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Bacterial and fungal growth on different plant litter in Mediterranean soils: Effects of C/N ratio and soil pH

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ABSTRACT

Plant litter represents an important source of nutrients and energy for soil microorganisms, but will also selectively affect which organism group, fungi or bacteria, that will be favoured during decomposition. The balance of fungal to bacterial growth will furthermore be affected by soil chemistry like pH. A laboratory experiment was carried out using two different Mediterranean forest soils differing in pH, adding five types of litter varying in C/N ratio from 15 to 75, including the major litter type from the two soils. Growth of bacteria (using the leucine incorporation technique) and fungi (using the acetate into ergosterol incorporation technique) was then followed during 6 weeks. The balance of fungal to bacterial growth was positively affected by litter with increasing C/N ratio, while the C availability, as judged by evolved CO_2, did not have any influence. Furthermore, low pH in the soil further favoured fungal growth, irrespective of the litter type. Despite differences in fungal to bacterial growth this appeared to have little influence on respiration rates from the added litter, suggesting functional redundancy. Our results highlight how both initial soil conditions (pH) and litter composition (C/N ratio) independently affects fungal and bacterial growth during decomposition.

1. Introduction

In the Mediterranean region, forests cover an area of over 85 million ha (estimated in 2010; FAO, 2010). Trees affect the soil environment in several ways through litter fall, labile C input, rhizodeposition, root turnover and effects on soil microclimate (Eviner and Chapin, 2003). Especially the quality of litter associated with different tree species influences the microbial community (Thoms et al., 2010; Aponte et al., 2014), since leaf litter is the main energy and nutrient source for soil microorganisms. Different microbial communities were also reported in soil under different tree species (Hackl et al., 2005; Thoms et al., 2010; Schweitzer et al., 2011).

Bacteria and fungi are the main decomposer groups involved in the recycle of soil organic matter. The environmental factors determining the importance of these two groups during decomposition processes are not completely understood, although for example the canonical effect of pH has been studied recently (Rousk and Bååth, 2011), with low pH being more conducive for fungal growth. The chemical composition of the substrate (e.g. the C/N ratio) is also predicted to be of importance, with higher C/N ratio of the litter being more conducive for fungal growth due to fungal hyphae having a higher C/N ratio than bacterial cells (Paustian and Schnürer, 1987; Bakken, 1985; Wallander et al., 2003) and the potential to translocate N to overcome limitation (Frey et al., 2003). However, N availability in itself is not always enough to explain differential growth of fungi and bacteria on plant litter, as shown by Rousk and Bååth (2007) after adding extra N to litter with originally different C/N content, suggesting that other chemical and physical conditions of different litter types will be of importance. Growth of fungi and bacteria during decomposition has mostly been studied on fairly easily available substrates, like glucose (Meidute e al., 2008; Reischke et al., 2014), manure (Maienza et al., 2014) or alfalfa and straw (Rousk and Bååth, 2007). Few studies have been focused on comparing different leaf litter (Rousk and Bååth, 2011).

The aim of our study was to investigate the influences of different litter types on fungal and bacterial growth in two forest soils, differing in pH. For this purpose, leaf litters belonging to three different Mediterranean forest systems (beech, holm oak and turkey oak forests) were added to two soils (beech and holm oak) from the same mountain area. Straw and alfalfa were also included as litter treatments having very different C/N ratios. Bacterial and

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fungal growth rate was measured over time using radioactive tracer incorporation techniques (leucine incorporation and acetate into ergosterol incorporation for bacteria and fungi, respectively) and compared with total activity (respiration) and changes in total biomass (SIR). We hypothesized that fungal growth would be relatively more important than bacterial growth in the soil with lower pH, as well on litter types with higher C/N ratio.

2. Material and methods

2.1. Soils

Soil was sampled during summer 2013 from a beech (*Fagus sylvatica* L.) and a holm oak (*Quercus ilex* L.) stand, using the top layer (0–5 cm) after removing litter. Both stands are located in the Matese mountain area (Apennines district, southern Italy). A more detailed description of the two forest stands is reported in Grosso et al. (2014). Soil cores, randomly collected at each stand, were pooled to obtain a representative sample. Soil samples were sieved (<2 mm) and stored at 4 °C until the laboratory analyses. The beech forest soil had a pH of 5.3 and 16.6% of organic C, while the holm oak soil had pH of 7.2 and 16.5% of organic C.

