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Microbial activities in soils of a Mediterranean ecosystem in different successional stages

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ABSTRACT

This study reports a comparative analysis of soil enzyme activities (β-glucosidase, protease, urease, arylsulphatase, phosphatase and fluorescein diacetate hydrolase), ATP, total N and organic matter contents in three vegetal successional stages (meadow, low shrubland and high maquis) of a Mediterranean ecosystem in the Natural Reserve of Castel Volturno (Campania, Italy). Because water availability is a major limiting factor of soil microbial activity in Mediterranean ecosystems, the analysis was performed in late spring (May), after the rainy period, and in early autumn (October), after the long dry summer.

A significant decrease in protease, arylsulphatase, urease and β-glucosidase activities was observed in meadow soil in the autumn sampling, probably due to the prolonged summer drought. Combining the values measured in the two sampling dates, the high maquis tended to have higher levels of enzymes activities than shrubland and meadow. Notably, high maquis had significantly higher phosphatase and arylsulphatase activities than shrubland and meadow and, in addition, a higher ATP content compared to meadow. Drastic changes were observed in EA/ATP ratios between the sampling periods in the meadow and shrubland, suggesting changes in the efficiency of microbial community more likely linked to climatic fluctuations than to the successional stage. The more stable EA/ATP ratio in the maquis probably reflects a constant contribution of microbial biomass to enzyme secretion.

In conclusion, our results point to an increase in soil microbial activity accompanying the succession from meadow to high maquis that probably reflects a parallel increase in soil functions. Nevertheless, spatial heterogeneity and, more important, temporal variations in soil activities often may obscure differences related to the plant cover type.

1. Introduction

It is well known that plant cover markedly affects the composition and activity of soil microbial community which in turn plays a key role in organic matter transformation and nutrient cycling in terrestrial ecosystems. As an example, microbial biomass, C and N mineralization rates and both dissolved organic carbon and nitrogen were found to be generally higher in *Betula pendula* (Roth.) than in *Picea abies* (L.) and *Pinus sylvestris* (L.) stands with originally similar soil in Northern Finland (Smolander and Kitunen, 2002). Moreover, the soil from *P. abies* stand had higher microbial activities than that from the *P. sylvestris* stand. A study in a Reserve area on the Tyrrhenian coast of Italy comprising different habitats ranging from mature *Quercus ilex* (L.) forest to Mediterranean maquis, *Pinus pinea* (L.) plantations and hydrophilous mixed back-dune forest showed that forest soils with a greater above-ground diversity harboured a larger microbial biomass, with higher respiratory efficiency and decomposition rates, than soils with homogeneous plant cover (Pinzari et al., 1999). Plant cover affects soil microbial activity even on a very small scale. In the patchwork of plants of a Mediterranean low maquis the soil microbial activity in the same cover-type stand was found to change under individual plants of different species (Papa et al., 2007).

The close interactions between soil microbial communities and plants suggest that the composition and activity of microbial communities change and evolve through the plant successional stages also as a consequence of changes in the physico-chemical status of soil. For example, the type and the abundance of covering affect the soil temperature and water content as well as the quality and quantity of litter during succession (Eviner, 2004). Nutrients, such as carbon and nitrogen, accumulate during primary succession; phosphorous, on the contrary, decline due to weathering and drainage loss (Crews et al., 1995; Aikio et al., 2000; Allison et al., 2007). Such changes strongly affect the activity of microflora involved in nutrient mineralization. Under this perspective, soil enzymes have been considered as "sensors" of soil biological

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activity and fertility (Skujins, 1978). It has been reported that the activities of soil enzymes involved in C and N mineralization declined in the organic soil with site age, while those related to P and S mineralization increased and peaked at an intermediate site age, respectively, along a nutrient gradient related to a chronosequence in an area between Franz Josefs glacier and the coast in New Zealand (Allison et al., 2007). Nevertheless, monitoring the diversity and activity of nitrate-reducing community in the rhyzosphere of *Poa alpina* (L.) on a glacier foreland in the Central Alps, Deiglmayr et al. (2006) found that nitrate reductase activity increased significantly in successional stages and was highly correlated with the organic carbon and NO_3^- availability. In Spain, under Mediterranean climate, higher enzyme activities were detected in soils with a more mature plant cover and higher organic carbon content than in degraded soils with low cover (Garcia et al., 2002). Similarly, the soil with the highest nutrient contents from pure high maquis, showed higher respiration and phosphatase, arylsulphatase and β-glucosidase activities than soils from a low maquis, *Q. ilex* wood and high maquis with pine presence (Rutigliano et al., 2004).

A large variability of composition and aspect characterizes the vegetation and its evolution in the Mediterranean area. Fire, more or less frequently recurring, grazing, tillage, management and the introduction of foreign plant species deeply affect the species composition of succession stages relative to the typical stages (grassland, low and high shrubland and finally the evergreen wood dominated by oak and pine), occurring in undisturbed areas (Polunin and Walters, 1985). Because of these multiple variables, the functioning of soil biota in Mediterranean ecosystems is still not fully understood. Compared to a relative wealth of data on soil microbial activity in successional stages in other ecosystems (Aikio et al., 2000; Chabrerie et al., 2003; Deiglmayr et al., 2006; Allison et al., 2007), there is currently very little information on this topic in the Mediterranean area of South Italy (Pinzari et al., 1999; Rutigliano et al., 2004). These studies compared Mediterranean biotopes covered with advanced successional stages (Pinzari et al., 1999) and subjected to management with the introduction of pine (Rutigliano et al., 2004).

