Geoderma 319 (2018) 156–166

Contents lists available at ScienceDirect

Geoderma

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

Distinct responses of soil bacterial and fungal communities to changes in fertilization regime and crop rotation

Chao Ai[b], Shuiqing Zhang[a,*], Xin Zhang[b], Doudou Guo[a], Wei Zhou[b], Shaomin Huang[a]

[a] Institute of Plant Nutrition and Environmental Resources Science, Henan Academy of Agricultural Sciences, Zhengzhou, 450002, PR China
[b] Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, 100081, PR China

ARTICLE INFO

Handling Editor: Yvan Capowiez

Keywords:
Bacteria
Crop rotation
Enzyme activity
Fungi
Long-term fertilization
PLS-PM

ABSTRACT

Nutrient availability and plant diversity are two important factors determining crop productivity in agricultural ecosystems, but little is known about the underlying mechanisms shaping microbial communities and their regulatory roles in soil biological activity and function. Here, we explored the impacts of fertilization regimes and crop rotations on soil physicochemical properties, crop yield and bacterial and fungal community structures in a 26-year field experiment. The critical determinants for regulating soil enzyme activity profiles involved in carbon (C), nitrogen (N) and phosphorus (P) cycling were identified by the partial least squares path model (PLS-PM). Long-term inorganic or organic fertilization significantly increased soil total N by 27%–77% and crop yield by 237%–419% and decreased soil pH by an average of 0.4 units when compared with non-fertilized control. Soil bacteria were more sensitive than fungi to the fertilization practices. Nutrient additions enriched copiotrophic taxa affiliated to the *Pseudomonadaceae* and *Cytophagaceae* bacterial families, but reduced some Acidobacteria such as subgroup 4 RB41, which was the most sensitive biomarker responding to no fertilization. Conversely, fungi were more active in response to crop conversion from wheat-maize to wheat-soybean rotation, leading to a 3-fold enhancement of an unclassified Sordariomycetes family in soybean-based rotation. PLS-PM revealed that fertilization-induced increases in soil enzyme activities were regulated by the bacterial community, while plant-driven alterations in yield, organic C input and soil aggregate-size distribution played an important role for fungal development, which, however, had no significant link to soil enzyme activity profiles. Our results suggest that different response patterns of soil bacteria and fungi to agricultural practices might have consequences for ecosystem function.

1. Introduction

Anthropogenic activities, especially agricultural production practices, strongly impact soil microbial ecosystems by changing the physicochemical properties of soil in the short- (Fernandes et al., 2011) and in the long-term (Hartmann et al., 2015), resulting in significantly altered soil processes (Ai et al., 2015; Qu et al., 2014). Fertilizer inputs (chemical or organic), pesticide application, planting diversity (e.g., crop rotations), and tillage practices affect soil microorganisms in different ways (Álvarez-Martín et al., 2016; Mbuthia et al., 2015). Among them, the influences of fertilization on soil microbiota are of great importance, as microorganisms have been identified as the key drivers of soil nutrient turnover and are therefore closely linked to soil fertility and crop yield (Mäder et al., 2002). Since 1998, the consumption of nitrogen (N), phosphorus (P) and potassium (K) fertilizers in China has increased by 49%, 19%, and 33%, respectively (Wu and Ma, 2015), resulting in a marked increase in soil nutrient content in numerous agricultural lands. In north-central China, 3 years of N fertilizer application (300 kg N hm^{-2}) increased soil nitrate (NO_3^-N) concentration by 8-fold and decreased soil pH by nearly 0.31 units (Zhao et al., 2014). However, the ecosystem-level influences of these fertilizer-induced alterations on the activity and function of various microbial taxa are unclear.

