3	Rhodococcus	Actinobacteria	41	92681	8,35
gi|653353442	alcohol dehydrogenase		Rhodococcus sp. UNC363MFTsu5.1	Rhodococcus	Actinobacteria	68	36570	5,54
gi|501196857	3,4-dihydroxy-2-butanone-4-phosphate synthase		Sorangium cellulosum	Sorangium	Proteobacteria	71	41733	5,49
gi|522820053	arsenical pump-driving ATPase		Sorangium cellulosum	Sorangium	Proteobacteria	31	64373	6,07
gi|544764883	flagellar motor protein MotB		Sphingobacterium sp. IITKGP-BTPF85	Sphingobacterium	Bacteroidetes	64	49273	6,29
gi|739623779	conjugative relaxase		Sphingobium sp. ba1	Sphingobium	Proteobacteria	74	108597	9,7
gi|739618297	peptidase M16		Sphingobium sp. ba1	Sphingobium	Proteobacteria	40	102043	5,7
gi|948029843	TonB-dependent receptor		Sphingobium sp. Leaf26	Sphingobium	Proteobacteria	45	108109	4,82
gi|518200743	cell division ATP-binding protein FtsE		Sphingomonas	Sphingomonas	Proteobacteria	64	25473	10,33
gi|728824480	phenylalanine--tRNA ligase subunit beta		Sphingomonas sp. 35-24ZXX	Sphingomonas	Proteobacteria	65	85468	5,4
gi|734982531	hypothetical protein	TonB-dependent receptor	Sphingomonas sp. ERG5	Sphingomonas	Proteobacteria	78	77436	5,81
gi|917661174	hypothetical protein	carboxylesterase	Sphingomonas sp. ERG5	Sphingomonas	Proteobacteria	59	53292	9,85
gi|947791995	ABC transporter		Sphingomonas sp. Leaf10	Sphingomonas	Proteobacteria	32	21203	10,09
gi|983347458	polysaccharide export protein		Sphingomonas sp. Leaf242	Sphingomonas	Proteobacteria	69	40773	8,71
gi|518345696	hypothetical protein	ATP synthase subunit B	Sphingomonas sp. Mn802worker	Sphingomonas	Proteobacteria	61	22232	5,89
gi|916484134	histidine kinase		Sphingomonas sp. PAMC 26605	Sphingomonas	Proteobacteria	56	104607	5,12
gi|94425218	TonB-dependent receptor		Sphingomonas sp. SKA58	Sphingomonas	Proteobacteria	56	104939	4,84
gi|826050203	restriction endonuclease subunit R, partial		Sphingomonas sp. Y57	Sphingomonas	Proteobacteria	62	94552	5,77

gi|966519746	ABC transporter ATP-binding protein		Sphingopyxis	Sphingopyxis	Proteobacteria	69	62069	9,4
gi|502785515	XRE family transcriptional regulator		Stackebrandtia nassauensis	Stackebrandtia	Actinobacteria	56	85348	5,08
gi|757609280	photosystem II D2 protein		Staphylococcus aureus	Staphylococcus	Firmicutes	144	39524	5,33
gi|486837945	hypothetical protein	membrane protein	Staphylococcus aureus	Staphylococcus	Firmicutes	68	125784	9,94
gi|757609117	ribulose 1,5-bisphosphate carboxylase		Staphylococcus aureus	Staphylococcus	Firmicutes	86	53248	6
gi|757608681	photosystem II chlorophyll-binding protein CP47		Staphylococcus aureus	Staphylococcus	Firmicutes	68	56063	6,27
gi|488684230	non-ribosomal peptide synthetase		Stigmatella aurantiaca	Stigmatella	Proteobacteria	67	366949	6,09
gi|503142255	molecular chaperone GroEL		Stigmatella aurantiaca	Stigmatella	Proteobacteria	35	57916	5,27
gi|497345378	bifunctional uroporphyrinogen-III C-methyltransferase		Streptococcus sp. AS14	Streptococcus	Firmicutes	38	52491	5,12
gi|663157499	transcriptional regulator		Streptomyces	Streptomyces	Actinobacteria	75	31920	6,57
gi|695833160	bifunctional N-acetylglucosamine-1-phosphate uridyltransferase		Streptomyces	Streptomyces	Actinobacteria	71	47719	5,63
gi|529241238	transcriptional regulator		Streptomyces collinus	Streptomyces	Actinobacteria	81	31202	5,76
gi|505422987	hypothetical protein	NF	Streptomyces fulvissimus	Streptomyces	Actinobacteria	63	37509	5,53
gi|764444508	DNA-binding protein		Streptomyces natalensis	Streptomyces	Actinobacteria	65	87184	5,69
gi|517382342	cytochrome P450 steroid C27-monooxygenase		Streptomyces sp. ATexAB-D23	Streptomyces	Actinobacteria	35	46337	5,13
gi|639146534	apolipoprotein N-acyltransferase		Streptomyces sp. AW19M42	Streptomyces	Actinobacteria	44	57137	9,5
gi|695867756	beta-glucosidase		Streptomyces sp. CcalMP-8W	Streptomyces	Actinobacteria	66	46569	5,31
gi|971858567	hypothetical protein	NF	Streptomyces sp. CdTB01	Streptomyces	Actinobacteria	64	33443	7,08
gi|971859822	hypothetical protein	NF	Streptomyces sp. CdTB01	Streptomyces	Actinobacteria	66	55642	4,54
gi|916399182	hypothetical protein	NF	Streptomyces sp. CNY243	Streptomyces	Actinobacteria	73	119567	6,11
gi|739903198	thioredoxin reductase		Streptomyces sp. GXT6	Streptomyces	Actinobacteria	84	35415	5,54
gi|953808522	hypothetical protein	NF	Streptomyces sp. MBT76	Streptomyces	Actinobacteria	56	297268	5,57
gi|746631115	TIGR02680 family protein		Streptomyces sp. MUSC 125	Streptomyces	Actinobacteria	56	150410	5,85
gi|926336027	ABC transporter		Streptomyces sp. NRRL B-1140	Streptomyces	Actinobacteria	57	64560	7,29

