CLIMATE CHANGE AND POSSIBLE IMPACTS ON SOIL

Unravelling complexity of soil microbial composition is crucial to preserve soil quality especially in the view of a climate change. This study presents some of the most advanced methods to study soil microbial communities.

1. Climate change and soil

In recent years concerns on climate change and in particular the increase of global temperature and the altered precipitations are inflaming the public debate among scientist, especially regarding the possible effect on the environment, animals, plants and biodiversity. Anthropogenic disturbances led to a rise in levels of atmospheric CO_2, with a consequent increase of temperatures and an effect on nutrient cycles (IPCC 2007). These effects along with altered levels of precipitations could affect the ecosystem equilibrium in the long term and in particular plant productivity.

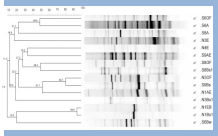
Microbial populations in soil are climate-dependent. Changes in soil microbial population could slightly influence plant growth and cause non-visible effects on agriculture system in the short-term; however they can dramatically change the soil ecosystem in the long run.

Key-features

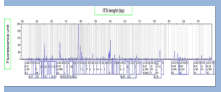
Environmental, climatic and anthropogenic factors are likely to modify the equilibrium of microbial communities in soil, with long-term consequences on the agricultural system.

As only a small part of the microbial community can be cultured on artificial media and microbial identification is time consuming, effective methods to detect, identify and analyse microbial populations are crucial.

To study the impact of temperature and the trophic relations among microorganisms we applied:

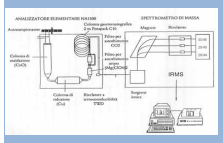


PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) – based on the electrophoretic mobility of DNA fragments; it is a semi-quantitative method used for the rapid comparison of samples.



ARISA (automated ribosomal intergenic spacer analysis) – it allows to compare communities coming from different soils, or to study the dynamics inside the same soil.

High-throughput sequencing technologies and RNA-Seq – they allow massively parallel sequencing approaches for analysing genomes at a higher resolution than previous tools.



IRMS (Isotope Ratio Mass Spectrometry) – it offers an accurate and precise measure of variations in the abundance of isotopic ratios of light elements (e.g $^{13}C/^{12}C$, $^{18}O/^{16}O$ etc.)

Soil processes involving soil microorganisms, such as mineralization, decomposition, nutrient cycling, could be affected by the abiotic changes in the climate, so altering the soil processes and influencing the organisms that take part in them. In this way climate change could influence the microbial community's structure and alter the level of interaction among microorganisms.

Drought on soil

Changes in soil temperature and precipitation levels could affect microorganisms' growth rate, their respiration and activity. Microorganisms could react differently to these changes, adapting their biology, evolving or favouring species already better adapted.

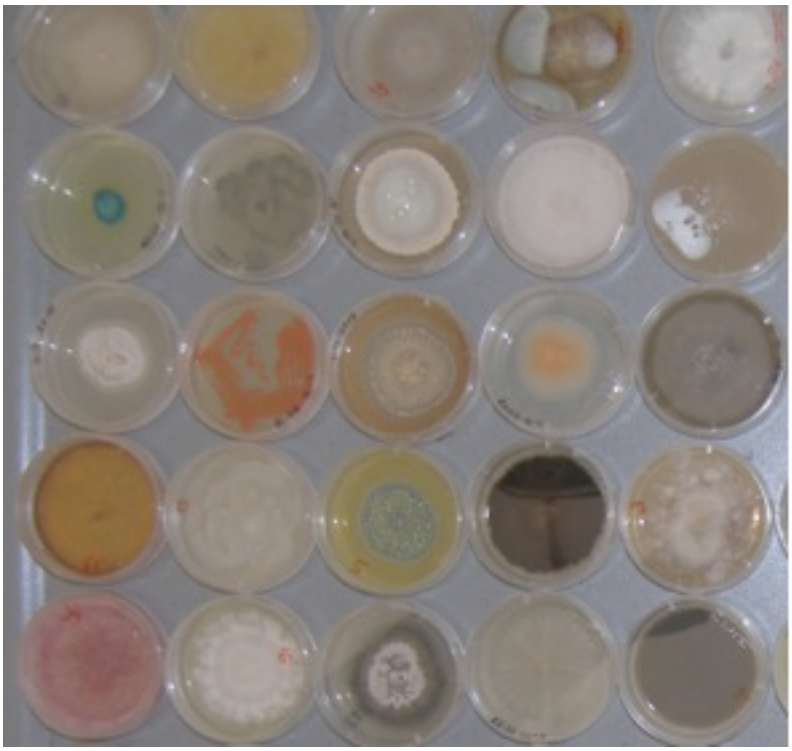
Based on the fact that the response of soil microorganisms is not well-known, it is important to investigate how the climatic parameters could influence the community composition, microbial activity, processing and turnover. In addition the sustainability of agricultural practices has to be taken in consideration to understand how the effect of climatic changes on soil ecology could have interactive effects on soil management practices.

