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Catabolic Profiles of Cultivable Microbial Communities in Forest Soils of Western Algeria along a Latitudinal Gradient

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Abstract

In Algeria, the soil degradation, more particularly the one related to the biological properties in the surface layers, is a major problem to the ecological balance and the development of forest massive. This phenomenon is considered to be the most important factor limiting the growth and productivity of forest plants in arid and semi-arid zones that can go as far as to almost sterilization of these soils. In this research, it is proposed to undertake the study on catabolic profiles of cultivable microbial communities of forest soils in western Algeria along a latitudinal gradient in some sampled surface horizons of the three zones, namely Tlemcen, Saida and Naama. Like most of soils in semi-arid and arid zones, they are soils with low content in organic matter and in clay, and most often the sandy to the sand-like texture.

The analysis of the catabolic profiles was carried out in five study plots of about 400 m^2 each selected in each study area, making a total of fifteen plots for the three zones (Tlemcen, Saida and Naama). On each plot, five samples of soil were taken randomly between 0 and 15 cm of depth. Catabolic profiles were determined following the modified protocol of Garland and Mills (1991).

The present research study show that it is in the Tlemcen zone, considered as sub-humid, that a greater diversity and catabolic wealth were found; and more down towards the arid zone, more that wealth and diversity decrease to a critical level for the Naama zone. The results showed that there is a significant effect of the bioclimatic stage on the soil biological wealth and diversity.

Keywords: Catabolic profiles, Arid and semi arid zones, Surface status, Soils, Forest

Introduction

The forest is dedicated by the Algerian constitution as an inalienable and imprescriptible national heritage which its safeguard and development constitute a major national objective. On a forest patrimony of 4.7 million hectares, the so-called economic and productive forests only cover about 1.2 million ha in Algeria. Moreover, Algerian forests are exceptionally rich in biodiversity and play a important role in terms of soils protection against wind and water erosions and of water resources conservation (DGF, 2007). In Algeria, the arid zone accounts for nearly 95% of the national territory with 80% representing the hyper-arid domain. In recent decades, the scarcity of water resources, drought, intensified human pressure on arid and semi-arid forest zones, as well as the recurrent fires have created conducive conditions to the soil degradation, deforestation and desertification.

Too often considered as a mineral environment, the soil is also a place of life. It harbors a very high diversity of species. Among these species, microorganisms are undoubtedly the most numerous and diverse. Composed of bacteria, archaebacteria and fungi, they perform essential functions such as biodegradation of organic matter, production of nutrients for plants, nitrogen fixation, pollutant degradation, etc. The biogeochemical cycles such as the carbon cycle, nitrogen cycle or phosphorus cycle are dependent (more than 90%) on microorganisms. They are responsible for the emission of greenhouse gases such as CO_2, N_2O and CH_4. However, despite this ecological importance, they remain very poorly known. This misunderstanding of the soil microbial diversity has many origins, but the main one is of a methodological aspect. Indeed, only 1% of the soil bacteria are cultivable, but until recently, the culture on a specific medium constituted the only method of characterization of the microbial species. This methodological latch was lifted thanks to the emerging of the molecular tools that permit the characterization and the count of microorganisms in their natural environment without going through the stage of the culture. Using these techniques, it has been shown that a single gram of soil can harbor up to 10,000 different bacterial species and nearly one billion bacteria (IRD, 2015).

Drought-type events, particularly their recurrence, are likely to have direct effect on soil microflora in its diversity and functionality and, probably beyond certain stress levels, to prevent a return to its initial status (Bouchet, 2008). However, the resistance and functional resilience capacities, of microbial communities in Mediterranean soils, to the stresses of climate change remain unknown. Several studies show the effect of the bioclimatic stage on the distribution of vegetation in the Algerian forests, but no study shows this effect on the distribution and abundance of microorganisms in the forest soils. In this work, the aim was to know an effect of the bioclimatic stage (temperature and precipitation) on the structure and the diversity of catabolic functions of cultivable microbial communities, determined by the Biolog® Ecoplates method (BIOLOG Inc., Hayward, CA). BIOLOG® ECO microplates are subdivided into 3 identical zones, each comprising 31 carbon sources and 1 control well without substrates. The oxidation of the substrates results in the proportional reduction of the colorless of nitro-tetrazolium blue to purple formazan. Real metabolic fingerprints, a kind of identity cards of the catabolic functions of the communities, can then be established and compared (Guénon, 2010).

