Seasonal Changes of Microbiological Properties in Steppe Soils from Degraded Arid Area in Tunisia

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There is growing interest in assessing soil quality using microbial properties in desertified areas. A study was conducted in arid soils subjected to desertification in the south of Tunisia to illustrate the effects of dominating steppes of Stipa tenacissima *and* Anthyllis sericea *subsp.* henoniana *on soil chemical, microbial, and biochemical properties. Soil samples were collected beneath the canopy of* S. tenacissima, A. sericea *subsp.* Henoniana, *and open areas in the rainy and dry seasons.*

These steppes showed higher values of soil organic carbon content (Corg). Microbial biomass carbon (Cmic) was also greater under steppes and reached $289\,\mu g\,C\,g^{-1}$ soil under Anthyllis canopies. Studied enzyme activities (dehydrogenase, phosphatase and β-glucosidase) increased greatly beneath steppes showing dehydrogenase activity of $159\,\mu g\,INTF\,g^{-1}$ soil d^{-1}, while it did not reach $36\,\mu g\,INTF\,g^{-1}$ soil d^{-1} in open areas. Conversely, lower values of C/N ratio and metabolic quotient (qCO_2), particularly in the vicinity of A. sericea *subsp.* henoniana.

The environmental conditions significantly affected studied properties: the soil organic carbon, microbial biomass, and enzyme activities were reduced in dry season, while C/N ratio and qCO_2 were higher.

Our results suggest the importance of vegetation cover in regulating soil microbial processes in arid degraded areas and that the wet season may play an important role in driving seasonal changes in microbial biomass and activity.

Keywords *A. sericea* subsp. *Henoniana*, arid soils, enzyme activities, microbial biomass, *S. tenacissima*, seasonal change

Introduction

Desertification is considered as a serious problem referring to land degradation in arid and semiarid areas (Albaladejo et al., 1998). Land degradation has a great influence on many soil quality attributes. Soil physico-chemical, microbiological and biochemical properties rapidly respond to soil quality and its degradation, especially the last two being the most sensitive (Ros et al., 2003). Microbial indicators represent the key elements to evaluate soil quality (Ros et al., 2003).

The soil microbial biomass, which is part of the active soil organic matter pool, can give an early indication of soil organic matter turnover (Powlson et al., 1987). According to Anderson (2003), the ratio of the percentage of soil Cmic to Corg (Cmic/Corg ratio) can be used as early warning indicator of the deterioration of soil

quality. The ratio of basal respiration to Cmic or qCO_2 is recognized as a valuable measure of the maintenance energy requirement of soil microbes (Anderson, 2003). The qCO_2 has been used as bioindicator of the effects of environmental factors on the soil microbial populations (Anderson and Domsch, 1993).

Enzyme activities are sensitive to quantitative changes in soil organic matter. In fact, the soil enzymes are known to play critical roles in organic matter decomposition and nutrient cycling and are considered as important components for biochemical functioning of soils (Patra et al., 2006; Makoi and Ndakidemi, 2008). Several enzyme activities are reported to be useful for assessing soil microbial activity (Nannipieri et al., 1990). Phosphatase and β-glucosidase (hydrolytic enzymes) activities have been widely studied because of their importance as indicators of soil organic matter changes (Gil-Sotres et al., 2005). Being exclusively intracellular and linked to intact and viable cells, dehydrogenase activity is considered as indicator of the presence of viable microorganisms (Trevors, 1984).

Soil degradation processes are closely linked to climatic conditions. It was reported that the seasonal variations control fluctuations in soil microbial biomass and activity and this control was specific to the studied site (Yao et al., 2011). In many studies, decreases in soil microbial density and activity have been reported in dry season (Bastida et al., 2006a; Devi and Yadava, 2006). However, some reports highlighted the positive influence of dry season on the soil microbial community (Yao et al., 2011). Therefore, it is important to assess the response of soil microbial processes to seasonal change.