First layer of a soil

2. Soil general description

Soil is one of the major habitat for microorganisms, each gram of soil may contain up to 10^{10} microbial cells. The majority of microorganisms lives in the first layer of soil, which represents a dynamic interface between plant and soil. Fungi and bacteria represent an essential functional component of terrestrial ecosystem, involved in a variety of bio-geochemical processes such as C (Carbon), N (Nitrogen), S (Sulfur) and Fe (Iron) cycling and they carry out important functions inside the soil, playing a key role in the food web chain, where they occupy the lowest level of the trophic chain.

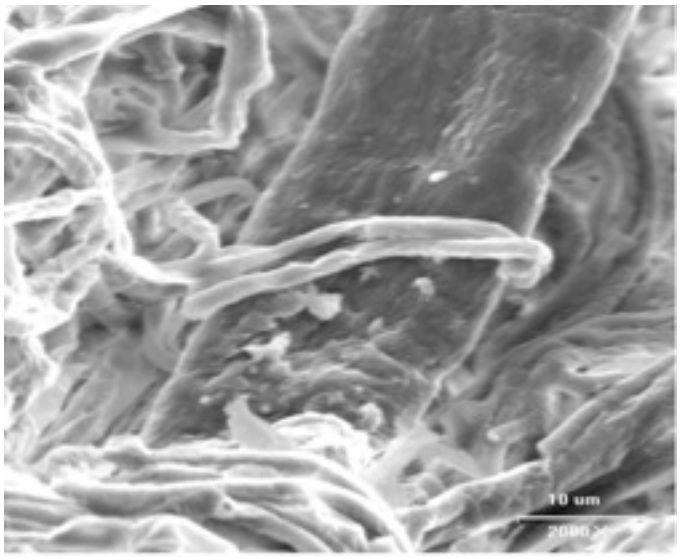
For this reason the protection and conservation of soil biodiversity have an economical and ecological impact and the soil monitoring represents a valuable approach to determine the soil variables affecting the biodiversity.



An example of cultivable fungi isolated in vineyard soil.

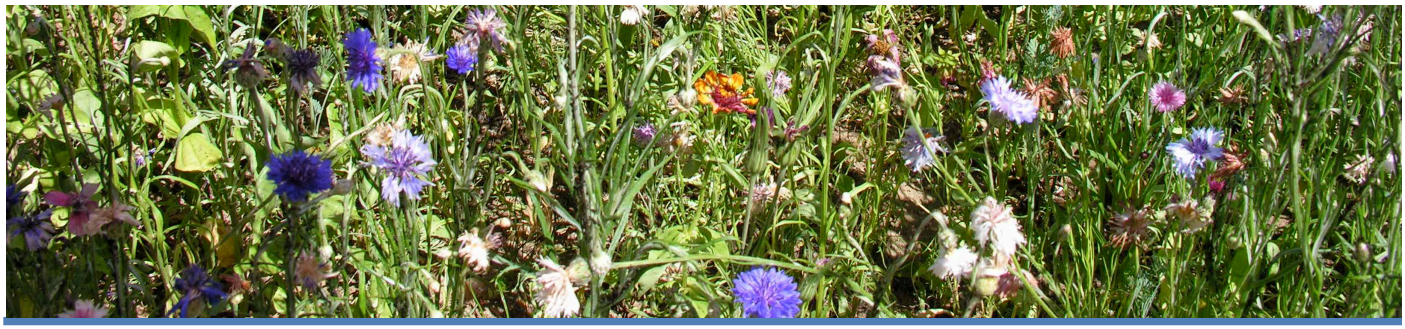
In particular, the study of the effect of abiotic and biotic factors in soil became a main topic in recent years. Environmental, climatic factors and anthropogenic disturbances could in fact modify the equilibrium of the microbial communities.

