Functional implications of the pH-trait distribution of the microbial community in a re-inoculation experiment across a pH gradient

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ABSTRACT

We compared the influence of the microbial community composition and the environmental conditions for the functioning — microbial growth and respiration — and trait distribution — bacterial pH tolerance — of soil microorganisms across a pH gradient. Sterilised soil microcosms, including pH 4.1, 5.2, 6.7 and 8.3, with added plant litter were inoculated with unsterilized soil in a factorial design and monitored during two months. The trait distribution — pH-tolerance — of bacterial communities converged with the pH of the soil environment. Still, the different inoculum communities could result in suboptimal pH-tolerance in all soil pH environments; inoculum communities derived from low pHs had lower than optimal pH-tolerance in high soil pH environments, and vice versa. The functioning of bacterial communities with trait distributions mismatched to the soil pH environment was impaired. The legacy of the initial bacterial trait distribution on bacterial pH tolerance and functioning was detected within one week and remained for two months in all soil pH environments. Fungal inoculum communities derived from low compared to high pHs resulted in higher fungal functioning. Thus, in contrast with bacteria there was no evidence that variation in pH-tolerance influenced fungal performance. Instead the fungal inoculum size appeared to explain these results. Bacteria dominated respiration in high pH while fungi dominated at low pH environments. Consequently, respiration was affected by how well-matched the bacterial trait distribution was to the pH of the soil environment at higher pHs. At low pH, the inoculum size of fungi appeared to determine the respiration.

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1. Introduction

Many important processes of the soil ecosystem are dominated by microorganisms, and it has been argued that a mechanistic understanding of soil functioning is only attainable with a clear understanding of the factors that structure microbial communities (Schmidt et al., 2011). With the emergence of low-cost molecular methods, large-scale systematic microbial biogeography is made possible (Handelsman, 2004; Martiny et al., 2006; Green et al., 2008; Nemergut et al., 2011). Important environmental factors that structure microbial communities include pH in terrestrial (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010a) and salinity in aquatic systems (Langenheder et al., 2003; Lozupone and Knight, 2007; Mohamed and Martiny, 2011).

Moving from correlations between community composition and the environment to the prediction of functional responses is an important next step (Balser and Firestone, 2005; Dumont and Murrell, 2005). Experimental approaches for reaching this goal have included the use of common garden experiments (Ingham et al., 1985; Langenheder et al., 2006) or reciprocal transplant studies (Reed and Martiny, 2007; McDaniel et al., 2014; Xun et al., 2015), often investigating if there is a "home-field advantage" in the performance of a process, such as the decomposition of plant litter (Strickland et al., 2009a, b; Carrillo et al., 2012; Freschet et al., 2012). The local environmental conditions can exert a powerful selective pressure structuring microbial communities, as evidenced by a strong convergence toward similar communities and functional outputs, such as photosynthesis for primary producers, or growth or respiration for heterotrophic communities (Venail et al., 2008; Allison, 2012), under identical environmental conditions (Langenheder et al., 2006). However, increasing interest has also arisen in the effect of the inoculum community used in reciprocal transplant studies (Reed and Martiny, 2007; Lindström and

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Langenheder, 2012). Such an effect implies that environmental changes concomitant with limited dispersal can structure the phylogenetic composition and, more importantly, can modulate the function of microbial communities. However, relating community patterns at the taxonomic level to ecosystem function is not straight-forward especially for microorganisms, whose functional variation within the taxonomic gradations we use can be large and frequently is unknown. A way forward, proposed by Green et al. (2008) and Allison (2012), is a trait-based approach analogous to that previously used for plants and animals (*e.g.* Grime, 1977; Southwood, 1977).

The composition of a community is determined by the properties, or traits, of individuals and species. As such, trait-based approaches are predictive about how organisms will respond to changes in their environment (Grime, 1977; Southwood, 1977; Norberg et al., 2001; Allison, 2012). However, the determination of traits is not an easy task for any group of organisms, let alone microorganisms, for which knowledge about the distribution of functional traits of most taxa in nature is limited (Green et al., 2008). While traits characterise a single organism or population, the group of species forming a community can be characterised by a distribution of traits (Norberg et al., 2001). Consequently, an alternative approach is to determine the trait variation of the entire community as one aggregate property, without distinguishing between individual populations (Norberg et al., 2001; Allison, 2012). This can be done by measuring the community tolerance or dependence on different environmental factors by measuring the intrinsic growth of the bacterial community at a range of different conditions. This approach of estimating community trait distributions with regard to temperature (Li and Dickie, 1987; Bárcenas-Moreno et al., 2009), tolerance to heavy metals (Blanck, 2002; Berg et al., 2012), and pH tolerance (Fernández-Calviño and Bååth, 2010; Fernández-Calviño et al., 2011a; Bååth and Kritzberg, 2015) has been applied in both soil and water. pH tolerance is easily estimated in environmental samples by measuring instantaneous growth of the bacterial community at different pH values, creating a unimodal trait distribution with fastest growth at the optimal pH (Bååth et al., 1992; Fernández-Calviño and Bååth, 2010). The optimum pH of growth is known to correlate with soil pH (Bååth, 1994; Fernández-Calviño et al., 2011a). Thus, in a set of reciprocal transplantation experiments with two soils, one with low and the other with high pH, inoculated with bacterial communities that were adapted to low or high pH, the relative importance of the soil pH environment and the community inoculum were compared in terms of bacterial community function, measured as growth rate (Pettersson and Bååth, 2004, 2013). The soil pH environment dominated both the resulting pH-trait distribution and the function of the resulting bacterial community. The nature of the inoculum, i.e. the initial trait distribution of the bacterial community, had a smaller effect. A significant influence on growth and trait distribution was only detected in the high pH soil.