In Tunisia, 65% of the area is affected by desertification, covering most arid and semi-arid lands. Considering that vegetation cover plays a key role in desertified areas, intensive studies concerning the degraded areas have been carried out, particularly in the fields of dynamics of vegetation dominating the arid regions in Tunisia (Noumi et al., 2010). However, very few have focused on soil quality. The aim of this study was therefore to assess the influence of dominating vegetation cover on soil quality in an arid area in Tunisia. We evaluated soil quality by measuring chemical (pH, Ec, Corg, and C/N ratio), microbial (Cmic, Cmi/Corg ratio, and qCO_2) and biochemical parameters (dehydrogenase, phosphatase, and β-glucosidase activities). Also, considering that the magnitude of the impact of seasonal change on soil microbiological properties showed different responses among studies, we assessed how seasonal change impacts the soil microbial community in these soils. Therefore, all studied soil properties were investigated in dry and rainy seasons.

Materials and Methods

Study Site and Soil Sampling

The studied site is located in an arid steppe in Matmata region (Gabès Governorate), in South-Eastern Tunisia. Its climate is arid Mediterranean and it receives an average of 200 mm rainfall a year. The region has been submitted to harmful degradation due to climatic conditions and livestock grazing. The ecosystem is dominated by steppes of *S. tenacissima* L. and *A. sericea* subsp. *henoniana* (Coss.) Maire with open vegetation-free areas, but there are a large number of other species with low soil coverage, such as *Ebenus pinnata* Aiton, *Genista microcephala* Coss. et Dur, and *Juniperus phoenicea* L.

For the study site, soil samples were collected: (a) beneath *S. tenacissima* canopy, (b) beneath *A. sericea* subsp. *henoniana* canopy, and (c) adjacent open areas outside

the canopies, at least 20 m far from the plants. Samplings were carried out in two different seasons: dry season (August) and rainy season (December) 2010 in order to study the influence of seasonal change. The average rainfall is 6 mm per month in the dry season and 78 mm per month in the wet season.

Soil samples were collected from the top 15 cm of soil and four replicates were taken for each sample. Before analysis, samples were stored at 4°C and sieved (mesh size 2 mm) to remove root particles and other organic debris.

Soil Analysis

Soil pH and electrical conductivity (EC) were determined in water saturated extract (Afnor, 1987) by pH meter and conductivity meter, respectively. The soil Corg was quantified by the Walkley and Black method (1934) and the total nitrogen was determined using the Kjeldahl method (Jackson, 1958).

Soil Cmic was evaluated using the fumigation–extraction method. According to Amato and Ladd (1988), measurements of ninhydrin reactive nitrogen released and extracted from soils with KCl, after a 10-day fumigation period, provide a useful sensitive assay of biomass carbon.

Soil respiration was determined by measuring CO_2 production as described by Ohlinger (1995) and the qCO_2 was determined as the ratio between soil respiration and the Cmic content.

For enzyme activities, phosphatase and β-glucosidase activities were determined as described by Caravaca et al. (2005). For phosphatase activity, samples received *p*-nitrophenyl phosphate (PNPP), while for β-glucosidase activity, they received *p*-nitrophenyl-β-D-glucopyranoside (PNG). The *p*-nitrophenol (PNP) formed was determined in a spectrophotometer at 398 nm (Tabatabai and Bremner, 1969). Dehydrogenase activity was determined by the method of Garcia et al. (1997). Samples were incubated with 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride (INT). The iodo-nitrotetrazolium formazan (INTF) formed was measured colorimetrically at 490 nm.

Statistical Analyses

Statistical analyses were performed with a SAS statistical package (SAS Statistical Institute, 1998). The data were subjected to ANOVA and the statistical effects of season and vegetation cover were tested. Comparisons among means were made using the Least Significant Difference test at the 5% levels of significance ($p \leq 0.05$).

Results

The results of two-factors ANOVA (vegetation cover and season) for chemical, microbial and biochemical properties are shown in Table 1. Both vegetation cover and season have significant effects ($p < 0.05$) on all studied parameters.