Several studies focused on the description of microorganisms living in a certain habitat to define the community structure. Other studies focused on the dynamics of a group of microorganisms belonging to the same species (population dynamics). Populations commonly studied belong to the biocontrol agents, plant pathogens, saprophytes and symbiontic species; their dynamics and their interaction with other organisms are in fact important defining the community's structure.



An example of mycoparasitism interaction between a pathogen (*Armillaria mellea*) and its antagonist (*Trichoderma atroviride).*

Microorganisms interact inside the soil at different levels; in some case interactions among microorganisms cause no effect. In mutualistic interactions microorganisms share reciprocal beneficial effects. In contrast, there are competitive interactions where microorganisms can produce toxic substances or compete for nutrients and space; in some cases the parts involved in this interaction are beneficial microorganisms, also called biocontrol agent (BCA), which act against plant pathogens. These different interactions influence the soil microbial dynamics and their study could allow the identification of new BCAs, which could be helpful in preserving soil quality.



3. Tools to study soil microorganisms

Numerous efforts have been done to determine the most appropriate methods for a deep analysis of the microbial communities and so to describe the complexity contained in the soil matrix. For this reason the cultivable-based methods have been coupled to non-cultivable-based approaches, which can better resolve the complexity of this environment.

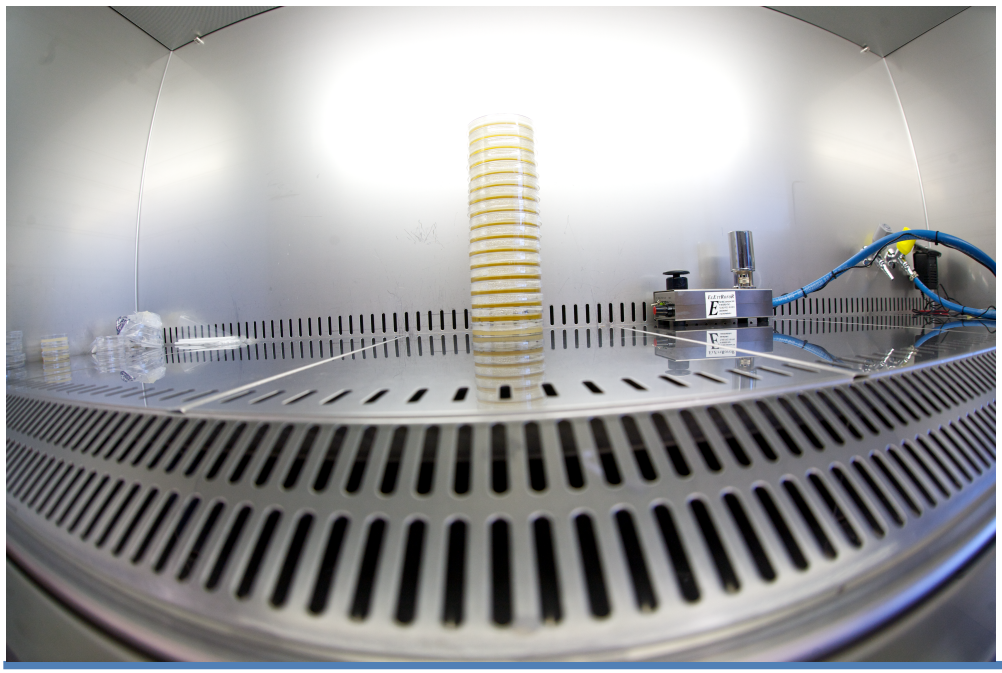
Only a small part of the microbial community is cultivable and the identification requires time and experience. In recent years numerous molecular approaches have been developed to investigate the microbial communities.

