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Trends in plant and soil microbial diversity associated with Mediterranean extensive cereal–fallow rotation agro-ecosystems

Helena Castro*, Lurdes Barrico, Susana Rodríguez-Echeverría, Helena Freitas

Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal

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ABSTRACT

This study examines plant and soil microbial diversity in a cereal–fallow rotation scheme in the cereal steppes of Castro Verde, Southern Portugal, which have an important conservation value as they provide habitat for many steppic birds with unfavorable conservation status. For that we monitored plant and soil microbial diversity during 4 years including all stages of the rotation cycle.

The structure and composition of plant and soil communities during wheat crop were different from those found in the fallow years, although the effect of wheat crop on soil bacteria was still noticeable in the 1st year of fallow. The main changes in the structure of microbial communities happened between the first and second year of fallow, probably due to changes in the quality and quantity of litter inputs. As expected, we observed an overall decrease in plant diversity in the wheat stage. Fallows had a positive effect on plant species diversity by allowing the maintenance of a seed bank and controlling the simplification of weed communities. However, the impact of fallow on microbial communities is more complex: bacterial diversity was higher during the wheat stage while fungal diversity was either higher or unchanged.

These results suggest that the rotation stage is the main driver of the diversity and composition of soil microbial communities, probably through changes in the plant community that deserve further examination. Inter-annual fluctuations in rainfall had a stronger impact on plant communities than on microbial communities indicating that the later are likely adapted to the characteristic fluctuation of annual rainfall in areas under Mediterranean climate.

1. Introduction

Agricultural management practices can severely impact the diversity of plant and soil microbial communities (Hole et al., 2005; Kiers et al., 2002). Several studies have documented the response of both plant and microbial communities to tillage, fertilization, herbicide application and crop rotation. Positive, negative or neutral effects of tillage, fertilizers or herbicides have all been reported for soil microbes (e.g., Acosta-Martínez et al., 2010; Lupwayi et al., 2010; Mathew et al., 2012; Sarathchandra et al., 2001). Similarly, the effects of tillage, fertilization or herbicide on plants are not always consistent and suggest that effects on plant communities are more likely to be observed on species composition than on plant species richness (e.g., Hyvönen and Salonen, 2002; Légère et al., 2005). However, few studies have evaluated the effect of vegetated fallow periods on plant and/or soil microbial communities, particularly under Mediterranean conditions. The term 'fallow' describes resting periods in agricultural lands in which the non-crop or dormant species are allowed to re-establish by natural succession after cropping. Vegetated fallow systems are traditional management practices aimed at restoring soil fertility and protecting the soil in areas where climate and low soil fertility constrain productivity and lead to a high risk of erosion.

Secondary succession during fallow leads to the development of annual plant communities that are determined by seed availability, due to soil seed bank or seed dispersal, time and climatic conditions (Pugnaire et al., 2006). Thus, fallow can increase plant diversity at local and regional scales. Higher plant diversity is associated with the dominance of several species in opposition to the dominance of a single species (Hyvönen and Salonen, 2002). This may result in benefits, for both crop production and diversity of other trophic levels (Marshall et al., 2003; Tscharntke et al., 2011). Higher weed diversity has been shown to be associated with a higher proportion of insect-pollinated species thereby extending food potential for other groups of organisms and creating a source of complexity for the agro-ecosystem (Kuussaari et al., 2011; Tscharntke et al., 2011).

* Corresponding author. Fax: +351 239855211.
E-mail address: hecastro@ci.uc.pt (H. Castro).

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Both plant diversity and community composition have been shown to influence soil-borne microbial communities, which are the basis of important ecosystem services, such as nutrient cycling and suppression of soil borne plant diseases (Bardgett and Wardle, 2010; Garbeva et al., 2004; Wardle et al., 2004). The effect of plants on soil microbes, however, is not consistent. Comparative studies report undetectable effects of plant diversity and community composition on soil microbial community (Buckley and Schmidt, 2003; Kielak et al., 2008) in former arable fields. Similarly, Hedlund et al. (2003) found inconsistent effects of plant diversity on soil microbial communities in grasslands. Plant species richness and composition did not have a significant effect on the below-ground microbial communities in a chronosequence secondary succession (Kuramae et al., 2011). It has also been suggested that soil microbial communities are influenced by long-term effects of cultivation which can overpower the effect of vegetation (Buckley and Schmidt, 2001). On the contrary, a study by Bezemer et al. (2006) found that manipulating plant species diversity had an effect upon soil microbial community structure. The cessation of the perturbation caused by agriculture may also lead to changes in the microbial community. Zornoza et al. (2009) detected changes in soil microbial community structure following the abandonment of cultivated terraces in Eastern Spain whereas in the study by Gomez-Montano et al. (2013) the microbial diversity remained unchanged, and in some cases decreased, with fallow time in Bolivian Altiplano soils.