Chemical Properties

Soil chemical properties were significantly influenced by vegetation cover and season (Table 1). All soils analyzed were characterized as sandy loam of alkaline pH. Compared with open areas, soil pH was lower beneath *Stipa* and *Anthyllis* canopies

Table 1. Results of two-factors Anova (vegetation cover and season) for chemical and microbiological properties

Factors	Season		Vegetation cover		Vegetation cover*Season	
	F^b	P^c	F^b	P^c	F^b	P^c
pH	7.28	0.0147	7.99	0.0033	0.34	0.7152
Ec^a	4.98	0.0386	13.91	0.0002	0.22	0.8051
$Corg^a$	12.31	0.0025	17.06	0.0001	0.89	0.4261
TN^a	18.69	0.0004	21.87	0.0001	1.21	0.3209
C/N ratio^a	6.40	0.0209	4.23	0.0311	0.25	0.7852
$Cmic^a$	80.03	0.0001	73.41	0.0001	8.98	0.0020
Cmic/Corg ratio^a	18.09	0.0005	17.26	0.0001	2.04	0.1585
RES	48.04	0.0001	1.65	0.2205	31.02	0.0001
$qCO_2{}^a$	36.98	0.0001	32.01	0.0001	5.85	0.0110
$DEHY^a$	32.24	0.0001	58.64	0.0001	3.89	0.0395
$PHOSP^a$	23.33	0.0001	31.43	0.0001	5.00	0.0187
ß-GLUC^a	59.67	0.0001	65.91	0.0001	8.06	0.0032

[a]Properties: Ec, electrical conductivity; Corg, soil organic carbon; TN, total nitrogen; C/N ratio, carbon/nitrogen ratio; Cmic, microbial biomass carbon; RES, soil respiration; qCO_2, respiratory quotient; DEHY, dehydrogenase activity; PHOSP, phosphatase activity; ß-GLUC, ß-glucosidase activity.
[b]F-ratio.
[c]p-values.

(Figure 1). However, soil Ec increased under studied canopies, particularly beneath *Anthyllis* canopies (Figure 1). The steppes of *S. tenacissima* and *A. sericea* subsp. *henoniana* showed higher values of soil Corg, in comparison with open areas (Figure 1). For C/N ratio, the open areas showed the highest values, while the steppes of *S. tenacissima* showed the lowest values.
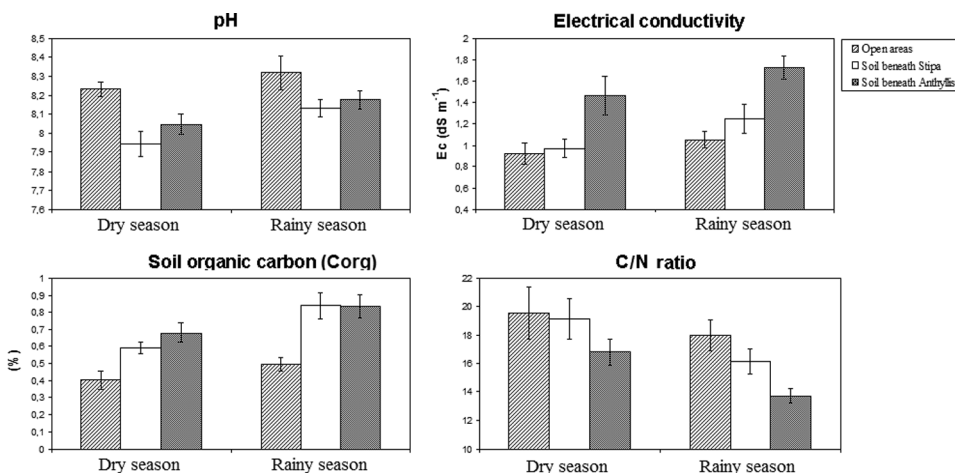
Figure 1. Soil pH, Electrical conductivity (Ec), soil organic carbon (Corg), and C/N ratio in the studied soils. Error lines correspond to standard deviation ($n = 4$).

Compared with dry season, soil pH, Ec and Corg showed significant higher values in rainy season. However, lower values were found for C/N ratio in rainy season.

We did not find a significant effect of season*vegetation cover interaction on soil chemical properties.

Microbiological Properties

We found significant effects of vegetation cover and season on Cmic and Cmic/Corg ratio (Table 1). The values were lower in open areas, compared to canopied soils. Also, the values were higher in rainy season compared to dry season (Figure 2).

