European Journal of Soil Biology 76 (2016) 9–18

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

The influence of soil properties on the size and structure of bacterial and fungal communities along a paddy soil chronosequence

Chen Liu [a, b], Nengfei Ding [b], Qinglin Fu [b], Philip C. Brookes [a], Jianming Xu [a, *], Bin Guo [b], Yichen Lin [b], Hua Li [b], Ningyu Li [b]

[a] Institute of Soil and Water Resources and Environmental Science, Zhejiang University, Hangzhou, 310058, PR China
[b] Institute of Environment, Resource, Soil and Fertilizer, Zhejiang Academy of Agricultural Sciences, Hangzhou, 310021, PR China

ARTICLE INFO

Article history:
Received 17 May 2016
Accepted 8 June 2016
Available online 6 July 2016

Handling editor: C.C. Tebbe

Keywords:
Paddy soil chronosequence
Bacterial community
Fungal community
Hiseq Illumina sequencing
PLFA

ABSTRACT

In agroecosystems, soil bacterial and fungal communities are crucial for soil and plant health because of their diverse metabolic functions. However, little is known about the effect of long term paddy management on microbial communities. This study was conducted to assess the responses of the soil bacterial and fungal communities to cultivation history along a paddy soil chronosequence by using phospholipid fatty acid (PLFA) profiling and Illumina HiSeq sequencing. Soil samples were collected from the paddy fields (50, 100, 300, 1000, and 2000 years of paddy use; P50-P2000-), the adjacent arable chronosequence (50, 100 and 300 years of land use; NP50-NP300) and mudflat (estuarine sediment, representing the parent material for paddy and arable soil reclamation). The results showed that soil microbial biomass PLFAs increased significantly with increasing soil organic C which accumulated more under paddy than arable management. Bacterial taxonomic groups assigned to *Proteobacteria* and *Acidobacteria* changed relatively in the transition from tidal wetland to agricultural land. The relative abundances of dominant bacterial phyla in paddy soil orderly changes with cultivation time. The dominant fungal phyla in all samples were *Ascomycota, Chytridiomycota, Basidiomycota, Glomeromycota* and *Zygomycota*, representing 18.50%, 18.46%, 10.02%, 7.34%, and 7.19%, respectively. The succession of fungal community structure was mainly associated with changes in *Ascomycota*. Correlation analysis showed that higher soil total carbon and nitrogen related to long-term cultivation were associated with lower *Proteobacteria* and *Ascomycota*, but higher *Verrucomicrobia*. Furthermore, different land use type differed significantly in their fungal composition, but likely had similar effects on the succession of bacterial composition, mainly the *Proteobacteria* and *Acidobacteria*. Our results indicate that orderly succession of soil bacterial and fungal communities occurred along the long-term development of paddy soil, which in turn was associated with changes in soil physicochemical properties over time.

1. Introduction

Rice is the most important food for more than 50% of the world's population. It is mostly grown under flooded lowland conditions in paddy fields. Paddy fields are habitats with a periodical cycle of flooding for 3–4months followed by drainage, resulting in a drastic switch between an aquatic and terrestrial environment [24]. In order to know the process of soil development, ecologists commonly translate spatial differences between soils into temporal differences by studying areas with a soil developmental gradient, or chronosequence, resulting from natural processes [23,50].

There are several reports relating to paddy soil formation. Paddy soil chronosequences have been reclaimed by constructing dams to facilitate the sedimentation of suspended material in coastal zones which have high Electrical Conductivities (ECs) [17,24]. A longer history of rice cultivation tends to result in more diversified horizons of paddy profiles in terms of distribution and redistribution patterns of total Fe and free Fe oxyhydrates [10]. The pH of paddy soils decreases from alkaline to neutral while the organic C concentrations and the maximum P sorption capacities in the paddy surface soil materials increase with increasing duration of rice cultivation [62]. Compared to arable cropping, paddy soil management associated with a dense plow pan decreased the leaching of DOC to the subsoil, which favors SOC accumulation in surface layers [29].

* Corresponding author.
E-mail address: jmxu@zju.edu.cn (J. Xu).

http://dx.doi.org/10.1016/j.ejsobi.2016.06.002
1164-5563/

In agroecosystems, soil microbial communities are critical for nutrient cycling, including organic matter decomposition and mineral weathering [51]. Bacteria generally dominate over fungi in paddy field soil during the flooding period [35], but organic matter decomposition by bacteria is slow due to low soil oxygen contents [43]. Knowledge of soil microbial variation under long term paddy management would provide valuable information for the sustainable management and improve our understanding of paddy soil evolution.

Possible variations in management under a specific cropping system may obscure the overall patterns of soil microbial succession over a long timescale. However, micro-organisms (e.g., bacteria, fungi, and protozoa) may not be sensitive to short-term consequences of conversion (disturbance, loss of habitat) but predominantly be affected by long-term changes in soil properties (probably loss of organic matter) [40]. Ancient paddy soil tend to accumulate more soil organic matter in the plowed soil layer with increased soil bacterial populations, higher metabolic activity, and greater functional diversity and genetic diversity than present day paddy soils [47]. The development of paddy soils with time has also been reflected in the increased activity of one particular subgroup of methanotrophs [22]. Fifty years of paddy management resulted in a higher abundance of nitrogen-fixing microbes than was found in the tidal wetland [25]. These results suggest that microbial variations in relation to soil development under long-term agricultural activities are possible. However, no comparison has been made in regard to soil formation occurring under dryland management conditions. Besides that, few studies have focused on the changes of fungal communities with soil development, which play a vital role in carbon and nitrogen cycling.

