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Differential responses of structural and functional aspects of soil microbes and nematodes to abiotic and biotic modifications of the soil environment

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ABSTRACT

We evaluated the effect of an abiotic and a biotic modification of soil environment on the structure and function of soil biological communities in a six month experiment established in a recently abandoned wheat field. Evaluations included PLFA and CLPP microbial profile as well as the nematode trophic structure and functional indices in plots that differed in irrigation (abiotic modification) and manure application (biotic modification). Four treatments were created: low water supply-manure fertilization, low water supply-no fertilization, high water supply-manure fertilization and high water supply-no fertilization. Two samplings were carried out: the first after 8 weeks of irrigation (March) and the second after 20 weeks (June).

The PLFA groups were mainly affected by the abiotic treatment (water supply), which acted either alone in June or in combination with the biotic treatment in March. In all plots, there was an increase of bacterial and to a lesser extent of fungal biomass from March to June, as well as a shift of the bacterial community towards Gram$^+$. The microbial catabolic profile was different in the two sampling occasions, whereas the microbial functional diversity (Shannon index) was not affected either by treatment or by sampling time. Nematode abundance was also higher in June in relation to March in all plots, whereas the response of nematode feeding groups to treatments was occasional and related to manure rather than to water supply.

1. Introduction

The soil environment is subject to a plethora of abiotic and biotic changes, either natural or induced by humans, and the response of soil components to them has been studied thoroughly. However, a question rarely addressed by soil biologists is whether the abiotic changes of the soil environment are more important for shaping soil communities than the biotic ones or vice versa. According to Whisenant (1999), in healthy and high resource wildlands, soil functioning is mainly controlled by biotic interactions, whereas in degraded lands, modifications of the abiotic environment are more important for soil functioning. Inspired by this hypothesis, we set up a field experiment focusing on the effects of different amounts of water supply (abiotic modification) and manure application (biotic modification) on the structure and function of soil microbial and nematode communities. The application of manure is considered a biotic intervention, because manure enhances microbial populations and triggers bottom-up alterations of the soil community. Our experiment was set on a recently abandoned Mediterranean field, which may be considered degraded, since Mediterranean soils are nutrient-poor (Parry et al., 2007), with high pH and low organic matter (Caliskan et al., 2008), low fertility and low decomposition rates (Delgado-Baquerizo et al., 2011), while they are fragile, shallow and with a distorted soil profile (Papatheodorou, 2008). Moreover, recently abandoned cultivated soils exhibit a reduced C content and a deficiency of N due to the lack of incorporation of organic material into soil (McLauchlan, 2006).

Microbial communities have attracted special interest for quantifying the impacts of biotic and abiotic changes of the soil environment, because they mediate many ecological processes in soil that are central to ecosystem functioning, such as nutrient cycling and litter decomposition, whereas they respond rapidly to environmental changes and stress (Winding et al., 2005). Changes in water content could impact the function and the structure of their communities, since dissimilar types of microorganisms are differentially affected by changing amounts of water potential (Todd et al., 1999; Griffiths et al., 2003; Drenovsky et al., 2004).

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Table 1
Soil physicochemical properties in the study area.

Soil properties	Mean (SE)
Clay (%)	37.75 (2.28)
Silt (%)	26.50 (2.64)
Sand (%)	35.75 (1.74)
Bulk density	1.48 (0.035)
pH (H_2O)	7.87 (0.15)
N-organic (%)	0.16 (0.04)
C-organic (%)	2.24 (0.72)
P (mg/100 g d.w.)	1.7 (0.21)
K (mg/g d.w.)	0.17 (0.01)

On the other hand, several studies have shown that the addition of manure is responsible for increases in soil microbial biomass and diversity (Chu et al., 2007; He et al., 2008). Apart from microbes, the ecological significance of nematodes as bioindicators for evaluating the impact of changing soil conditions has also been highlighted in numerous studies. According to Fiscus and Neher (2002), nematodes reflect changes in ecological structure and function of soils in ways more predictable and efficient than other soil flora or fauna. Nematode responses to soil moisture changes are reported to vary among trophic groups and across ecosystem types (Todd et al., 1999; and references therein), while amendments involving organic matter inputs, such as manure application, may change the trophic structure of their communities reflecting changes in the decomposition pathways (Tsiafouli et al., 2006, 2007). Although closely linked within the soil food web, microbes and nematodes exhibit different modes of life, different sensitivity to soil disturbances (Kapagianni et al., 2010), and their responses to changing soil conditions are not synchronized (Papatheodorou et al., 2004).