These approaches are based on direct sequencing of nucleic acids or on the amplification of specific genes, characterized by a length or sequence heterogeneity allowing to discriminate between different genera and in some case between different species. Among all the available molecular techniques some of them were chosen to study microbial soil communities and will be described here following.

3.1 Cultivable-dependent approach for microorganisms quantification and isolation

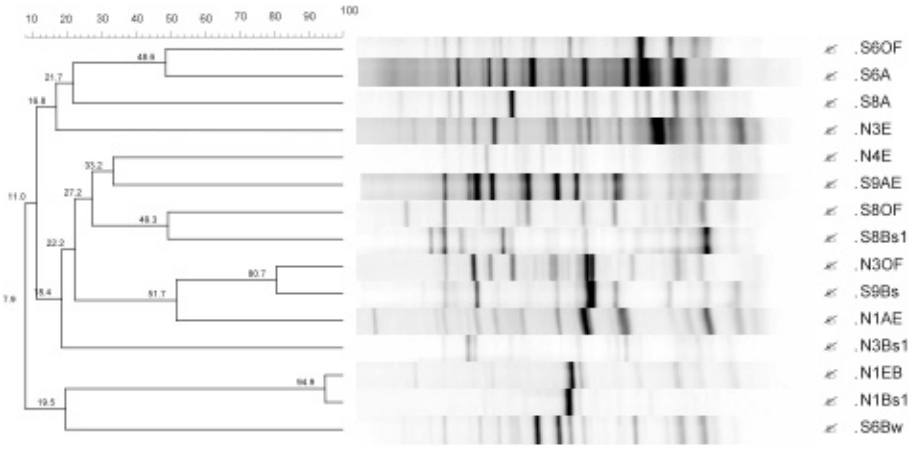
Microbiogical analysis represents a first approach to estimate the quantity of total bacteria and fungi and for the quantification of species considered of particular interest, such as biocontrol agents or microorganisms indicating the soil quality. Microbiological experiments are carried out in sterile condition, under sterile benches. The starting soil is firstly resuspended in saline solution and then serially diluted and plated on different cultural agar, such as potato dextrose agar (PDA), malt extract agar (MEA), tryptic soy agar (TSA), Luria agar (LA), or using broth culture made of the same extract.

Then specific antibacteria or antifungi can be added to isolate fungi or bacteria respectively. Cultivable-dependent technique enable to isolate a small portion of the total population (0.1-10%), but still remain an important approach for the study of the morphology of fungal and bacterial cells and for the investigation of the activity of biocontrol agents.



Laminal flow hood and petri dishes.

3.2 PCR-DGGE (Polymerase chain reaction-denaturing gradient gel electrophoresis)

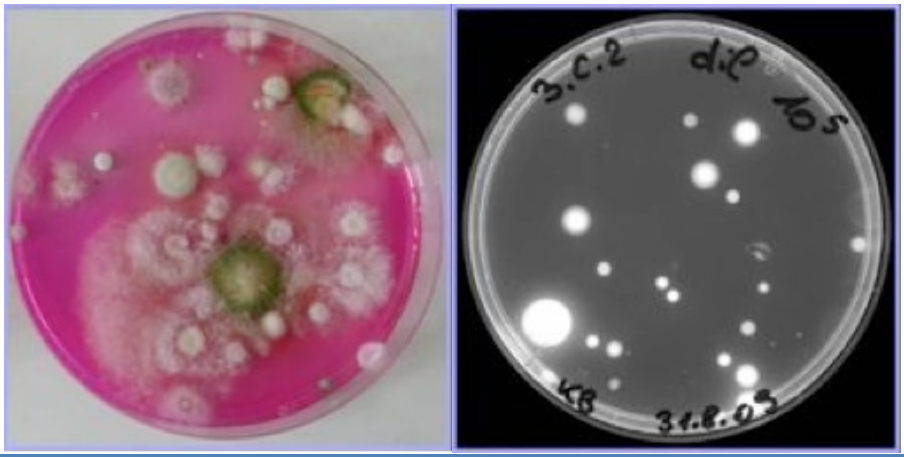


PCR-DGGE banding patterns of fungal populations of different horizons from forest soils.

