
PhD THESIS

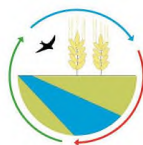
Global change impact on soil property, functioning and provided ecosystem services

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INTRODUCTION

Soil environment is a critical compartment of terrestrial ecosystem that face global change pressures (Gaillard et al., 2015; Tang et al., 2016; Jitareanu et al., 2006; Liu et al., 2018). As a non-renewables resource (Marcon et al., 2018; Rusu et al., 2017; Szajdak et al., 2002; Tecon and Or, 2017) and as a significant provider with ecosystem services (Peng et al., 2015) that sustain life and human's development, it requires special attention to could find solutions in mitigating and/or adapting at drivers of global change. At moment there is a general knowledge gap on how drivers of global change could modify or alter soil properties (Allen et al., 2011; Bach et al., 2018; Rusu et al., 2014) with implication on its functioning (Noronha et al., 2017) and provision with ecosystem services (Kourtey et al., 2002).

Soil well-functioning is assured by abiotic and biotic properties of soil. While knowledge on how global change could modify soil physicochemical properties are available (Holstein et al., 2009; Altonen et al., 2019), there are little information on how soil microbiota could be changed or damaged by global change derived pressures (Gaillard et al., 2015). Soil microbiota is a key element of soil ecosystem, mediating numerous reactions in soil (chemical, biochemical, etc.). Representative actions of soil microbiota refer at nutrients cycling, soil organic matter decomposition, pollutants degradation, etc. Through reactions that perform, they are connected as contributors in soil ecosystem services provision as support, regulation, and provision (Surda et al., 2015; Szajdak et al., 2016).

Supposing that changes in soil microbiota will lead in soil properties and functioning modification that has consequences on soil ecosystem services provision, the major goal of this thesis was to find answers on how global change drivers could impact soil properties, functioning and provided ecosystem services with special attention on soil microbiota implications.

THESIS STRUCTURE

This thesis integrates seven chapters of that one refers at identified challenges of soil ecosystem (*literature review section*), the second one (*personal contributions*) present this thesis objectives, followed by chapters dedicated for applied research methods description, obtained results presentation and discussions, conclusions and thesis novelty underlying.

Briefly the *literature review section* tackle subjects of soil ecosystem, global change, soil properties change under challenges of global change, soil microbiota and soil ecosystem services linkage between abiotic and biotic factors under challenges of global change drivers.

The second chapter present the three main *objectives* of this thesis. It is followed by the chapter dedicated for *sampling sites and sampling design description*. In this part of the thesis are detailed studied areas geographical descriptions, sampling procedures and applied laboratory experiments applied, as: soil column experiments; rhizo-box experiments; and climate change chamber experiments.

The fourth chapter of thesis is dedicated to ***applied methods description***. It consists four major parts, of that the first is related to soil microbiota structure and abundance assessment; the second present the applied tools for soil microbiota functional and physiological profile assessment. In the third part of this sections are detailed applied methods for soil biochemical (soil extracellular enzymatic activity assessment) and chemical properties assessment. The last part of this section id dedicated to applied geostatistical tools for soil properties distribution and prediction assessment. Specifically, five geostatistical interpolation tools are presented. These are kriging, inverse distance to a power, modified Shepard's method, minimum curvature method and radial basis function methods interpolations.

Thesis chapter five include thesis obtained ***results and related discussions***. This have six major parts that refer as follows at: studied biomes soil microbiota structure and abundance; abiotic factors influence on soil microbiota; biotic factors influence on soil microbiota: plant allelopathy and root exudates; land use and management influence on soil functioning; climate change drivers influence on soil functioning; and finally, global change drivers potential impact on soil functioning and provided ecosystem services with involvement of soil microbiota.

The next chapter summarize ***conclusions and recommendations*** obtained after performed studies and brief recommendations. These are followed by the last section where are highlighted the ***novelty aspects of the thesis***.

PURPOSE AND OBJECTIVES OF RESEARCH

The general objective of thesis is to “***assess how global change impact soil properties, functioning and provided ecosystem services***”. Targeted specific objectives are, as follows:

2.1. Evaluate current soil quality and properties from different geospatial and climatic region with special attention on soil microbiota community structure and abundance.

