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SOIL MICROBIAL RESPIRATION BENEATH *STIPA TENACISSIMA* L. AND IN SURROUNDING BARE SOIL

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Abstract

NOVOSÁDOVÁ, I., RUIZ-SINOGA, J. D., ZÁHORA, J., FIŠEROVÁ, H.: *Soil microbial respiration beneath Stipa tenacissima L. and in surrounding bare soil.* Acta univ. agric. et silvic. Mendel. Brun., 2011, LIX, No. 1, pp. 183–190

Open steppes dominated by *Stipa tenacissima* L. constitute one of the most representative ecosystems of the semi-arid zones of Eastern Mediterranean Basin (Iberian Peninsula, North of Africa). Ecosystem functioning of these steppes is strongly related to the spatial pattern of grass tussocks. Soils beneath *Stipa tenacissima* L. grass show different fertility and different microclimatic conditions than in surrounding bare soil. The objective of this study was to assess the effect of *Stipa tenacissima* L. on the key soil microbial activities under controlled incubation conditions (basal and potential respiration). Basal and potential microbial respirations in the soils beneath *Stipa tenacissima* L. were, in general, not significantly different from the bare soils. The differences were less than 10%. Significantly less ethylene produced by microbial activity in soils beneath *Stipa tenacissima* L. after the addition of glucose could indicate the dependence of rhizospheric microbial communities on available carbon compounds. It can be concluded, that the soil respiration in semi-arid Mediterranean ecosystems is not necessarily associated with the patchy plant distribution and that some microbial activities characteristics can be unexpectedly homogenous.

Stipa tenacissima L., basal and potential microbial respirations, ethylene

Arid and semi-arid regions form approximately 40% of the Earth's land surface (Meigs, 1953; Bochet *et al.*, 1999). These lands are often fragile, easily eroded and highly degraded because of the interaction of harsh climatic conditions and human impact (Bochet *et al.*, 1999). The vegetation cover of these regions is often spatially discontinuous and mainly composed of perennial shrubby species (Schlesinger *et al.*, 1994). Tussock grasses can form mounds of organic debris and fine soil particles, which retain more water (Sánchez and Puigdefábregas, 1994) and have higher nutrient content than the soil between tussocks (Jackskon and Caldwell, 1992). The competition for water and resources between plants and microorganisms is strong and mediated trough an enormous variety of exudates and resource depletion intended to regulate soil microbial communities in the rhizosphere, control herbivory, encourage beneficial symbioses, and change chemical and physical properties in soil (Armas et Pugnaire, 2005).

Open steppes dominated by *Stipa tenacissima* L. (alpha grass) constitute one of the most representative ecosystems on the semi-arid zones of Eastern Mediterranean basin (Maestre *et al.*, 2007). Alpha grass steppes cover 32 000 km^2 in the western Mediterranean basin. These are the remains of an estimated 86 500 km^2 area covered by this species some decades ago (Le Houérou, 1995). Many authors, who were interested for patchy plant communities in arid and semi-arid regions, have described interactions among individual plants and soil properties. They have suggested that the plants of such environments locally improve the chemical, micro-climatic and physical status of the soil (Bochet *et al.*, 1999). Soil beneath alpha grass show higher fertility and improved microclimatic conditions, favouring the formation of „resource island" (Maestre *et al.*, 2007).

These promote the establihment and growth of vascular plants, mosses and lichens. The nutrient release processes have a fundamental role in ecosystem functioning, particulary in Mediterranean areas, where nutrient availability, mainly nitrogen and carbon, represents a limiting factor together with water availability (Hanley and Fenner, 2001). Soil N availability, for examples, has been found to affect plant water use efficiency (Sardans *et al.*, 2008a).

The present study is focused on the effect of *Stipa tenacissima* L. on soil microbial respiration in Mediterranean environment, in terms of *ex situ* microbial transformation of soil carbon, in order to characterize the key soil microbial activities which could strongly affect carbon turnover in soil and hereby soil fertility and soil organic matter "quality". These microbial activities could at unsuitable agricultural practices with adverse environmental conditions induce unfavorable hydrological tempo-spatial response.

MATERIALS AND METHODS

Soil microbial respiration and some important soil characteristics were measured in the laboratory in incubated root-free soil samples which were collected in July 2008 and in February 2009 either beneath tufts of grass *Stipa tenacissima* L. or in surrounding bare soil in open steppe dominated by the alpha grass in a Mediterranean area of southern Spain.