PCR-DGGE is one of the most extensively used molecular approaches to study diversity in microbial ecology. This method is based on the electrophoretic mobility of DNA fragments of similar length but with different base-pair sequences using a poly-acrylamide gel containing a denaturing gradient of urea and formamide.

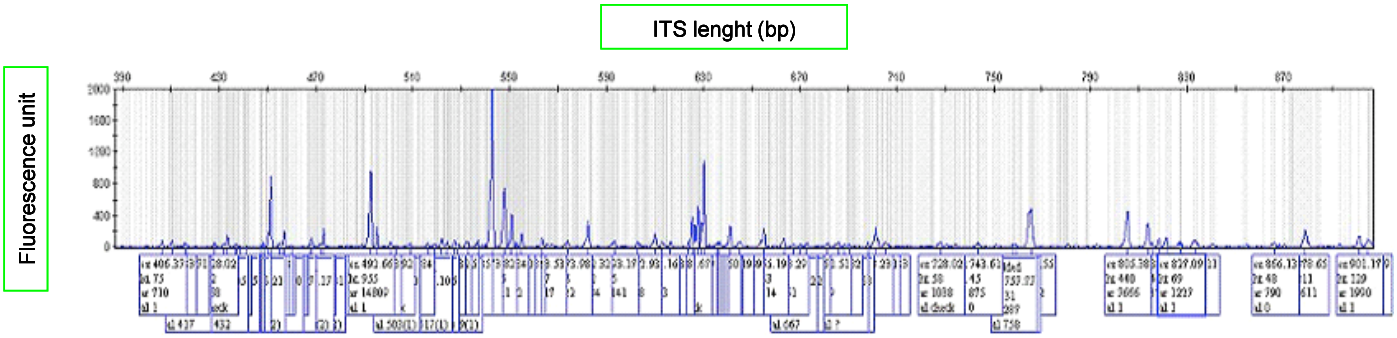
The sequences of amplified DNA fragments migrate at different positions in the gel generating an array of bands. Each band in a DGGE fingerprinting is referable to a single microorganism, although heterogeneity within a single species has been reported. The excision of selected bands followed by amplification and sequencing can yield a taxonomic identity of the bands and determine the species composition. The bands intensity correspond to some degree with the frequency of species into the sample and, in this context, PCR-DGGE can be regarded as a semi-quantitative method used for the rapid comparison of samples, identifying any similarities or differences in composition or diversity. These features made the DGGE a suitable method to elucidate and characterize microbial populations such as bacteria, fungus or nematode in different environments, or to monitore how indigenous microbial communities respond to environmental changes.

In our laboratories, DGGE method has been applied to characterise microbial populations in forest and agricultural soils. We also use DGGE to detect the effects of organic and conventional agricultural managements in the microbial community structure and biodiversity in soil vineyards. Others works regards the monitoring of dynamic populations of native biological control agents (*Trichoderma* spp. and *Pseudomonas* spp.) in agricultural soils and the structure and composition of soil fungal communities in extreme environment (alpine glacier).



Population of antagonists obtained in soils: Semi selective media with rose Bengal for *Trichoderma* spp. (left) and King media plate for *Pseudomonas fluorescens* (right).

3.3 Automated Ribosomal Intergenic Spacer Analysis (ARISA)



Example of ARISA electropherogram; each peak represents the ITS lenght of a single or groups of organisms.