Through this thesis it was proposed to collect data about soil microbiota heterogeneity from different climatic and geospatial regions from Romania, based on land use type (forest, grassland, agricultural land, vineyard, orchard). Different interpolators were tested as geostatistical estimators for prediction of microbiota community structure distribution and other defining properties of soils on studied areas surfaces. Considering soil chemistry and overlying vegetation, geospatial distribution of microbial community structure and abundance were realized with aim to understand factors that regulate their distribution in different soil systems. Changes of soil microbiota composition with soil layer depth (until 50 cm) was also considered

2.2. Assessment of global change impact on soil microbiota and their response level at these drivers. Relationship were established between microbiota community structure and drivers of global change as anthropogenic pressures (soil management strategies, pollution, etc.) and different climatic factors (regular and anomalous variation). Lab scale experiments on whole community and isolates were performed considering their exposure under controlled conditions to major

identified global change drivers. Differentiations between microbiota richness in bulk soils and rhizosphere of crops (maize, wheat) and vegetables (tomato) were studied

2.3. Link soil microbiota and soil properties with soil functioning and estimate the potential impact on soil provided ecosystem services. Connections between supporting services interceded by soil microbiota and influence of global change drivers were established for major nutrients and organic components of soil substrate. Impact of global change drivers on soil microbiota liaised soil ecosystem regulating services as soil potential contaminants degradation were studied.

RESEARCH METHODOLOGY

Soil microbiota components, through their heterogeneity, high taxonomic diversity and metabolic potential, are key elements in mediation of numerous biogeochemical cycles and reactions in soil (Cojniak et al., 2015). Thus, microbiota is considered as a representative indicator of soil ecological processes status (Rusu et al., 2009). In this thesis was measured the abundance and community structure of soil microbiota using phospholipids derived fatty acids (PLFA) approach (Vestal et al., 1989; Cojniak et al., 2015). Neutral lipids derived fatty acids were used for assessment of shifts in different phenotypic groups while glycolipids derived fatty acids for assessment of development level of major bacterial and fungal groups. This was done with the reason to understand changes on soil microbiota (community structure, abundance, cell membranes fatty acids, etc.) due to pressures of global change drivers. Importance to fill this gap of knowledge is given by that potential changes in soil microbiota could modify soil functioning and provision with ecosystem services. Using of these soil microbiome indicators could contribute in establishment of an early warning system of potential losses of these soil services and goods.

Whereas effects of global change drivers on soil physicochemical properties are observed in time, soil microbiota respond to any environmental changes in a much shorter period (Wu et al., 2017). Phenotypic measurement of soil community structure and abundance offers numerical information about the dominant members of soil micro-biodiversity. Thus, functional assessment of soil microbiota community under challenges of global change driver are required to could better understand their functioning under external pressures, and to predict soil functions and ecosystem services provision in that they are involved. To achieve proposed objectives of this thesis, soil microbiota functioning and physiological profile were assessed based on (a) average metabolic response and community metabolic diversity analysis through sole carbon, nitrogen and phosphorous source consumption (CLPP, community level physiological profiling); and on (b) basal (BR) and substrate induced respiration (SIR) rate analysis.

Soil biochemical and chemical properties were also assessed for a better understanding

of soil processes, functions and provisions with ecosystem services. Soil enzymes are important components that act as catalysts in soil organic matter decomposition and nutrient cycling. Compared with soil microbiota structure or with physiological characteristics, which refer at potential activities, enzymes are directly connected with activities resulted from thus of potential in the moment of sampling. Through this thesis enzymatic activities related to organic matter decomposition and nutrient cycling were studied. These enzymes were: catalase, phosphatase, dehydrogenase, invertase, amylase, urease, aryl sulfatase, phenol oxidase, and β - glucosidase. Titrimetric and microplate assays approaches were applied for their assessment. Microplate assays involve four major steps: activation of well plate, enzyme reaction and extraction, and quantitation.

For proper assessment of global change drivers impact on soil functioning and provided ecosystem services, several other physicochemical properties of soil were analyzed also during this thesis. For proper interpretation of obtained data about soil properties different geostatistical models were applied. These models quantified soil property parameters spatial distribution and variability in accordance with spatial scale of the monitored area, and with distance between sampling points. Also, modeling semi variograms of spatial pattern was applied. Tested geostatistical models were kriging interpolation, inverse distance to a power, modified Shepard's method, minimum curvature method, and radial basis function.