gi|973384776	cytoplasmic protein		Streptomyces sp. NRRL F-5122	Streptomyces	Actinobacteria	61	44596	9,01
gi|671538676	esterase		Streptomyces sp. NRRL F-5123	Streptomyces	Actinobacteria	56	39675	10,21
gi|664207804	deacylase		Streptomyces sp. NRRL F-5135	Streptomyces	Actinobacteria	32	47855	5,6
gi|702847549	Matrixin		Streptomyces sp. NRRL F-525	Streptomyces	Actinobacteria	84	82040	5,45
gi|663179125	penicillin-binding protein 2		Streptomyces sp. NRRL WC-3626	Streptomyces	Actinobacteria	65	83017	9
gi|663185060	aminotransferase class V		Streptomyces sp. NRRL WC-3626	Streptomyces	Actinobacteria	65	42953	5,18
gi|664506320	transcriptional regulator		Streptomyces sp. NRRL WC-3725	Streptomyces	Actinobacteria	81	31705	6,96
gi|951115425	ABC transporter		Streptomyces sp. Root1310	Streptomyces	Actinobacteria	35	34094	9,48
gi|916255647	protein kinase		Streptomyces sp. TOR3209	Streptomyces	Actinobacteria	57	137376	5,91
gi|926363231	hypothetical protein	NF	Streptomyces sp. WM6372	Streptomyces	Actinobacteria	67	35392	10,06
gi|302474014	IS630 family transposase		Streptomyces viridochromogenes DSM 40736	Streptomyces	Actinobacteria	35	17965	9,74
gi|558544122	hypothetical protein N566_17870, partial	polyketide synthase	Streptomycetaceae bacterium MP113-05	Streptomyces	Actinobacteria	57	150370	5,88
gi|499174842	photosystem II D2 protein		Synechocystis	Synechocystis	Cyanobacteria	144	39467	5,35
gi|502891453	cell wall arabinan synthesis protein		Tsukamurella paurometabola	Tsukamurella	Actinobacteria	74	110340	9,49
gi|495108259	peptidase M23		Variovorax	Variovorax	Proteobacteria	38	17952	9,6
gi|503308046	non-ribosomal peptide synthetase		Variovorax paradoxus	Variovorax	Proteobacteria	75	192650	5,75
gi|495102899	universal stress protein UspA		Variovorax sp. CF313	Variovorax	Proteobacteria	56	15252	7,1
gi|797065421	hypothetical protein	NF	Williamsia sp. ARP1	Williamsia	Actinobacteria	58	34439	5,14
gi|946903272	oxidoreductase		Xanthomonas sp. Leaf131	Xanthomonas	Proteobacteria	96	45595	8,65
gi|946925574	ABC transporter		Xanthomonas sp. Leaf148	Xanthomonas	Proteobacteria	64	22840	8,65
gi|961355845	late control protein		Xanthomonas translucens	Xanthomonas	Proteobacteria	38	35945	8,96

Annex XI: Full list of identified protein and relative genus in the rhizosphere of *V.vinifera* in a vineyard subjected to integrated pest management. In green line were reported the protein commonly expressed in the two soil samples by the same genus, while in yellow line were reported commonly genus that expressed different proteins. Protein accession number in orange reported the protein involved in the regulation of nitrogen compound metabolic process, while protein accession number in blue reported protein involved in phosphorus metabolic process.

Protein accession number	**protein description**	**Blast results**	**Reference organism**	**Genus**	**Phylum**	**prot_ score**	**prot_ mass**	**prot _pi**
gi|1000237337	hypothetical protein UZ17_ACD001002116	NF	Acidobacteria bacterium OLB17	Acidobacteria	Acidobacteria	60	27889	5,43
gi|117648342	hypothetical protein Acel_0671	NF	Acidothermus cellulolyticus 11B	Acidothermus	Actinobacteria	50	11383	11,8
gi|947758754	MFS transporter		Acidovorax sp.	Acidovorax	Proteobacteria	45	41015	10,65
gi|916641990	hypothetical protein	NF	Acidovorax sp. JHL-9	Acidovorax	Proteobacteria	61	57353	5,77
gi|947502282	secretion protein HlyD		Acidovorax sp. Leaf78	Acidovorax	Proteobacteria	77	40973	9,79
gi|1011112161	hypothetical protein	Outer membrane protein	Acinetobacter sp. BMW17	Acinetobacter	Proteobacteria	224	37416	4,79
gi|491321813	class II glutamine amidotransferase		Acinetobacter sp. CIP 53.82	Acinetobacter	Proteobacteria	58	32324	5,81
gi|491317843	outer membrane protein omp38		Acinetobacter sp. CIP 53.82	Acinetobacter	Proteobacteria	184	37963	5,02
gi|359836986	yhcZ-like uncharacterized transcriptional regulatory protein		Actinoplanes sp. SE50/110	Actinoplanes	Actinobacteria	80	89119	9,47
gi|506282604	non-ribosomal peptide synthetase		Actinosynnema mirum	Actinosynnema	Actinobacteria	66	869268	5,5
gi|502426901	ABC transporter		Actinosynnema mirum	Actinosynnema	Actinobacteria	81	35370	6,34
gi|492876585	phosphate ABC transporter substrate-binding protein PstS		Afipia sp.	Afipia	Proteobacteria	112	35846	8,86
gi|488805021	phosphate ABC transporter substrate-binding protein PstS		Afipia felis	Afipia	Proteobacteria	38	35802	8,7
gi|639257240	membrane protein		Afipia sp. OHSU_II-C1	Afipia	Proteobacteria	72	25041	5,85
gi|947630558	hypothetical protein	Porin	Afipia sp. Root123D2	Afipia	Proteobacteria	75	50779	5,45
gi|503401495	regulatory protein RepA		Agrobacterium sp. H13-3	Agrobacterium	Proteobacteria	43	80109	6,34
gi|504766130	Tsukamurella		alpha proteobacterium HIMB5	alpha proteobacterium HIMB5	Proteobacteria	40	33177	6,64
gi|502994772	mannose-6-phosphate isomerase, class I		Amycolatopsis mediterranei	Amycolatopsis	Actinobacteria	65	44160	5,14

gi|521090623	MerR family transcriptional regulator		Amycolatopsis sp. ATCC 39116	Amycolatopsis	Actinobacteria	84	12872	7,79
gi|219953757	conserved hypothetical protein		Anaeromyxobacter dehalogenans 2CP-1	Anaeromyxobacter	Proteobacteria	69	40543	12,21
gi|947969229	glycosyl hydrolase family 15		Arthrobacter sp. Soil736	Arthrobacter	Actinobacteria	68	72040	5,52
gi|557821538	hypothetical protein	tetratricopeptide repeat family protein	Asticcacaulis sp. AC402	Asticcacaulis	Proteobacteria	71	102731	6,06
gi|910018990	hypothetical protein	NF	Azospirillum sp. B4	Azospirillum	Proteobacteria	63	30706	4,94
gi|757147818	NADPH:quinone reductase		Azospirillum sp. B506	Azospirillum	Proteobacteria	63	36157	6,01
gi|769892663	subtype I-C CRISPR-associated endonuclease Cas1		Bacillus sp.	Bacillus	Firmicutes	32	39399	9,55
gi|446101673	hypothetical protein	NF	Bacillus cereus group	Bacillus	Firmicutes	54	14318	6,64
gi|505407291	tRNA 2-selenouridine synthase		Bacillus sp. 1NLA3E	Bacillus	Firmicutes	41	41139	6,06
gi|654963952	spore gernimation protein		Bacillus sp. 278922_107	Bacillus	Firmicutes	31	42270	9,38
gi|515721482	carbamate kinase		Bacillus sp. FJAT-13831	Bacillus	Firmicutes	32	34868	5,08
gi|654948184	spore gernimation protein GerC		Bacillus sp. FJAT-14578	Bacillus	Firmicutes	61	44710	5,69
gi|924342542	pyridine nucleotide-disulfide oxidoreductase		Bacillus sp. FJAT-21945	Bacillus	Firmicutes	55	19763	6,07
gi|951401321	hypothetical protein	membrane protein	Bacillus sp. FJAT-25496	Bacillus	Firmicutes	31	62629	5,16
gi|951390900	pyridine nucleotide-disulfide oxidoreductase		Bacillus sp. FJAT-25547	Bacillus	Firmicutes	55	19728	5,54
gi|921233208	hypothetical protein	thymidylate synthase	Bacillus sp. FJAT-27997	Bacillus	Firmicutes	46	11177	5,19
gi|929002849	amidase		Bacillus sp. FJAT-28004	Bacillus	Firmicutes	63	51525	4,94
gi|938927747	hypothetical protein	Integrase	Bacillus sp. JCM 19041	Bacillus	Firmicutes	39	40965	9,51
gi|857575121	respiratory nitrate reductase alpha chain		Bacillus sp. LF1	Bacillus	Firmicutes	33	139742	6,45
gi|651591216	hypothetical protein	NF	Bacillus sp. NSP9.1	Bacillus	Firmicutes	39	17457	6,59
gi|751606029	hydrolase Cof		Bacillus sp. OxB-1	Bacillus	Firmicutes	66	29910	4,75
gi|806495947	multidrug ABC transporter ATP-binding protein		Bacillus sp. TH008	Bacillus	Firmicutes	42	26762	6,23
gi|651536048	transcriptional regulator		Bacillus sp. UNC41MFS5	Bacillus	Firmicutes	35	16194	7,88
gi|916981883	hypothetical protein	NF	Bacillus sp. UNC437CL72CviS29	Bacillus	Firmicutes	36	18972	4,64

