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Seasonal controls on grassland microbial biogeography: Are they governed by plants, abiotic properties or both?☆

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ABSTRACT

Temporal dynamics create unique and often ephemeral conditions that can influence soil microbial biogeography at different spatial scales. This study investigated the relation between decimeter to meter spatial variability of soil microbial community structure, plant diversity, and soil properties at six dates from April through November. We also explored the robustness of these interactions over time. An historically unfertilized, unplowed grassland in southwest Germany was selected to characterize how seasonal variability in the composition of plant communities and substrate quality changed the biogeography of soil microorganisms at the plot scale (10 m × 10 m). Microbial community spatial structure was positively correlated with the local environment, i.e. physical and chemical soil properties, in spring and autumn, while the density and diversity of plants had an additional effect in the summer period. Spatial relationships among plant and microbial communities were detected only in the early summer and autumn periods when aboveground biomass increase was most rapid and its influence on soil microbial communities was greatest due to increased demand by plants for nutrients. Individual properties exhibited varying degrees of spatial structure over the season. Differential responses of Gram positive and Gram negative bacterial communities to seasonal shifts in soil nutrients were detected. We concluded that spatial distribution patterns of soil microorganisms change over a season and that chemical soil properties are more important controlling factors than plant density and diversity. Finer spatial resolution, such as the mm to cm scale, as well as taxonomic resolution of microbial groups, could help determine the importance of plant species density, composition, and growth stage in shaping microbial community composition and spatial patterns.

1. Introduction

All natural systems are temporally and spatially bounded and the defined spatial organization observed in many ecosystems suggests that spatial organization is of functional importance (Legendre et al., 2005). In terrestrial systems many studies have shown that soil microbial communities are structured at several spatial scales (Franklin and Mills, 2003; Ritz et al., 2004; Bru et al., 2011; Keil et al., 2011), indicating effects of environmental drivers such as land use and abiotic conditions. For example, Franklin and Mills (2003) found multi-scale variations in microbial community spatial structure (from 30 cm to >6 m) with high spatial heterogeneity due to soil properties, in a wheat field study using DNA fingerprinting. Ritz et al. (2004), in an unimproved grassland study,

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observed a high degree of spatial variation in community-level microbiological properties, but were not able to characterize overarching controlling factors. Keil et al. (2011), in contrast, found that ammonia-oxidizing and denitrifying microorganisms were spatially structured in soils from 10 m × 10 m grassland plots. This was confirmed in a study by Berner et al. (2011), who found that spatial heterogeneity in grasslands at scales of 1–14 m was related to land use intensity; i.e., fertilization, mowing frequency, and grazing practices. Indeed, many studies indicate a close link between above and belowground components in terrestrial ecosystems (Reynolds et al., 2003; Zak et al., 2003; Wardle et al., 2004; van der Heijden et al., 2008). Plants may affect the soil microbial community directly via nutrient and water uptake, litter input, and root exudates, or indirectly, by changing composition or abundance of the decomposer community. Microbes may also have direct or indirect effects on plants; thus, understanding the patterns of interaction between plant and soil microbial communities is critical. However, the degree of coupling between plants and microbial communities has been hard to quantify in grasslands, probably due to the very high plant density (Ritz et al., 2004) and/or high plant species richness (Zak et al., 2003; Nunan et al., 2005). It is also possible that these interactions occur at scales that have not yet been identified.

The picture that emerges from the existing literature is that microbial communities are subjected to many external structuring influences and that the relative importance of these influences is both context and microbial group dependent (Martiny et al., 2006). Furthermore, many of the relationships are not particularly strong and it is therefore legitimate to ask whether they persist over time and through seasons. The vast majority of microbial spatial or biogeographic studies have been carried out at a single time point and those studies which have combined spatial and temporal approaches have yielded conflicting results. Zak et al. (2003), in a long term study, found that microbial composition and function were influenced by plant diversity, while Grayston et al. (2001) found plant productivity, temperature, and moisture to have the strongest effects on soil microbial community structure. However, Habekost et al. (2008) observed that distribution patterns of microbial communities in grassland soils changed with time, mainly in response to plant performance. Only a few studies have been carried out at the plot scale in grasslands or agricultural fields over multiple time points (Grayston et al., 2001; Habekost et al., 2008; Kulmatiski and Beard, 2011; Lauber et al., 2013). Coupled spatial characterization with temporal variability of soil microbial communities has been less often explored.