RESEARCH RESULTS

As soil microbiota is considered an essential element of soil ecosystem, their abundance variation between studied biomes was assessed. Variations both in structure as well abundance were observed for studied sampling sites.

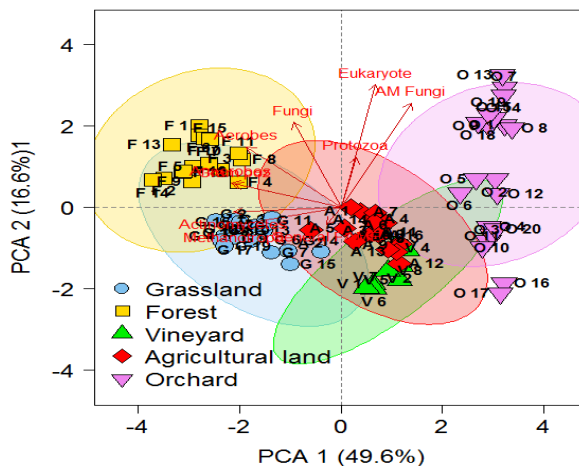


Figure 1. Principal component analysis of soil microbiota community abundance related with different land use.

Principal component analysis **Figure 1.**, explained the total variance of 66.2 % ($p = 0.001$) between microbiota abundance of different biomes, of that PC1 explain 49.9 % and PC2 16.6 %. The differences were relevant especially in case of bacteria where large squared cosine was obtained for samples collected from forest (0.909) followed by those collected from orchard (0.855), agricultural (0.731), grassland (0.728) and vineyard (0.716) lands, respectively.

At microbiota community structure level, the average value of total bacteria followed the following pattern within biomes: forest ($342.1 \text{ nmol}\cdot\text{g}^{-1} \text{ dw}$) > grassland ($240.2 \text{ nmol}\cdot\text{g}^{-1} \text{ dw}$) > orchard ($141.1 \text{ nmol}\cdot\text{g}^{-1} \text{ dw}$) > vineyard ($137.8 \text{ nmol}\cdot\text{g}^{-1} \text{ dw}$) > agricultural land ($106.3 \text{ nmol}\cdot\text{g}^{-1} \text{ dw}$). In the five studied biome the average value of fungal abundance, expressed as sum of arbuscular mycorrhizal fungi, saprotrophic fungi, ectomycorrhizal fungi and other general fungi, vary between 3.2 and 6.3 $\text{nmol}\cdot\text{g}^{-1} \text{ dw}$. Higher values were detected in soil samples collected from forest and orchard.