Traditional approaches to the study of total microbial biomass in soils cannot separate fungi and bacteria. Community based methodologies such as phospholipid fatty acid (PLFA) can circumvent the problems [16,55]. The use of gene sequencing permits the analysis of bacterial and fungal community separately without culturing [18]. Despite the substantial differences in read length and sequencing protocols with 454-pyrosequencing, Illumina metabarcoding can provide similar estimates of microbial communities, at lower prices [30,45]. These techniques provide useful tools for understanding how soil bacterial and fungal communities change with paddy soil formation.

Therefore, the objectives of this study were (1) to test the effects of paddy management over long time scales on the biomass and composition of bacterial and fungal communities, and (2) to reveal the association between microbial succession and environmental factors. To achieve the objectives, soil samples were taken from a paddy soil chronosequence. We also made comparisons with non-paddy soils under arable cropping and tidal wetland sites which typically represent the parent material for agricultural land reclamation. We determined the differences in the biomass of bacterial and fungal communities by PLFA analyses. We also used Hiseq sequencing to estimate bacterial and fungal composition and diversity to assess the impact of paddy soil formation.

2. Materials and methods

2.1. Site description and soil chronosequence recognition

The study area was located in Cixi, Zhejiang Province, China (Fig. 1). The mean annual temperature is 16.3 °C and the mean annual precipitation is 1325 mm. The deposited materials originated from the nearby Yangtze River as evidenced by geochemistry studies [19]. Farmland was created during the past 2000 years through the reclamation of marine sediments by construction of protective dikes. The chronosequences were established according to the Cixi County Annals and the construction dates of different dikes in the study area. In the paddy fields (50, 100, 300, 1000, and 2000 years of paddy use;-P50-P2000), rice was cultivated once a year in the flooding season from April to October and rice intercrops (wheat, barley and vegetables) were planted in winter. The adjacent non-paddy chronosequence (50, 100 and 300 years of land use; NP50–NP300) was exclusively used for non-flooded crop production. The reference site (RF) was situated at the mudflat (estuarine sediment, representing the parent material for agricultural land reclamation). We cannot assume constant management at all the sites during the past 50–2000 years, but changes in the rates, and methods of application of fertilizers and pesticides, once developed, were similar between the fields. A detailed description of this chronosequence of paddy soils is given by Cheng et al. [10].

2.2. Sample preparation and analysis of basic soil property

Field sampling was conducted in March 2011 shortly after the harvest of the arable crops, before the beginning of rice transplanting to make the soils more comparable, since agricultural practices, e.g. fertilizer applications to plants, had not been applied at the date of sampling. The background information of field sites are listed in Table 1.

At every site, three plots were established, with a distance of at least 50 m from each other. A soil auger was used to sample the 0–20 cm depth of soil, excluding the surface litter. Five soil samples were mixed from each plot in plastic bags and transported to the laboratory on ice. A portion of soil sampled from each plot, which was free of major debris, was freeze-dried and stored at −70 °C until PLFA and Hiseq analyses. The standard procedure for final preparation of soil samples involved sieving <5 mm, air-drying at ambient temperature and storing in the dark for later use.

All analyses were done according to the protocols of the Agricultural Chemistry Committee of China [2]. In brief, soil pH and EC were measured at a soil:water ratio of 1:2.5. The sand content was obtained by wet sieving (>0.2 mm), and silt and clay were determined by sedimentation rates. Total C (Ctot) was determined by oxidation with dichromate; total N (Ntot) was measured by the Kjeldahl method; total P (Ptot) was determined following a wet acid digestion procedure with perchloric and sulfuric acid, and then measured by the molybdenum blue method. All measurements are the mean of three replicate analyses.

2.3. Soil PLFA analysis

Lipid extraction and PLFA analyses were performed using a modified Bligh and Dyer-method [56]. Briefly, freeze-dried soil (5.0 g) was extracted using a single-phase chloroform-methanol-citrate buffer system (15.2 ml at a 1:2:0.8 vol basis). The PLFA fraction was isolated by a silica acid column (Waters Inc., Ireland). Following methylation of the phospholipids, the methyl esters of PLFAs were separated and identified using a gas chromatograph (7890A; Agilent, USA) fitted with a MIDI Sherlock-Microbial Identification System (Version 4.5; MIDI, Newark). The GC temperature progression was set by the MIDI software. The fatty acid 19:0 was added as an internal standard before methylation. Fatty acids were measured according to Wu et al. [55]. The fatty acids 15:0, 17:0, i15:0, i16:0, i17:0, a15:0, a17:0, 16:1ω7c, 18:1ω7c, cy17:0 and cy19:0 were chosen to represent bacterial PLFAs. The PLFA 18:2ω6,9c was used as a fungal biomarker [15,16,60]. Mole percentages of PLFAs are shown in Table S1. For each sample, total, bacterial and fungal PLFAs were expressed as nmol PLFA g^{-1} soil.