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Structure and activity of soil microbial communities in three Mediterranean forests

Felicia Grosso[a], Paola Iovieno[b], Anna Alfani[c], Flavia De Nicola[a,*]

[a] Dipartimento di Scienze e Tecnologie, Università degli Studi del Sannio, via F. De Sanctis SNC, 82100 Benevento, Italy
[b] Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca Orticoltura e Florovivaismo, Via Cavalleggeri 25, 84098 Pontecagnano-Faiano, SA, Italy
[c] Dipartimento di Chimica e Biologia "Adolfo Zambelli", Universitàdegli Studi di Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, SA, Italy

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ABSTRACT

The effect of plant cover on soil microbial community structure and activity was investigated in forest ecosystems dominated by holm oak (*Quercus ilex*), Turkey oak (*Quercus cerris*) and beech (*Fagus sylvatica*), in two seasons (autumn and early summer). Microbial community structure was investigated by phospholipid fatty acid (PLFA) profile and ergosterol determination. Microbial community activity was assessed by fluorescein diacetate hydrolisys, β-glucosidase and cellulase activities. The Turkey oak forest showed the lowest soil microbial biomass, both as total and specific PLFA markers. PLFA profile showed a different microbial community structure among forest soils, mainly between the two oak systems. Enzyme activities were affected by soil organic carbon content, with the lowest values measured in Turkey oak. A seasonal effect both on microbial biomass and on enzyme activity was generally observed. Among the investigated forest systems, the beech forest appeared to store more carbon both in aboveground biomass and in soil.

1. Introduction

Plant cover, especially the dominant tree species, can influence multiple properties of soils such as pH, organic matter quality and quantity (litter and root exudates), soil structure and microclimate (Ayres et al., 2009) and through these changes it can indirectly affect soil microbial community (Prescott and Grayston, 2013). Thus, soil microorganisms represent a link between dominant vegetation and ecosystem processes, taking on crucial importance in global carbon sequestration and nutrient cycling in forest ecosystems.

Soil microbial biomass, estimated as 10^3–10^4 kg ha^{-1} of surface soils (Fierer et al., 2007), is dominated by bacteria and fungi that play a key role in nutrient cycles. It is commonly believed that the first are mainly responsible for decomposition of easily available substrates, while the second ones are essential for the decomposition of more complex organic macromolecules derived from plant materials like cellulose and lignin (Rousk and Bååth, 2011). Enzymes are the main responsible of biological soil processes such as the recycling of elements (Abellan et al., 2011). The study of enzymatic activities in soil is a useful tool for assessing the function diversity of soil microbial communities. Soil enzyme activities are widely used to evaluate the functionality of microbial communities, being sensitive to several environmental factors and disturbance (Gianfreda et al., 2005).

The effect of plant cover on soil microbial community structure was pointed up by the different phospholipid fatty acid (PLFA) profiles between soils under white spruce and aspen (Hannam et al., 2006), as well as among soils covered by spruce, birch and pine (Priha et al., 2001). It is known that plant litter may drive the abundance and the composition of soil fungal and bacterial communities (Prescott and Grayston, 2013). Additionally, plant cover can affect also the activity of extracellular enzymes (Rutigliano et al., 2004; Steinauer et al., 2015). Microbial community composition is known to affect ecosystem process rates (Reed and Martiny, 2007; Strickland et al., 2009), thus the study of the structure and function of soil microbial communities is of paramount importance to understand forest ecosystem responses to environmental changes. In the Mediterranean area, the studies on the link between plant cover and soil microbial community composition and function are scarce (Rutigliano et al., 2004, 2009), especially in forest ecosystems (Goberna et al., 2005; Iovieno et al., 2010; Lucas-Borja et al., 2012a,b).

We aim to compare the soil microbial community structure and function in forest ecosystems typical of Mediterranean area, differing for dominant tree cover and altitude in order to test the hypothesis that different carbon inputs and microclimatic conditions affect microbial

* Corresponding author.
E-mail address: fdenicol@unisannio.it (F. De Nicola).

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community composition and activity. At this aim, along an altitudinal gradient three forest stands with different dominant species, an evergreen (holm oak) and two deciduous ones (Turkey oak and beech) were selected, being the most represented forest systems in the Italian peninsula (Vacchiano et al., 2012). Soil microbial community activity was investigated by the measurement of fluorescein diacetate hydrolytic activity, β-glucosidase and cellulase activities. Soil microbial community structure was analysed by PLFA profile and ergosterol determination. Moreover, in order to detect the effect of seasonality, microbial enzyme activities, PLFA pattern and ergosterol content were compared between two sampling campaigns carried out in autumn and early summer.

2. Material and methods

2.1. Soil sampling

In autumn 2012 and early summer 2013, soil samples were collected in three forests located on Matese Mountains (Campania, southern Italy): a Turkey oak (*Quercus cerris* L.) stand (41°16′ N, 14°24′ E, 200 m a.s.l.); H, a holm oak (*Quercus ilex* L.) stand (41°21′ N, 14°24′ E, 700 m a.s.l.); T, B, a beech (*Fagus sylvatica* L.) stand (41°25′ N, 14°28′ E, 1300 m a.s.l.). The investigated stands differed in density (10, 27, and 25 stem/plot respectively for T, H and B forest) and slope (17, 64 and 6%, respectively for T, H and B forest), although all were on calcareous parent material covered with pyroclastic materials; for a full description see Grosso et al. (2014).