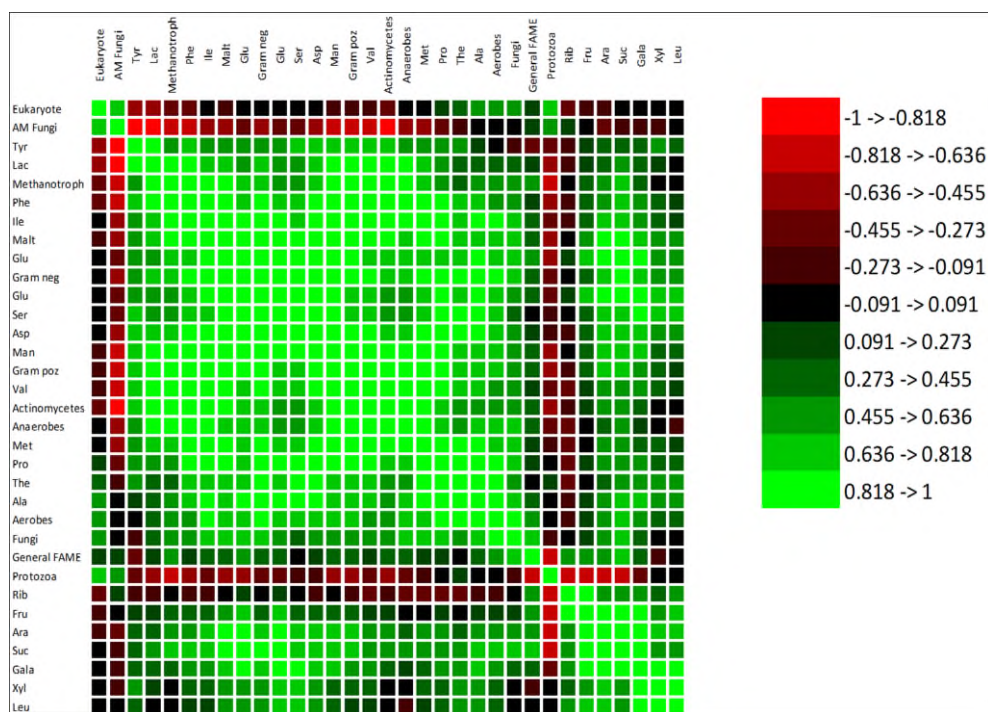


Figure 2: *Pearson correlation matrix of soil microbiota structure and soil carbohydrates and amino acids content.*

Measured soil properties differ significantly through biomes environment. In grassland positive influence of organic carbon (0.377) and C:N ratio (0.547) on fungal and bacteria communities was observed, while temperature (0.619) negatively influenced protozoa community. In forest moisture (0.585) have a positive impact on fungal community, while temperature (0.612) and organic carbon (0.478) affected eukaryotes in negative

way. In vineyard could be observed that C:N ratio (1.107) has a negative impact on bacterial communities. Agricultural lands soil properties as pH (1.286), C:N ratio (0.611), moisture (0.754) and organic carbon (0.601) content influenced negatively bacterial and fungal communities. In orchard, bacterial communities were influenced positively by C:N ratio (0.266), moisture (0.187), soil organic matter (0.536) and organic carbon. Pearson correlation matrix **Figure 2** has shown poor correlation or no correlation between protozoa, eukaryotes, fungi and general FAME and carbohydrates and amino acids content of soil, while gram - negative bacteria and gram - positive bacteria strongly correlates with most of these compounds.

Management practices influence both soil microbiota structure as well soil properties. Principal component analysis on the total PLFA biomass reveal clear separations between samples of open field and greenhouse soil see **Figure 3**.

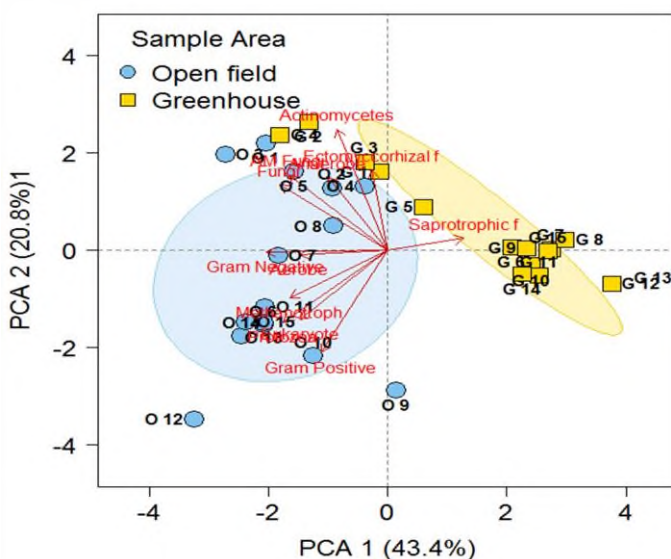


Figure 3. Principal component analysis of soil microbiota structure in open field and greenhouse soil samples

The principal components PC1 and PC2 explained 43.4 and 20.8 % of the variance. These results are similar with those presented by (Babin, 2019; Bonanomi, 2016) and (Legrand, 2018), where different soil management practices impact on soil microbiota was stated.

Correspondence analysis biplot separate clearly conventional treatment by tillage treatment (**Figure 4**). The first two principal components accounted for 42.1 and 17.3 % of the total sample variance, respectively. Measured data shows that soil microbiota community components as gram positive (0.546) and negative bacteria (0.821), methanotroph bacteria (0.781), eukaryotes (0.511) and fungal community (0.502 - 0.492) converged at conventional management while protozoa (0.84) and aerobic bacteria (0.601) converge at tillage management (Figure 4).

