BIOLOGICAL AND BIOCHEMICAL PARAMETERS DISTINGUISHING SOIL MICROSITES UNDER DIFFERENT MEDITERRANEAN SHRUB SPECIES

E.M. PAPATHEODOROU[1], N. MONOKROUSOS[1], D. CHALKOS[1], G. KARRIS[1], M. ARGYROPOULOU[2], D. VOKOU[1], J. DIAMANTOPOULOS[1] AND G.P. STAMOU[1]

1-Department of Ecology, School of Biology, Aristotle Univ. of Thessaloniki, Greece, 540 06
2-Department of Zoology, School of Biology, Aristotle Univ. of Thessaloniki, Greece, 540 06

Introduction

In the Mediterranean region, the stress season associated with high temperatures and drought lasts for about 6-8 months and influences strongly the growth of vegetation. Moreover, Mediterranean soils are considered of limited fertility and development (Connacher & Sala 1998). This is generally attributed to the rather low litter input, which is the result of the limited biomass production and concomitant litter accumulation. In addition, the low humidity that prevails for a considerable part of the year conditions the short periods of activity of soil organisms (Stamou 1998).

Environmental stress in Mediterranean areas is associated with temporal and spatial heterogeneity of soils properties, particularly those related with high sand content, shallow soil depth, and low water availability (Roy et al. 1995, Roberts et al. 2001). More specifically, heterogeneity in space creates microsites that offer a considerable number of potential habitats for colonization to the various soil organisms. Climate, soil, and litter related heterogeneities superimpose on this micro-topographic heterogeneity resulting into fine-grained spatial structures (Stamou 1998). It is expected that procedures linked to organic matter transformations and nutrient release will considerably differ among such microsites.

Since the major part of organic matter decomposition and nutrient transformations in soil are mediated by microbes (Swift et a1.1979), it is imperative to know the structure and activity of microbial biomass, if the nutrient status of an ecosystem is to be assessed (Eijsackers 2001). In this paper, we aimed to investigate whether shrubs belonging to different species, life forms, and strategies (evergreen or seasonally dimorphic) create soil microhabitats that clearly differ in their biological and biochemical parameters. These parameters were recorded within the upper soil layer, under the canopy of shrubs (up to 5 cm in depth under the litter), within the stress period, in September 2000.

Materials and Methods

Site characteristics

The study area is located in Northern Greece (40°20' N latitude and 23°12' E longitude) in

the Prefecture of Halkidiki, between the villages of Petralona and Eleochoria. Parent material consists of alluvium deposits derived from the nearby limestone hills. *Quercus coccifera*, *Globularia alypum*, *Juniperus oxycedrus, Thymus capitatus, and Erica* sp., are the dominant woody species with quite similar distribution in the vegetation. Goats (about 500 herds) lightly graze throughout the area. Shrubs have a maximum height of about 1-m.

The vegetation of the study area consists of species that represent both types of the major strategies associated with woody species of Mediterranean-type ecosystems, i.e. evergreen *sclerophyllous* and *phryganic* (drought deciduous or seasonally dimorphic) shrubs. In addition, the vegetation consists to a large degree of one-species-patches, interspersed by grass-covered areas, rather than mixed patches of shrubs belonging to different species. Given its composition and spatial structure, the study area is most suitable for our research objectives.

Concerning the climatic characters of the area, data from the nearby village of Petralona (1981-1990) show a mean monthly temperature of 19°C, and overall mean annual rainfall of 411 mm. As shown in the ombrothermic graph for Petralona (Fig. 1), the drought period lasts for 7 months, from April to October.