gi|516366654	hypothetical protein	ABC transporter	Bacillus sp. ZYK	Bacillus	Firmicutes	77	71452	6,54
gi|296326991	germination protein, Ger(x)C family protein		Bacillus thuringiensis BMB171	Bacillus	Firmicutes	54	44527	8,95
gi|228810026	Decarboxylase		Bacillus thuringiensis serovar monterrey BGSC 4AJ1	Bacillus	Firmicutes	43	71008	5,1
gi|1000278704	cytochrome c class I		Bacteroidetes bacterium OLB9	Bacteroidetes	Bacteroidetes	34	52034	7,24
gi|948050713	double-strand break repair protein AddB		Bosea sp. Leaf344	Bosea	Proteobacteria	62	110639	6,04
gi|996003717	hypothetical protein AXW83_13100	ABC transporter substrate-binding protein	Bosea sp. PAMC 26642	Bosea	Proteobacteria	75	58150	8,71
gi|504308464	hypothetical protein	NF	Bradyrhizobium japonicum	Bradyrhizobium	Proteobacteria	92	40331	11,06
gi|653555505	hypothetical protein	Porin	Bradyrhizobium sp. Ai1a-2	Bradyrhizobium	Proteobacteria	79	56373	8,18
gi|500991217	polymerase		Bradyrhizobium sp. BTAi1	Bradyrhizobium	Proteobacteria	81	54386	8,49
gi|992051729	peptidase		Bradyrhizobium sp. CCH5-F6	Bradyrhizobium	Proteobacteria	75	38030	7,96
gi|500951593	transcriptional regulator		Bradyrhizobium sp. ORS 278	Bradyrhizobium	Proteobacteria	88	17848	10,34
gi|493661417	hypothetical protein	decarboxylase	Bradyrhizobium sp. ORS 285	Bradyrhizobium	Proteobacteria	44	58134	6,86
gi|493662131	Organic solvent tolerance protein OstA (ImpA)		Bradyrhizobium sp. ORS 285	Bradyrhizobium	Proteobacteria	76	91953	7,94
gi|493664379	transcriptional regulator		Bradyrhizobium sp. ORS 285	Bradyrhizobium	Proteobacteria	88	18138	10,52
gi|493661490	peptidase		Bradyrhizobium sp. ORS 285	Bradyrhizobium	Proteobacteria	35	42486	8,82
gi|496248207	5-aminolevulinate synthase		Bradyrhizobium sp. STM 3809	Bradyrhizobium	Proteobacteria	80	44529	5,89
gi|656045715	hypothetical protein	NF	Bradyrhizobium sp. th.b2	Bradyrhizobium	Proteobacteria	90	40181	10,68
gi|653530744	hypothetical protein	NF	Bradyrhizobium sp. WSM1743	Bradyrhizobium	Proteobacteria	64	59932	7,71
gi|495410734	ABC transporter ATP-binding protein		Bradyrhizobium sp. YR681	Bradyrhizobium	Proteobacteria	79	58646	7,21
gi|647376848	glucose-methanol-choline oxidoreductase		Brevibacterium sp. VCM10	Brevibacterium	Actinobacteria	60	55564	4,67
gi|328845914	short chain dehydrogenase family protein		Brevundimonas diminuta ATCC 11568	Brevundimonas	Proteobacteria	35	30322	5,41
gi|946727154	hypothetical protein	NF	Brevundimonas sp. Leaf280	Brevundimonas	Proteobacteria	61	15152	11,16
gi|947787639	alanine acetyltransferase		Brevundimonas sp. Root1279	Brevundimonas	Proteobacteria	71	21810	8,18
gi|492898323	sensor histidine kinase		Burkholderia sp.	Burkholderia	Proteobacteria	86	38464	6,06

gi|1000895238	short-chain dehydrogenase		Burkholderia sp.	Burkholderia	Proteobacteria	30	35635	10,24
gi|740962859	hypothetical protein	NF	Burkholderia sp. ABCPW 111	Burkholderia	Proteobacteria	33	135402	6,51
gi|740930983	exodeoxyribonuclease V subunit alpha		Burkholderia sp. lig30	Burkholderia	Proteobacteria	86	79534	6,25
gi|984155405	hypothetical protein AWB71_01394	acyl-CoA dehydrogenase-related protein	Burkholderia sp. LMG 29314	Burkholderia	Proteobacteria	63	36524	5,72
gi|747210628	polyphosphate kinase 2		Burkholderia sp. MR1	Burkholderia	Proteobacteria	36	32336	9,22
gi|506944064	molybdopterin oxidoreductase		Burkholderia sp. RPE64	Burkholderia	Proteobacteria	88	70905	5,96
gi|495627132	pantetheine-phosphate adenylyltransferase		Burkholderia sp.	Burkholderia	Proteobacteria	34	18921	6,3
gi|748637748	2-nitropropane dioxygenase		Burkholderiales bacterium 1_1_47	Burkholderia	Proteobacteria	35	43561	8,01
gi|374665489	hypothetical protein BurJ1DRAFT_1404	NF	Burkholderiales bacterium JOSHI_001	Burkholderia	Proteobacteria	68	150835	10,14
gi|818891013	3-deoxy-D-manno-octulosonate 8-phosphate phosphatase, YrbI family		candidate division Kazan bacterium GW2011_GWC1_52_13	candidate division Kazan bacterium GW2011_GWC1_52_13	Bacteria candidate phyla	54	43109	9,68
gi|952349132	bifunctional 5		candidate division NC10 bacterium CSP1-5	candidate division NC10 bacterium CSP1-5	Bacteria candidate phyla	74	30467	9,17
gi|931379683	hypothetical protein AMJ44_00255	DNA polymerase I	candidate division WOR_1 bacterium DG_54_3	candidate division WOR_1 bacterium DG_54_3	Bacteria candidate phyla	43	101779	8,9
gi|931375769	hypothetical protein AMJ44_08340	riboflavin biosynthesis protein RibF	candidate division WOR_1 bacterium DG_54_3	candidate division WOR_1 bacterium DG_54_3	Bacteria candidate phyla	60	33509	10,66
gi|973121479	cell division protein FtsX, partial		candidate division WS6 bacterium 34_10	candidate division WS6 bacterium 34_10	Bacteria candidate phyla	74	74001	4,73
gi|530551546	3-deoxy-manno-octulosonate-8-phosphatase		candidate division Zixibacteria bacterium RBG-1	candidate division Zixibacteria bacterium RBG-1	Bacteria candidate phyla	52	18871	7,68
gi|909617018	hypothetical protein	CoA-substrate-specific enzyme activase	Candidatus Solibacter usitatus	Candidatus Solibacter usitatus	Acidobacteria	70	111483	6,21
gi|502434787	mycothiol conjugate amidase Mca		Catenulispora acidiphila	Catenulispora	Actinobacteria	46	34976	4,83
gi|256357854	conserved hypothetical protein		Catenulispora acidiphila DSM 44928	Catenulispora	Actinobacteria	82	36584	5,86
gi|494944039	IclR family transcriptional regulator		Caulobacter sp. AP07	Caulobacter	Proteobacteria	74	29490	7,88
gi|835377200	preprotein translocase subunit SecA		Chryseobacterium sp. CF356	Chryseobacterium	Bacteroidetes	44	116255	5,7
gi|646318083	subtype I-C CRISPR-associated endonuclease Cas1		Clostridiales bacterium VE202-28	Clostridium	Firmicutes	32	39352	8,96
gi|524619873	putative uncharacterized protein		Clostridium sp. CAG:356	Clostridium	Firmicutes	65	21503	8,74
gi|524469645	predicted protein		Clostridium sp. CAG:413	Clostridium	Firmicutes	46	57387	4,99