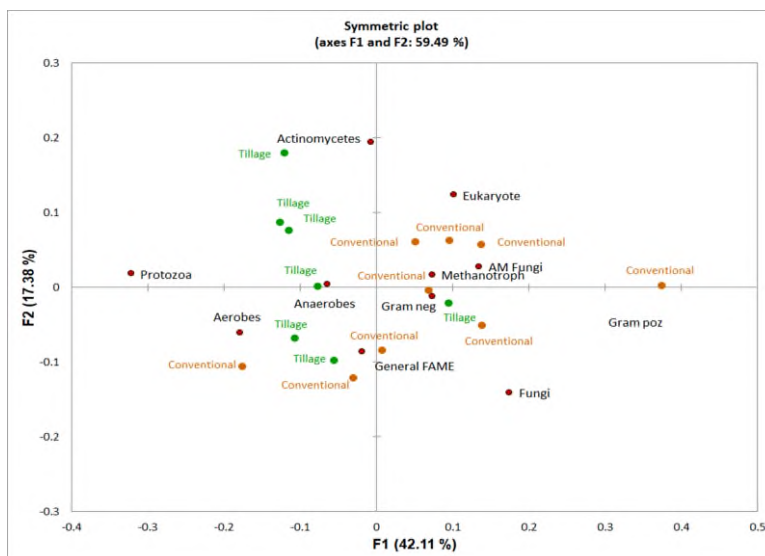


Figure 4. Correspondence analysis of applied management practices impact on soil microbiota.

Through this work, soil samples from the different seasons were collected. Seasonal differences were obtained in case of all studied biomes. All components of soil microbiota changed with season. Generally, higher abundance of microbiota components was measured in period of summer - fall (**Figure 5**), excepting gram positive and gram-negative bacteria whose abundance were higher in spring season in case of all studied biomes.

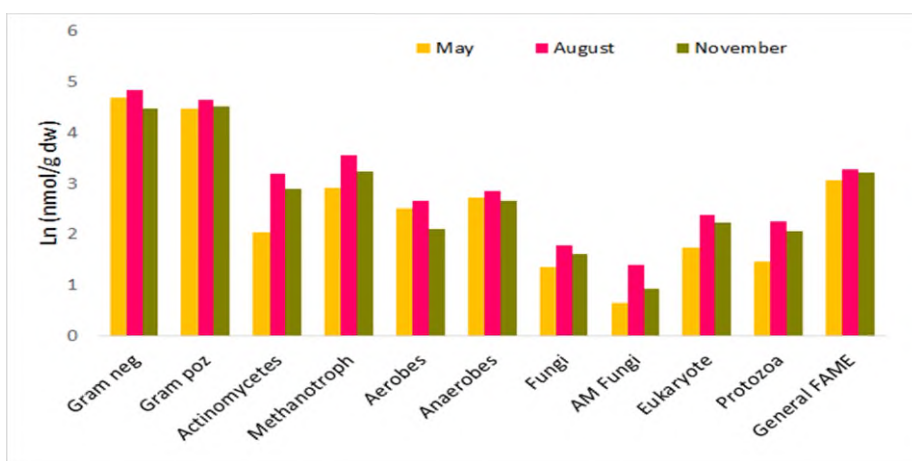


Figure 5: PLFA ratios for studied soil management practices.

Drought and flooding simulated column and climate change chambers experiments revealed that soil microbiota is more sensitive at soil moisture content (**Figure 6**).

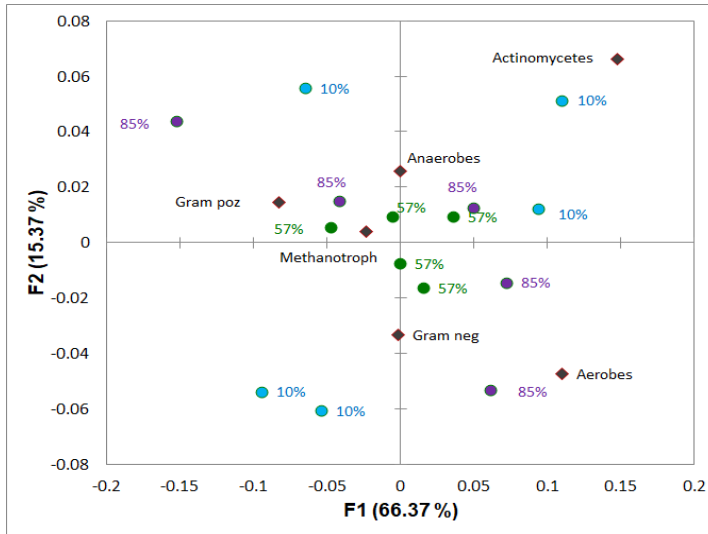


Figure 6: Canonical correspondence analysis of simulated drought and flooding conditions impact on soil microbiota abundance.