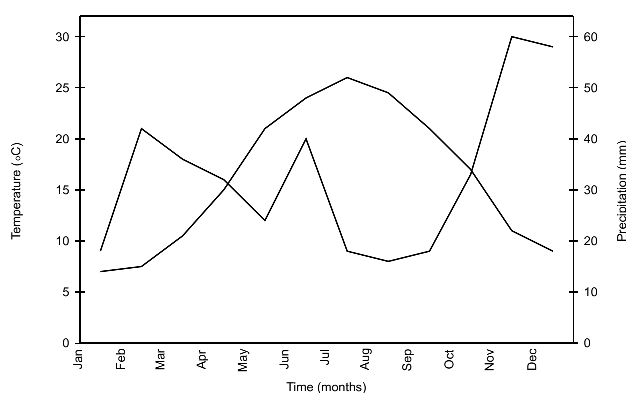

Figure 1. Standard ombrothermic graph for the area (data from the meteorological station of Petralona, for the period 1981-1990)

Sampling and biochemical analyses

The upper 5-cm of soil under the canopy of the five dominant shrubs as well as from the grass covered areas between them was sampled by means of a cylindrical corer (diameter 7.5 cm) in mid-September 2000. Five replicate samples were collected from each of the six microsites. The soils were analyzed for C-microbial (method of Jenkinson & Powlson 1976, with the modifications recommended by Ross 1990), CO_2-evolution at 10°C, fungal biomass (as estimated by the ergosterol method; Djajakirana et a1. 1996), bacterial substrate utilization at 28°C, for 120 h (used as an index of bacterial activity), by use of GN Biolog plates (a modified BIOLOG assay; Vahjen et a1. 1995), rate of C-mineralisation at 28°C, C-organic, N-organic and N-inorganic (NH_4 and NO_3). The size of the bacterial population was estimated in nutrient agar cultures, where diluted soil solution was added. After their incubation for 24 h at 30°C, the number of bacteria colonies was counted.

Results and Discussions

The size of bacterial populations was 2 X 10^4, on average. Lowest population size was recorded in samples from *Erica* sp. microsite and highest from *Q. coccifera* microsite (Fig. 2).

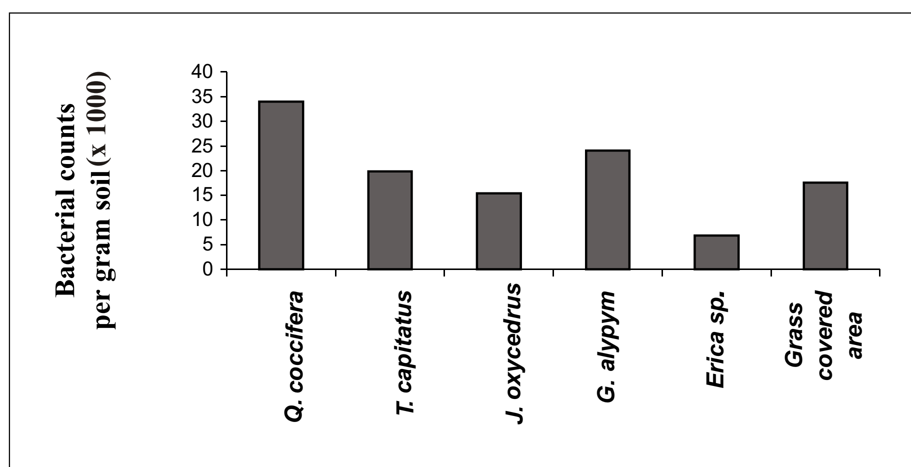

Figure 2. Average bacterial population size in the different microsites

The ninety-six different substrates/wells of the Biolog plates were grouped into six categories, i.e. polymers, carbohydrates, carboxylic acids, amines, amino acids and miscellaneous. Mean utilization per substrate type representing bacterial activity is given in Table 1. One-way ANOVA revealed significant differences among microsites. Low activity values were recorded in the soil samples from the grass covered area and under *Erica* sp. and *J. oxycedrus* canopies, whereas the highest values were recorded in samples taken under the canopy of *G. alypum*. Intermediate to high values were associated with soil under *Q. coccifera* and *T. capitatus* canopies.

Table 1. Mean bacterial utilization per substrate type (standard error) by use of the Biolog method. Different letters, in vertical lines refer to statistically significant differences among microsites.