gi|524641938	chromosome partition protein Smc		Clostridium sp. CAG:43	Clostridium	Firmicutes	83	136218	5,18
gi|524663709	uronate isomerase 2		Clostridium sp. CAG:62	Clostridium	Firmicutes	45	53854	5,1
gi|523996023	two-component sensor histidine kinase		Clostridium sp. CAG:715	Clostridium	Firmicutes	37	48467	7,11
gi|749562446	hypothetical protein	NF	Clostridium sp. CL-6	Clostridium	Firmicutes	49	14429	5,14
gi|496292442	chromosome partitioning protein ParA		Clostridium sp. D5	Clostridium	Firmicutes	49	29863	6,14
gi|759622522	two-component sensor histidine kinase		Comamonas sp. B-9	Comamonas	Proteobacteria	60	47414	6,02
gi|516499224	hypothetical protein	NF	Curtobacterium flaccumfaciens	Curtobacterium	Actinobacteria	65	16824	9,8
gi|947635400	hypothetical protein	NF	Deinococcus sp. Leaf326	Deinococcus	Deinococcus-thermus	77	339004	9,9
gi|504716818	hydantoinase		Desulfosporosinus meridiei	Desulfosporosinus	Firmicutes	62	62707	5,22
gi|737111764	molecular chaperone GroEL		Devosia sp. LC5	Devosia	Proteobacteria	87	56870	4,96
gi|944723498	hypothetical protein ASF04_26215	NF	Duganella sp. Leaf61	Duganella	Proteobacteria	70	67787	5,53
gi|645054776	molecular chaperone GroEL		Ensifer	Ensifer	Proteobacteria	32	57764	5,03
gi|950177511	hypothetical protein	NF	Ensifer sp. Root142	Ensifer	Proteobacteria	39	18293	9,6
gi|651620310	hypothetical protein	NF	Ensifer sp. TW10	Ensifer	Proteobacteria	65	119680	6,55
gi|505144868	DUF1446 domain-containing protein		Fibrella aestuarina	Fibrella	Bacteroidetes	77	46497	5,66
gi|395436879	acyl-CoA oxidase		Flavobacterium sp. F52	Flavobacterium	Bacteroidetes	69	85541	5,9
gi|917745155	carbamoyltransferase HypF		Flavobacterium sp. KMS	Flavobacterium	Bacteroidetes	38	85254	8,8
gi|947403384	50S ribosomal protein L11		Flavobacterium sp. Leaf359	Flavobacterium	Bacteroidetes	38	15350	9,64
gi|949905834	TonB-dependent receptor		Flavobacterium sp. Root901	Flavobacterium	Bacteroidetes	78	82824	5,86
gi|947391936	NDP-hexose 4-ketoreductase		Frigoribacterium sp. Leaf164	Frigoribacterium	Actinobacteria	68	91108	5,47
gi|946878616	XRE family transcriptional regulator		Frigoribacterium sp. Leaf263	Frigoribacterium	Actinobacteria	41	32104	6,19
gi|663115269	hypothetical protein	NF	Glycomyces sp. NRRL B-16210	Glycomyces	Actinobacteria	41	22173	4,77
gi|736211358	hypothetical protein	ATP-binding protein	Herbaspirillum sp. B501	Herbaspirillum	Proteobacteria	45	54319	6,26
gi|972944152	RNA-splicing ligase RtcB		Hymenobacter sp. DG5B	Hymenobacter	Bacteroidetes	49	51187	8,27

gi|808084406	RNA-splicing ligase RtcB		Hymenobacter sp. MIMtkLc17	Hymenobacter	Bacteroidetes	46	51248	7,8
gi|551361186	malic enzyme		Ideonella sp. B508-1	Ideonella	Proteobacteria	66	82548	5,91
gi|551359555	multidrug transporter		Ideonella sp. B508-1	Ideonella	Proteobacteria	35	109882	5,94
gi|503259067	23S rRNA (guanosine(2251)-2~-O)-methyltransferase RlmB		Intrasporangium calvum	Intrasporangium	Actinobacteria	56	33582	10,07
gi|737851323	hypothetical protein	NF	Janthinobacterium sp. RA13	Janthinobacterium	Proteobacteria	39	328695	4,46
gi|501035236	twitching motility protein PilT		Kineococcus radiotolerans	Kineococcus	Actinobacteria	38	41862	6,44
gi|917207570	hypothetical protein	phosphatase	Kitasatospora sp. MBT63	Kitasatospora	Actinobacteria	39	82470	5,36
gi|917306257	CHAP domain-containing protein		Kutzneria albida	Kutzneria	Actinobacteria	84	47572	5,34
gi|578007737	hypothetical protein KALB_2627	NF	Kutzneria albida DSM 43870	Kutzneria	Actinobacteria	99	49822	5,01
gi|946896370	hypothetical protein	NF	Leifsonia sp.	Leifsonia	Actinobacteria	103	36244	6,27
gi|947506795	haloacid dehalogenase		Leifsonia sp. Leaf325	Leifsonia	Actinobacteria	65	29654	4,58
gi|702088295	hypothetical protein LF41_1176	NF	Lysobacter dokdonensis DS-58	Lysobacter	Proteobacteria	32	7067	4,83
gi|691635502	chemotaxis protein		Massilia sp. JS1662	Massilia	Proteobacteria	79	82259	5,48
gi|947672025	formate dehydrogenase		Massilia sp. Leaf139	Massilia	Proteobacteria	40	83139	6,12
gi|503661011	valine--tRNA ligase		Mesorhizobium opportunistum	Mesorhizobium	Proteobacteria	35	104588	5,13
gi|563076641	hypothetical protein X755_06985	NF	Mesorhizobium sp. LNJC405B00	Mesorhizobium	Proteobacteria	37	22028	6,75
gi|685091620	Proteases secretion ATP-binding protein PrtD		Mesorhizobium sp. ORS3324	Mesorhizobium	Proteobacteria	89	61336	9,29
gi|835625928	AMP-binding protein		Methylibium sp. YR605	Methylibium	Proteobacteria	62	62617	6,3
gi|498369207	hypothetical protein	NF	Methylobacterium mesophilicum	Methylobacterium	Proteobacteria	90	53736	7,05
gi|501288264	MexE family multidrug efflux RND transporter periplasmic adaptor subunit		Methylobacterium sp. 4-46	Methylobacterium	Proteobacteria	84	44194	10,71
gi|501287217	ATPase		Methylobacterium sp. 4-46	Methylobacterium	Proteobacteria	80	60598	5,55
gi|651602114	chloride channel protein		Methylobacterium sp. 77	Methylobacterium	Proteobacteria	62	45905	9,2
gi|914805467	ATPase		Methylobacterium sp. ARG-1	Methylobacterium	Proteobacteria	79	84039	6,49
gi|959936765	hypothetical protein	NF	Methylobacterium sp. GXS13	Methylobacterium	Proteobacteria	61	35777	10,4