In order to quantify the structural and functional responses of microbial and nematode communities to changing soil moisture regime and manure application, we analyzed the PLFA and CLPP soil profiles as well as the nematode trophic structure and functional indices. We hypothesized that microbes and nematodes would respond differently to these alterations of the soil environment. Furthermore, within the context of Whisenant's (1999) hypothesis, we explored whether in a low-resource environment the abiotic treatment is of more crucial importance than the biotic one on shaping the microbial and nematode communities.

2. Materials and methods

2.1. Study site

The study was conducted in Koila Kozanis (West Macedonia, North Greece), between the city of Kozani and Via Egnatia. According to 40 year climatic data from the metereological station of Kozani, the mean annual temperature and rainfall is 13 °C and 497.1 mm, respectively. The wet period lasts from October to May and the dry one from June to September.

Soil physicochemical properties of our experimental area (Table 1) were determined as described in Section 2.3. The soil is classified as calcaric lithosol (FAO) with a clay loam texture and alkaline pH, exhibiting low amounts of organic C, organic N and extractable P, compared to other Mediterranean grasslands (Monokrousos et al., 2004; Papatheodorou and Stamou, 2004). Therefore, it can be considered a low-resource land as originally expected.

2.2. Experimental design and sampling

The experiment was set in a recently abandoned (2 years) wheat field. Sixteen experimental plots (1 m × 1 m each) receiving different treatments regarding water supply and manure were interspersed following a randomized complete design (4 treatments × 4 replicate plots), while large untreated empty spaces were left between them. Once every week, eight plots were sprayed with 7 L of water and the other eight with 20 L of water. These two levels of water supply correspond to 50% and 150% respectively of the mean monthly precipitation of the wet period of the year in the area, estimated on the basis of 40-year climatic data of the nearby meteorological station. For protection from precipitation, all plots were sheltered with plastic shield in a form of a gable roof. The two plastic surfaces that formed the roof triangle were touching the ground, so that water was shed effectively and side effects of rain were avoided as much as possible. Channels were dug around each plot to prevent surface runoff. At each irrigation level, four of the plots received cattle manure (4 kg/m^2) at the beginning of the experiment, whereas in the rest four no manure was applied. Overall, four treatments were established: 50-F (low water supply-manure fertilization), 50-UF (low water supply-no fertilization), 150-F (high water supply-manure fertilization), and 150-UF (high water supply-no fertilization).

The experiment lasted from January to June 2009. The manure was applied once in the middle of January, it was incorporated into the soil with a mattock, and the irrigation started. Two samplings were carried out; the first in the middle of March (after 8 weeks of watering) and the second in the middle of June (after 20 weeks of watering). To study nematodes, a composite sample was taken from each plot, consisting of three soil cores 3 cm in diameter and 20 cm in depth. To determine the microbial community structure (PLFA) and its catabolic profile (Ecoplates), we took three samples per plot, to account for within-plot heterogeneity, by an auger 7.5 cm in diameter and 20 cm in depth.

2.3. Soil physicochemical variables

Before the beginning of the experiment, ten soil samples were randomly collected from the experimental area by a soil auger (7.5 cm × 20 cm), for the determination of soil texture, pH and the amounts of C, N, P and K. Soil texture was estimated by the Bouyoucos (1962) method. Soil organic C was determined by a wet oxidation–titration procedure using an acid dichromate system (Allen, 1974). Soil organic N was measured by the Kjeldahl method. To estimate extractable P, we used the method of Olsen et al. (1954), as specified by Allen (1974). The concentration of K was determined in soil extracts by an atomic absorption spectrophotometer. Finally, pH was measured in a 1:5 soil/water solution.

2.4. Phospholipid fatty acid analysis

Extraction of phospholipids from soil samples was done according to Bossio et al. (1998) with slight modifications described in Spyrou et al. (2009). The steps of the procedure are briefly described as follows: (i) extraction of lipids, (ii) separation of phospholipids by column chromatography, (iii) methylation of esterified fatty acids in the phospholipid fraction, (iv) GC analysis into a Hewlett Packard HP 5890 Series II Gas Chromatograph equipped with FID detector and connected with a 5% phenyl methylsiloxane fused silica capillary column (HP-5MS 26 m length × 0.320 mm i.d., film thickness 0.25 μm). Further confirmation of the different components of the standard fatty acid methyl esters mixtures was achieved by analysis in a GC–MS Agilent 5973 system operated in the same temperature program and mass spectra identification based on the NIST 98 library data base (Spyrou et al., 2009). Electron energy in electron impact was 70 eV, while temperatures of the source, quadrapole, and interface were 230 °C, 150 °C, and 280 °C, respectively. Fatty acids were quantified (nmol g^{-1}) by calibration against standard solutions of the internal standard 19:0 ME. For this, a six-point calibration curve was constructed in the range of 25–200 μg ml^{-1}