	Polymers	Carbo-hydrates	Carbo-xylic acids	Amines	Aminoacids	Misce-llaneous
Grass covered area	0.233 ±0.16 **a,b**	0.250 ±0.06 **a,b**	0.190 ±0.08 **a**	0.054 ±0.07 **a,e**	0.100 ±0.06 **a**	0.071 ±0.08 **a**
***Erica* sp.**	0.081 ±0.10 **b**	0.113 ±0.05 **b,c**	0.155 ±0.07 **a**	0.044 ±0.07 **a**	0.112 ±0.06 **a**	0.044 ±0.07 **a**
Globularia alypum	0.626 ±0.20 **c**	0.617 ±0.10 **d**	0.528 ±0.10 **b**	0.214 ±0.15 **b,c**	0.533 ±0.10 **b**	0.474 ±0.14 **b**
Thymus capitatus	0.417 ±0.17 **a,c**	0.345 ±0.07 **a,e**	0.463 ±0.09 **b**	0.210 ±0.12 **b,e,c**	0.413 ±0.09 **b**	0.165 ±0.10 **a,c**
Quercus coccifera	0.447 ±0.20 **a,c**	0.399 ±0.08 **e**	0.414 ±0.08 **b**	0.215 ±0.13 **c**	0.550 ±0.10 **b**	0.276 ±0.11 **c**
Juniperus oxycedrus	0.179 ±0.14 **a,b**	0.107 ±0.04 **c**	0.087 ±0.06 **a**	0.056 ±0.09 **a,b**	0.171 ±0.08 **a**	0.095 ±0.07 **a**

In order to estimate the functional diversity of the bacterial communities, we used Shannon and Evenness indices on the basis of the number and amount of the utilized substrates. Bacterial communities did not differ significantly with respect to diversity; H' varied within the range 1.215 to 1.822. The same holds true for Evenness; E varied within the range 58.4 to 79.2 %. It is obvious that the differences in bacterial activity among microsites cannot be explained in terms of differences in the structure of the bacterial community.

Table 2. Diversity and Evenness indices of the soil bacterial community at the six mixrosites.

Microsites	Diversity $H' = - \ p_i \ln p_I$ p_i = proportional colour development of the *i*th well over total colour development of all wells of a plate	Evenness $E = H'/\ln S$ H'= index of diversity S = number of wells with colour development
Grass-covered area	1.457	0.748
Juniperus oxycedrus	1.215	0.584
Globularia alypum	1.572	0.792
Quercus coccifera	1.822	0.711
Thymus capitatus	1.753	0.761
Erica sp	1.234	0.589

Since data analysis did not reveal differences in the structure of the bacterial community among microsites, we examined whether the latter could be distinguished on the basis of the other biochemical parameters examined, i.e. CO_2 evolution, fungal biomass, organic and inorganic nitrogen, C-microbes and carbon mineralisation. To reach this goal, discriminant analysis was performed.

The analysis revealed highly significant discrimination of microsites ($p < 0.01$) (Fig. 3). In relation to the first axis of the DA, samples taken from the grass covered microsite along with those taken under the canopy of *Erica* are oriented towards the left end-point while the rest of the samples tend to occupy the right end-point of the axis.

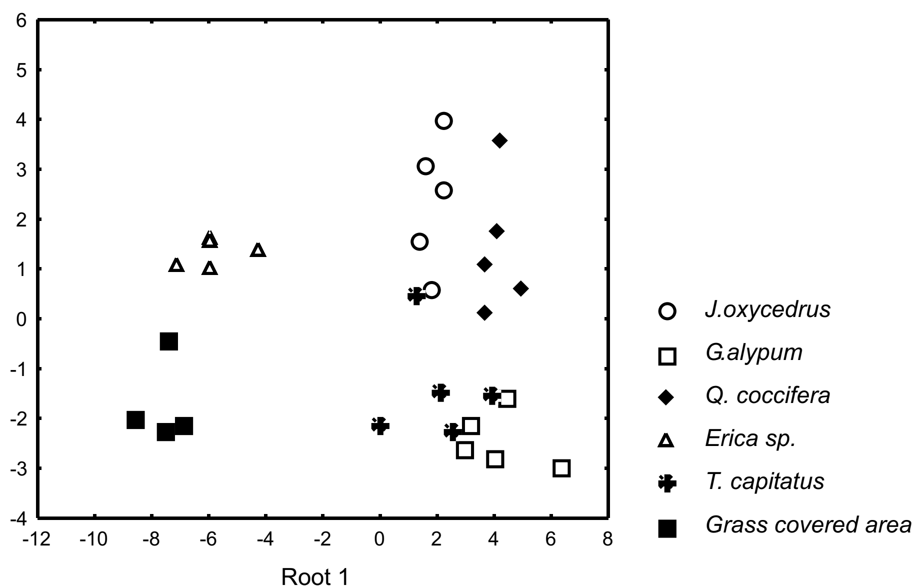

Figure 3. Results of discriminant analysis (DA) based on the soil biochemical parameters (except that of substrate utilization by bacteria).