2. Materials and Methods

2.1. Presentation of the study areas

In the present study, the studied zones were on latitudinal gradient from sub-humid to arid following the bioclimatic stage of the western Algeria.

2.1.1 Plots of Tlemcen (Sub-humid area)

Tlemcen is located on the north-west coast of the country with an area of 9017.69 Km². The study stations in this area are located at the reserve of Moutas (Figure 1), at 26 km of south-west of the town of Tlemcen, being part of the Hafir forest, the highest and most wooded area of the Tlemcen Mountains. The reserve occupies an area of 2156 ha on a perimeter of 15 km, characterized by typically mountainous relief. The altitude is between 1310 m at Djebel Atiem and 1017m at Sidi Messaoud (Baba ali, 2014). It is on a rugged relief with a good exhibition and view. The dominant slopes are from 12.5 to 25%. The sandstones gave rise to brown forest soils of sandy loamy texture, often evolved in fersiallitic soils (Gaouar, 1998). The climate is Mediterranean, marked by a summer drought that manifests as early as June. Only 7.2% of the 607 mm / year of rain falls during the summer season (period 1970-2014). The average maximum temperature of the hottest month is 31.6°C while the minimum temperature of the coldest month is 2.8 ° C. The Emberger Q2's rainfall index (Emberger, 1939) is 84.5, which allows this climate to be classified in the low sub-humid with a fresh variant. The snow covering is also present on the whole massif above an altitude of 1200 m, the number of snow days varies from 7 to 25 with an average of layer thick of 10 to 20 cm (Benabdeli, 1996).

2.1.2 Plots of Saïda (Semi arid area)

The town of Saïda is located in the western of Algeria, it covers an area of 6 613 km^2. The study stations of Saïda zone are located in the forest of Djaafra Chéraga (Figure 1) occupying a total area of 10 177 ha and located between the mountains of Saida and Dj. Hadid, Oum graf and El-Assa (B.N.E.D.E.R, 1992). Currently divided into 13 cantons, the forest is about 20 to 25 km to the West of the Saïda town. From a climate point of view, the forest of Djaafra Chéraga has a semi-arid climate with a drought period of almost 6 months stretching from May to mid-October and cold in winter with more abundant rainfall. The average rainfall in this area is about 348 mm / year. The temperature varies from -7 ° C in December to 46 ° C in the month of July. The dominant winds are of south and very dry (Sirocco) (ANDI, 2013). According to Lucas (1952), the territory of the wilaya of Saïda consists mainly of secondary terrains generally of Jurassic and Cretaceous sandstones with hardness varying based on the degree of consolidation as well as limestone, marly or dolomitic layers. The depressions and valleys are covered by continental terrains (Fluvial and wind) of tertiary-age often undifferentiated (Mio-Pliocene) and Quaternary soils.

2.1.3 Plots of Naama (Arid area)

The town of Naama is located between the Tellian Atlas and the Saharan Atlas covering an area of 29,514.14 Km^2. The study stations in the Naama zone (Figure 1) are located in the Mekalis Forest and the Green Belt. The total area of these plots was 4,961 ha with latitude of 33°.03'43"97 N and longitude of 33 °.03'43"97 N. The climatic parameters used were those of Mechéria meteorological station (West Algeria), located in the study area. With a mean value of annual rainfall of 200 mm, the rainfall pattern is FSWS (Fall, Spring, Winter, Summer), favorable to vegetative activity despite the length of the drought period from April to October. The Emberger’s rainfall quotient is 20, leading the study zone to be classified in the lower fresh arid bioclimatic stage (Alcaraz, 1969). In general, the climate year of the wilaya is divided into two major seasons: a cool and relatively humid season that extends from November to April and, a dry and hot season from May to October. The maximum average temperature varies between 35.1°C and 47.6°C and the average annual precipitation fluctuates between 150 and 300 mm per year (ANDI, 2013).