Field sites

The experimental plots were located in the province Almería in Sierra de los Filabres Mountains near the village Gérgal (southeast Spain) in the small catchment, which is situated between 1 090–1 165 m a. s. l. The area with extent of 82 000 m^2 is affected by soil degradation. The climate is semiarid Mediterranean. The mean annual rainfall is of about 240 mm mostly concentrated in autumn and spring. The mean annual temperature is 13.9 °C. The studied soil has a loam to sandy clay texture and is classified as Lithosol (FAO-ISRIC and ISSS, 1998). The vegetation of these areas is an open steppe dominated by *Stipa tenacissima* L.

Soil sampling

In July 2008 and in February 2009 representative soil samples from the top 10 cm were taken beneath grass tussock of *Stipa tenacissima* L. and pairwise from neighboring bare soil using a special hand spade. Four soil sub-samples were randomly collected per each sampling plot. Individual plots were chosen to represent different slope exposure (Ge1 – SE, Ge3 – E, and Ge5 – W) and catchment position (Ge2 near the border of the small catchment Ge4 at the bottom of valleys) see Tab. I. Each mixed sample was placed in a plastic bag in the field and kept cool until processed in the laboratory. Initial processing included sieving the samples through a 2 mm mesh and determining the gravimetric moisture content on subsamples after drying at 105 °C. The samples were stored at 4 °C until analysis.

Soil analyses

Soil water holding capacity (WHC) was determined after soil sampling by soaking the soil samples in water for 2h and then draining for 2h. (Dykyjová *et al.*, 1989). Total carbon content was determined according to Konovová and Belčiková method, adapted from Novak. Total nitrogen content was determined by distillation method according to Kjehldahl (expressed in %). Calcium, potassium and magnesium contents were determined by the method according to Melich II and using GBC 933 Atomic absorption spectrometer and the results were expressed as milligrams per kilogram of soil. Soil pH was measured on the 211 Microprocessor pH meter and was determined as an exchange of KCl extract. Phosphorus content was measured by flow analysis on the Spekol 210 according to ISO 11263:1994.

Experimental design

One week before laboratory incubations the native soil moisture in composite soil samples were reestablished to restore and to stabilize their microbial activities during a short-term preincubation. Respiration measurements were evaluated during a 37-day aerobic incubation after adjusting water content to 60% WHC:

a) Basal respiration – addition of distilled water (Šantrůčková, 1993a).
b) Potential respiration with the glucose solution – addition of glucose corresponded to the addition of 1% C (indicated as potential microbial activities with "glucose").
c) Potential respiration with the glucose + peptone solution – addition of both compounds corresponded to the addition of 1% C and 0,04% N (indicated as potential microbial activities with "peptone").

I: *Geographic coordinates of the sampling plots*

Sampling plot	Latitude	Longitude	Altitude
Ge 1	37°08'37,92"	2°31'24,60"	1 131 m
Ge 2	37°08'34,08"	2°31'26,70"	1 133 m
Ge 3	37°08'32,64"	2°31'25,62"	1 125 m
Ge 4	37°08'32,94"	2°31'24,24"	1 113 m
Ge 5	37°08'29,76"	2°31'21,96"	1 105 m

1: *Localization of the sampling plots in the small catchment near the village Gérgal (southeast Spain) (Google Earth, 2010)*

2: *Patchy plant distribution in open steppe dominated by Stipa tenacissima L. in Sierra de los Filabres Mountains (southern view from the sampling plot Ge2)*

Soil respiration was determined in 70 g soil subsamples incubated in the dark at 23 °C in ceramic pots inserted in jars sealed with rubber (latex) membrane twelve hours before headspace gas sampling. Soil subsamples were located between two sand layers (at the top and bottom of the pot; the depth of each sand layer was 0.5 cm) to make water percolation possible. After two weeks (at the day 16) of incubation simulated rainfall was proceeded in order to be as close as possible to natural conditions (the amount of percolating water corresponded to 120 ml per 70 g DW soil). After it initial moisture content was readjusted on the weight base. Both, ceramic pots and sand were repeatedly washed out before incubation experiment with acid (0.1 M HCl) and alkali (0.1 M NaOH) and than multiply with distilled water to prevent enrichment soil subsamples with some undesirable compounds during the laboratory

incubation. Ceramic pots with soil subsamples were periodically weighed and initial moisture contents restored.