gi|947811185	cytochrome c		Methylobacterium sp. Leaf104	Methylobacterium	Proteobacteria	47	53410	5,81
gi|518942120	hypothetical protein	recombinase RecQ	Microbacterium sp. 11MF	Microbacterium	Actinobacteria	62	62212	5,77
gi|914779622	carboxylesterase		Microbacterium sp. GCS4	Microbacterium	Actinobacteria	66	52039	5,26
gi|947566590	hypothetical protein	NF	Microbacterium sp. Leaf203	Microbacterium	Actinobacteria	90	305932	4,86
gi|947195725	GCN5 family acetyltransferase		Microbacterium sp. Root166	Microbacterium	Actinobacteria	61	23615	9,75
gi|773084753	hypothetical protein RS85_02891	NF	Microbacterium sp. SA39	Microbacterium	Actinobacteria	61	109018	4,61
gi|738381048	hypothetical protein	NF	Microbispora sp. ATCC PTA-5024	Microbispora	Actinobacteria	32	16538	5,3
gi|495482518	hypothetical protein	TPR repeat-containing protein	Microcystis sp. T1-4	Microcystis	Cyanobacteria	78	44375	4,35
gi|494134540	hypothetical protein	NF	Micromonospora sp. ATCC 39149	Micromonospora	Actinobacteria	44	17936	10,25
gi|759608906	MerR family transcriptional regulator		Micromonospora sp. M42	Micromonospora	Actinobacteria	86	14627	9,29
gi|495782796	hypothetical protein	NF	Mucilaginibacter paludis	Mucilaginibacter	Bacteroidetes	54	35271	7,05
gi|495784907	hypothetical protein	BadF/BadG/BcrA/BcrD ATPase family protein	Mucilaginibacter paludis	Mucilaginibacter	Bacteroidetes	54	31969	9,13
gi|565996269	membrane protein		Mycobacterium sp.	Mycobacterium	Actinobacteria	61	104226	5,64
gi|489988287	D-alanine--poly(phosphoribitol) ligase subunit 1		Mycobacterium smegmatis	Mycobacterium	Actinobacteria	67	804441	4,95
gi|500045893	non-ribosomal peptide synthetase		Mycobacterium smegmatis	Mycobacterium	Actinobacteria	65	805815	4,95
gi|521714005	PPE family protein		Mycobacterium sp. 012931	Mycobacterium	Actinobacteria	45	14251	5,26
gi|947900595	cytochrome c oxidase subunit II		Mycobacterium sp. Root135	Mycobacterium	Actinobacteria	39	28173	5,99
gi|950024335	sodium:solute symporter		Mycobacterium sp. Soil538	Mycobacterium	Actinobacteria	61	52652	9,01
gi|656075527	FAD-dependent oxidoreductase		Mycobacterium sp. URHD0025	Mycobacterium	Actinobacteria	65	56730	6,5
gi|499875781	histidine kinase		Myxococcus xanthus	Myxococcus	Proteobacteria	83	95265	5,87
gi|503988718	sulfatase		Niastella koreensis	Niastella	Bacteroidetes	37	79975	9,25
gi|753207093	hopanoid biosynthesis associated RND transporter like protein HpnN		Nitrosospira sp. NpAV	Nitrosospira	Proteobacteria	62	96854	5,31
gi|738546996	8-oxoguanine deaminase		Nocardia sp. BMG111209	Nocardia	Actinobacteria	78	47138	5,5
gi|926406132	MerR family transcriptional regulator		Nocardia sp. NRRL S-836	Nocardia	Actinobacteria	97	14460	5,74

gi|948247919	sodium:proton antiporter		Nocardioides sp. Root122	Nocardioides	Actinobacteria	66	65213	5,12
gi|947942592	hypothetical protein	glycosyl transferase family 2	Nocardioides sp. Soil774	Nocardioides	Actinobacteria	75	107461	5,69
gi|837749391	2~-5~ RNA ligase		Nocardiopsis sp. RV163	Nocardiopsis	Actinobacteria	47	21458	8,42
gi|898242226	hypothetical protein	NF	Nocardiopsis sp. SBT366	Nocardiopsis	Actinobacteria	40	14488	9,46
gi|943675027	glycosyl transferase family 1		Nonomuraea sp. NBRC 110462	Nonomuraea	Actinobacteria	75	44448	10,04
gi|943865890	hypothetical protein	NF	Nonomuraea sp. NBRC 110462	Nonomuraea	Actinobacteria	74	24499	10,88
gi|953512881	hypothetical protein NOS3756_45570	3-octaprenyl-4hydroxybenzoate decarboxylase	Nostoc sp. NIES-3756	Nostoc	Cyanobacteria	80	55546	5,41
gi|499308552	glycosyl transferase family 2		Nostoc sp. PCC 7120	Nostoc	Cyanobacteria	63	40515	8,99
gi|504952341	hypothetical protein	NF	Nostoc sp. PCC 7524	Nostoc	Cyanobacteria	42	72602	8,93
gi|550926497	conjugal transfer protein TraK		Novosphingobium sp. B-7	Novosphingobium	Proteobacteria	32	27900	5,41
gi|1011446500	hypothetical protein	NF	Novosphingobium sp. CCH12-A3	Novosphingobium	Proteobacteria	77	56484	5,97
gi|1011451089	AAA family ATPase		Novosphingobium sp. CCH12-A3	Novosphingobium	Proteobacteria	78	106976	7,16
gi|922777375	non-ribosomal peptide synthetase		Paenibacillus sp. FJAT-27812	Paenibacillus	Firmicutes	62	286715	5,26
gi|947373617	hypothetical protein	NF	Paenibacillus sp. Leaf72	Paenibacillus	Firmicutes	88	35655	8,66
gi|948043581	glycoside hydrolase		Paenibacillus sp. Root52	Paenibacillus	Firmicutes	46	75638	6,15
gi|655107801	hypothetical protein	NF	Paenibacillus sp. URHA0014	Paenibacillus	Firmicutes	41	90737	5,66
gi|500777924	osmotically inducible protein C		Parvibaculum lavamentivorans	Parvibaculum	Proteobacteria	74	43312	5,98
gi|948224817	sodium:proton antiporter		Phycicoccus sp. Soil748	Phycicoccus	Actinobacteria	66	67561	5,75
gi|495147384	aldo/keto reductase		Polaromonas sp. CF318	Polaromonas	Proteobacteria	87	36128	6,8
gi|490462327	hypothetical protein	NF	Porphyromonas endodontalis	Porphyromonas	Bacteroidetes	50	52693	9,58
gi|520787521	molybdopterin-synthase adenylyltransferase MoeB		Pseudomonas sp.	Pseudomonas	Proteobacteria	46	27058	5,66
gi|523668802	hypothetical protein	glycosyl hydrolase	Pseudomonas sp.	Pseudomonas	Proteobacteria	40	34423	5,53
gi|919793257	non-ribosomal peptide synthetase, partial		Pseudomonas sp. 250J	Pseudomonas	Proteobacteria	70	331649	5,58