For soil respiration, no significant effect of vegetation cover was detected. However, significant effect of season was observed and the highest values were detected in dry season. A significant effect of vegetation cover on the qCO_2 was found and values significantly decreased from open areas, which have the lowest microbial biomass, to canopied soils which have the greatest microbial biomass. There was also a significant variation in qCO_2 between dry and rainy seasons (Figure 2), being the highest in dry season, and lowest in rainy season (Figure 2).

A significant effect of vegetation cover*season interaction was detected for soil Cmic and qCO_2.

Biochemical Properties

Dehydrogenase, phosphatase, and β-glucosidaese activities were significantly influenced by vegetation cover and season (Table 1). For phosphatase activity, soils beneath *Stipa* and *Anthyllis* canopies behaved similarly and were clearly higher than open area (Figure 3). For Dehydrogenase and β-glucosidaese activities, soils beneath *Anthyllis* canopies showed higher values than soils beneath *Stipa* canopies, whereas the lowest values were observed in open areas (Figure 3).
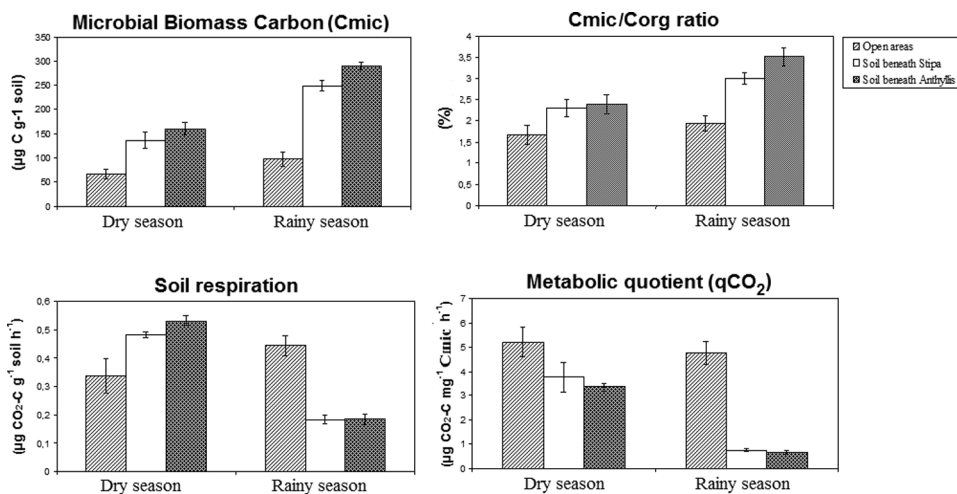

Figure 2. Soil microbial biomass carbon (Cmic), soil respiration, metabolic quotient (qCO_2), and Cmic/Corg ratio in the studied soils. Error lines correspond to standard deviation ($n = 4$).
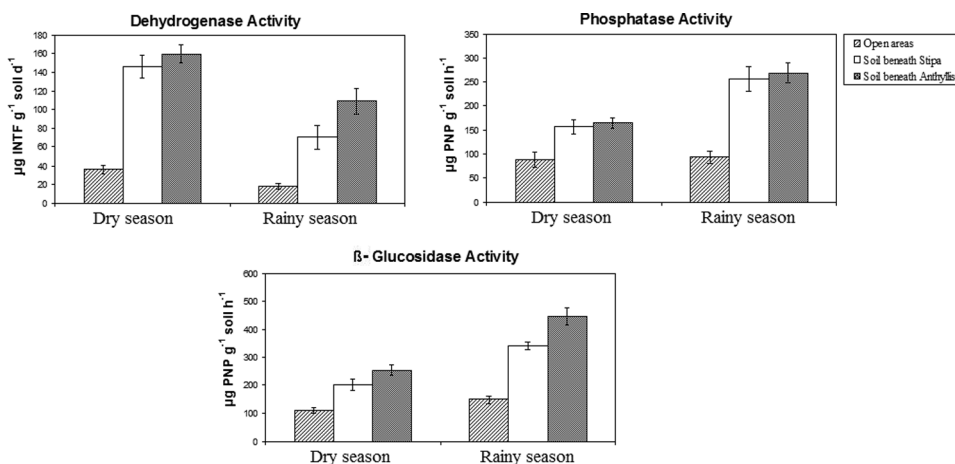
Figure 3. Soil dehydrogenase, phosphatase, and ß-glucosidase activities in the studied soils. Error lines correspond to standard deviation ($n = 4$).