Soil respiration

Measurement of microbial respiratory activity in soil was based on the quantities of CO_2- C evolved for a specific period of time related to a one gram of dry soil (Šantrůčková, 1993b). Soil respiration was measured at 1st, 2nd, 3rd, 8th, 9th, 10th, 15th, 17th, 21st, 24th, 30th and 37th days of incubation; the cumulative production of CO_2- C was calculated by linear interpolation for resting days. Gas samples (2 cm^3) were withdrawn from the headspace via a stainless steel needle with nylon syringe after twelve hours through a latex membrane. The syringes with the gas samples were immediately stuck into the designated rubber stoppers. After that the jars were opened and the initial moisture of the soil subsamples was restored. Carbon dioxide was analyzed on a gas chromatograph CHROM 5 (Czech Republic) equipped with a kathanometer with 1.5 m long stainless steel column filled with Poropak Q, and a FID detector. Carrier gas (hydrogen) was maintained at 40 $ml.min^{-1}$. Column temperature was 170 °C. Other collected gas samples (ethylene) were analyzed by gas chromatography (Fisson Instrument) with capillary column 24 m long HP-PLOT/Al_2O_3. Injection temperature was 230 °C, detector temperature was 200 °C and column temperature was 40 °C. Results were expressed as µg of CO_2- C in 1 g of DM. Statistical evaluation was performed by means variance analysis (ANOVA $P > 0.05$).

RESULTS AND DISCUSSION

Soil characteristics

The main characteristics of the soil beneath *Stipa tenacissima* L. and of the bare soils soil are shown in Table II. The measured data documented that the presence of grass tussock enhanced soil pH, the total carbon and nitrogen content as well as contents of calcium, potassium and magnesium. Such data supported positive interactions among individual plants and soil properties based on higher inputs of different forms of carbohydrates either in form of plant litter or in form of rhizospheric deposition (Bochet *et al.*, 1999; Maestre *et al.*, 2007). Only the content of phosphorus remained unaffected.

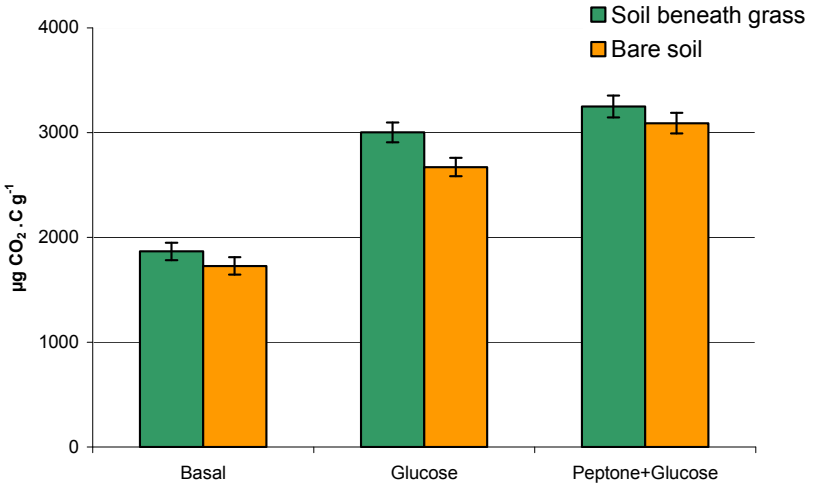
Soil microbial respiration

The basal respiration measured during incubation assay under favourable conditions for microbial growth is usually seen as the overall rate of decomposition of native soil organic matter. The basal soil respirations in combination with the respiration of soil microorganisms after stimulation by readily available carbon, or combined carbon and nitrogen source in a ratio of 25:1 (potential soil respiration) are a very good indicator of soil biological activity. (Šimek, 2004).

In our experiment, the rate of basal and potential microbial respirations was always slightly higher in

II: *Soil characteristics (0–10 cm) of the soil samples from experimental localities*

	pH	Combustible substances	P	K	Mg	Ca	N_t	C_{org}
		%	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	$mg.kg^{-1}$	%	%
Bare soil	6.5	4.61	9	59	132	1162	0.115	2.31
S. tenacissima	6.7	5.93	9	98	152	1362	0.158	2.97