The goal of this study was to resolve some of this uncertainty by a detailed investigation of spatial patterns in microbial community structure to learn how the relationships between microbial communities and their local environment persist over time. Edaphic factors have been shown to exert the strongest influences on microbial community composition at regional and continental scales (Fierer and Jackson, 2006; Lauber et al., 2008; Dequiedt et al., 2011; Griffiths et al., 2011; Sayer et al., 2013). A physically homogeneous grassland plot was used for this study, however. This provided an opportunity to assess what other factors could be identified at specific dates as drivers of spatial relationships of the microbial community to both the local soil environment and to changes in the plant community. One 10 m × 10 m plot in a grassland characterized by low land use was intensively sampled over a complete growing season, from early April, before plants had begun to actively grow, until November of that year when plant growth had ceased after a hard frost. Sampling times were selected to coincide with stages of plant growth in the permanent grassland; replicate samples were separated by 50 cm. Using a combination of conventional and spatial statistical approaches, we characterized above- and below-ground communities both temporally and spatially for each date. Our aim was to learn whether or not changes in microbial abundance, in microbial community structure, or in distributions of plants and microorganisms could be temporally and spatially distinguished.

We hypothesized that (i) by a temporally and spatially intensive examination of an unimproved grassland at the plot scale (10 m × 10 m) we could distinguish spatial changes in microbial biogeography, and (ii) this sampling approach would clarify the degree to which the microbial spatial structures we observed could be correlated with stages of plant growth and soil abiotic properties. We expected also to gain insight into the persistence of microbial spatial structure and the relationships of microbial communities with their environment.

2. Materials & methods

2.1. Site description

The present study is part of a larger, interdisciplinary project of the German Biodiversity Exploratories (Fischer et al., 2010). Our study site is located near the village of Wittlingen, Baden-Württemberg, 48°25′0.01″ N, 9°30′0.00″ E, in the Swabian Alb, a limestone middle mountain range in southwest Germany. The study site is AEG31, within which a 10 m × 10 m grassland plot was established. Annual precipitation in 2011, the year in which this study was done, was 810 mm and average temperature was 8.1 °C (Appendix A: Fig. A1). The study site is managed at low intensity: no fertilizer is applied, it is mown once per year, and is briefly grazed by sheep for 1–2 weeks typically in late summer or early autumn. The soil type at the site is characterized as a Rendzic Leptosol (FAO classification), a calcareous, shallow AC-soil (typically 10 cm depth), with an average pH of 6.7, containing total 0.66 mg g^{-1} carbon (C) and 0.07 mg g^{-1} nitrogen (N). C/N ratios, pH, and soil texture were uniform over the sampling period.

2.2. Sample design

A 10 m × 10 m plot was established within this grassland and divided into 30 subplots (each 2 m × 1.67 m). Within each subplot six pairs of sample locations were randomly assigned, with one pair sampled at each of six dates over the growing season (Appendix A: Fig. A2). Each sample pair per subplot for a given date was separated by 50 cm to provide appropriate lag distances for later geostatistical analyses (Appendix A: Fig. A2). Sixty samples were collected at each date (two individual sample locations per subplot × 30 subplots). A total of 360 soil samples were collected over the season. Each sample location was assigned unique *x* and *y* coordinates with respect to the boundaries of the plot. Samples were collected in 2011: on April 5th at the beginning of the vegetation period, May 17th during the main growth phase, June 27th at around peak plant biomass, August 16th two weeks after the grassland was mown, October 5th, nine weeks after mowing and two weeks after it was lightly grazed, and November 21st after the first frost.

2.3. Sampling – aboveground

On each sampling date, before soil core samples were collected, 20 cm × 20 cm grids were centered over each of the sixty individual sampling points. Vegetation data and above ground biomass were collected from all grids. Above-ground biomass was harvested by cutting all plants at ground level. Biomass samples were sorted into litter (dead leaves and plant matter on the soil surface), grasses (Poaceae), legumes, forbs, bryophytes and *Rhinanthus minor*. The latter was separated because this species parasitizes other plants,