Metabolic activity of soil microbiota was evaluated from soil samples where different management practices were applied.

In **Figure 7** could be evaluated the consumption pattern of six substrates categories (carbohydrates, carboxylic acids, amino acids, amines, phenolic compounds, and polymers) by microbiota from soils where management as pesticides addition (PA), crop rotation (CR), greenhouse (GH), open field (OF), conventional tillage (CT) and NT (no - tillage) management were applied. Average well color development (AWCD) vary within 0.624 - 3.254 for studied inoculated well plates. According with Figure 7 it could be noticed that less metabolic activity was within soil microbiota where pesticides (e.g. cypermethrin) were applied. In this case the metabolic activity was lower with 34 %. It was followed by greenhouse management where the microbiota metabolic activity was lower with 27 %. High metabolic activity was registered in open field and crop rotation managed soil samples. Under conventional tillage and no - tillage management soil microbiota presented a medium metabolic activity. Carbohydrates were the most utilized substrates whereas amides and amines the less ones. These data were in correlation with soil extracellular enzymatic activities where lower amount enzymes involved in nutrient cycling were determined in soil samples as follows: OF > NT > CR > CT > GC > PA. No significant variation was observed in case of phosphatase and aryl sulphatase between the six treatments studied. Determined metabolic indexes as Shannon diversity index (H'), Shannon evenness index (E) and substrate richness index (R) varied within 1.6 - 4.8, 0.71 - 0.95, and 21.2 - 32.4, respectively after 36 h incubation.

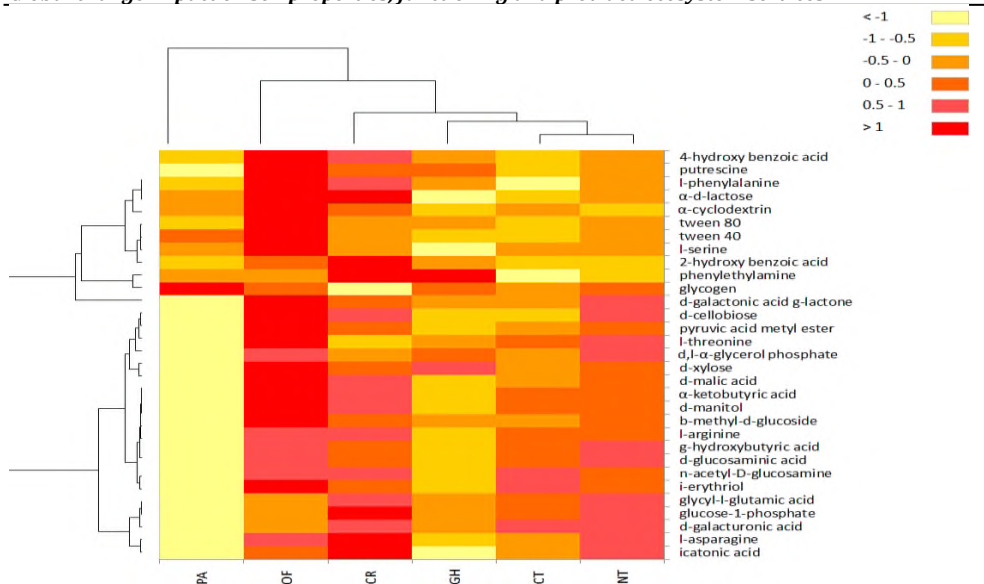


Figure 7: Soil microbiota metabolic activity under different agriculture management practices.

For experiments involving climate change chambers that simulated extreme temperature events or, extreme flooding or drought events low metabolic activity was measured for microbiota of soil samples exposed at low temperature (-20 °C) and flood conditions (80 % WHC) while drought condition registered the highest metabolic activity (AWCD, 1.3 - 4.29) (Figure 8).

