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# Mechanisms of soil acidification reducing bacterial diversity

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ABSTRACT

A central goal in soil microbial ecology research is to identify the biodiversity patterns and reveal the underlying mechanisms. Long-term soil acidification is known to reduce soil bacterial diversity, but the mechanisms responsible for this pattern have not been well explored. Soil acidification may reduce bacterial richness through ecological filtering (EF). In contrast, two types of processes may promote the maintenance of bacterial richness: species may adapt to the acidic pressure through evolution, and endemic species already adapted to the acidic pressure can colonize the acidified soils through dispersal. To identify the relative contribution of EF and evolution/dispersal (ED), we collected soils with a pH range of 4–7 from different ecosystems, conducted an acidification experiment with a similar pH range in a neutral soil, and proposed a conceptual framework that could distinguish the three potential types of mechanism (neither EF nor ED operate; EF operates alone; ED counteracts some effect of EF). We found that the entire bacterial domain was driven by the third type of mechanism, with ED counteracting about 42.4% (95% confidence interval: 32.7–50.4%) effect of EF. Meanwhile, different bacterial phyla/classes were governed by different types of mechanisms, and the dominant was the third type. Our results highlight the importance of both ecological and evolutionary mechanisms for regulating soil bacterial communities under environmental changes.



## 1. Introduction

Soil microbial ecology research is still at the descriptive stage (Horner-Devine et al., 2004; Martiny et al., 2006). Most previous studies usually aimed to reveal various types of biodiversity patterns, but the underlying mechanisms were often not fully explored (Prosser et al., 2007; Hanson et al., 2012). While precipitation and temperature are the primary drivers of plant diversity across different terrestrial ecosystems, soil pH has been found to be a key factor shaping bacterial diversity (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010). For example, Fierer and Jackson (2006) found that soil pH alone accounts for more than 70% variation in bacterial diversity across different terrestrial ecosystems, and other ecological factors, such as precipitation and soil nitrogen content, account for only a small part of variation. They also found that as soil pH increases (from acidic to alkaline), bacterial diversity first increases then declines. The relationship between soil pH and bacterial diversity is arguably one of the most important patterns in microbial ecology (Fierer and Jackson, 2006; Jones et al., 2009).

It is generally agreed that all terrestrial ecosystems and their biological components are originated from the ocean (Nisbet and Sleep, 2001; Martin et al., 2008). Because the pH of the primitive ocean was nearly neutral, the original soil bacterial communities should be adapted to the neutral environment. While the soil pH of some terrestrial ecosystems remained nearly neutral, that of some others gradually acidified/alkalized in the long-term ecosystem development process (Wu, 1994; Fierer and Jackson, 2006). This natural acidification/alkalization process was driven by the interactions among climate, organisms and soil, such as by the decomposition of the plant detritus (Chapin et al., 2011). The intracellular pH of most microorganisms is generally within one pH unit of being neutral (Madigan et al., 1997), and the acidification/alkalization process has resulted in declined soil bacterial diversity.

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However, the ecological/evolutionary mechanisms of soil pH affecting bacterial diversity have yet been explored.

In this study, we aim to explore the mechanisms in the process of long-term soil acidification reducing bacterial diversity across different land ecosystems. Several processes could contribute to the relationship between soil bacterial diversity and the natural acidification pressure. On the one hand, soil acidification will decrease bacterial diversity through ecological filtering (EF), because the species not adapted to the acidic pressure will be driven to extinction. On the other hand, some species may adapt to the acidification pressure through evolutionary processes. Meanwhile, there are some species already adapted to the acidification pressure in other habitats, and they may disperse into the acidified soils and successfully establish populations (Martiny et al., 2006; Hanson et al., 2012). Overall, both evolution and dispersal (ED) may promote the maintenance of bacterial diversity, counteracting the effect of EF. Therefore, there were three potential types of mechanism for different bacterial groups under the soil acidification pressure: I) neither EF nor ED changes bacterial diversity; II) EF alone reduces bacterial diversity; III) ED counteracts some effect of EF. Logically, the bacterial groups driven by the second type of mechanism will be most vulnerable under the acidification pressure, because they show no adaptive characteristics. The groups driven by the first and third types of mechanism will be relatively resistant to changes in pH.

To identify the relative contribution of EF and ED for various soil bacterial taxonomic groups, we first collected soils with a pH range of 4–7 from different terrestrial ecosystems across China. This natural acidification process took place at such a large spatiotemporal scale that both EF and ED could be responsible for the pattern of soil acidification declining bacterial diversity. We also conducted an acidification experiment with a similar pH range from a neutral soil. This experimental acidification process took place at a small spatiotemporal scale such that EF alone could be the primary driver of the pattern of bacterial diversity, especially for the diversity of bacterial OTUs (operational taxonomic units) defined as 16S rRNA gene clusters with larger than 97% sequence similarity (Stackebrandt and Goebel, 1994). The difference between the natural and experimental biodiversity patterns should be primarily caused by ED. Thus, this conceptual framework allows us to distinguish the three potential types of mechanism by comparing the patterns observed in the two types of studies. Here we applied it to identify the mechanism type for the entire bacterial domain as well as 13 dominant phyla/classes.

