



# The effects of soil microbial and physiochemical properties on resistance and resilience to copper perturbation across China



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## ABSTRACT

There has been a debate on the relationship between soil microbial diversity and soil resilience. Moreover, the key soil properties that drive soil resistance and resilience are seldom known. Therefore, we conducted an integrative study with the aim of investigating the effects of soil microbial diversity and abundance along with soil physiochemical properties on soil resistance and resilience. A total of 24 soil samples were collected throughout China from the north (Harbin, N45°45'56"; E126°38'42") to the south (Xishuangbanna, N22°0'22"; E100°47'44"). The soil microbial diversity based on bacterial 16S rRNA gene fragments was determined using terminal restriction fragment length polymorphism. The 16S rRNA gene was quantified using real-time PCR. Soil physiochemical properties, including soil pH, total carbon and nitrogen concentrations; sand and clay proportions; and soil cation exchange capacity, were also determined. Soil resistance and resilience were determined by measuring the substrate induced respiration (SIR) rate one day and sixty days after the application of 100 mg kg<sup>-1</sup> Cu<sup>2+</sup> perturbation, respectively. The results showed that there was no significant correlation between soil microbial diversity and soil resistance and resilience of SIR to Cu<sup>2+</sup> perturbation. The resistance was positively correlated with soil pH, while the resilience was negatively correlated with the proportion of sand. Both correlations were significant ( $P < 0.05$ ). Because the soil pH exhibited a spatial variation that decreased from north to south in China, the soil resistance showed a similar trend. The exponential and polynomial regression models were optimal for pH-resistance and sand proportion-resilience relationships, respectively. Our results suggest that soil microbial functional resistance and resilience is decided by soil properties that immobilize heavy metals rather than by microbial diversity and abundance.

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## 1. Introduction

Pollution with heavy metals caused by industrial and agricultural activities inevitably inhibits soil microbial functions, impairs the soil ecosystem and can lead to serious land degradation (Mao et al. 2015; Wang et al. 2015). Moreover, heavy metals in the soil may enter the food chain and produce harmful effects on human health (Roy and McDonald 2015). Soil is important in the earth system as a regulator of the biological, hydrological and geochemical systems and also as a source of goods and services to humankind (Robinson et al. 2013). Therefore, there is great concern about whether the soil ecosystem has the ability to maintain functional stability after pollution with heavy metals, such as Cu, Zn, Cd, Pb and Hg (Griffiths and Philippot 2013).

Soil resistance and resilience are two major aspects of stability (McCann 2000). Resistance refers to the ability of soil to withstand the immediate effects of perturbations, while resilience refers to the ability to recover from perturbations. Because soil microbial functions, such as the degradation of organic substrates and nitrogen conversion, play a crucial role in materials cycling (Guo et al. 2013), resistance and resilience of soil microbial functions are considered to be important indicators of soil health and quality (Zhang et al. 2010). Understanding the key factors that drive soil microbial functional resistance and resilience is of great significance for assessing the impact of heavy metal pollution on the soil ecosystem. However, the key factors that drive soil resistance and resilience are unclear.

There has been a long debate on the effects of biodiversity on the functional stability of an ecosystem and the diversity-stability relationship. The insurance effect and negative co-variance effect provide conceptual supports for a positive relationship between diversity and stability (Lehman and Tilman 2000). However, empirical studies provide conflicting results, including results with positive relationships and no relationships. For example, Griffiths et al. (2000) found that

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soil was less resilient to heating and copper perturbations with decreasing biodiversity, while Wertz et al. (2007) found that reducing the diversity of denitrifying and nitrite-oxidizing communities did not impair the resistance and resilience of denitrifiers and nitrite oxidizers following a disturbance. In these studies, dilution and fumigation methods were applied to produce a gradient of diversity for laboratory tests of the relationship between microbial diversity and soil resistance and resilience. However, these methods were considered to destroy the interactions between species by eliminating minor groups of microorganisms or even keystone species, exerting a larger effect on ecosystem functions more than diversity itself could bring about (Deng 2012). In addition, microbial diversity might gradually recover after it was artificially reduced, and no differences between diversity treatments were obtained. We therefore propose to study the diversity–stability relationship without artificial manipulation of the diversity gradient.

In addition to soil microbial diversity, soil physiochemical properties, such as soil pH, clay content and organic matter, can largely affect the toxicity of pollutants and the activity of microorganisms (Park et al. 2011); thus, they may influence soil resistance and resilience. Gregory et al. (2009) found that soil resilience was positively correlated with the organic matter content and clay content, while Ng et al. (2015) showed that the addition of compost did not alter the resistance and resilience of soil functions to drying and re-wetting. Nevertheless, the effects of soil physiochemical properties other than the organic matter content on soil resistance and resilience were seldom studied. An integrative study on a series of soil properties would be more useful to determine the key properties that have decisive effects on soil resistance or resilience.