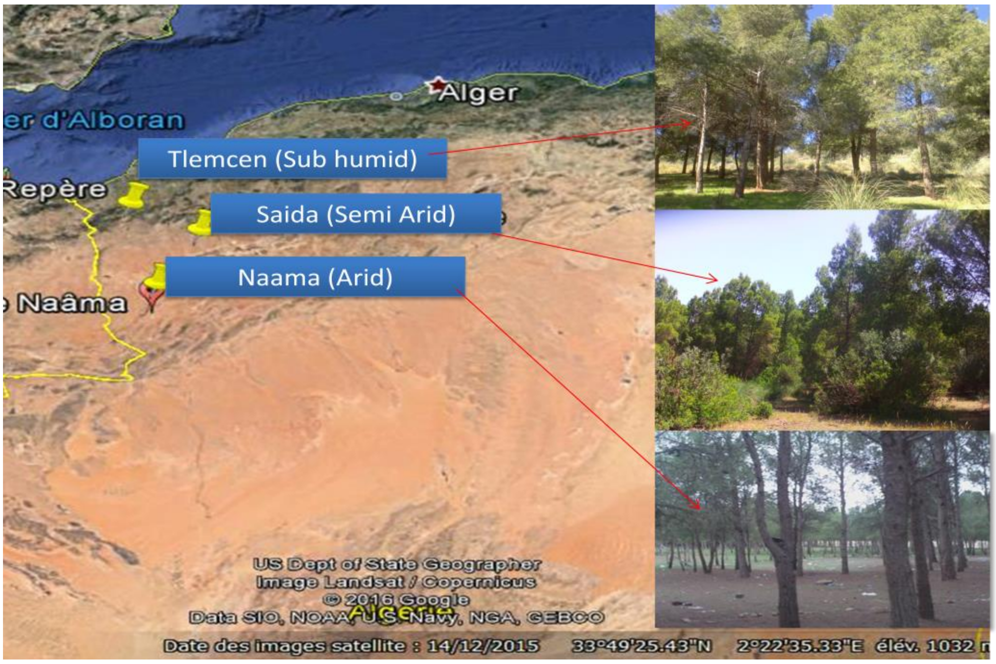

Figure 1: Location of the study zones for Algeria

2.2 Sampling of soils

Five study plots of about 400 m2 each were selected in each study area (zone), making a total of 15 plots for the three zones (Tlemcen, Saida and Naama). On each plot, five soil samples were taken randomly between 0 and 15 cm deep. The samples were then mixed to obtain a composite sample per plot. The samples were then on field sieved to 2 mm, packaged, stored in ice box and transported to the laboratory for analysis. An aliquot of the composite sample was kept fresh (4 ° C) waiting for the microbiological analyses performed within 10 days of samples collection. Most of chemical analyzes were carried out on another air-dried aliquot (Guénon, 2010).

2.3. Catabolic profiles of cultivable microbial communities

Catabolic profiles were determined according to the modified protocol of Garland and Mills (1991). Briefly, 5 grams (dry equivalent) of fresh soil were suspended in 50 ml of a 0.1% sodium pyrophosphate sterile solution (pH 7) and then stirred for 20 minutes on an orbital shaker. The suspension was then centrifuged (500 g, 10 min, 4°C.) and then diluted 50 times in sterile saline solution (0.85% NaCl). The wells of the Ecolog® microplate were inoculated with 125 µl of the diluted suspension. The microplates were incubated at 22 ° C for 6 days.

The optical density development of each well was recorded twice a day at a wavelength of 590 nm (Elisa 960 Metertech® spectrophotometer) until the end of the development of the average optic density of the 31 wells. The incubation time for which the average optical density of all substrates reached a value of 0.4 was calculated according to the method of Garland and Mills (1991) for each sample. That incubation time was used to extrapolate the optical density of each substrate.

2.4. Statistical analyses

The catabolic profiles were studied by a principal component analysis (PCA) calculated on a covariance matrix. The PCA arranges the soil samples on a 2-dimensional factor map. These dimensions (or main components) are selected to explain the maximum variance. A circle of correlations corresponding to a projection on the factorial map in the form of vectors of the catabolized carbon substrates makes it possible to interpret the ordination of the samples. The permutation multivariate variance analysis (PERMANOVA) was used to test the effects of sources of variation on catabolic profiles. In the case of a significant effect of the factor, the differences between the groups are tested in pairs by the Monte Carlo permutation test (999 permutations). These analyzes were carried out on the Primer-e software version 6 (Primer-E Ltd, UK) (Anderson *et al*., 2008).

