Soil Biology & Biochemistry 109 (2017) 214–226

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Spatial and temporal dynamics of nitrogen fixing, nitrifying and denitrifying microbes in an unfertilized grassland soil

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ARTICLE INFO

Article history:
Received 31 August 2016
Received in revised form
7 November 2016
Accepted 10 November 2016
Available online 16 November 2016

Keywords:
Ammonia-oxidation
Denitrification
Potential enzyme activity
Biogeography
Niche-partitioning
Spatio-temporal variability

ABSTRACT

The microbial groups of nitrogen fixers, ammonia oxidizers, and denitrifiers largely drive the inorganic nitrogen cycle in temperate terrestrial ecosystems. Their spatial and temporal dynamics, however, vary depending on the studied scale. The present study aimed to fill a knowledge gap by providing an explicit picture of spatial and temporal dynamics of a subset of these soil microorganisms at the plot scale. We selected an unfertilized perennial grassland, where nitrogen cycling is considered to be efficient and tightly coupled to plant growth. At six times over one growing season 60 soil samples were taken from a 10 m × 10 m area and abundances of marker genes for total archaea and bacteria (16S rRNA), nitrogen fixing bacteria (*nifH*), ammonia oxidizing archaea (*amoA* AOA) and bacteria (*amoA* AOB), and denitrifying bacteria (*nirS*, *nirK* and *nosZ*) were determined by qPCR. Potential nitrification activity (PNA) and denitrifying enzyme activity (DEA) were determined. Seasonal changes in abundance patterns of marker genes were detected, and were associated with changes in substrate availability associated with plant growth stages. Potential nitrification and denitrification enzyme activities were strongly spatially structured at the studied scale, corresponding to periods of rapid plant growth, June and October, and their spatial distributions were similar, providing visual evidence of highly localized spatial and temporal conditions at this scale. Temporal variability in the N-cycling communities versus the stability of their respective potential activities provided evidence of both short-lived temporal niche partitioning and a degree of microbial functional redundancy. Our results indicate that in an unfertilized grassland, at the meter scale, abundances of microbial N-cycling organisms can exhibit transient changes, while nitrogen cycling processes remain stable.

1. Introduction

Soils are challenging environments to study because of their extreme structural and microbial heterogeneity, and yet soil microorganisms are important drivers of soil quality and ecosystem function, depending both on local microbial adaptation and on interactions with plants and other soil biota (Bardgett, 2005). Recent estimates indicate that in addition to a large number of fungi, protists, and other micro-eukaryotes, one gram of soil may harbor more than one million bacterial and archaeal species (Paul, 2014). This enormous biodiversity is a result of multiple interfaces with differing biogeochemical properties that are formed in soil as a result of interactions between microbes and their abiotic environment (Totsche et al., 2011). Not surprisingly, the issue of scale has become a critical topic in microbial soil ecology. Different influences on the soil microbiome and its functions have been identified depending on the scale under investigation (Franklin and Mills, 2009). Spatial studies of influences on the composition of the

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http://dx.doi.org/10.1016/j.soilbio.2016.11.011
0038-0717/

microbial community have to date been done mainly at the scale of fields (Hallin et al., 2009; Enwall et al., 2010) and regions (Philippot et al., 2009; Drenovsky et al., 2010). At field scales, typically in the range of hectares, cultivation regimes such as fertilizer application, tillage practices, landscape gradients, land use history, edaphic factors such as soil texture and pH, and vegetation composition affect community composition and spatial distribution of microbes by, for example, influencing their access to nutrients and moisture (Ettema and Wardle, 2002; Ritz et al., 2004; Martiny et al., 2006). At regional and landscape scales, typically in the range of km, factors such as soil type, climate, and precipitation regimes influence the composition of microbial communities and their functional traits through differences in soil physicochemical properties (Lauber et al., 2008; Bru et al., 2011; Dequiedt et al., 2011). Both field and regional scales are characterized by heterogeneity of vegetation, soil, microclimate, land-use history, and in the case of regional scales, of underlying geology. As scale increases, the interactions of factors such as soil type, climate, land management, or pollution, rather than of individual compounds, contribute to the composition of the soil microbiome (Grayston et al., 2001; Bardgett et al., 2005; Fierer and Jackson, 2006). In contrast, the plot scale, ranging typically from centimeter to meter, is characterized by homogeneity of these factors. For example, Grundmann et al. (2001), using a modeling approach, demonstrated significant differences in microbial communities which catalyze processes of nitrification in different soil compartments at the sub-millimeter scale. At this scale, individual substrates or physicochemical properties have been identified as drivers of microbial community development. In a multi-scale study, Franklin and Mills (2003) demonstrated that small variations in soil properties at scales from 30 cm to greater than 6 m contributed to shaping subsets of microbial communities in soil. Thus, studies at these small spatial scales make it possible to detect influences that may be obscured under more heterogeneous conditions, but which must be identified in order to understand interactions among microorganisms.

