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Microbial functional diversity in a mediterranean forest soil: impact of soil nitrogen availability

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Beneficial or negative effects of N deposition on forest soil are strongly linked to the activity of microbial biomass and enzyme activities because they regulate soil quality and functioning due to their involvement in organic matter dynamics, nutrient cycling and decomposition processes. Moreover, because the ability of an ecosystem to withstand serious disturbances may depend in part on the microbial component of the system, by characterizing microbial functional diversity we may be able to better understand and manipulate ecosystem processes. Changes in the biodiversity of the soil microbial community are likely to be important in relation to maintenance of soil ecosystem function because the microbial communities influence the potential of soils for enzyme-mediated substrate catalysis.

Objective of this study was to evaluate how soil N availability affected microbial functional diversity in a 4 months laboratory experiment.

The incubation experiment was carried out with an organo-mineral soil collected in a Quercus cerris forest at the Roccarespampani site (Central Italy, Viterbo). All samples were incubated at 28°C and were kept to a water content between 55 and 65% of the water holding capacity. Different amount of N (NH4NO3) were added as solution once a week in order to mimic the N wet deposition and to let microbial community deal with a slow increase in time of inorganic N content. The amount of nutrient solutions was chosen depending on the average soil-water loss due to evaporation in one week. The total amount of N-NH4NO3 was chosen to be comparable with the range of N depositions currently reported in European forests, i.e. between 1 and 75 kg N ha-1 y-1. The total amount added at the end of incubation varied from 0, 10, 25, 50 and 75 kg N ha-1. Distilled water was added in the control soil in order to provide the same amount of solution as the treated soils. In order to discriminate the effect of N, the NH4NO3 solutions were adjusted to soil pH and phosphorus was added in order to prevent any nutrient limitation effect.

In this experiment microbial functional diversity was assessed at the community level with two independent approaches: the first one uses soil hydrolytic and oxidative enzymes and the second one C substrates utilization rates with the MicroResp system.

The activities of important soil enzymes involved in organic matter and nutrient transformations were determined using a fluorimetric approach: beta-glucosidase, alfa-glucosidase, beta-xylosidase and beta-cellobiohydrolase activities are key enzymes in the cellulose and starch degradation; N-acetyl--glucosaminidase and leucine-aminopeptidase activities are involved in N cycling through chitin degradation, a major source of mineralizable N in soil and peptides release; acid phosphatase is crucial in organic P transformation; butyric esterase is an indicator of the physiological performance of microbial biomass in soil. (Poly)phenol oxidative activity was determined spectrophotometrically as an indicator of lignin and lignin-like substances polymerization and depolymerization. All enzymes were assessed at the beginning of the incubation and after 6, 13, 26, 42, 55, 83 and 118 days.

For the MicroResp method C substrates for the analysis of Community Level Physiological Profile (CLPP) were selected depending on their ecological relevance and the objective of the experiment. C sources include four carbohydrates (Alpha-D-glucose, N-acetyl-Glucosamine, D-Galactose, D-fructose), four amino acids (L-arabionose, L-leucine, L-arginine, Glycine), five carboxylic acid (Malic acid, citric acid, Oxalic acid, L-aspartic acid and gamma-amino-butyric acid) and two phenolic acids (vanillic acid and syringic acid). MicroResp analysis was performed at the beginning and at the end of the incubation.

Discriminant function analysis and Shannon diversity index were used to determine microbial functional diversity

with the two different approaches.