3. Results

3.1 Catabolic profiles of soils

The different catabolic profiles of the microbial communities of the three study zones are shown in Figure 2. A significant difference in microbial catabolic profiles was observed between the different zones, resulting in an increase in AWCD (Average Well Color Development), the wealth and catabolic diversity as well as the fairness in the sub-humid zone so that these values begin to descend into the semi-arid and the arid whereby values are quite low. These results show that there is a difference between the AWCD and the catabolic diversity between the three zones. However, for catabolic wealth, there is a similarity between the sub-humid zone and Semi arid, but remains different from the arid zone. There was equitably noted an intermediate stage represented by the semi-arid zone and a difference between the sub-humid and arid zone. The results obtained also showed that catabolic wealth and equitability were positively correlated.

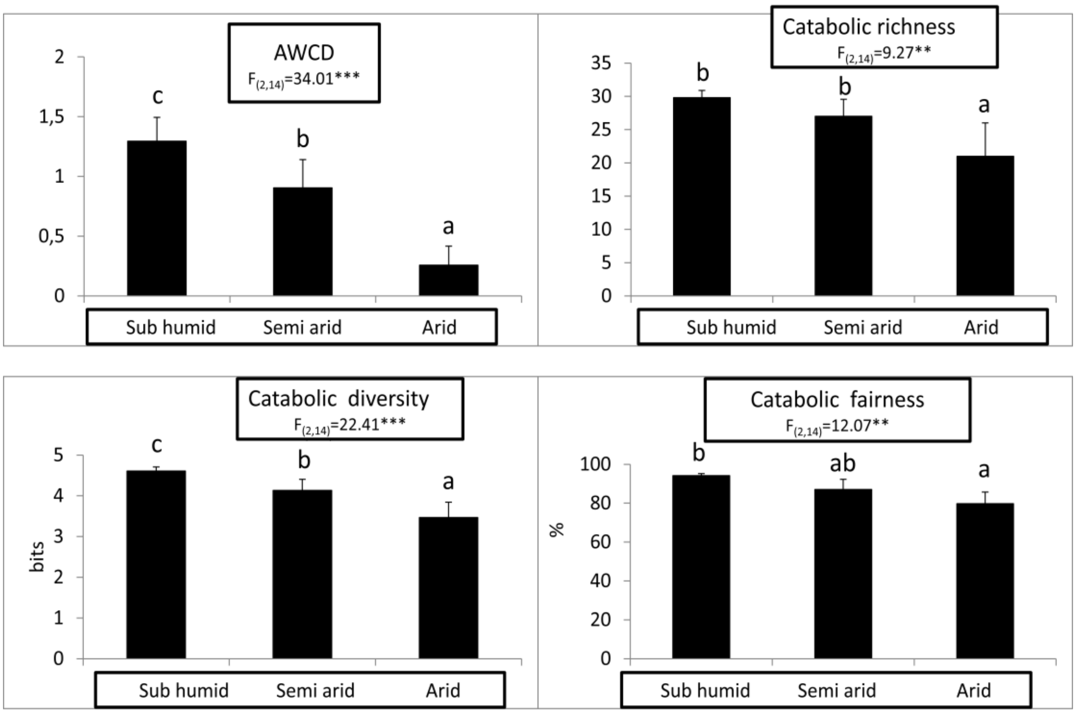

Figure 2: Catabolic profiles of microbial communities

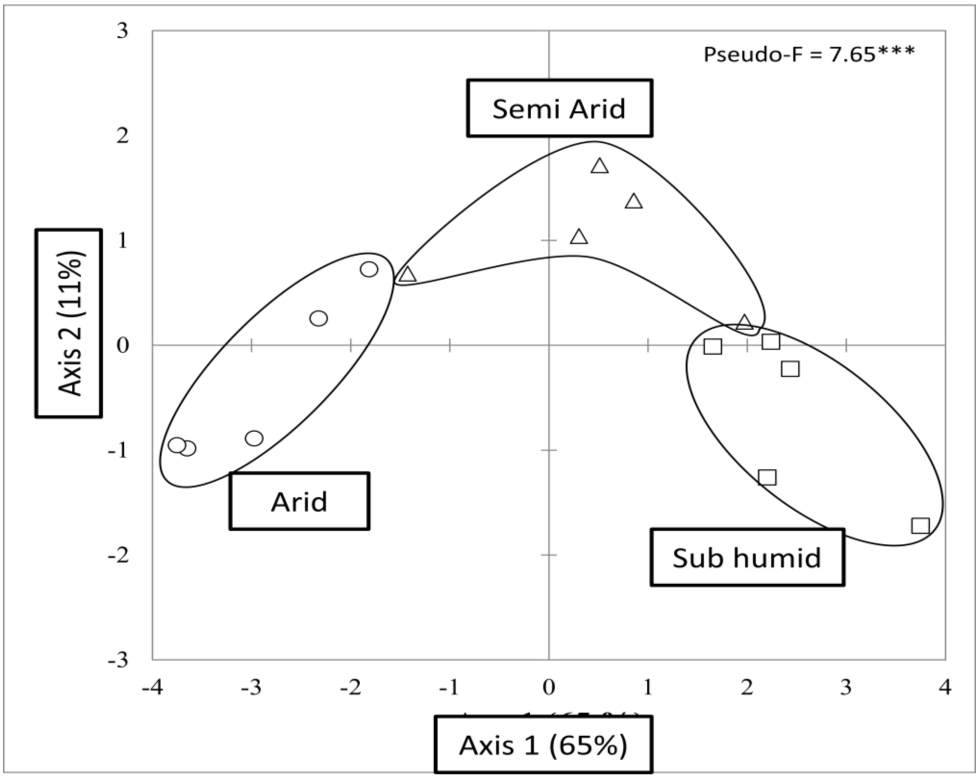

Figure 3: Diagram factorial (axes 1 and 2) generated by PCA on the Biolog® profiles

The PCA generated from the catabolic profiles (Figure 3) revealed a significant change in the functional structure of the microbial communities following a gradient of aridity. Axis 1 with 65% inertia clearly separates the catabolic profiles from the arid and sub-humid zones, and this proved that there is a significant difference between the two microbial communities in terms of wealth and diversity. This axis also showed that there is an intermediate stage which is the semi-arid zone, the one close to the sub-humid and arid zones. In overall, it was noted that it is the gradient of aridity that makes it possible to discriminate the different study areas.

4. Discussion

