



## Original article

# Manure substitution of mineral fertilizers increased functional stability through changing structure and physiology of microbial communities



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## ARTICLE INFO

## Article history:

Received 21 June 2016

Received in revised form

6 October 2016

Accepted 7 October 2016

Handling Editor: C.C. Tebbe

## Keywords:

Soil microbial resilience

Microbial community structure

Soil aggregation

Soil organic matter

Organic amendment

Fertilization

## ABSTRACT

Soil function, such as decomposition of organic materials, is of crucial importance to sustain soil fertility and may be enhanced through soil management. We hypothesized that manure amendment would increase soil functional stability more effectively than mineral fertilization when soil nutrients were not limited. By using a 22-yr field experiment, the objectives were 1) to determine the effects of manure substitution and reduction of mineral fertilizers on soil physio-chemical properties, soil microbial community structure, and soil biological functional stability; 2) to isolate the effects of organic amendment from those of mineral fertilization on soil biologic functional stability; and 3) to elucidate the controlling mechanisms on the soil functional stability. Soils were sampled from the field treatments, no fertilization (CK), mineral N, P and K (NPK), two doses of NPK (2NPK), manure amendment (OM) and OM in combination with NPK (NPK + OM). The nutrient inputs were similar in treatments OM and 2NPK. The functional stability was quantified by measuring the decomposition rate of crop litter added to the soils following Cu addition and heating. Soil nutrients, organic carbon and pH increased due to mineral fertilization and organic amendment. The principal component analysis of phospholipid fatty acid (PLFA) profiles demonstrated that the structure of soil microbial communities shifted between the mineral-fertilized soils and manure-amended soils and the shifts were not due to nutrient limitation because the soil microbial communities were not separated between the treatments of NPK and 2NPK. The manure amendment enhanced the resistance and resilience to Cu and heating more than the mineral fertilization, to a larger extent in treatment NPK + OM than in treatment OM. The resistance and resilience to Cu addition was positively correlated with soil organic matter, soil aggregate stability, while only the resistance to heating was positively correlated to soil aggregate stability. Moreover, the resistance and resilience were correlated with the shifts of functional and physiological structure of soil microbial communities due to long-term manure amendment and mineral fertilization. In conclusion, the partial substitution of mineral fertilizers with manure (NPK + OM) increased soil functional stability to heavy metal pollution and global warming through altered structure and physiology of soil microbial communities due to improved soil aggregation with higher soil organic matter.

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## 1. Introduction

Healthy soils are intrinsically able to adapt to either natural or anthropogenic perturbations in order to deliver soil functions for

sustaining ecosystem goods and services [1]. The ability is defined as functional stability and comprises both resistance and resilience [2,3]. Soil microbes play a central role in determining soil functions such as soil organic matter decomposition, nutrient transformation and soil structure formation and stabilization. Therefore, soil biological stability is quantified by measuring changes in the short-term microbial functional groups or specific functions in response to experimental perturbation to understand the controlling mechanisms and the effects of land use and soil management [4].

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Soil biological stability is related to many soil properties, among which soil organic matter and soil aggregation are most important soil properties and to a less extent clay and soil pH [5–9]. As a consequence, soil management can influence soil biological stability as being observed using paired long-term experiments [8,10,11]. Mineral fertilization has greatly contributed to crop production and food security, but excessive mineral fertilization leads to decreasing on soil quality and environmental pollution. Organic amendment and substitution of mineral fertilizers are increasingly recommended for the development of sustainable agriculture to sustain crop yield and soil quality while minimizing environmental issues [12,13]. However, to our knowledge there is only one study which reported that the microbial functional stability of a tropical sandy clay loam soil to heat was enhanced most effectively in the treatment of 36-y application of farmyard manure with mineral N, P and K compared with the treatments of mineral fertilization only and no fertilization [11]. Meanwhile, the effects of organic amendment have not been isolated and the controlling factors are largely unknown.

Soil biological stability is hypothesized to be primarily generated by inherent diversity of soil microbial communities with functional redundancy (the insurance hypothesis) [14] or by soil resource heterogeneity (the resource heterogeneity hypothesis) [15]. Both hypotheses have been intensively validated, but no consensus has been reached [3,10,16–18]. Griffiths et al. [5], proposed soil biological stability is likely governed by the physiochemical structure of the soil through its effect on microbial community composition and microbial physiology. Many long-term studies have demonstrated that soil microbial communities changed under mineral fertilization and organic amendment [7,19–22]. Since the growth of soil microorganisms is mainly limited by C or N [23] and also sensitive to application of P and K [24], the factors controlling the shifts of soil microbial communities may be then different between organic amended soils and mineral-fertilized soils. Therefore, understanding the controlling factors is critical to isolate the effects of organic amendment and mineral fertilization on soil biological stability.