The principal discriminating factor is the fungal biomass. Low values of fungal biomass were recorded in samples from the grass-covered microsite and under *Erica* canopy and high values in all other microsites. In relation to the second axis, two groups were also distinguished. Samples from the canopies of the sclerophyllous shrubs (*J. oxycedrus*, *Q. coccifera,* and *Erica* sp.) tend to occupy the upper part of the graph, whereas samples from the phryganic species (*G. alypum*, *T. capitatus*) as well as from the grassy patches tend to be ordinated towards the lower part of the graph. The factors overriding this upper-bottom discrimination of samples are organic nitrogen and C-microbial; higher values for both parameters were recorded under the sclerophyllous shrubs.

Conclusions

Compared with other ecosystem types (Donegan et al. 2001), much lower sizes of bacterial and fungal[1] populations were recorded in our study area. This can be attributed to the following reasons: (i) because our measurements were done during the least favorable time of the year for a Mediterranean ecosystem, i.e. during the hot, dry period, and (ii) because the vegetation cover in our study area was much lower. In contrast, when comparing the bacterial activity that we recorded to that found in a more xeric Mediterranean area (Ekschmitt et al. 1999), it was proved higher on average. Bacterial activity under high temperatures and low soil humidity can be associated with resistant to water stress Mediterranean microflora (Van Gestel et a1. 1993).

Results of our work show that the ability of the soil bacterial community to metabolise carbon sources differs among microsites. However, taking into consideration the values of evenness (mean value equals 70%), we can infer that trophic specialisation among bacterial populations is rather low. This may be due to the fact that cosmopolitan and easily adaptable bacterial populations are evenly distributed in the examined microsites. The low

activity recorded in the grass-covered microsite can be explained in terms of the lower input of organic material since an association is expected between the bacteria distribution and soil organic matter (Wood 1995).

Apart from the Biolog tests, the other biochemical parameters examined also displayed highly significant discriminatory capacity. Fungal biomass, N-organic and C-microbial contributes most to distinguishing the microsites. Samples taken under *Erica* canopy and those from the grass-covered area differ from all others; they are associated with low fungal biomass.

Most *Erica* species grow on poor or acidic soils (Cruz & Moreno 2001). This may explain the ordination of samples under *Erica* canopy close to those from the grass-covered area. We can conclude, therefore, that the low fungal biomass is a reliable indication of poorly developed soils. Hendrikson et al. (1985) showed that moisture content is positively correlated with microbial biomass on forest floors. It is probable that shrubby species, which create more favorable humidity conditions in the soil than their grass and *Erica* neighbors, enhance fungal activity.

Samples collected under the canopies of the phryganic species and from the grass-covered area differ from those taken under the canopies of the evergreen species in exhibiting lower concentrations of N-organic and C-microbial. It is worth noticing that the litter of *Thymus capitatus*, one of the two phryganic species in the study site, is rich in essential oil (Vokou & Margaris 1986). It is found that essential oils increase the size of the soil bacterial population by manifold, favouring bacteria that can use them as a carbon and energy source.

For instance, the essential oil of the dwarf shrub *Lavandula stoechas*, another component of phryganic ecosystems, was found to induce threefold increase of the size of the bacterial population (Vokou et al. in press). However, soil samples taken under *T. capitatus* were ordinated close to those taken from the other, non-aromatic phryganic species, *G. alypum*. It seems, therefore that the discriminatory power of the strategy type (evergreen shrubs versus drought deciduous shrubs) is higher than that of the litter properties.

It is worth noticing that the soil under *J. oxycedrus* proved similar to those under *Erica* sp. and the grass covered area when considering the Biolog-test data; nevertheless, in terms of the other biochemical parameters examined, the soil under *J. oxycedrus* proved similar to *Q. coccifera*.