Fallow is traditionally used in the cereal steppes of Castro Verde, Southern Portugal, which have an important conservation value as they provide habitat for many steppic birds with unfavorable conservation status such as the great bustard (*Otis tarda* L.), the little bustard (*Tetrax tetrax* L.), and the lesser kestrel (*Falco naumanni* Fleischer) constituting one of the last refuges for these species (Moreira et al., 2005). Some management practices targeting biodiversity conservation, such as maintaining a minimum amount of farmland under fallow and limiting grazing during the nesting period have had a positive effect on key bird species' population numbers (Alcazar, 2013). However, the effect of these practices on other trophic levels is unknown. This study examines plant and soil microbial diversity in a cereal–fallow rotation scheme. For that we monitored plant and soil microbial diversity during 4 years including both stages of the rotation cycle in this area under Mediterranean climate. Besides the crop/fallow cycle, the seasonality and unpredictability in precipitation characteristic of the Mediterranean climate are known to strongly determine plant composition by influencing germination, duration of vegetative growth and seed production (Espigares and Peco, 1993; Montalvo et al., 1993; Peco and Espigares, 1994), and to affect microbial communities (Gallardo et al., 2000).

We hypothesize that: (1) crop stage, combining a change in vegetation cover, soil disturbance and fertilizer inputs, will have a strong effect on plant and soil microbial communities with vegetated fallows having a positive impact on plant species diversity by allowing the maintenance of a seed bank that buffers the negative impact of crop years, (2) plant and soil microbial communities will be influenced most by inter-annual fluctuations in precipitation than by time of fallow because crop/fallow rotation is a cyclic practice that has been carried out for centuries and it is expected that both plant and soil microbial communities have been filtered by a long management history.

2. Methods

2.1. Study area

The sampling for this study took place in the cereal steppes of Castro Verde, Southern Portugal.Two farms within the cereal steppes of Castro Verde, Southern Portugal, were selected for this study: Vale Gonçalinho, at about 37°44′11.03″N and 8°1′53.79″W, and S. Marcos at about 37°41′85.29″N and 7°55′15.70″W. Climate is Mediterranean with mean annual temperature of 16.6 °C and mean annual rainfall around 520 mm with large inter-annual fluctuation. The years of sampling varied in the amount of rainfall during late winter-early spring (February to April). 2010, 2011 and 2013 were wet years (170%, 138% and 146% of the average rainfall, respectively) and 2012 was a dry year (39%). The air temperature for the same period also varied among years and was lower in 2012 (12.3 °C) than in the remaining years (2010, 14.0 °C; 2011, 14.5 °C and 2013, 13.1 °C). The dominant soils are poor and shallow lithosols of non-calcareous schist with high stoniness.

At the selected farms current and past land use is traditional cereal/fallow rotation. In the rotation system, the farm is divided into fields and each field is under different phases of the rotation cycle, creating a mosaic of fallow and cultivated fields. For 1–2 years a field is under cereal cultivation (wheat, oat), after which land is left fallow for a period of 2–4 years. During fallow vegetation is allowed to grow from natural sources (seed bank and/or seed rain) and animals, usually sheep, are allowed to feed on this vegetation. Both farms are owned by a NGO devoted to nature conservation (LPN) and are managed with the aim of preserving endangered bird species. Special practices for that purpose include limiting grazing during bird nesting period (spring), crop harvesting is done after the end of the nesting period (late June), and wheat stubbles are not removed.

At each farm an area of 4–6 ha was selected where we sampled vegetation and soil over 4 years (2010–2013). In both areas fields were sowed with wheat in 2010 and left under fallow in 2011, 2012 and 2013. In both farms conservation tillage was used and wheat seeding was done by direct drilling. Fertilizers were applied twice, first in October, at the time of seeding (N = 14 kg/ha P = 42 kg/ha) and again in January (N = 40 kg/ha) at S. Marcos farm and only once at V. Gonçalinho farm, in March (N = 40.5 kg/ha, CaO = 6 kg/ha).

2.2. Vegetation survey

Vegetation data was taken by delimitating six randomly distributed plots of 4 m^2 in each farm. In each plot we recorded plant species presence and percentage cover, and the cover of the functional groups legumes, grasses and forbs, as well as, the percentage of soil that was not covered by vegetation (bare soil) and the percentage that was covered by previous year dead plant material (litter). Additionally, we recorded average vegetation height, by taking 5–10 measurements per plot.

2.3. Soil sampling and analyses

2.3.1. Soil sampling

In the same plots used for vegetation sampling we took 4 soil cores (up to 10 cm deep) that were mixed to create a composite soil sample per plot. A subsample of soil for DNA extraction was taken and frozen at −20 °C. The remaining soil was air-dried and subsamples taken for chemical analyses.

2.3.2. Soil properties

Soil carbon and nitrogen were determined by Dumas method and phosphorus was determined by Olsen method at UTAD. Soil calcium, magnesium and potassium contents were determined by atomic absorption following extraction with ammonium acetate (Thomas, 1982). Soil organic matter was determined by loss-on-ignition after 3 h at 360 °C on oven dried soil samples.