The aim of this study was to analyze soil enzyme activities involved in the main element cycles and the soil ATP content in earlier successional stages (meadow, low shrubland and high maquis) of the Mediterranean ecosystem. Among the soil enzymes investigated, protease and urease, that hydrolyze the peptide bond between amino acids and urea, respectively, and β-glucosidase, breaking down labile cellulose, are involved in N and C cycling, while arylsulphatase and phosphatase participate in S and P cycles. Fluorescein diacetate hydrolase (FDA) activity, on the other hand, is an index of overall enzyme activity, because this compound is a substrate for a wide diversity of hydrolysing enzymes (Perucci, 1992). ATP content is used as an index of microbial biomass (Jenkinson, 1988; Nannipieri et al., 1990). Since water availability is a major limiting factor for soil microbial activity in Mediterranean ecosystems, we analyzed samples collected in late spring (May) after the rainy period and in early autumn (October) following the dry summer. This work is part of a larger project involving other research groups dealing with net N mineralization, net and potential nitrification and denitrification (Rutigliano et al., 2009), pattern of biomass allocation, role of leguminous species as determinants of N input to the soil (De Marco et al., 2008), fungal diversity (Persiani et al. unpublished data).

2. Material and methods

2.1. Study site and sampling

The study was carried out within the Natural Reserve of Castel Volturno, a flat coastal area in Southern Italy (40° 55′ N; 14° 1′ E). The topography of the area is characterized by dunes of alluvial deposits with an elevation of 6–9 m above sea level, loose siliceous-calcareous sand of marine origin and pyroclastic products.

The area has a typically Mediterranean climate with a mean annual temperature of 18.6 °C and an average annual rainfall of 680 mm. Lang aridity index (annual precipitation in mm/annual mean temperature in °C) (Larker, 1993) is 37. Precipitation occurs mostly in autumn and winter. Winters are mild (the mean temperature of the coldest month is 10.6 °C) and the summers hot and dry (the mean temperature of the hottest month is 28.0 °C).

The soil exhibits a sandy texture (coarse and fine sand are more than 90% of the dry weight) and, as a consequence, has a low water-holding capacity (WHC = 40–60, H_20 g/100 g dry weight depending on plant cover and organic matter content). The reaction is sub-alkaline (pH around 8).

As concerns the vegetation, the Reserve includes stands of *Q. ilex* and *P. pinea*, and stands of high and low maquis often including small areas with herbaceous plants. Further information on Reserve vegetation can be found in Esposito et al. (1998).

Three plant cover typologies were chosen: 1) high maquis (height: 2.5–3 m; covering: about 100%) dominated by *Q. ilex* and *Phillyrea angustifolia* (L.), also containing *Pistacia lentiscus*, *Myrtus communis* (L.) and *Pinus halepensis* (Miller); 2) low maquis (height: 30–40 cm; covering: about 70%) with shrubs mainly of *Cistus incanus*, (L.) *Cistus salvifolius* (L.) and *Rosmarinus officinalis*; 3) meadow with a herbaceous cover of Fabaceae and Poaceae (covering in the spring: 80–90%). So, within the study area at sea level, four groups of three plots (high maquis, low maquis and meadow) were identified. The three plots (each of them of about 20 m^2) characterized by different plant cover were chosen one close to another. The interval among the four groups was variable, because dependent on contemporary presence of all three types of covering.

In each plot 12 cores of the upper soil layer (0–5 cm) were collected randomly and pooled to form the soil sample used for the analyses after sieving through a 2-mm-mesh sieve. The samplings were made in two different periods of the year: a) in the spring, when the Mediterranean species have the maximum growth rate and b) in the early autumn, when they have more or less completely stopped growing because of prolonged summer aridity. This shifting is particularly pronounced for the herbaceous plants of the meadow, for which spring and autumn are the growing and senescent period, respectively; moreover, these also are the periods of higher and lower soil activity, respectively (Papa et al., 2002).

3. ATP content and enzyme assays

ATP content was extracted from sieved soil according to Ciardi and Nannipieri (1990) and assayed by the bioluminescence test system. Before extraction, the soil sample was moistened to 50% of its water-holding capacity (WHC), as suggested by Nannipieri et al. (1990) when the content of ATP in soil is considered an index of microbial biomass, and incubated for seven days in the dark at 25 °C in closed containers; two small beakers containing soda lime and water were put near the soil in order to avoid soil desiccation and trap the CO_2 derived from microbial respiration.

ATP content was measured with a luminometer Bio-Orbit OY 1253 (Turku) using an integration mode (10 s integration period).

3.1. Alkaline phosphatase

Alkaline phosphatase was determined spectrophotometrically according to Eivazi and Tabatabai (1977). 0.5 g of fresh soil was treated with 2 ml modified universal buffer stock solution (MUB) pH 11 and 0.5 ml of 15 mM *p*-nitrophenyl phosphate. The mixture was stirred and incubated at 37 °C for 1 h, treated with 0.5 ml of