Crop rotation such as diversification with legume crops is an alternative strategy to maintain soil quality and crop productivity when compared with monocultural cropping patterns (Tiemann et al., 2015). Crops impact the soil microbiota by shaping their composition and diversity through root exudates, plant residues and symbiotic association, or directly altering soil carbon (C) input, nutrient availability and soil structure (e.g., texture and aggregates distribution) (Ai et al., 2015; Graaff et al., 2010; Su et al., 2017; Trivedi et al., 2015). Legumes with their high N content and enhanced ecosystem N inputs associated with biological N fixation are regarded as a preferential alternative to rotate with other crop species (Murugan and Kumar, 2013). However, the

* Corresponding author at: No. 116, Agricultural Road, Zhengzhou, Henan Province, 450002, PR China.
E-mail address: zhangshuiqing@hnagri.org.cn (S. Zhang).

https://doi.org/10.1016/j.geoderma.2018.01.010
Received 5 September 2017; Received in revised form 4 January 2018; Accepted 11 January 2018

existing results of taxon-specific responses of soil microbial communities to legume crop planting are ambiguous, especially for bacteria and fungi. The presence of legume crops significantly increased fungal biomass and fungal/bacterial ratio (Murugan and Kumar, 2013), whereas Tiemann et al. (2015) found lower fungal/bacterial ratio in higher diversity rotations and a significant negative correlation with crop diversity index. Furthermore, the trend of soil fungal/bacterial ratio under various legume-based rotation systems was not consistent (Qin et al., 2017).

Bacteria and fungi in soil generally account for > 90% of the total soil microbial biomass (Six et al., 2006), and they are dominant regulators of soil organic matter decomposition and nutrient dynamics. Previous studies have shown that the ratio of bacterial and fungal biomass was highly sensitive to soil disturbance, with higher ratios associated with increased tillage practices (Six et al., 2006), increased N fertilizer input (de Vries et al., 2006), and decreased soil acidity (Khan et al., 2016). Moreover, the quality of soil organic matter also alters bacterial/fungal dominance, with labile organic substrate (low C/N) favoring bacteria and recalcitrant organic matter (high C/N) favoring fungi (Andresen et al., 2014; Waring et al., 2013).

Long-term field experiments would provide valuable information about the sustainability of intensive agriculture, where large amounts of fertilizers are imported into the system each year. Furthermore, the influence of plant diversity such as crop rotation on soil microbial properties and crop yield are also dependent on time (Qin et al., 2017). In this study, we evaluated the effects of fertilization regime and crop rotation system on soil bacterial and fungal communities based on a long-term (26-year) fertilizer experiment where wheat-maize rotation was further divided into wheat-maize and wheat-soybean rotations since 2012. MiSeq sequencing of 16S and 18S rRNA genes and microbial fluorometric assays were used to estimate microbial community structures and soil enzyme activities, respectively. Our objectives were (*i*) to determine whether soil bacteria and fungi had different responses to variations in crop rotation system and fertilization practice, (*ii*) to identify which taxa significantly changed in these shifts, and (*iii*) to assess the relative contribution of those variables to changes in soil microbial activities and function. We hypothesized that there were two distinctive response patterns, with bacteria being more sensitive to fertilization regimes and fungi being more sensitive to rotation conversion.

2. Material and methods

2.1. Field design and soil sampling

A long-term field fertilizer experiment was initiated in 1990 in Zhengzhou (34.28° N, 112.30° E), Henan Province, China, where wheat-maize rotation is the common cropping system. The site has a temperate and monsoonal type climate with annual average temperature and precipitation of 14.4 °C and 640 mm, respectively. The experimental soil is derived from alluvial sediments of the Yellow River and is classified as Aquic Ustochrept according to U.S. soil taxonomy. It is a typical soil in the North China Plain with a sandy loam texture (about 16.5% clay, 20.8% silt, and 62.7% sand) in the plough layer (0–20 cm). The field was not fertilized in the two years prior to the start of the experiment to ensure homogeneity. At the start of the experiment, the soil had a pH (H_2O) of 8.60, 6.7 g kg^{-1} organic C, 0.65 g kg^{-1} total N, 0.64 g kg^{-1} total P, 16.9 g kg^{-1} total K, 76.6 mg kg^{-1} alkaline hydrolysable N, 6.5 mg kg^{-1} available P (Olsen-P) and 71.7 mg kg^{-1} available K (ammonia acetate extractable).