At each forest stand, 5 adjacent plots (5 × 5 m each) were selected. In each plot, 8 surface (0–5 cm) soil cores were randomly collected with a plastic shovel after litter removing, and pooled to obtain a homogenous and representative sample. Soil samples from each plot were separately analysed, at both autumn and summer sampling. Soils were sieved (2 mm) and stored until the laboratory analyses, at 4 °C to assess microbial enzyme activity or at −20 °C to determine the PLFA and ergosterol content. All analyses were carried out on three laboratory replicates per plot (n = 5 plots × 3 replicates × 2 samplings = 30 analysed samples, for each stand).

2.2. Soil physical and chemical analyses

Soil water content was determined by weighing before and after oven drying (105 °C until constant weight), and water holding capacity (WHC) via the gravimetric method after water saturation and drainage of gravitational water followed by oven-drying. Soil pH was determined in a water suspension (1:50, w/v soil:water) via the potentiometric method, and organic carbon content (C_{org}) was measured on air-dried soil samples, according to the Walkley-Black method (Walkley and Black, 1934) based on organic carbon oxidation by dichromate to carbon dioxide. Oven-dried samples were analysed for total carbon and nitrogen (CHNS-O analyzer, Thermo Flash EA 1112).

2.3. Phospholipid fatty acid (PLFA) profile

Soil samples (1 g) were extracted in 10 mL of Bligh and Dyer reagent (chloroform:methanol:citrate buffer, 1:2:0.8, v/v/v). The lipid extract was fractionated into neutral, glycol- and polar lipids (phospholipids) on a silicic acid column (Frostegård et al., 1993). The phospholipids were methylated and the resulting fatty acid methyl esters separated by a capillary column (HP 5MS UI 60 m × 0.25 mm) and identified by a gas chromatograph equipped with a flame ionization detector (GC-FID 7890A Agilent Technologies). The temperature program started at 80 °C for 1 min; raised at 20 °C min^{-1} up to 160 °C followed by a further increase of 5 °C min^{-1} until the final temperature of 280 °C, and held to a total time of 50 min. For quantification, the internal standard methyl nonadecanoate fatty acid-FAME (19:0) was added before the methylation step in each sample. All glassware was heated at 400 °C overnight to remove lipid contaminants. Fatty acids were designated by the total number of carbon atoms followed by the number of double bonds and their position (ω indicates that the carbon chain is numbered starting from the methyl end of the molecule). General assignments associate letters c and t to the presence of *cis* and *trans* configurations, respectively; letters i and a indicate iso and anteiso ramifications, respectively; a methyl ramification in unidentified position is represented by br; 10Me indicates the presence of methyl group on the tenth carbon atom; finally cy indicates cyclopropane (Bååth and Anderson, 2003; Frostegård et al., 1993).

The sum of i14:0, i15:0, a15:0, 15:0, i16:0, 10Me16:0, i17:0, a17:0, cy17:0, 17:0, br18:0, 10Me17:0, 18:1ω7, 10Me18:0 and cy19:0 was used as indicator of bacterial biomass (Bååth and Anderson, 2003). The PLFA 18:2ω6,9 was used as indicator of fungal biomass (Bååth and Anderson, 2003). The PLFA 16:1ω5 was used as marker of vesicular-arbuscular mycorrhizae (Olsson, 1999) and the PLFA 10Me18:0 was used as marker of actinomycetes (Kroppenstedt, 1985).

2.4. Ergosterol content

Soil samples (1 g) were extracted with 4 mL 10% KOH in methanol, sonicated (LB S1 6Lt, FALC instrument) for 15 min, and incubated at 70 °C for 90 min. After the heat treatment, samples were mixed with 1 mL of distilled water and 2 mL of cyclohexane and centrifuged and the top phase removed. The remaining solution was washed once with 2 mL cyclohexane and cyclohexane fractions were combined and dried under a gentle nitrogen stream at 40 °C. The dried extracts were dissolved in methanol at 40 °C and filtered (Grant and West, 1986). The samples were analysed by reverse-phase HPLC (Finningan Surveyor, Thermo Scientific instrument) with 100% methanol at a flow rate of 1 mL min^{-1} as mobile phase, and 282 nm as wavelength for the photodiode array detector.

2.5. Enzyme activities

Enzymatic activities were analysed within few days after sampling: 1) fluorescein diacetate hydrolytic activity (FDAase) was determined according to Schnürer and Rosswall (1982), incubating (at 25 °C) the soil samples with 3, 6-diacetyl fluorescein as substrate and measuring the absorbance of the released fluorescein at 490 nm; 2) β-glucosidase activity (GLUase) was determined according to Tabatabai(1994) incubating (at 37 °C) soil samples with *p*-nitrophenyl β-D-glucopyranoside as substrate and measuring the absorbance of released *p*-nitrophenol (PNP) at 398 nm; 3) carboxymethyl-cellulase activity (CMCase) was determined according to Schinner and Von Mersi (1990) incubating (at 50 °C) the soil samples with carboxymethyl sodium salt solution as substrate and measuring the absorbance of the released sugars at 690 nm.

All spectrophotometric measurements were carried out by a BioMate-3 spectrophotometer (Thermo Scientific, USA).

2.6. Statistical analysis

The significance of differences in physico-chemical parameters, enzymatic activities, PLFA and ergosterol contents were tested by Two-way ANOVA, on normalised data, followed by Tukey post hoc test, with the three forest stands and seasonality as fixed factors. Correlations among the studied parameters were evaluated by the Spearman's test, according to the non-normal distribution of the data, assessed using the Shapiro-Wilk test. The statistical analyses were performed using SigmaPlot 12.0 Systat Software Inc. package. Moreover, the PLFA data (mol%) were processed, separately for the two samplings, by Principal Component Analysis (PCA) using MVSP 3.1 (Multi Variate Statistical Package, Kovach Computing Services); the significance of differences of scores among forest stands were tested by One-way ANOVA and One-way ANOVA on Ranks, respectively for normally and non-normally