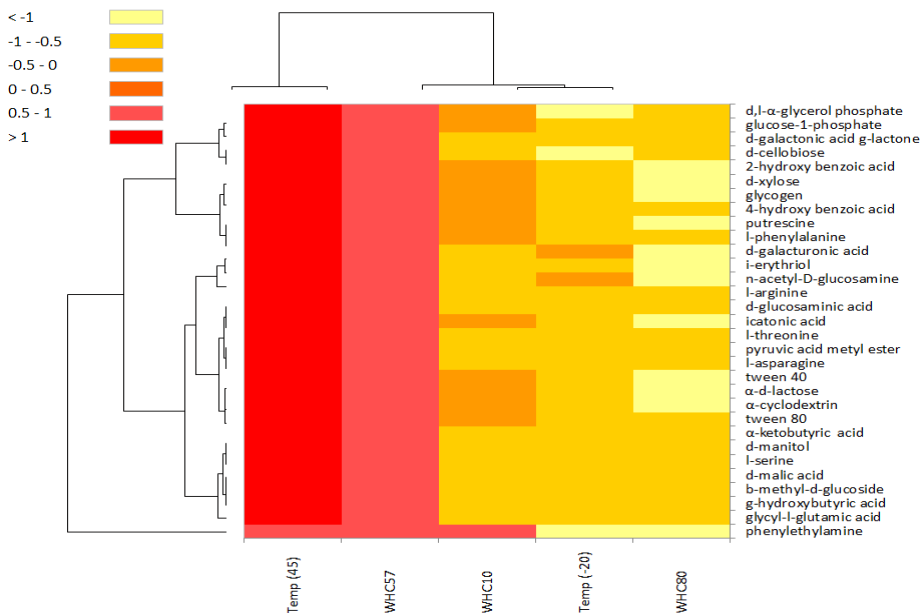


Figure 8: Soil microbiota metabolic activity under different climate change driver exposure.

GENERAL CONCLUSIONS

Through this thesis global change pressures on soil ecosystem was studied. Because soil microbiota is involved in numerous soil processes throughout numerous soil ecosystem services are provided, special attention was accorded to them. To achieve this thesis general objective as find answer on “*how global change impact soil properties, functioning and provided ecosystem services*” research and experiments connected to the mentioned objectives were performed (2.1., 2.2., 2.3., chapter 2). Obtained results connected to each specific objectives of thesis could be summarized as follows:

1st objective. *Assessment of current soil quality and properties from different geospatial and climatic region with special attention on soil microbiota community structure and abundance*

- Through this thesis soil samples of five biomes were studied from different geospatial regions of Transylvania. Specifically, these referred at forest, grassland, vineyard, orchard and agricultural land. Clear differentiation within soil microbiota abundance and structure of the five studied biomes was determined (66.2 %, PCA).
- In all soil samples bacterial dominance was evidenced within soil microbiodiversity ($G+/G-$, $aerobe/anaerobe > 0.5$; $F/B > 0.1$).
- Abiotic properties influence on soil microbiota community structure and abundance was explained by redundancy analysis for all studied biomes (RDA, $F1$: 63.5 – 79.7 %). Plant allelopathy and root exudates could modify soil microbiota distribution.

2nd objective. *Assessment of global change impact on soil microbiota and their response level at these drivers*

- Land conversion (open field → greenhouse) change soil microbiota composition: greenhouse shape soil microbiome (PCA1: 43.5 %). Stress indexes related to microbiota abundance and structure were higher in greenhouse soils than open field.
- Tillage types impact soil microbiota components, major influences being observed on protozoa, actinomycetes and aerobic bacteria communities. These was explained by correspondence analysis with 42.1 %. Sensitivity test on each phenotypic class sustained this observation.
- Seasonal variation changes soil microbiota structure. Artificial climate chamber experiments shown fungal and bacterial community shifts at extreme negative temperatures. However, experiments revealed also microbiota components could resilience at -24 – -20 °C temperatures. Climate chamber experiments at high temperatures (> 35 °C) revealed bacterial resistance.

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- Column experiments with simulated drought and flood conditions shown higher sensitivity of soil microbiota at soil moisture.

3rd objective. *Link soil microbiota and soil properties with soil functioning and estimate the potential impact on soil provided ecosystem services.*

- Soil metabolic activity could be lowered by intense management practiced on agricultural lands.
- Soil microbiota metabolism is strongly impacted when pesticides are used in land fields (metabolic activity is lower with 34 %). Low Shannon diversity index suggest that microbiota abundance could be lowered even by some species losses due to pesticides application.
- Considering simulated extreme climate events high metabolic activity of soil microbiota was observed when soil samples were exposed at high temperatures (45 °C). Flooding condition or freeze conditions lower significantly soil microbiota metabolic activity.

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