## 2. Materials and methods

### 2.1. Sampling

To understand the effect of natural soil acidification on bacterial diversity over a large spatiotemporal scale, we collected soil samples (with pH 4–7) at 7 sites along the latitude of 43°N and at 17 sites along the longitude of 116°E in the Mainland of China in August 2009 (Fig. S1; Table S1). At each sampling site, we collected four soil cores (10 cm depth, 3.5 cm diameter) from four locations that were at least 3 m apart. The four soil cores were then thoroughly mixed.

To examine the effect of soil acidification on bacterial diversity over a small spatiotemporal scale, we performed a ten-year soil acidification experiment at the intersection point of the latitudinal and the longitudinal transects (43°N, 116°E; Fig. S1), with a similar pH gradient (4–7) as observed in the soil samples collected from the two transects. The soil at the interaction point was neutral (~6.90). The experimental design has been described elsewhere (Zhang et al., 2011). In brief, the study was conducted in a typical steppe ecosystem near the Inner Mongolia Grassland Ecosystem Research Station in China, which lies between 43°26′–44°08′ N and 116°04′–117°05′ E at an average elevation of 1200 m. A continental middle temperate semiarid climate dominates the area, and is characterized by a cold, dry winter and a warm, moist summer. The region is characterized by a dark chest soil. The dominant plant species accounting for >80% of the total aboveground plant biomass in the area are *Leymus chinensis* (Trin.) Tzvel., *Stipa grandis* P. Smirn., *Agropyron cristatum* (L.) Gaertn. and *Achnatherum sibiricum* (L.) Keng. The experimental site (400 m × 600 m), constructed in 1980, was surrounded by an iron fence to exclude animal grazing. In early July each year from 2000 to 2009, $NH_4NO_3$ was added homogeneously to plots (5 m × 5 m) with a 1-m buffer zone at rates of 0, 1.75, 5.25, 10.5, 17.5, and 28 g N/($m^2$·yr), respectively. It has been demonstrated that N addition affected soil bacterial communities primarily through decreasing soil pH in this steppe ecosystem and that the increase in soil nitrogen content only had a small effect (Zhang et al., 2011, 2013, 2014; Zhang and Han, 2012). Each treatment was replicated in three plots. All 18 plots were distributed across an area of 55 m × 110 m in a randomized block design. In late August 2009, four soil cores (10 cm depth, 3.5 cm diameter) were collected at four random locations from each plot and thoroughly mixed.

The pH was measured in 1:2.5 (*W*/*V*) suspensions of soil in distilled water. DNA was extracted from 0.5 g of mixed soil using the FastDNA SPIN kit for soil (Qbiogene, Carlsbad, CA) according to the manufacturer's instructions, except that 350 μL of DNA elution solution was used to elute the DNA in the tenth procedure instead of 50 μL. The DNA solution was stored at −20 °C.

### 2.2. Measurement of bacterial composition and analysis of pyrosequence data

The method of 454 pyrosequencing was used to measure the bacterial composition of each soil sample. The primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 338R (5′-TGC TGC CTC CCG TAG GAG T-3′) were used to amplify the fragment of 16S rRNA gene from soil DNA. In order to measure bacterial composition of all 42 samples in one run, a unique 10-mer tag for each soil DNA sample was added to the 5′-end of the primer 338R (Hamady et al., 2008). Each 20-μl PCR mixture contained 4 μl FastPfu Buffer (5×; Transgen), 2 μl of 2.5 mM dNTPs, 0.4 μl of each primer (5 μM), 0.8 μl of DNA template, and 0.4 μl of FastPfu Polymerase (Transgen). The PCR protocol was 95 °C for 2 min (denature); 25 cycles of 95 °C for 30 s (denature), 55 °C for 30 s (anneal), 72 °C for 30 s (elongate); and 72 °C for 5 min. Three PCRs were performed for each sample. The combined products were purified by agarose gel electrophoresis and recovered. The recovered products were quantified with PicoGreen using a TBS-380 Mini-Fluorometer, and equal molar concentrations of PCR products for each sample were pooled. The pooled products were sequenced in a Roche 454 Genome Sequencer FLX Titanium system at Shanghai Majorbio Bio-pharm Technology Co., Ltd.

The pyrosequence reads were analyzed using the Mothur software package (Version 1.19) (Schloss et al., 2009). The reads were first assigned to samples according to their tags, and those <150 bp in length or with ambiguous characters were removed. The chimeric sequences were excluded by the chimera.uchime command with default parameters. The first 150 bp of the remaining reads were aligned to the Silva database (Version 106) (Pruesse et al., 2007), and non-bacterial reads were further removed. To minimize the influence of unequal sampling on the subsequently calculated indexes, 3007 reads were randomly selected from each sample for analysis. All the 126,294 (3007 × 42) sequences were clustered into OTUs based on 97% similarity (Stackebrandt and Goebel, 1994). The OTU number of each sample was used to represent the richness of the entire bacterial domain. A different