Microbial communities also show distinct and differing response patterns in time. As a consequence, the concept of highly localized and concentrated areas of microbial activity, known as hotspots (Parkin, 1987; Nunan et al., 2003) has been expanded to include hot moments (Groffman et al., 2009; Kuzyakov and Blagodatskaya, 2015). The duration of hot moments is highly variable, and changes among members of the microbial community vary depending on the choice of observed time scale. On the scale of hours to days, activity patterns of microbial communities (Schmidt et al., 2007) and sometimes even community structure (Cruz-Martinez et al., 2012) have been detected, while over longer time periods, clear shifts in microbial community structure can occur (Grayston et al., 2001; Bardgett et al., 2005; Dandie et al., 2008; Habekost et al., 2008; Lauber et al., 2013).

Plants can be considered as both architects of spatial heterogeneity and as drivers of temporal heterogeneity in soils. For example, growing roots change the physico-chemical environment of soils, thereby introducing small scale heterogeneity. In grassland soils, as a result of intensive plant growth, plants can change the physico-chemical conditions of the entire upper 10 cm of a soil (Mueller et al., 2013). During periods of vegetative growth, plant-derived exudates and availability of labile carbon act as drivers of microbial community structure and function (Houlden et al., 2008; Kuzyakov and Blagodatskaya, 2015), while during plants' senescent phase, plant litter and decaying root material become the most important supply of carbon for microbial communities. Therefore, both the amount and quality of carbon from exudates and litter vary substantially over the season, and this variation strongly influences microbial performance in soil (Chapin et al., 2002; Wardle et al., 2004; Houlden et al., 2008; Kuzyakov and Xu, 2013).

Previous studies in which both temporal and spatial dynamics have been investigated have often focused on either phylogenetic aspects of microbial communities (De Boer and Kowalchuk, 2001; Gubry-Rangin et al., 2011; Pasternak et al., 2013; Graf et al., 2014) or on a single functional group of microorganisms involved in soil N cycling, such as denitrifiers (Dandie et al., 2008; Groffman, 2012). In particular, studies of small-scale, seasonal variations in grassland microbial communities are lacking, especially those which comprehensively address changes in the abundances of microorganisms involved in different soil N-cycling processes together with their potential activities. Our goal was to fill a knowledge gap in the relationships between abundance and function in the soil nitrogen cycling microbial community at this scale. We selected an unfertilized perennial grassland with high plant diversity, where nitrogen cycling is considered to be highly efficient and tightly coupled to plant growth (Culman et al., 2010). In an initial study on this plot, we provided a biogeographical overview of microbial communities by documenting their small-scale spatial and temporal variability in relation to abiotic soil characteristics and plant biomass using PLFA analysis (Regan et al., 2014). This was followed by a characterization of spatial interactions between archaeal ammonia-oxidizers and nitrite-oxidizing bacteria, a specific group of organisms involved in two tightly coupled steps in N-cycling (Stempfhuber et al., 2016).

In this study we provide an explicit picture of spatial and temporal changes in abundances of nitrogen fixing bacteria, ammonia oxidizers (archaeal and bacterial) and bacterial denitrifiers as well as the potential enzyme activities of the latter two in order to address the following questions: 1) To what degree are different functional groups of microbes involved in the N-cycle spatially correlated with each other at the plot scale and how do these correlations change over a season? 2) Can the observed patterns be related to changes in abiotic characteristics such as soil moisture and N availability or to changes in plant growth associated with land management and the resulting changes in substrate availability? 3) What can the observed patterns tell us about grassland ecosystem N-cycling processes at the studied scale?

Spatial and temporal changes in microbial populations involved in N-fixation (*nifH*), ammonia-oxidation (AOA and AOB), and denitrification (*nirK*, *nirS*, and *nosZ*), and total bacterial and archaeal abundances as well as potential nitrification and denitrification activities were investigated at the plot scale of 10 m × 10 m at six dates over one growing season. Sampling times were selected to coincide with plant growth stages and management activities, from before active plant growth had started in the spring until after plant senescence following frost in autumn. Data were analyzed for geostatistical relationships with previously published soil biogeochemical data on nutrient distributions and changes in biomass of plant functional groups (Regan et al., 2014). While it is known that archaeal *nifH* (Francis et al., 2007; Dos Santos et al., 2012) archaeal *nirK* (Bartossek et al., 2010; Long et al., 2015), and archaeal *nosZ* (Rusch, 2013) have also been identified, they have to date most often been studied in manipulated or extreme environments, and less often in temperate, unfertilized grasslands. Their exact mechanisms and routes of archaeal denitrification are also still being investigated (Wallenstein et al., 2006; Lund et al., 2012; Pajares and Bohannan, 2016). They were therefore not included in this study.

2. Materials and methods

2.1. Site description

The present study is part of a larger, interdisciplinary project of the German Biodiversity Exploratories (Fischer et al., 2010). The study site is located in the Schwäbische Alb, a limestone middle