Automated ribosomal intergenic spacer analysis (ARISA) is based on the amplification, through labelled primers, of the 18S-28S internal spacer (ITS) of the fungal rRNA and of the bacterial intergenic spacer (IGS) between the 16S and 23S genes; these regions are highly polymorphic in length so empowering to discriminate different genera and in some cases to reach the species level. The amplicons are loaded on a capillary gel were denatured fragments are separated.

The output is an electropherogram composed by different peaks, each of them representing one or more species. Each species could be represented by one or more peak, considering that this region is present inside each organism in more than one copy and that could be polymorphic in length.

The peaks are then converted in table of presence-absence and with the fluorescence associated to each peak, which represent the relative quantity of each of them. This data are then used for further statistical analysis. This method does not allow identifying the species associated to each peak, but gives a general information about the community and it is a powerful method to compare communities coming from different soils, or to study the microbial dynamics inside the same soil in time under the effect of multiple factors.

Different soils in a microcosm experiment.

3.4 High-throughput sequencing technologies and RNA sequencing (RNA-Seq)

New sequencing technologies offer the acquisition of gigabases of sequence information in a short time, and constitute a precious non-cultivable dependent approach for studying soil microorganisms. The first next-generation high-throughput sequencing technology 454 GS20 pyrosequencing platform became available in 2005, and was followed by the Illumina and SOLiD, sequencers, which appeared in 2007.

RNA-Seq is a recently developed technique that uses these massively parallel sequencing approaches for analyzing genomes at higher resolution than is available with Sanger sequencing- and microarray-based methods.

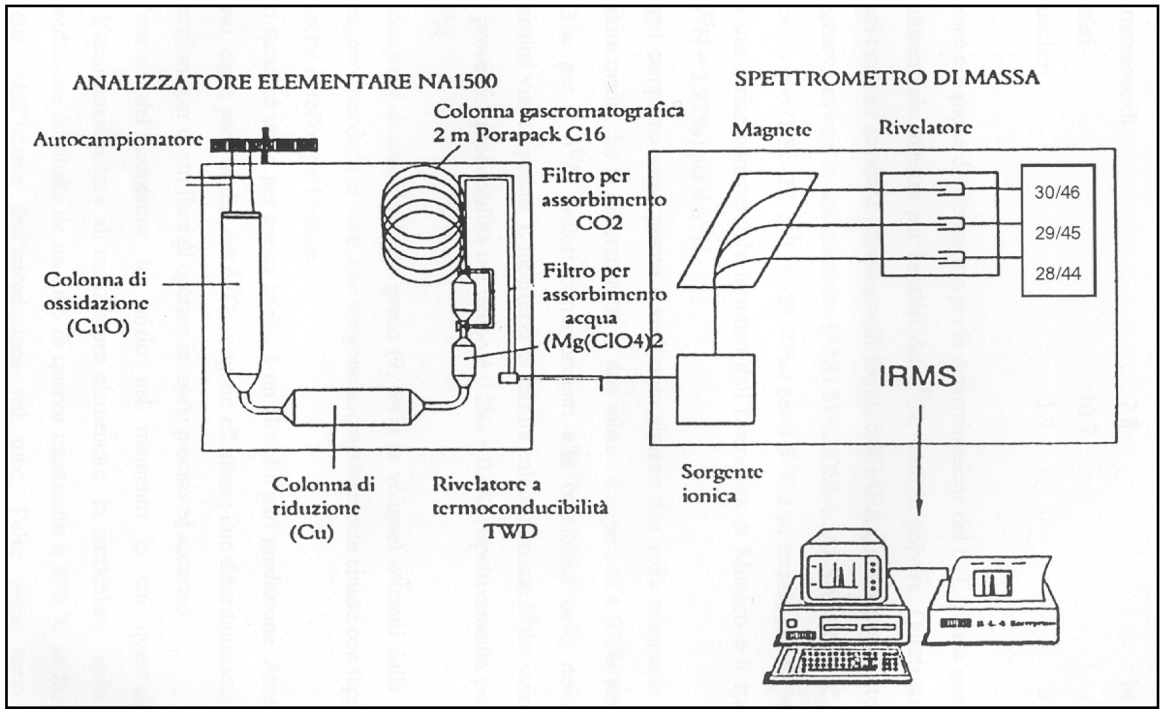
In the RNA-Seq method, complementary DNAs (cDNAS) generated from the concerned RNA are directly sequenced using the mentioned next-generation technologies. The reads obtained can then be aligned to a reference genome for the construction of a whole-genome transcriptome map. Nevertheless, since the length of the reads obtained with these methods is very short (80-100 pb with Illumina sequencer), reconstructing a comprehensive transcriptome is a challenge, and it requires computing systems with