Soil microbial communities may change in different mechanisms between long-term mineral fertilization and organic amendment. Long-term mineral fertilization changes soil pH and nutrient environments and then influences soil microbial communities [20,25]. For example, long-term application of mineral N in excess can reduce soil pH, to which soil bacteria are more sensitive to change than fungi and actinomycetes [19,26]. In addition, long-term mineral fertilization can increase soil organic matter due to increased crop production and crop litter inputs [27]. Thus soil microbial communities may shift with the increase in soil organic matter and its effect on soil water and temperature and soil aggregation [21,28–30]. Therefore, if soil pH, soil nutrient and soil organic matter do not change with increasing amounts of mineral fertilizers in combination, soil microbial communities may not change. In contrast, long-term application of organic materials, e.g. crop straw and manure, may favor the growth of cellulolytic microorganisms, such as fungi, in relation to those that were not able to degrade cellulose [20,31,32]. Therefore, it is expected that the quality and quantity of soil organic matter will increase with increasing amount of organic amendments, leading to continuous shifts in soil microbial communities. In such conditions, the effects of organic amendment and mineral fertilization on soil biological stability can be isolated and the redundancy and resource heterogeneity hypothesis of functional stability can be tested.

In this study, we aimed to isolate the effects of manure amendment on soil biological functional stability from those of mineral fertilization by using a 22-yr field experiment. The mineral N, P and K fertilizers were applied in combination and the nutrient

inputs were not less than under manure amendment than under mineral fertilization to avoid the effects due to nutrient limits. The specific objectives were 1) to determine the effects of manure substitution and reduction of mineral fertilizers on soil physiochemical properties, soil microbial community structure, and soil biological functional stability; 2) to isolate the effects of organic amendment from those of mineral fertilization on soil biological functional stability; and 3) to elucidate the controlling mechanisms on the soil functional stability. The aboveground biomass was removed to eliminate the additional effects. We hypothesized that manure amendment would increase functional stability compared with balanced mineral fertilization and soil organic matter would play a crucial role in controlling soil microbial communities and functional stability.

## 2. Materials and methods

### 2.1. Study site description

The research site was located at the Institute of Red Soil, Jiangxi Province, China (116°26' E, 28°37' N, and about 26 m above sea level). The area has a typical sub-tropical humid climate, with mean annual temperature of 17.5 °C, ranging from 5.5 °C in January to 29.9 °C in July. Annual rainfall averaged 1727 mm with majority of the precipitation falls between March to July. The soil was derived from Quaternary red clay and classified as Ultisol according to USDA soil taxonomy [33].

The long-term fertilization experiment was established in 1986 to determine the influence of the increasing use of mineral fertilizers on crop yield and soil properties compared with the traditional agriculture with only manure amendment. The 2NPK treatment represented the conventional agriculture with high inputs of mineral fertilizers in balance, while the treatments of NPK, OM and NPK + OM represented those alternative models of manure substitution and reduction of mineral fertilizers.

The field experiment was arranged following a randomized complete block design with three replicates. Each experimental plot was 22.22 m<sup>2</sup>. Five treatments from the experiment were selected for this study considering different nutrient inputs (Table 1). The treatments were no fertilization control (CK), application of mineral N, P and K fertilizers (NPK) and that with double doses of NPK rates (2NPK), organic manure alone (OM), and NPK plus organic manure (NPK + OM). The amounts of N, P and K fertilizers in NPK were adopted by local farmers in 1980s and were projected to be doubled for high yield since then. The amount of N input fixed to be equivalent between OM and 2NPK though the measured value was slightly different. The mineral fertilizers used were urea, calcium magnesium phosphate and potassium chloride and the application rate was 60, 13 and 50 kg ha<sup>-1</sup> for N, P and K at each growing season, respectively. The organic manure was pig manure applied at 15.0 t ha<sup>-1</sup> y<sup>-1</sup> (dry weight), which had 23.9% of total C, 2.0% of total N, 0.7% of total P and 0.7% of total K. Two thirds of inorganic N fertilizer, all P and K fertilizers as well as pig manure were applied as basal fertilization, and the rest of N fertilizer was applied as top dressing. The cropping system was double maize (*Zea mays* L.) cropping with winter fallow each year. Approximately 37.8% and 14.4% of annual rainfall occurred for the first (April to July) and second maize growing season (July to October), respectively, and irrigation was applied if necessary.

### 2.2. Soil sampling and analysis

Surface soils (0–20 cm) were collected at five random points in each plot to make a composite sample in March 2008. The soil samples were immediately shipped to the laboratory and

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