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Change in deep soil microbial communities due to long-term fertilization

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ABSTRACT

Soil microbes play critical roles in ecosystem function. Although the effects of agriculture management on microbial communities in topsoil have been well studied, few studies have examined such impacts in subsoil. To determine and compare the effects of long-term fertilizations on the microbial community in topsoil (0–0.2 m) and subsoil (0.2–3 m), we applied high-throughput pyrosequencing to investigate bacterial and archaeal communities in the 0–3m soil profile in a long-term fertilizer experiment started in 1990 in an irrigated farmland in arid zone. The following fertilizer treatments were compared with no fertilizer treatment (CK): inorganic fertilizer alone (CF) and inorganic fertilizer combined with wheat straw (CF/OM). Actinobacteria and Proteobacteria were the predominant phyla (48–65% of abundance) in topsoil, while Proteobacteria was the overwhelmingly dominant phyla (16–84%) in subsoil. The most abundant class in topsoil was Alphaproteobacteria (9–11%) and that in subsoil was Gammaproteobacteria (3–51%). Fertilizer applications changed microbial community structure throughout the profile, e.g. the relative abundances of nitrifying bacteria and Gammaproteobacteria increased while that of Actinobacteria and Deltaproteobacteria decreased. In contrast to the similar community structure in the topsoil for the two fertilizer treatments, there was a clear differentiation of the subsoil community. The order Xanthomonadales, order Nitrosomonadales, Gemmatimonadetes and Crenarchaeota were more abundant in the CF compared with CF/OM treatment; whereas the order Methylococcales, order Enterobacteriales, order Pseudomonadales and Nitrospirae were more abundant in CF/OM treatment. Total nitrogen had the greatest impact on microbial community structure in topsoil while organic carbon had the greatest impact on microbial community structure in subsoil. Our results suggested that long-term fertilizer applications altered nitrogen, carbon availability as well as electrical conductivity throughout the profile. However, this resulted in community differentiation only in deep soil. The mechanism underlain should have been that deep soil was altered by substances leached in from above, while the topsoil was altered by direct fertilization and irrigation.

1. Introduction

Soil microbes play key roles in ecosystems (van der Heijden et al., 2008). However, most studies on microbial communities have focused on the top 0.2 m of the soil column or less, where the microbial biomass is greatest. Although the subsoil (below 0.2 m) usually has low nutrient content and microbial biomass, its volume is large due to the depth of the soil profile – as a result, it still has a large population of microbes. Previous research has showed significant changes in microbial communities with soil depth (Hartmann et al., 2009; Will et al., 2010; Eilers et al., 2012), and suggested that soil microbes in subsoil have very important effects on soil formation, ecosystem biochemistry and pollutant degradation, as well as in maintaining the quality of groundwater (Madsen, 1995; Fierer et al., 2003b).

Microbial communities are strongly shaped by soil properties (Hansel et al., 2008; Chu et al., 2010; Eilers et al., 2012) such as the availability of carbon (C) and nutrients, pH value and salinity. In agricultural ecosystems, different management practices can change the microbial community structure by changing soil properties, and then exert either a positive or negative impact on soil

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quality or even ecological stability. Research has shown that overuse of nitrogen (N) fertilizer can result in loss of soil organic carbon (SOC), reduced microbial biodiversity, enhanced nitrification and the leaching of nitrate nitrogen (NO_3^-–N) which increases groundwater pollution (Nowinski et al., 2008; Seneviratne, 2009; Ramirez et al., 2010). In contrast, agricultural management based on the combined application of chemical and organic fertilizer may have positive effects on microbial diversity, biomass and activity, and helps to maintain or increase the SOC content (Birkhofer and Bezemer, 2008; Papatheodorou et al., 2008; Truu et al., 2008). Different fertilizer applications add substances of varying quality and quantity into soil, and then immediately affect the soil microbial communities in topsoil. The soluble nutrients contained in these substances can also move from topsoil to deeper soil layers during irrigation (Stowe et al., 2010). Previous study showed that microbial mineralization was significantly more sensitive to increases in nutrient availability (addition of N or P) in subsoil than in topsoil (Fierer et al., 2003a). Meanwhile, the supply of fresh plant-derived carbon to the subsoil would stimulate the microbial mineralization of old carbon (Fontaine et al., 2007). Therefore, those substances added to subsoil during irrigation should have an inevitable impact on the microbial communities and their ecological functions in deep soil, which may in turn exert critical influences on soil formation and long-term soil carbon sequestration (Eilers et al., 2012). It could not be ignored given much greater volume of subsoil than that of the topsoil. However, most research has concentrated on topsoil due to its immediate impact on plants, and the influences of agricultural management practices on the microbial communities in the deep soil remain poorly understood. In particular, little is known about the relationship between these communities and soil properties in the deep soil profile.