Illumina HiSeq 2000 Sequencing System.

large memories and/or many cores to run parallels algorithms. However, RNA-Seq can be successfully used for the quantification of transcripts levels, confirm or revise previously annotated 5′ and 3′ ends of genes, or map exon/intron limits.

RNA Seq provides a powerful tool in metatranscriptomics studies which will allow understanding the timing and regulation of complex microbial processes within soil communities, as well as how the communities respond to changing conditions.

3.5 Isotope Ratio Mass Spectrometry (IRMS)



IRMS workflow.

The difficulties to study the active interaction among microorganisms (direct parasitism or active assimilation of metabolites/degradation products) and to monitor the different microorganisms into the soil might be overcome using labelled microorganisms by stable isotopes.

Labelled microorganisms by ^{13}C isotope can be monitored in the environment and, if they are plundered by another organisms, labelled residues can be detected into its predator by Isotope Ratio Mass Spectrometry (IRMS) analysis.

IRMS offers an accurate and precise measure of variations (0.1‰) in the abundance of isotopic ratios (δ values) of light elements such as $^{13}C/^{12}C$, $^{18}O/^{16}O$, $D/^{1}H$, $^{15}N/^{14}N$, and $^{34}S/^{32}S$.

We set the protocols to detect the active degraders of *Armillaria mellea*, the causal agent of root rots of several woody plants. Two different microorganisms were tested to identify its ability to active degrade of the pathogen, ***Trichoderma atroviride*** SC1 and ***Pseudomonas fluorescens*** Pf-5, which represent BCAs species present in nearly all soils.

Trichoderma atroviride, strain SC1

A fungus known as mycoparasite, competitor for nutrients or space, inducer of resistance and competing for space and nutrients and antibiosis.

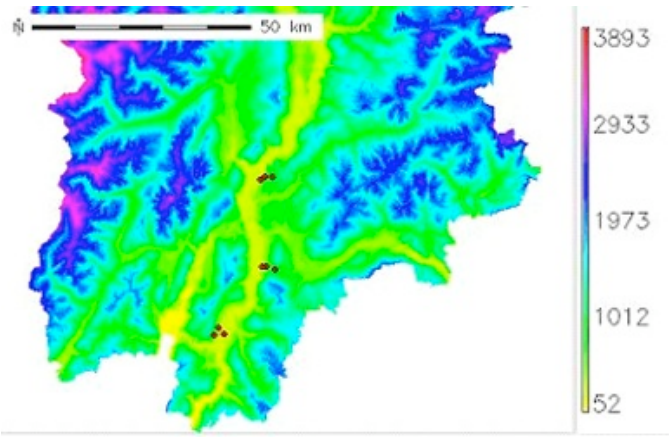
Pseudomonas fluorescens, strain Pf-5

A bacterium able to produce antibiotics and to control several plant diseases.

4. Soil microorganisms and temperature: the case of selected vineyards in Trentino

Temperature is one of the most important environmental factors regulating the activity and determining the composition of bacterial and fungal communities. Temperature shifts can affect microbial activity, processing and turnover causing the microbial community to shift in favour of species adapted respectively to higher or lower temperatures and faster or slower growth rate. Changes in temperature affect heterotrophic respiration and induce a modification in the ability to metabolize substrates. These effects could be the result of a direct action of the temperature on soil community or an indirect result of the temperature effect on plant. For this reason we investigated how microbial communities living in vineyard soil could be impacted by temperature changes.