In this study, we built on the results of previous work but refined the comparison of the influence of the inoculum community (initial trait distribution) and the environmental conditions (soil pH) on microbial community traits and functions. Our assessment included a more comprehensive range of environmental conditions (soil pH range, and number of pH levels) and microbial functions (respiration, and productivity measured as microbial growth and biomass accumulation) than previously used. We also assessed fungal responses in addition to those of bacteria. We performed a full factorial reciprocal inoculation experiment including soils with different pH levels (pH 4.1, 5.2, 6.7, 8.3), in which sterilised soil microcosms were inoculated with unsterilized soil. We monitored function and community pH-trait distributions over 2 months. Our approach was based essentially on the conceptual framework proposed by Webb et al. (2010). We estimated the initial bacterial trait-distribution for each soil as the pH tolerance of the bacterial community in soils inoculated with a community from a matching soil pH. We used an "environmental gradient" of different soil pH environments, with the different pHs acting as "performance filters" resulting in communities with a "filtered trait distribution" (*sensu* Webb et al., 2010). Finally, we related the response of the trait distribution of the community to a set of functions.

We hypothesized that (H1) the bacterial pH-tolerance (trait distribution) of all inoculated communities would converge at the pH of the soil environment, but (H2) that the different initial trait distributions would modulate the resulting trait distributions; inoculum communities derived from acid soil resulting in lower pH tolerance and inoculum communities derived from alkaline soil resulting in higher pH tolerance. Further, we hypothesized (H3) that a mismatch of the trait distribution with the environment would result in functional consequences, so that soils inoculated with a community derived from a soil with the same pH as that of the soil environment it was inoculated into would function "optimally", whereas communities derived from soils with a pH different from that of the environment it was inoculated into would function less well there. In addition, based on results from previous experiments (Pettersson and Bååth, 2004, 2013), we hypothesized (H4) that differences between trait distributions of bacterial communities formed from different inoculum communities would be larger in high pH environments compared to low pH environments. It has been shown that fungal species can grow well in environments representing a wider pH range than can bacteria (Wheeler et al., 1991). Thus, we hypothesized (H5) that differences in function (growth) between communities derived from different pH (inoculum communities) would be more pronounced for bacteria than for fungi.

2. Materials and methods

2.1. Soil collection and sterilization

As previously described (Aciego Pietri and Brookes, 2008), a one-time, uneven application of chalk to an arable field in the mid 19th century resulted in a pH-gradient ranging from 4.0 to 8.3 within 200 m, called the Hoosfield Acid Strip. Environmental factors other than pH vary minimally over the strip, as exemplified by a stable organic C content (ca. 0.9%) and C:N ratio (ca. 9) between pH 4.5 and 8.3. Below pH 4.5 there was a small drop in C to about 0.8%, probably related to a reduced plant input at that location due to impeded crop growth (Rousk et al., 2010b). The soil is classified as Typic Paleudalf (USDA) or Chromic Luvisol (FAO).

Soil was sampled at different pH levels along the gradient in April 2008 and homogenized into composite samples having a pH of 4.1, 5.2, 6.7 and 8.3. After sieving (<2.8 mm), water content was adjusted to 40% of water holding capacity. Aliquots of soils to be used as inoculum communities (initial trait distributions) were stored at 5 °C. The soil samples were then inserted into heat resistant plastic bags, and autoclaved at 100 °C for 1 h. This step was repeated after three days at room temperature. We used repeated heating at a lower temperature than the normal operating temperature for an autoclave to avoid possible toxic products due to high temperatures during sterilization. The water content was gravimetrically controlled after the last treatment, and adjusted if needed.

2.2. Experiment

The sterile soil samples (80 g fresh weight) were weighed into 200 mL closed plastic vials, with three replicates for each of the