In the present study, a total of 24 soil samples were collected across China. These soils were different according to their microbial and physiochemical properties. The glucose-induced respiration rate of soil was adopted as a representative of microbial function to study soil resistance and resilience following copper ( $\text{Cu}^{2+}$ ) perturbation (Wada and Toyota 2007; Deng et al. 2009). We applied  $\text{Cu}^{2+}$  as a perturbation because it is one of the most important heavy metal pollutants and is a model type of

perturbation in studies on soil resistance and resilience (Griffiths and Philippot 2013). Our previous study showed that resilience of microbial functions occurred with the aging of  $\text{Cu}^{2+}$  and development of resistant microorganisms (Deng et al. 2009). We therefore hypothesize that soil resistance and resilience might be governed by soil physiochemical properties. We aimed to determine the key factors that drive soil resistance and resilience among soil physiochemical properties, along with soil microbial community diversity and abundance, and to set up models that quantify the relationship between key factors and soil resistance and resilience.

## 2. Material and methods

### 2.1. Soil sampling

Soil samples were collected from 24 sites across China in April 2013 (Fig. 1). The sampling sites covered a wide geographic range from  $\text{N}45^{\circ}45'56''$  to  $\text{N}22^{\circ}0'22''$  and from  $\text{E}91^{\circ}06'44''$  to  $\text{E}126^{\circ}38'42''$ , with average annual temperatures and precipitation varying between  $3.5^{\circ}\text{C}$  and  $21.9^{\circ}\text{C}$  and 300 mm and 1946 mm, respectively. Each sampling site was located in woodland dominated by one vegetation type (Supplementary Table S1). In each site, surface soil samples (0–20 cm) from three randomly selected plots ( $0.5\text{ m} \times 0.5\text{ m}$ ) spaced approximately 10 m apart were collected and mixed to represent a site (Zhang et al. 2010). The collected soil samples were sieved through a 2-mm diameter mesh and subsequently either stored at  $4^{\circ}\text{C}$  for less than two weeks before the resistance and resilience experiments or air dried for physiochemical analysis.

### 2.2. Soil physiochemical properties

The soil physiochemical properties were analysed using routine methods (Lu 2000). The soil pH was measured using a glass/calomel electrode (Model 206-C, Shanghai San-Xin Instrument Co. China) at a soil:water ratio of 1:2.5 (w/v). Soil organic carbon (SOC) was

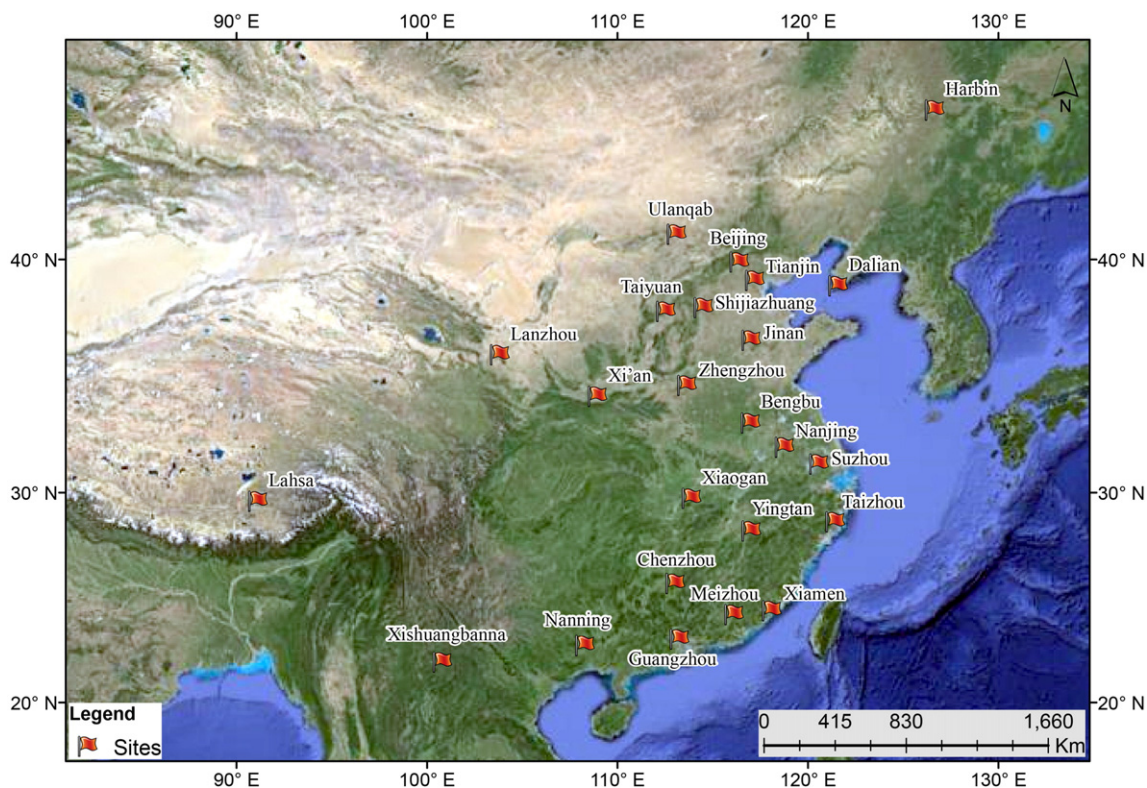


Fig. 1. The locations of sampling sites in China (ArcGIS 10.1, ESRI, USA).

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