From the start of experiment until June 2012, the experiment had winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.) rotations with a randomized block design (Zhang et al., 2016). The plot size was 100 m^2. The three fertilization treatments (three replicates per treatment) included no fertilizer (CK), mineral N, P and K combination (NPK), and mineral fertilizers plus organic manure (NPKM). For the NPK treatment, fertilizer N, P and K were applied in the form of urea (352.5 kg N hm^{-2} per year), superphosphate (176.25 kg P_2O_5 hm^{-2} per year) and potassium chloride (176.25 kg K_2O hm^{-2} per year), respectively. The source of organic manure was horse dung (1990–1999) or cow dung (since 2000) containing 6.1–21.4 g N kg^{-1}, 2.3–10.6 g P kg^{-1}, and 2.3–15.9 g K kg^{-1} (Dry weight). The amount of manure applied in the NPKM treatment was calculated based on the N concentration in manures so as to maintain the same total N input as in the NPK treatment and keep the ratio of N in manures to chemical fertilizer N as 7:3. For all fertilizer N, P and K and manure, 46.8% and 53.2% were applied in the wheat and maize seasons, respectively. All organic manure and fertilizer P and K were applied as basal fertilization, while two-thirds of N was applied as basal fertilization and one-third as topdressing for both wheat and maize.

To further illustrate the influence of crop type on soil fertility and crop productivity, a major change was made in the crop rotation after wheat harvest in May 2012. Maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) were grown on equally split half-size plots, respectively. Thus, the experiment included two crop rotations (i.e., wheat-maize (WM) and wheat-soybean (WS)) from 2012 onwards. Winter wheat seeds were sown in strips around 20 October and harvested in late May of the following year. Summer maize and soybean seeds were sown in holes between the wheat strips in early June and harvested in early October. The tillage method consisted of rotary tillage (20 cm depth) in autumn after maize harvest. Wheat was irrigated 2–3 times and maize (or soybean) was irrigated 1–2 times depending on precipitation. The volume of water used for each of irrigation was 800 to 1500 m^3 hm^{-2}. Herbicides and pesticides were applied to control weeds and insect growth, respectively. Wheat, maize and soybean were harvested to the soil surface level, thus the aboveground crop residues were completely removed and the straw and stubble left in the field were negligible. Crop yield was measured as dry weight of grains by harvesting each plot.

In October 8, 2016, 5 soil cores (6-cm diameter) were taken from each plot as a composite sample at 0–20-cm depth after maize and soybean harvest. Soil samples were transported to the laboratory on ice, sieved (2 mm), and then stored at room temperature for chemical analysis, at 4 °C for extracellular enzyme analysis (within 1 week), and at −70 °C for molecular analysis. In addition, the field-moist soils were gently broken apart along the natural breakpoints and passed through a 5-mm sieve for particle-size fractionation.

2.2. Soil chemical analysis and aggregate preparation

Soil pH was measured with a compound electrode (PE-10, Sartorius, Germany) using a soil to water ratio of 1:2.5. Soil organic C (SOC) was determined by oxidizing organic C with potassium dichromate ($K_2Cr_2O_7$), and total N by Kjeldahl digestion. Available P was determined by the Olsen method (Olsen and Sommers, 1982) and available K was analyzed by ammonium displacement of the exchangeable cations.

The physical procedure used for aggregate separation was adopted from Manna et al. (2007) with modifications. Fresh soil was wet sieved to obtain six aggregate size fractions: large macroaggregates (> 2 mm), small macroaggregates (1–2 mm, 0.5–1 mm, 0.25–0.5 mm), microaggregates (0.053–0.25 mm), and silt and clay size particles (< 0.053 mm). In brief, each equivalent of 100 g dry mass of fresh soil was then transferred to a nest of sieves with mesh size of 2, 1, 0.5, 0.25, and 0.053 mm. Soil-water suspension was dispersed by moving the sieve 3 cm vertically 30 times during a period of 1 min. Subsequently, the aggregate fractions (> 2 mm, 1–2 mm, 0.5–1 mm, 0.25–0.5 mm and 0.053–0.25 mm) were collected into an aluminum pan. The smallest fraction (< 0.053 mm) was centrifuged for 10 min at 3000 rpm and the pellet backwashed to an aluminum pan. Each soil fraction was dried at 65 °C and weighed.