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Response of soil microorganisms after converting a saline desert to arable land in central Asia

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ABSTRACT

Uncultivated saline lands are reserve resources for arable land without environmental degradation. A field experiment was conducted in a continuously cultivated farm station in Xinjiang province, northwest China, including a saline-desert land. The objective was to investigate the effects of different cotton cultivation periods from 1 to 16 years on soil chemical and soil biological properties. Cultivation led to a decrease in soil electrical conductivity and reduced soil pH from 8.9 in uncultivated land to 7.9. Soil organic C showed a small and variable increase after cultivation. After year 1 of cultivation, the contents of muramic acid, galactosamine and glucosamine were all roughly 30% lower, similarly to those of PLFA in comparison with the uncultivated soil. Further cultivation led to a continuous non-linear 40% increase until year 16 in comparison with the uncultivated soil, which mainly occurred within five years of cultivation. The ratio of fungal C to bacterial C, based on fungal glucosamine and bacterial muramic acid, varied around 0.7 in the uncultivated soil and around 1.4 from the second year on after cultivation. Microbial biomass C showed a 100% increase to contents around 300 $\mu g\, g^{-1}$ soil from year 4 to 16 in comparison with the initial values. The microbial biomass C to SOC ratio ranged from 2 to 6% without clear cultivation effects. The ergosterol to microbial biomass C ratio increased from values around 0.04% during the first 3 years to values around 0.2% from year 4 on. Also, the Gram-negative to Gram-positive bacterial PLFA ratio increased with increasing cultivation time, whereas the microbial biomass C to total PLFA ratio varied in a relatively small range around 8.9 $\mu g\, nmol^{-1}$.

1. Introduction

Salinity affects more than 20% of the irrigated land and soil salinity is a major problem that limits agricultural development across the world (Qadir et al., 2000; Setia et al., 2013). High concentrations of salt can induce negative effects not only on soil physical and chemical properties but also on plant growth and yield (Keren, 2000; Levy, 2000). Recently, the demand for food has been increasing, with less land available and little scope for arable land expansion. Uncultivated saline lands are reserve resources for arable land without environmental degradation. To utilize these saline lands, many agricultural practices are adopted to improve soil quality, including tillage, drip irrigation and fertilization. Conversion of uncultivated saline land to arable land changes soil properties and soil microorganisms (Yuan et al., 2007; Kamble et al., 2014). Understanding the shift of soil microbial processes of land-use change may help to keep agricultural systems stable in the long-term (Yao et al., 2000; de Vries et al., 2012).

As important regulators in soil processes, soil microorganisms participate in nutrient mineralization, structure formation, and the capture and transport of nutrients to plants. Soil microorganisms are sensitive to changing environmental conditions, including salinity. Increasing of soil salinity may cause a significant decline in microbial biomass (Batra and Manna, 1997; Rietz and Haynes, 2003). When salinity stress was reduced, sensitive species increased, such as fungi (Sardinha et al., 2003) or Gram-negative bacteria (Schimel et al., 2007), causing respective shifts in the microbial community structure. Even in very saline soils, activity and growth of sensitive functional groups can rapidly increase when the electrical conductivity is reduced and substrate availability is increased (Muhammad et al., 2006; Wichern et al., 2006). These shifts in microbial biomass and community structure may help to understand the cultivation effects and may serve as early and sensitive indicators for future changes (Powlson et al., 1987; Joergensen and Emmerling, 2006).

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The soil microbial community consists of two main functional groups with distinct roles in microbial processes, i.e. fungi and bacteria (de Vries et al., 2006). Archaea and protozoa contribute only small percentages to the soil microbial biomass (Joergensen and Emmerling, 2006). Molecular biomarkers are mainly derived from cell-membrane components, such as ergosterol (Djajakirana et al., 1996) and PLFA (Frostegård et al., 1993) or amino sugars (Amelung, 2001). The cell-wall components fungal glucosamine (GlcN) and bacterial muramic acid (MurN) are highly specific for the two main functional microbial groups, but accumulate as microbial residues in soil organic matter (Amelung, 2001; Appuhn and Joergensen, 2006). Ergosterol occurs only in fungi, but not in all, especially not in arbuscular mycorrhizal fungi (AMF)(Olsson et al., 2003), but also not in some Zygomycota (Weete and Gandhi, 1999).

Linoleic acid (18:2ω6,9) has been often used as an indicator for total fungal biomass (Frostegård et al., 2011), but occurs only in low concentrations in AMF (Olsson et al., 1995; Olsson and Johansen, 2000) and Zygomycota (Klamer and Bååth, 2004). Oleic acid (18:1ω9) is especially common in Zygomycota (Stahl and Klug, 1996; Klamer and Bååth, 2004) and 16:1ω5 in AMF (Olsson et al., 1995). Several specific PLFA exist also for Gram-negative and Gram-positive bacteria. High growth rates in the presence of readily available substrate are usually associated with Gram-negative bacteria, whereas Gram-positive bacteria are characterized by slow growth rates and adaptation to conditions of poor nutrient supply (Fierer et al., 2003).

In the current field experiment, the objective was to investigate the effects of different cotton cultivation periods from 1 to 16 years on soil chemical and soil biological properties. The hypotheses were that a cultivation-induced decline in salinity and soil pH (a) generally increases the formation of microbial residues and microbial biomass, (b) causes specific shifts toward fungi and Gram-negative bacteria, and (c) shows a non-linear response with increasing cultivation time.