2.2. Litter sampling and analyses

Leaf litters were collected in the two forest stands above and in a turkey oak (*Quercus cerris* L.) stand also located in the same area (see Grosso et al., 2014 for a description of this forest stand). Litters were sampled between autumn 2012 and winter 2014 by litter traps (1 m × 1 m) placed randomly in the forest stands. Annual leaf-litter forest productivity was 337, 638 and 171 g/m^2 in holm oak, beech and turkey oak stands. Litters were dried (75 °C) to constant weight, and pH in water suspension (1:50, w/v, litter:water) was determined. Holm oak, beech and turkey oak litters showed pH values in a sub-acid range (5.6, 5.8 and 4.9). Oven-dried leaf litter was ground into a fine powered by an agate mortar and pestle (Fritsch Analysette Pulverisette 0), and analyzed (CHNS-O analyzer, Thermo Flash EA 1112) for total carbon and nitrogen. C/N ratios of the litter types were: holm oak 42, beech 43, turkey oak 34. We also used wheat straw and alfalfa litters as positive controls, since straw, with high C/N (75), has been shown to favour fungal growth and alfalfa, with low C/N ratio (15), bacterial growth (Rousk and Bååth, 2007). pH for straw was 6.8 and for alfalfa 5.9.

2.3. Experimental set-up

Five dry litters (holm oak, beech, turkey oak, straw and alfalfa) were ball-milled and sieved to recover the fraction in the range of 250 μm–1 mm. The five litter types were added both to the beech and holm oak soils. A soil sample without litter addition was used as control for each soil. All the six treatments (5 litter additions and 1 control) were replicated three times per soil. Each soil replicate (25 g moist soil) were mixed with litter (0.5 g) and incubated in plastic containers with lids at room temperature (approx. 21 °C) at darkness for 42 days. The amendment rate is similar as earlier used by Henriksen and Breland (1999) and Kamble and Bååth (2014) for straw. At time intervals, basal respiration (over 27 days), microbial biomass (over 28 days), and bacterial and fungal growth (over 42 days) were analyzed.

2.4. Basal respiration and microbial biomass

Basal respiration was measured on 1 g of soil in 20 ml vials with gas tight rubber seals. The vials were flushed with pressurized air before sealing and incubated overnight at room temperature. CO_2 released was measured by gas chromatography (6500 GC system, YL Instrument).

Microbial biomass was determined by the Substrate Induced Respiration (SIR) method (Anderson and Domsch, 1978). Soil (1 g) was mixed with glucose-talcum (10 mg; 4:1 w/w), flushed with pressurized air and incubated for 2 h at room temperature. CO_2 released by microorganism respiration was then measured by gas chromatography as above.

2.5. Bacterial and fungal growth

Microbial growth rates were measured by incorporation techniques based on the addition of tracer amounts of radioactively labelled precursors, which will be incorporated into macromolecules synthesized during microorganism growth. Bacterial growth was estimated by the ^{3}H-Leucine incorporation method adapted for soil (Bååth et al., 2001). Bacteria were extracted by vortexing soil (1 g) with distilled water (20 ml), and after centrifugation (1000 x g) the supernatant with extracted bacteria was recovered (1.5 ml into microcentrifugation vials). This bacterial suspension was mixed with L-4,5-^{3}H-Leucine (2 μl, 37 MBq ml^{-1}, 1.48–2.22 TBq mmol^{-1}, Perkin Elmer) together with non-radioactive Leu (final Leu concentration 275 nM) and incubated for 2 h at room temperature. Bacterial growth was stopped by adding 75 μl of 100% trichloroacetic acid. Removal of non-incorporated Leu by centrifugation and subsequent measurement of radioactivity on a scintillation counter was as described by Bååth et al. (2001).

Soil fungal growth was measured by the ^{14}C-acetate incorporation into ergosterol method (Bååth, 2001). Soil (1 g) was mixed with distilled water (1.95 ml), unlabelled acetate (30 μl, 16 mM) and [1^{-14}C] acetic acid (20 μl, sodium salt; 7.4 MBq ml^{-1} and 2.04 GBq mmol^{-1}; Perkin Elmer), resulting in a final acetate concentration of 220 μM. After incubation for 4 h at room temperature, fungal growth was stopped by adding 5% formalin and the samples were centrifuged. The supernatant was removed and 10% KOH in methanol was added to the samples. After sonication (15 min), the samples were incubated for 1 h at 70 °C to extract ergosterol. Ergosterol was purified by phase separation, measured by HPLC (Elite LaChrome, Hitachi) to detect fungal biomass (Grant and West, 1986) and collected using an autosampler. Finally, samples were mixed with scintillation cocktail for scintillation counting analysis.

2.6. Statistical analysis

To compare the effect of litter additions for the different soils, the results are expressed as delta values, i.e. the values in the control soil (without litter addition) were subtracted at each measurement time. Cumulative values were calculated for basal respiration, bacterial and fungal growth for the whole period of soil incubation. Differences between cumulative values were tested by a two-way ANOVA with soil and different litter types as fixed factors, followed by Holm-Sidak test for comparison between groups. To differentiate between soil effects and C/N of the litter types on the cumulative fungal to bacterial growth ratio, ANCOVA on the log transformed ratios were made, with C/N ratio of the litter types as a continues factor and soil type as the fixed one.

3. Results

3.1. Respiration rate and microbial growth

The respiration rate increased after all litter additions in both soils (Fig. 1). The respiration was highest after alfalfa addition and lowest after beech litter amendment in both soils. Respiration