This study focused on an irrigated farmland in a typical arid region (the southern edge of the Gurbantonggut Desert), where the irrigation is not only applied to supply water to crops but also to leach salts out. Our preceding research showed that cultivation and fertilizer significantly changed soil properties in topsoil as well as in subsoil, and soil properties responses in subsoil were distinctly different from that in topsoil (Li et al., 2010, 2013). In the current study, we selected two typical fertilizer treatments (inorganic fertilizer alone and inorganic fertilizer combined with wheat straw) to compare with no fertilizer treatment, and applied high-throughput pyrosequencing to examine the shifts of bacterial and archaeal communities with depth. The objective was to determine the responses of microbial communities to long-term fertilizer applications within the soil profile and the link between the communities and soil properties. We hypothesise that changes in microbial communities in the subsoil should be determined by received leached-in fertilization effects, which will differ from topsoil that is influenced by direct fertilization.

2. Materials and methods

2.1. Study site and experimental design

The experiments were conducted in the Fukang Station of Desert Ecology, Chinese Academy of Sciences, located in the hinterland of the Eurasia continent (44°17′N, 87°56′E and altitude 461 m). The station is situated at the southern edge of Junggar Basin and is only 8 km from the southern edge of Gurbantonggut Desert and 20 km at the northern foot of the Tianshan Mountains. The region is a typical temperate desert with varying soil salinity and is influenced by a continental, arid, temperate climate with an annual precipitation of only 164 mm and an annual pan evaporation >1000 mm. The soil in this region is Aridosol, under the group of Calciorthid (USDA Soil Taxonomy). The soil texture is silt loam and with silt content at 60–84% (Li et al., 2010). Runoff from mountains is used to irrigate farmland and create oasis in this arid land, which results in high groundwater level and serious salinization (Wang et al., 2002). The total salt content and pH were 21–90 g kg^{-1} and 8.4–9.0 respectively, and the soil organic matter was 0.6–1.7% at 0–0.2 m depth. Irrigated farmland is the prevailing land-use type in this location.

The long-term experiment on soil fertility started in 1990. Winter wheat was planted in September each year and harvested at the end of June or July of the following year. The experiment included 11 treatments in 48 plots. Each treatment had at least three replicates and the plot size was 33 m^2. The following two treatments, given their similar application rates of inorganic fertilizers with local commercial applications, were selected to compare with no fertilizer application (CK): inorganic fertilizer alone (CF) and inorganic fertilizer combined with winter wheat straw (CF/OM). Each treatment had three replicates. The application rate for the CF treatment was 300 kg ha^{-1} N, 66 kg ha^{-1} phosphorus (P) and 50 kg ha^{-1} potassium (K) each year. In the CF/OM treatment, wheat straw (390, 4.9, 0.7 and 14.3 g kg^{-1} as total C, N, P and K, respectively) was applied at approximately 5.4 t ha^{-1} each year on the basis of the same application rate of N and P fertilizer as the CF treatment. Since wheat straw could supply K effectively due to its high K content, there was no K fertilizer application in the CF/OM treatment (Wang et al., 2002). Half of recommended N along with all P and K were given as a basal application at 1–2 d before sowing and the remaining half of N in March (when snow starts to melt) of the following year in the form of urea, calcium superphosphate and potassium sulfate. The wheat straw with grain was manually harvested, leaving a short stubble height (5 cm). After harvesting the plots were manually plowed to a depth of 20 cm each year. Meanwhile, fresh wheat straw was incorporated immediately by harrowing. The mean grain yields for the CK, CF and CF/OM treatments were 870, 4240 and 4450 kg ha^{-1} y^{-1} (Li et al., 2013). This region requires extra irrigation to leach salts due to natural salinization resulting from low precipitation and high evaporation. Flood irrigation of 6000 m^3 ha^{-1} y^{-1} is common in local commercial practice to ensure crop yield. A detailed description of the irrigation can be found in Li et al. (2013).

2.2. Soil sampling and selected soil properties

Soil samples were collected at the end of June 2010 (after the winter wheat harvest). The sampling depth was 0–3 m. Five sample points were chosen randomly in each plot of CF, CF/OM and CK treatments. Soil samples for chemical and microbiological analysis were taken vertically using an auger at the following depth intervals (m): 0–0.2, 0.2–0.4, 0.4–0.6, 0.6–1, 1–1.5, 1.5–2, 2–2.5 and 2.5–3 m. Samples taken at the same depth in each plot were mixed to obtain a representative sample. Extractable ammonium N (NH_4^+–N) and NO_3^-–N contents were assessed by shaking 5 g of field-moist soil with 50 mL of 0.01 M $Cacl_2$ for 30 min. The soil extracts were then filtered and frozen at −20 °C until NH_4^+–N and NO_3^-–N levels were measured in the AA3 flow injection analyzers (FIA SFA CFA, Germany). Samples for other soil properties were air-dried and ground further to pass a 0.25 mm sieve for determination of soil physical and chemical properties. SOC and total N contents were measured using a Total Organic Carbon/Total Nitrogen analyzer (Multi C/N 3100, Analytik Jena, Germany), and total P was determined by acid melt–molybdenum, antimony, and scandium colorimetry. The pH and electrical conductivity (EC) were measured using potentiometry and the conductivity method, respectively (at a soil:water ratio of 1:5).