gi|914742919	diguanylate cyclase		Pseudomonas sp. 250J	Pseudomonas	Proteobacteria	32	139246	5,54
gi|495714489	hypothetical protein	NF	Pseudomonas sp. Ag1	Pseudomonas	Proteobacteria	96	191124	5,98
gi|520790573	hypothetical protein	NF	Pseudomonas sp. CFII64	Pseudomonas	Proteobacteria	36	91311	5,1
gi|495204880	3-methylcrotonyl-CoA carboxylase subunit alpha		Pseudomonas sp. GM17	Pseudomonas	Proteobacteria	62	69698	5,81
gi|495295107	glycosyl hydrolase		Pseudomonas sp. GM55	Pseudomonas	Proteobacteria	40	34483	5,7
gi|497298792	transcriptional regulator		Pseudomonas sp. M1	Pseudomonas	Proteobacteria	64	52659	5,42
gi|939347783	non-ribosomal peptide synthetase		Pseudomonas sp. NBRC 111143	Pseudomonas	Proteobacteria	83	566411	5,4
gi|953984713	LysR family transcriptional regulator		Pseudomonas sp. TTU2014-080ASC	Pseudomonas	Proteobacteria	38	34461	9,32
gi|953991462	NADH-quinone oxidoreductase subunit G		Pseudomonas sp. TTU2014-096BSC	Pseudomonas	Proteobacteria	39	97951	5,64
gi|653605016	chromosome segregation protein SMC		Pseudomonas sp. URHB0015	Pseudomonas	Proteobacteria	87	135624	5,48
gi|504907593	NADH:flavin oxidoreductase		Pseudomonas sp. UW4	Pseudomonas	Proteobacteria	86	44756	9,63
gi|519305603	glycosyl transferase family protein		Pseudomonas syringae pv. actinidiae ICMP 19096	Pseudomonas	Proteobacteria	196	34069	5,9
gi|948221616	citryl-CoA lyase		Pseudoxanthomonas sp. Root630	Pseudoxanthomonas	Proteobacteria	66	30618	6,55
gi|308919926	NAD dependent epimerase/dehydratase family protein		Ralstonia sp. 5_7_47FAA	Ralstonia	Proteobacteria	60	36540	11,5
gi|947808185	hypothetical protein	NF	Rhizobacter sp. Root404	Rhizobacter	Proteobacteria	61	38693	6,33
gi|489644364	PAS domain-containing sensor histidine kinase		Rhizobium leguminosarum	Rhizobium	Proteobacteria	60	112477	5,2
gi|1011473817	hypothetical protein	NF	Rhizobium sp. BR10423	Rhizobium	Proteobacteria	56	10575	5,75
gi|739269671	peptide ABC transporter ATP-binding protein		Rhizobium sp. CF097	Rhizobium	Proteobacteria	36	28225	6,32
gi|493751506	ABC transporter ATP-binding protein		Rhizobium sp. IRBG74	Rhizobium	Proteobacteria	75	62967	7,18
gi|820887492	phosphatidylinositol kinase		Rhizobium sp. LC145	Rhizobium	Proteobacteria	86	45830	5,32
gi|1011769624	ATP-binding protein		Rhizobium sp. Leaf371	Rhizobium	Proteobacteria	40	31015	4,95
gi|739344918	hypothetical protein	NF	Rhizobium sp. YR295	Rhizobium	Proteobacteria	37	80516	7,67
gi|947418896	glycoside hydrolase family 3		Rhodanobacter sp. Root480	Rhodanobacter	Proteobacteria	61	93897	6,46
gi|490042852	ATP-dependent dsDNA exonuclease SbcC		Rhodococcus erythropolis	Rhodococcus	Actinobacteria	65	105725	5,18

gi|490038412	uracil permease		Rhodococcus erythropolis	Rhodococcus	Actinobacteria	76	47183	8,44
gi|495992757	chromosome segregation protein SMC		Rhodococcus sp. AW25M09	Rhodococcus	Actinobacteria	91	132058	4,99
gi|938937820	hypothetical protein	NF	Rhodococcus sp. HA99	Rhodococcus	Actinobacteria	92	38186	9,97
gi|846871202	Heavy metal translocating P-type ATPase		Rhodococcus sp. RD6.2	Rhodococcus	Actinobacteria	84	82358	5
gi|504239320	hypothetical protein	NF	Rubrivivax gelatinosus	Rubrivivax	Proteobacteria	63	49998	5,98
gi|1001989170	hypothetical protein TH61_05675	NF	Rufibacter sp. DG15C	Rufibacter	Bacteroidetes	35	30451	5,09
gi|772725198	hypothetical protein	NF	Saccharothrix sp. ST-888	Saccharothrix	Actinobacteria	37	18344	11,44
gi|919126200	type I polyketide synthase		Saccharothrix sp. ST-888	Saccharothrix	Actinobacteria	97	287674	5,29
gi|764627440	hypothetical protein	NF	Skermanella aerolata	Skermanella	Proteobacteria	49	15829	5,13
gi|522818948	penicillin-binding protein		Sorangium cellulosum	Sorangium	Proteobacteria	93	92653	9,55
gi|501190326	AraC family transcriptional regulator		Sorangium cellulosum	Sorangium	Proteobacteria	38	33274	11,07
gi|161162045	polyketide synthase		Sorangium cellulosum So ce56	Sorangium	Proteobacteria	64	542201	6,1
gi|663817923	glycosyl transferase		Sphingobium sp. DC-2	Sphingobium	Proteobacteria	62	43556	10,03
gi|948029843	TonB-dependent receptor		Sphingobium sp. Leaf26	Sphingobium	Proteobacteria	42	108109	4,82
gi|998162096	hypothetical protein K663_14440	Carbamoyl phosphate synthase	Sphingobium sp. MI1205	Sphingobium	Proteobacteria	66	36955	5,24
gi|730270323	glycosidase		Sphingomonas sp. Ant H11	Sphingomonas	Proteobacteria	33	19336	9,49
gi|734979353	hypothetical protein	MerR family transcriptional regulator	Sphingomonas sp. ERG5	Sphingomonas	Proteobacteria	79	13047	9,73
gi|947760998	3-hydroxyacyl-CoA dehydrogenase		Sphingomonas sp. Leaf33	Sphingomonas	Proteobacteria	35	29693	5,82
gi|916359191	2-hydroxyacid dehydrogenase		Sphingomonas sp. Mn802worker	Sphingomonas	Proteobacteria	85	49339	5,66
gi|497904998	GlcNAc-PI de-N-acetylase		Sphingomonas sp. PAMC 26621	Sphingomonas	Proteobacteria	92	23822	11,5
gi|739679147	hypothetical protein	NF	Sphingomonas sp. RIT328	Sphingomonas	Proteobacteria	79	12599	8,15
gi|497506595	hypothetical protein	NF	Sphingomonas sp. SKA58	Sphingomonas	Proteobacteria	43	83235	5,54
gi|920554579	hypothetical protein	NF	Sphingomonas sp. Y57	Sphingomonas	Proteobacteria	54	42010	5,14
gi|947679918	hypothetical protein	NF	Sphingopyxis sp. Root1497	Sphingopyxis	Proteobacteria	44	35026	8,78