The degradation of the vegetation cove is generally the first visible symptom of the desertification, but this status of degradation is often accompanied or preceded by great disturbances in the physicochemical and biological properties of the soil (Requena et al., 2001, Cardoso and Kuyper, 2006, Siddiqui and Pichtel, 2008). However, these properties widely determine the quality and fertility, therefore the productive capacity of the soil. From this degradation of the soil, it results in a drastic reduction of the microbial potential. In the Mediterranean basin, weather patterns predict an increase in summer droughts and an increase in temperatures (Gibelin and Déqué, 2003). This global trend would be accompanied by a higher frequency of extreme events such as rainfall and drought (IPCC, 2007). These extreme events, their intensity (duration, frequency ...) and the suddenness with which they occur are likely to directly affect the microflora of the soil in abundance, diversity and functionality and, probably beyond certain thresholds, to not allow a return to a close status to the initial status (Bouchet, 2008) (Guénon, 2010). By their metabolism, micro-organisms develop biosynthetic elements necessary for their reproduction. The availability of substrates and the biosynthesis conditions in a given ecosystem may vary depending on several biotope-specific factors.

The results of this work show that the specific soils to each zone have different points with regard to the profiles of the bacterial populations. The bacterial communities of the sub-humid zone remain very distant from the profiles obtained for the soil of the arid zone. The complexity of the bacterial communities existing in the sub-humid soil is also much more important in terms of wealth and diversity than in the arid zone. This difference can be explained first by the climatic factors, particularly the temperature and precipitation prevailing in each zone. In fact the Tlemcen zone is characterized by 500 mm annually with an average temperature between 6.4 °C (January) and 26.4 °C (July and August); for the arid zone represented by Naama the maximum average temperature oscillates between 35.1 °C and 47.6 °C and the average annual rainfall fluctuates between 150 and 300 mm per year; and for the semi-arid, an intermediate zone, an average rainfall of about 348 mm / year is recorded and the temperature varies from -7 °C in December to 45 °C in July (ANDI, 2013).