2. Materials and method

2.1. Sites

The study site is located in the Cotton Experimental Station (44°17′57″N, 86°22′6″E, 400 m a.s.l.) of Xinjiang Academy of Agricultural Sciences in Manasi County, Xinjiang, northwestern China. The annual average temperature is 7.2 °C and annual total rainfall is 180 to 270 mm. The total evaporation is 1000–1500 mm. The groundwater table of is more than 3 m below ground. The temperature and precipitation of the study area in years of 2011–2013 can be find in Wang et al. (2014). The farmer use drip irrigation to grow crops, e.g. cotton and maize in this area. The total amount of irrigation for cotton per growing-season is 5250 m^3/hm^2. The water is supplied eight to ten times during the growth stages. Cotton (*Gossypium hirsutum*L.) has been widely grown in sodium-sulfuric saline soils in the station since 1995 under drip irrigation with mulch film, resulting in a gradient of years after cultivation which were marked as 0 (uncultivated land), 1, 2, 3, 5, 6, 7 and 16. Eight sites were set up according to years after cultivation and each site had three replicates in a completely randomized design. At the end of every cotton growth season, cotton straw was crushed and returned to the field as organic fertilizer. In 2011, cotton (*G. hirsutum*L., Xinluzao 18) was planted in April. KH_2PO_4 (72 kg P ha^{-1} and 65 kg K ha^{-1}) and urea (345 kg N ha^{-1}) were fertilized and plowed as starter fertilizers before cotton was planted. In the growth season from June to August, dissolvable fertilizers (17 kg P ha^{-1} as KH_2PO_4 and 345 kg N ha^{-1} as urea) were utilized as fertigation with drip irrigation.

2.2. Soil sampling and analysis

Using a stainless steel corer, soil samples were collected from 0 to 20 soil layer on October 09, 2011. Each sample was thoroughly mixed with 4 positions (2 within the cotton planting row and 2 between the cotton planting rows) and immediately sieved through a 2 mm mesh. All soil samples were divided into several parts. Fresh soil samples were immediately stored at −80 °C, −20 °C and 4 °C, respectively, for measuring microbial biomass and community. The remaining soil was air-dried at room temperature for measuring electrical conductivity, pH, and soil organic C (SOC). Cotton lint yield (7 sites of cotton field) was surveyed and estimated before harvest in September 2011. Cotton lint yield was calculated on the basis of surveying the number of plants, the number of peaches and the weight of fibers per peach in 2 m × 3 m along eight cotton rows.

Electrical conductivity and pH were measured in water at 1 to 5 ratios (10 g air-dried soil in 50 ml distilled water). SOC was measured according to ferrous iron titration with digestion of soil samples by $K_2Cr_2O_7$ and H_2SO_4 at 170 ± 5 °C preheated paraffin (Yeomans and Bremner, 1988).

2.3. Amino sugars

Muramic acid (MurN), glucosamine (GlcN), and galactosamine (GalN) were determined according to Appuhn et al. (2004) as described by Indorf et al. (2011) using OPA (*o*-phthalaldehyd) derivatisation. Moist samples of 2 g soil were hydrolysed for 6 h with 10 ml 6 M HCl at 105 °C. Chromatographic separations were performed on a Hyperclone C_{18} column (125 mm length × 4 mm diameter) at 35 °C, using a Dionex (Germering, Germany) P 580 gradient pump, a Dionex Ultimate WPS—3000TSL analytical autosampler with in-line split-loop injection and thermostat and a Dionex RF 2000 fluorescence detector set at 445 nm emission and 330 nm excitation wavelengths. Fungal C was calculated by subtracting bacterial GlcN from total GlcN as an index for fungal residues, assuming that MurN and GlcN occur at a 1 to 2 molar ratio in bacterial cells (Engelking et al., 2007): mmol fungal C g^{-1} dry weight = (mmol GlcN − 2 × mmol MurN) × 9. Bacterial C was calculated as an index for bacterial residues by multiplying the concentration of MurN by 45 (Appuhn and Joergensen, 2006). Microbial residue C was estimated as the sum of fungal C and bacterial C.

2.4. Soil microbial biomass indices

Microbial biomass C was estimated by the fumigation-extraction method, using 0.5 M K_2SO_4 as extractant (Vance et al., 1987), as described by Khan et al. (2009). Organic C in the 0.5 M K_2SO_4 extracts was measured using an automatic analyzer (Multi N/C 2100, Analytik Jena, Germany). Microbial biomass C was calculated as E_C/k_{EC}, where E_C = (organic C extracted from fumigated soils) − (organic C extracted from non-fumigated soils) and k_{EC} = 0.45 (Wu et al., 1990).

The fungal cell-membrane component ergosterol was extracted from 2 g soil with 100 ml ethanol by oscillated shaking at 250 rpm for 30 min according to Djajakirana et al. (1996). Ergosterol was determined by reversed-phase HPLC with 100% methanol as the mobile phase and detected at a wavelength of 282 nm.

PLFAs were measured using a minor modification to the method described by Frostegård et al. (1991, 1993) in detail. PLFAs were extracted from 5 g soils with a mixture (1/2/0.8 v/v/v) of chloroform, methanol, and citrate buffer (0.15 M, pH 4.0). Mixtures were shaken for 2 h at 200 rpm and afterwards centrifuged for 10 min at 2500 rpm. The upper aqueous layer was removed to tubes. Another mixture (2/5/2.5 v/v/v) of chloroform, methanol,