The studied enzyme activities were affected by season and the highest values were detected in rainy season, while dehydrogenase activity was higher in dry season than in rainy season. Also, there was a significant interaction effect between vegetation cover and season for these enzyme activities (Table 1).

Discussion

Knowing that soil quality in degraded soils is affected by many biotic and abiotic factors, its improvement is a complicated process. The results of this study and others in arid soils (Caravaca et al., 2005; Bastida et al., 2007) indicated that vegetation cover has improved soil chemical and biological properties. In our investigation, the steppes of *S. tenacissima* and *A. sericea* subsp. *henoniana* showed significant effects on soil chemical properties, compared with open areas: they have contradictory effects on soil pH and Ec, by decreasing pH and increasing Ec in the studied soils. The tendency for the pH to decrease beneath plant canopies may be caused by the larger exudation of acidic root compounds (Garcia et al., 2005).

According to Pascual et al. (2000), the low level of organic matter in arid degraded soils constrainsts soil quality. In our study, the highest soil Corg content shown by the soils, beneath the steppes of *S. tenacissima* and *A. sericea* subsp. *henoniana* canopies, gives a particular importance of these steppes for the fertility of the studied arid soils. Also, we identified a significant decrease in soil C/N ratio beneath studied canopies, particularly beneath the steppes of *A. sericea* subsp. *henoniana*, which demonstrated increased soil nitrogen content. Being a legume, this species can improve soil nitrogen content through symbiotic fixation of nitrogen with nitrogen fixing bacteria and mycorrhizae (Zakhia et al., 2004).

The presence of steppes of *S. tenacissima* and *A. sericea* subsp. *henoniana* improved the density of microbial populations in soil as their high Cmic reflected. Results obtained agree with those obtained by Garcia et al. (2005) and demonstrated the favorable role of vegetation cover for soil microbes. Also, the higher Cmic/Corg ratio in the studied steppes may be explained by the higher availability of organic matter to support soil microbial populations with time (Jia et al., 2010).

The decrease of qCO_2 values beneath *Stipa* and *Anthyllis* steppes in this arid area demonstrated the substrate utilization efficiency of the soil microbial communities and the improved rhizosphere health (Wardle and Ghani, 1995), whereas the higher qCO_2 values in open areas indicated their poor soil health.

Dehydrogenase in soil is an oxidoreductase enzyme which catalyses biological oxidoreductive reactions in microorganisms (Toseland et al., 2005). It is often used to determine microbial activity in soil (Nannipieri et al., 1990). Therefore, the increase of this enzyme activity beneath *Stipa* and *Anthyllis* steppes may indicate their role in promoting soil microbial processes. This was also in agreement with the findings obtained by Garcia et al. (2005).

The increase of the phosphatase activity beneath *Stipa* and *Anthyllis* steppes is possibly a response to their higher phosphorus requirements (Caldwell, 2006). The β-glucosidase activity increased beneath *Stipa* and *Anthyllis* steppes. Considered as available substrates for this enzyme, the exudates of these species are known to enhance β-glucosidase activity (Caravaca et al., 2005). The activity of these extra-cellular enzymes is of vital importance for microbial growth in soil as shown by Bastida et al. (2007).

The highest effect of vegetation cover on microbial and biochemical properties was induced by *A. sericea* subsp. *henoniana*. Many authors reported the improvement of soil microbial density and activity in the rhizosphere of leguminous plants (Traoré et al., 2007; Cao et al., 2008). Being a legume and following their ability to develop symbiotic associations with both rhizobial bacteria and mycorrhizal fungi, *Anthyllis* steppes will improve the fertility of soil by accumulating organic matter. Compared with *Stipa* steppes, the greatest soil Cmic and Cmic/Corg ratio and the lowest C/N ratio showed the active organic matter in the rhizosphere of this legume. The highest ability of *Anthyllis* steppes to enhance microbial communities could be attributed to differences in the quality and quantity of plant litter and/or root exudates (Traoré et al., 2007). Also, the greatest enzyme activities beneath *Anthyllis* steppes confirmed the importance of this legume in improving soil microbial activities (Delay et al., 1994).