Several authors have shown that ecological factors affecting a given microbial ecosystem are numerous and act simultaneously. In the case where biocenosis is subjected to conditions of pH, temperature, extreme osmotic pressure, the selection is effected by removing certain individuals from the population, thereby altering its structure. Among the different parameters that climate brings together, temperature and humidity are factors known to have an impact on the dynamics and structure of microbial communities. Temperature is one of the most important factors in the growth and survival of microorganisms (Rosso et al., 1995) and has been recognized as an important environmental variable influencing population structure (Pettersson & Baath 2003; 2009). The conclusions of several studies in different ecosystems have shown that moisture can promote the growth and microbial diversity (Leyden et al., 1987; Butenschoen et al., 2011). However, under certain conditions, an excessive increase in moisture may slow microbial activity.

The availability of substrates, which vary according to the area studied, may also explain these differences. The soil provides the microbial community with important sources of carbon from plant debris such as leaves or woody tissue. The tree barks, made up of dead cells rich in cellulose, also provide nutrients that can potentiate high microbial activity. The zone of Tlemcen is very rich in trees and herbaceous vegetation, whereas in the forests of the arid zone it is only found Aleppo pine with very open formations and the absence of the other strata which could provide the essential of the soil resources; and this may explain this significant difference in catabolic profiles between the two zones. It is also possible that the large differences measured between the catabolic profiles reflect the limiting role of the quantity and the quality of the organic matter especially in the arid zone.

For the sub-humid zone represented by Tlemcen, the importance of wealth, diversity and microbial equitability may be the results of good resistance to low intensity and non-recurring stresses in a short time of these microbial communities. This is all the more ecologically relevant as it is well established that greater catabolic diversity and equitability in soil would translate resistance to stress and disturbance and lead to better resilience (Degens et al., 2001). This could also be the result of the presence of nitrates in soils which, through increased net nitrification, facilitated the return of catabolic profiles, filling the high microbial nitrogen demand. Some authors have also shown that repeated fires accelerate the return of net nitrification and catabolic profiles after fire. Calderón *et al*. (2001) suggested that a change in the N mineralization process and therefore N availability may be related to changes in catabolic profiles. Moreover, the availability of resources, which, when abundant, allows a better regularity of use of the substrates and therefore higher values of functional diversity (Degens et al., 2001). The work of Degens et al. (2000) showed a strong link between soil carbon content and the catabolic capacities of microbial communities.

Regarding the semi-arid and arid zones, the low rate of diversity and catabolic wealth can be explained by the different stresses of high intensity and recurrence in a very short time, particularly the hydric and thermal stress for the arid zone, are responsible for the structuring of catabolic profiles in this zone. Some authors have shown that the change in the functional structure of microbial communities after 4 cycles of water stress reveals a change in the catabolic capacities of stressed communities (Schimel et al., 1999), which may be likely, according to Mikha *et al.* (2005), to lead to a decrease in the mineralization rates of C, and thus of respiratory activity. Inversely, a strong history of stress modifies these relationships in favor of both resistant and resilient respiratory functions, suggesting possible changes of the structure of communities with high or diversified catabolic potentialities (Schimel et al., 1999).

Conclusion

Because of their diversity, activity and abundance, microorganisms ensure stability within a biological community, thus playing a key role in the balance of ecosystems. The results of the present study showed that catabolic diversity and wealth follow a gradient of aridity. In fact, the catabolic profiles of microbial communities are structured by the temperature and the rainfall that dominate in these regions following the bioclimatic stage of these zones. It is the climatic factors that determine the distribution of soil micro-organisms via the resources and qualities of these soils. A significant and long-term increase in temperature can lead to a regression or irreversible disappearance of the microbial communities of these soils. What is happening in the arid zone reflects the status of the studied soils in the future in the semi-arid and sub-humid zones because of the climatic changes that will disrupt the ecological balance of these sensitive and vulnerable areas. If nothing is done to protect these soils, there is a definitive risk of desertification in all these zones.

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