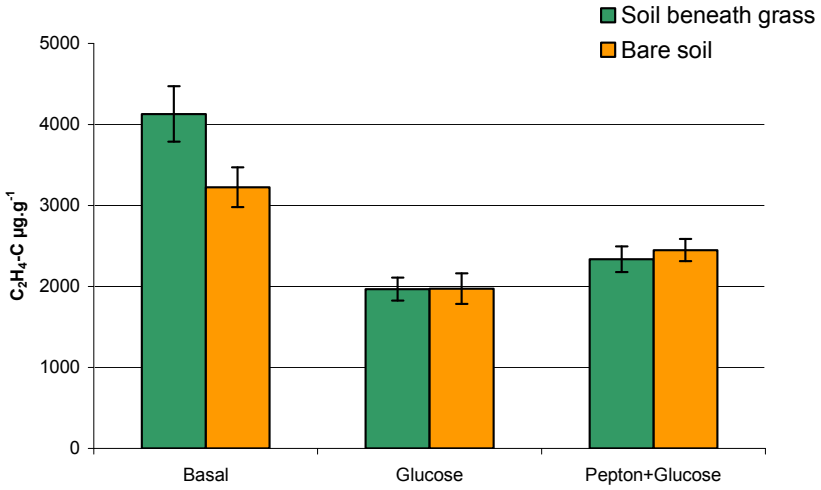
3: *Cumulative basal and potential respiration (after the addition of peptone and glucose) during 37 days of incubation. Mean values from 4 replicates are given.*

samples taken up below tussocks of esparto grass than in samples collected from bare soil; however, this difference was statistically insignificant.

The differences between plant-covered soil and bare soil in cumulative values of CO_2- C production were less than 10%. We can conclude that the addition of peptone together with glucose stimulated the cumulative respiration during the 37 days more than the addition of solely glucose (Fig. 3).

High differences between the cumulative values of potential respirations after the addition of easily degradable organic matter and the basal respiration documented that such stimulations play there a key role for microbial activities and hereby also for higher mineralization of organic matter and better access of plants to nutrients. On the other hand the high value indicates higher stability of resting soil organic matter.

The physiological efficiency of ethylene is high. Most physiological responses occur in the range of 0.01–1 $\mu l.l^{-1}$ ethylene concentration (Burg, 1968). More information is needed to properly asses gained results which showed opposite pattern as the soil microbial respiration. The production of ethylene represents only a negligible amount compared with the amount of CO_2-C production. On the other hand, the ethylene production during incubation experiments could be a valuable indicator of the inappropriate conditions for soil microbial activities. Results were expressed as µg of C_2H_4-C in 1 g of DM. Statistical evaluation was performed by means variance analysis (ANOVA $P > 0.05$).



4: *Ethylene production during 37 days of incubation. Mean values from 4 replicates are given.*

Ethylene production

There is widely not sufficient information about the soil microbial production of ethylene. In plants, ethylene generally affects the processes of growth and development. Its physiological effects, chemical, biochemical properties and biosynthesis have been studied by a number of authors (Bleecker and Kende, 2000). Gaseous ethylene is released from the intercellular spaces into the environment where its concentration is in equilibrium with the amount of ethylene dissolved in the cytoplasm. The 1-aminocyklopropan-1-carboxylic acid (ACC), which comes from the amino acid L-methionine, is considered to be the immediate precursor of ethylene (Adams and Yang, 1979).

No statistically significant differences were found out in contents of ethylene produced within the whole incubation period from soil samples taken either directly below tussocks of esparto grass or from the soil without the vegetation cover.

Significantly less ethylene produced by microbial activity in soils beneath *Stipa tenacissima* L. after the addition of glucose indicates probably the dependence of rhizospheric microbial communities on available carbon compounds mainly from root exudates.

It can be concluded, similarly as published Goberna *et al.* (2007), that the distribution of soil microbial properties in semi-arid Mediterranean ecosystems is not necessarily associated with the patchy plant distribution and that some microbial activities characteristics can be unexpectedly homogenous.

SUMMARY

The objective of this study was to assess the effect of *Stipa tenacissima* L. on the key soil microbial activities (basal and potential respiration) under controlled incubation conditions. The experimental plots were located in the province Almería in Sierra de los Filabres Mountains near the village Gérgal (southeast Spain). The representative soil samples from the top 10 cm were taken beneath grass tussock and from bare soil in July 2008 and in February 2009. Soil samples were incubated after moisturizing with distilled water (basal microbial activities) and then moisturized with the glucose (potential microbial activities with glucose 1% C) and with the mixture of glucose and peptone solution (potential microbial activities with glucose and pepton 1% C + 0.04% N). The CO_2-C, evolved under controlled conditions (60% WHC, 23 °C) during a 37-day aerobic incubation was determined. Ethylene production, basal and potential microbial respirations in the soils beneath *Stipa tenacissima* L. were, in general, not statistically different from the bare soils. Significantly less ethylene produced by microbial activity in soils beneath *Stipa tenacissima* L. after the addition of glucose indicate the dependence of rhizospheric microbial communities on available carbon compounds. It can be concluded, that the soil respiration in semi-arid Mediterranean ecosystems is not necessarily associated with the patchy plant distribution and that some microbial activities characteristics can be unexpectedly homogenous.

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