In conclusion, the mean utilisation of different groups of substrates differs among microsites in spite of the fact that bacterial communities associated with them do not differ in their structure. In addition, fungal biomass can be used to discriminate poor soils from relatively rich soils, while N-organic and C-microbial can distinguish soils under phryganic species from those under evergreen species.

Acknowledgements

This research was supported by PENED program (99/164), Greek Ministry of Development, Secretariat of Research and Technology.

[1] Ten times less

References

Conacher A.J. and Sala M. 1998. Land degradation in Mediterranean environments of the world. John Wiley & Sons, Chichester.

Cruz A. and Moreno J.M. 2001. Lignotuber size of Erica Australis and its relationship with soil resources. J. Veg. Sci. 12: 373-384.

Djajakirana G., Joergensen R.C. and Meyer Â. 1996. Ergosterol and microbial biomass relationship in soil. Biol. Fertil. Soils 22: 299-304.

Donegan K, Watrudb L.S., Seidlerb R. J., Sharon P., Shiroyamab M. T., Porteousb A. and Di Giovanni G. 2001. Soil and litter organisms in Pacific northwest forests under different management practices. Appl. Soil Ecol. 18 (2): 159-175.

Eijsackers H. 2001. A future for soil ecology? Connecting the system levels: moving from genomes to ecosystems. Opening Lecture to the XIII ICSZ "Biodiversity of soil organisms and ecosystem functioning". Eur. J. Soil. Biol. 37: 213-220.

Ekschmitt K., Bakonyi G., Bongers M., Bongers T., Bostrom S., Dogan H., Harnson A., Kallimanis A., Nagy P., O'Donnell A.G., Sohlenius B., Stamou G.P. and Wolters V. 1999. Effects of the nematofauna on microbial energy and matter transformation rates in European grassland soils. Plant & Soil 212: 45-61.

Hendrickson O.Q., Chatarpaul L. and Robinson J.B. 1985. Effects of two methods of timber harvesting on microbial processes in forest soil. Soil Sci. Soc. Am. J. 49: 739-746.

Jenkinson D.S. and Powlson D.S. 1976. The effects of biocidal treatments on metabolism in soil. V. Soil Biol. & Biochem. 8: 209-213.

Roberts N., Meadows M.E. and Dodson J.R. 2001. The history of Mediterranean type environments: climate, culture and landscape The Holocene Vol. 11, no 6, pp. 631-634.

Ross D.J. 1990. Measurements of microbial biomass C and N in grassland soils by fumigation-incubation procedures: Influence of inoculums size and the control. Soil Biol. Biochem. 3: 289-294.

Roy J., Aronson J. and di Castri F. 1995 Timescales of Biological Responses to Water constraints. The case of mediterranean biota. SPB Academic Publishing Amsterdam.

Stamou G.P. 1998. Arthropods of Mediterranean-Type Ecosystems. Springer-Verlag.

Swift M.J., Heal O.W. and Anderson J.M. 1979. Decomposition in Terrestrial Ecosystems. Blackwell, Scientific, Oxford, UK.

Vahjen W., Munch J.C. and Tebbe C.C. 1995. Carbon source utilization of soil extracted micro organisms as a tool to detect the effect of soil supplemented with genetically engineered and non engineered Conebacterium glutamicum and a recombinant peptid at the community level. FEMS Microb. Ecol. 18: 317-328.

Van Gestel M., Merckx R. and Vlassak Ê. 1993. Microbial biomass responses to soil drying and rewetting: the fate of fast- and slow-growing micro organisms in soils from different climates. Soil Biol. Biochem. 25: 109-123.

Vokou D., Margaris N.S. 1986. Variation of volatile oil concentration of Mediterranean aromatic shrubs, Thymus capitatus Hoffmag et Link, Satureja thymbra L., Teucrium polium L., and Rosmarinus officinalis L. Int. J. Biomet. 30:147-156.

Vokou D., Chalkos D., Yangou M., Karamanlidou G. 2002. Activation of soil respiration and shift of the microbial population balance in soil as a response to Lavandula stoechas essential oil. J. Chem. Ecol. (in press).

Wood M. 1995 Environmental soil biology (2nd edition) Blackie Academic & professional. London.