We also found that total soil chemical, microbial, and biochemical properties varied with season. In general, soil Cmic was high in December (rainy season) and low in August (dry season). Several studies on soil microbial properties reported that maximum value of microbial biomass is obtained in rainy period and minimum in dry period (Devi and Yadava, 2006), which are in conformity with our report. Also, the ratio of Cmic/Corg in soil showed a significant increase in rainy season compared to dry one. These findings indicated that soil microbial communities are more metabolically active and soil carbon turnover is greater in the rainy season than in dry season. The tendency of metabolic quotient to increase in August may be attributable to the stressful conditions with temperature increase (Bastida et al., 2006b). The fact that C/N ratio varied considerably between the seasons with the great increases in total microbial biomass from August to December would suggest that perhaps nitrogen availability exert as large a control on the microbial community in this nitrogen poor soils in which nitrogen availability largely regulates soil microbial processes (Cleveland et al., 2004).

Enzyme assays can indicate microbial response to environmental change (Moorhead and Sinsabaugh, 2000). The increase of dehydrogenase activity in dry season demonstrated that the activity of this enzyme is related to the stressful environmental conditions and namely the increase of temperature. This entails the increase of metabolic activity of microorganisms in summer that diverts more energy

to maintenance (Bastida et al., 2006a). On the contrary, phosphatase and
β-glucosidase activities decreased greatly in dry season. These results seem to agree
with the ideas of Li and Sarah (2003). In their view, these enzymes play a relevant
role in the decomposition of plant remains and their activities decrease when organic
matter is reduced in soils in dry season.

Our results demonstrated also that season would modulate the magnitude of
Stipa and *Anthyllis* steppe effect on the studied soil properties; In fact, rainy season
helped the studied steppes to improve soil microbial processes beneath their canopy,
while dry season impeded it. It was reported that the shed of root exudates, root
decay, and fresh litter is more enhanced in the wet season, which could be responsible
to easily decomposable organic substrate for soil microorganism growth (Joergensen
et al., 1994). These mechanisms could account for the influence of seasonal conditions
on the effect of vegetation on microbial communities.

Therefore, it appears that environmental conditions in rainy season were more
favorable for microbial growth, as shown by the increase of microbial biomass and
the enhanced enzyme activities (phosphatase and β-glucosidase activities) to satisfy
the greater nutritional requirements. While in dry season, high metabolic activity
(metabolic quotient and dehydrogenase activity) demonstrated that microorganisms
diverted more energy to maintenance (Garcia and Hernandez, 1996). The magnitude
of seasonal change to affect soil microbial properties had been assessed widely in bib-
liography and many authors confirmed our results. Other findings have reported that
microbial density and activity decrease in the dry season (Devi and Yadava, 2006).

Conclusion

The present study demonstrated the effects of *S. tenacissima* and *A. sericea* subsp.
henoniana steppes on soil microbiological processes in two distinct environmental
seasons (rainy and dry season) in the arid soils in Tunisia. The rates of change of
studied soil parameters demonstrated that soil microbial and biochemical properties
are more sensitive indicators of climate change than total soil organic matter.

Our results indicated that *Stipa* and *Anthyllis* steppes influence soil chemical,
microbial and biochemical properties, and therefore its quality. This has great
ecological significance due to the role of these steppes in improving soil quality in
the studied degraded area. Microbial communities were more active and had greater
biomass beneath *Anthyllis* steppes, which highlights its role in future restoration
programs of degraded areas.

Furthermore, the studied soil properties were sensitive to seasonal change. Dry
season presented markedly smaller and less active microbial communities. Therefore,
our findings showed that microbial populations change seasonally and thereby
influence soil quality. More studies are needed to better assess the soil quality in
the arid degraded soils, particularly for rehabilitation programs.

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