gi|648182768	teichoic acid biosynthesis protein B		Staphylococcus sp.	Staphylococcus	Firmicutes	59	42940	9,38
gi|600856940	fibronectin-binding protein A, partial		Staphylococcus aureus DAR1980	Staphylococcus	Firmicutes	54	50745	4,32
gi|242349787	hypothetical protein HMPREF0793_1012	NF	Staphylococcus caprae M23864:W1	Staphylococcus	Firmicutes	37	27213	9,31
gi|57636186	hypothetical protein SERP2104	NF	Staphylococcus epidermidis RP62A	Staphylococcus	Firmicutes	54	6617	5,46
gi|512477603	molecular chaperone Hsp33		Staphylococcus sp. HGB0015	Staphylococcus	Firmicutes	32	31858	4,88
gi|495543735	thiol reductant ABC exporter subunit CydD		Stenotrophomonas sp. SKA14	Stenotrophomonas	Proteobacteria	75	61736	7,18
gi|488695855	histidine kinase		Stigmatella aurantiaca	Stigmatella	Proteobacteria	39	77804	6,24
gi|446656446	macrolide ABC transporter ATP-binding protein		Streptococcus oralis	Streptococcus	Firmicutes	79	25773	6,25
gi|499342207	transcriptional regulator		Streptomyces sp.	Streptomyces	Actinobacteria	87	29201	6,01
gi|926312028	hypothetical protein	NF	Streptomyces sp.	Streptomyces	Actinobacteria	87	41758	11,56
gi|490084990	magnesium-transporting ATPase		Streptomyces sp.	Streptomyces	Actinobacteria	35	84806	5,63
gi|529246222	lysine--tRNA ligase		Streptomyces collinus	Streptomyces	Actinobacteria	62	118865	8,53
gi|529244478	two-component sensor histidine kinase		Streptomyces collinus	Streptomyces	Actinobacteria	84	56850	5,73
gi|505469470	beta-galactosidase		Streptomyces davawensis	Streptomyces	Actinobacteria	61	142331	5,96
gi|505424187	hypothetical protein	NF	Streptomyces fulvissimus	Streptomyces	Actinobacteria	81	42824	7,98
gi|748773185	hypothetical protein	NF	Streptomyces sp. 150FB	Streptomyces	Actinobacteria	81	42672	8,98
gi|518264683	dihydropteroate synthase		Streptomyces sp. AA0539	Streptomyces	Actinobacteria	62	30830	6,27
gi|754598858	glycosyl transferase		Streptomyces sp. AcH 505	Streptomyces	Actinobacteria	63	40784	9,53
gi|926433974	type I polyketide synthase		Streptomyces sp. AS58	Streptomyces	Actinobacteria	82	287939	5,31
gi|966524178	hypothetical protein APS67_05951	NF	Streptomyces sp. AVP053U2	Streptomyces	Actinobacteria	71	49407	12,5
gi|639146534	apolipoprotein N-acyltransferase		Streptomyces sp. AW19M42	Streptomyces	Actinobacteria	45	57137	9,5
gi|517382554	hypothetical protein	NADH:ubiquinone oxidoreductase	Streptomyces sp. BoleA5	Streptomyces	Actinobacteria	65	52602	8,95
gi|517387267	hypothetical protein	peptidase M4 family protein	Streptomyces sp. BoleA5	Streptomyces	Actinobacteria	62	130320	5,67
gi|822676122	hypothetical protein	helix-turn-helix transcriptional regulator	Streptomyces sp. CNQ-509	Streptomyces	Actinobacteria	87	108260	5,46

gi|654254777	hypothetical protein	NF	Streptomyces sp. CNQ329	Streptomyces	Actinobacteria	63	61070	4,94
gi|517673511	hypothetical protein	NF	Streptomyces sp. CNS335	Streptomyces	Actinobacteria	72	90839	5,93
gi|916745181	ATP-dependent RNA helicase HrpA		Streptomyces sp. CNT360	Streptomyces	Actinobacteria	50	150167	8,62
gi|517677883	hypothetical protein	NF	Streptomyces sp. CNT372	Streptomyces	Actinobacteria	75	75587	5,99
gi|654991446	biotin carboxylase		Streptomyces sp. DpondAA-B6	Streptomyces	Actinobacteria	41	48557	4,95
gi|516766239	Na+/H+ antiporter		Streptomyces sp. FxanaC1	Streptomyces	Actinobacteria	72	57248	6,04
gi|648478522	phosphoenolpyruvate synthase		Streptomyces sp. FxanaC1	Streptomyces	Actinobacteria	63	100582	6,41
gi|512659419	hypothetical protein	NF	Streptomyces sp. HPH0547	Streptomyces	Actinobacteria	79	515670	5,37
gi|926356568	hypothetical protein	NF	Streptomyces sp. IGB124	Streptomyces	Actinobacteria	63	48745	8,22
gi|920664738	hypothetical protein	NF	Streptomyces sp. KE1	Streptomyces	Actinobacteria	78	75874	6,41
gi|496014496	membrane protein		Streptomyces sp. Mg1	Streptomyces	Actinobacteria	60	78925	10,93
gi|517366042	non-ribosomal peptide synthetase		Streptomyces sp. MspMP-M5	Streptomyces	Actinobacteria	78	663893	5,9
gi|517361223	Na+/H+ antiporter		Streptomyces sp. MspMP-M5	Streptomyces	Actinobacteria	72	56712	6,06
gi|815004581	phosphotransferase enzyme family protein		Streptomyces sp. MUSC136T	Streptomyces	Actinobacteria	72	79449	10,28
gi|943915427	hypothetical protein	NF	Streptomyces sp. NBRC 110028	Streptomyces	Actinobacteria	99	2643129	5,62
gi|927900129	hypothetical protein	NF	Streptomyces sp. NRRL B-24085	Streptomyces	Actinobacteria	62	20555	5,64
gi|663322191	short-chain dehydrogenase/reductase		Streptomyces sp. NRRL B-3229	Streptomyces	Actinobacteria	39	26263	6,43
gi|917200721	carbonate dehydratase		Streptomyces sp. NRRL F-2664	Streptomyces	Actinobacteria	83	78354	9,01
gi|664329930	hypothetical protein	NF	Streptomyces sp. NRRL F-2747	Streptomyces	Actinobacteria	78	40498	7,13
gi|973290143	hypothetical protein ADL22_06325	NF	Streptomyces sp. NRRL F-4489	Streptomyces	Actinobacteria	82	42773	11,07
gi|664258558	histidine kinase		Streptomyces sp. NRRL F-5008	Streptomyces	Actinobacteria	62	60239	5,25
gi|917239715	polyketide synthase		Streptomyces sp. NRRL F-5053	Streptomyces	Actinobacteria	61	250762	5,35
gi|973384776	cytoplasmic protein		Streptomyces sp. NRRL F-5122	Streptomyces	Actinobacteria	44	44596	9,01
gi|973385084	diguanylate cyclase		Streptomyces sp. NRRL F-5122	Streptomyces	Actinobacteria	65	88692	5,33