Grapevine represents one of the most important crops in Trentino region, with high economic importance, so the preservation of the soil quality and microbial biodiversity is crucial in order to get an optimum production. Considering that the crop is cultivated in a wide range of altitude we investigated the effect of altitude considered as an analogue of soil temperature shifts in different seasons of the year. Nine vineyard fields distributed over three altitudinal gradient (200 m, 450 m and 700 m respectively) were selected. Soil samples were collected in two consecutively years in winter (February) and summer (July), representing temperatures registered in soil and so relevant to investigate the seasonal effect.



Digital elevation map of Trentino region made by GRASS 6.2.2.

Microbial community's dynamics were investigated through ARISA. Soil samples were first extracted through commercial kit, quantified and amplified using specific primers identified for fungal ITS and bacterial IGS regions. The ARISA results showed that the bacterial communities, present at 700 m differ from those found at 200 m,

elucidating the presence of an altitude effect on the microbial profile.

In the case of the fungal structure an altitudinal effect was not so evident. The physicochemical pattern is also strongly affecting and explaining some differences in the microbial communities structure, in fact soils differing in microelements (Al, Fe, Si, Mn, B), macroelements (N, P, K, Na, Ca, Mg, S) and heavy metals (Cu, Zn, Ni, Pb) contents are characterized by different bacterial and fungal communities.

Vineyard fields in summer and winter.

5. Gene expression profiling by massively parallel sequencing induced by *Armillaria mellea* and *Trichoderma atroviride* in an artificial

RNA-Seq technologies constitute a very powerful tool for studying the expression profile of a microbial community. We applied RNA-Seq for the study of a controlled artificial soil ecosystem and the effect that cause on the transcriptome profile of this system the arrival of a pathogen, *Armillaria mellea* and its antagonist *Trichoderma atroviride* SC1. We created an artificial sandy soil, which was inoculated with a total of eleven different species that are listed in Table 1. All the microorganisms chosen are normally found in natural soils and their complete genome sequence are available in public databases. Five soil microcosms were inoculated with the selected microorganisms' species. One of them was also inoculated with *T. atroviride* SC1, another with *A. mellea*, and the other with both fungi. The other two microcosms were used as controls. Total RNA was extracted from the five different microcosms after 24 hours of incubation at 20°C, and enrichment in mRNA was also done before sequencing with Illumina HiSeq 2000. Results released from this analysis will help understanding the soil system as a 'superorganism' and see how a pathogen and its antagonist interfere in its gene expression profile.

Source	Species
DSM 5975	*Azorhizobium caulinodans*
IASMA	*Bacillus subtilis subsp. subtilis* 168
DSM2839	*Ralstonia metallidurans* CH34
ATCC BAA477	*Pseudomonas f luorescens* Pf-5
CBS 513.88	*Aspergillus niger*
DSM 1075	*Penicillum chrysogenum*
FGSC 9935	*Fusarium oxysporum* fsp. *lycopersici*
CBS 767	*Debaryomyces hansenii*
CBS 6054	*Pichia stipitis*
DSM 70576	*Schizosaccharomyces pombe* 972h
S288C	*Saccharomyces cerevisiae*

Table 1. Microorganisms inoculated in the artificial soil microcosm.

New genes involved in pathogenicity might be discovered, as well as others will be annotated in the already sequenced genomes.

In addition, *A. mellea* is the only species in the system not sequenced, being its complete genome sequencing a complement part of the Envirochange project.

Armillaria mellea growth in Petri dishes or directly growing on grapevine roots collected in vineyard.

To know more

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THE ENVIROCHANGE PROJECT

The EnviroChange project focuses on global change and sustainable management of agriculture in highly developed mountain environment.
It aims at assessing the short-term biological, environmental and economic impact of climatic change on agriculture at the level of the Trentino region particularly on quality and pest management that are more likely to be influenced by climate change in the short term. The final aim is to preserve and improve the quality of life of habitants, protecting environment and biodiversity for the future generations, as well as to represent a model for sustainable development of mountain areas.

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