gi|918338677	hypothetical protein	NF	Streptomyces sp. NRRL F-5123	Streptomyces	Actinobacteria	83	184136	5,49
gi|664383559	hypothetical protein	NF	Streptomyces sp. NRRL F-5126	Streptomyces	Actinobacteria	86	159580	7,44
gi|702840848	hypothetical protein	phytoene synthase	Streptomyces sp. NRRL F-525	Streptomyces	Actinobacteria	75	33621	5,94
gi|663311700	hypothetical protein	helix-turn-helix transcriptional regulator	Streptomyces sp. NRRL F-6131	Streptomyces	Actinobacteria	77	104598	7,14
gi|917163475	hypothetical protein	helix-turn-helix transcriptional regulator	Streptomyces sp. NRRL F-6131	Streptomyces	Actinobacteria	75	112170	6,23
gi|663420820	hypothetical protein	polynucleotide adenyltransferase	Streptomyces sp. NRRL S-1448	Streptomyces	Actinobacteria	62	22040	9,88
gi|664469082	hypothetical protein	NF	Streptomyces sp. NRRL S-1813	Streptomyces	Actinobacteria	75	22559	5,89
gi|664269847	insecticidal toxin complex protein		Streptomyces sp. NRRL S-337	Streptomyces	Actinobacteria	80	360602	5,35
gi|664336511	non-ribosomal peptide synthetase		Streptomyces sp. NRRL S-37	Streptomyces	Actinobacteria	69	308903	5,87
gi|664334376	serine/threonine protein kinase		Streptomyces sp. NRRL S-37	Streptomyces	Actinobacteria	62	54781	9,91
gi|917197208	maleylacetate reductase		Streptomyces sp. NRRL S-474	Streptomyces	Actinobacteria	64	35388	5,34
gi|664261348	hypothetical protein	helicase	Streptomyces sp. NRRL S-920	Streptomyces	Actinobacteria	81	130837	6,23
gi|664465992	aldo/keto reductase		Streptomyces sp. NRRL WC-3744	Streptomyces	Actinobacteria	77	35195	5,5
gi|664481143	hypothetical protein	helix-turn-helix transcriptional regulator	Streptomyces sp. NRRL WC-3773	Streptomyces	Actinobacteria	70	102657	6,07
gi|478745967	putative membrane protein		Streptomyces sp. PAMC 26508	Streptomyces	Actinobacteria	95	16567	11,14
gi|852466222	hypothetical protein QR97_33660	NF	Streptomyces sp. PBH53	Streptomyces	Actinobacteria	66	164012	6,26
gi|606221058	peptide synthetase		Streptomyces sp. PCS3-D2	Streptomyces	Actinobacteria	85	62537	5,35
gi|951116668	hypothetical protein	serine protease	Streptomyces sp. Root1310	Streptomyces	Actinobacteria	63	77770	5,84
gi|917823392	hypothetical protein	NF	Streptomyces sp. RSD-27	Streptomyces	Actinobacteria	67	66965	5,77
gi|517334627	ABC transporter ATP-binding protein		Streptomyces sp. ScaeMP-e10	Streptomyces	Actinobacteria	74	58901	7,85
gi|654982758	hypothetical protein	NF	Streptomyces sp. TAA204	Streptomyces	Actinobacteria	78	44027	9,82
gi|654987005	hypothetical protein	NF	Streptomyces sp. TAA486	Streptomyces	Actinobacteria	54	45714	5,22
gi|332745525	putative molybdopterin biosynthesis protein		Streptomyces sp. Tu6071	Streptomyces	Actinobacteria	69	56699	4,93
gi|332745404	hypothetical protein STTU_3058	NF	Streptomyces sp. Tu6071	Streptomyces	Actinobacteria	45	28044	11,92

gi|697207029	hypothetical protein	NF	Streptomyces sp. URHA0041	Streptomyces	Actinobacteria	67	13966	5,1
gi|925433393	hypothetical protein ADK55_06360	NF	Streptomyces sp. WM4235	Streptomyces	Actinobacteria	83	111990	9,51
gi|797202279	DNA primase		Streptomyces sp. WMMB 714	Streptomyces	Actinobacteria	76	69546	6,22
gi|493421600	hypothetical protein	NF	Streptomyces turgidiscabies	Streptomyces	Actinobacteria	84	168450	5,29
gi|917163278	uridylyltransferase		Streptomyces sp. NRRL F-6131	Streptomyces	Actinobacteria	80	88034	6,35
gi|328881659	Transcriptional regulator, MerR family		Streptomyces venezuelae ATCC 10712	Streptomyces	Actinobacteria	83	14475	10,61
gi|51855300	ComE-like competence protein		Symbiobacterium thermophilum IAM 14863	Symbiobacterium	Firmicutes	68	86420	10,39
gi|921079705	sodium:proton antiporter		Tetrasphaera japonica	Tetrasphaera	Actinobacteria	72	67600	6,03
gi|946887613	glycerophosphodiester phosphodiesterase		Tetrasphaera sp. Soil756	Tetrasphaera	Actinobacteria	63	65817	5,45
gi|495108259	peptidase M23		Variovorax sp.	Variovorax	Proteobacteria	37	17952	9,6
gi|564756399	membrane protein		Williamsia sp. D3	Williamsia	Actinobacteria	96	88076	5,11
gi|919134440	hypothetical protein	GGDEF domain-containing protein	Xanthomonas sp. GPE 39	Xanthomonas	Proteobacteria	63	113393	9,28
gi|946904936	two-component system response regulator		Xanthomonas sp. Leaf131	Xanthomonas	Proteobacteria	62	50630	6,48
gi|946931271	hypothetical protein	NF	Xanthomonas sp. Leaf148	Xanthomonas	Proteobacteria	41	11895	8,57
gi|941954498	hypothetical protein	GGDEF domain-containing response regulator	Xanthomonas sp. Mitacek01	Xanthomonas	Proteobacteria	77	75599	4,8
gi|961355845	late control protein		Xanthomonas translucens	Xanthomonas	Proteobacteria	37	35945	8,96

Listo of Pubblication

1. Gamalero E., Marzachì C., Galetto L., Veratti F., Massa N., Bona E., **Novello G.**, Glick B.R., Ali S., Cantamessa S., D'Agostino G. and Berta G. (2016). An 1-Aminocyclopropane-1-carboxylate (ACC) deaminase-expressing endophyte increases plant resistance to flavescence dorée phytoplasma infection. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology*, DOI: 10.1080/11263504.2016.1174172

2. Bona E., Cantamessa S., Pavan M., **Novello G.,** Massa N., Rocchetti A., Berta G. and Gamalero E. (2016). Sensitivity of Candida albicans to essential oils: are they an alternative to antifungal agents?. *Journal of Applied Microbiology,* doi:10.1111/jam.13282

3. **Novello G.,** Gamalero E., Bona E., Boatti L., Mignone F., Massa N., Cesaro P., Lingua G. and Berta G. Exploring the rhizospheric microbiome of *Vitis vinifera* cv. Pinot noir in an integrated pest management vineyard. UNDER REVIEW

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