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Bacterial community structure in atrazine treated reforested farmland in Wuying China

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ABSTRACT

The Grain for Green (GFG) Project in China is currently the largest environmental rehabilitation project aimed at turning low-yielding farm land to forests and pastures. Such conversion of land use type also promotes remediation of the polluted environment. Soil microbes reflect soil function and are therefore considered an essential component of ecosystem restoration. To evaluate the environmental effects of converting atrazine polluted farmland to secondary forest, we determined soil chemical properties, soil bacterial communities and their responses to three types of land use (primary forest, PF; secondary forest, SF; farm land, FL) in Wuying, China. Our results showed that soil organic matter significantly decreases in the order PF > SF > FL. Bacterial 16S rRNA gene 454 pyrosequencing revealed that the soil bacterial diversity level remained unchanged. However between FL and the two forested sites, we observed an increase of Actinobacteria, β-proteobacteria and Firmicutes; and a decrease of Acidobacteria and Verrucomicrobia, while in SF the bacterial community structure was similar to PF. We conclude that reforestation permits bacterial community, resetting from FL back to a state that resembles natural conditions. In addition, 20 years of natural attenuation degraded soil atrazine residues in SF but traces remained in the soil. Reforestation generally resulted in favorable ecological impacts on soil quality and the bacterial community compared with active farm fields.

1. Introduction

In 1999, the Chinese government launched the Grain for Green (GFG) Project, a state campaign to restore ecological balance by returning low-yielding farmland to forests and pastures. With a budget of approximately 40 billion US dollars and a targeted area of 146 700 km^2, GFG is one of the largest environmental rehabilitation projects in the world, and has drawn much attention both in China and internationally (Uchida et al., 2005; Qiu et al., 2011).

Microbes perform most of the biogeochemical functions in soil and are therefore considered an essential component of ecosystem restoration (Harris et al., 2009). On the other hand, the structure of the soil microbial community is significantly influenced by many factors, such as ground vegetation (Carney and Matson, 2006), land use (Jangid et al., 2008), soil properties (Fierer and Jackson, 2006; Rajaniemi and Allison, 2009; Bach et al., 2010), etc., Agricultural practices, such as the use of fertilizers and pesticides, greatly alter soil characteristics (Matson et al., 1997). Atrazine, 2-chloro-4-ethylamino-6-isopropylamino-s-triazine, is widely used in China to control broad-leafed weeds, and it may cause pollution of the environment due to its high mobility and persistence in soil and water (Wang et al., 2010). Furthermore, atrazine is harmful to many organisms and to human health (dos Santos and Martinez, 2014). Residues of atrazine and its metabolites have been detected in the soil, surface water and groundwater of agricultural areas years after application (Zaya et al., 2011). Thus, despite reestablishment of forests, atrazine may persist in formerly farmed soils for a long time. Few attempts have been made to assess the residual concentrations and effects of atrazine in a conserved secondary forest, especially the influence of atrazine residues on the soil microbial community.

Studies of the effects of the GFG Project on microbial ecology have not been exhaustive. Soil microbial diversity and community composition are important indicators of soil health, which is influenced by factors such as pH, nutrients, organic matter and the application of chemical additives (Smith et al., 2000; Kennedy and

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Stubbs, 2006). Analyses using phospholipid fatty acid (PLFA) and denaturing gradient gel electrophoresis (DGGE) technologies have revealed a high level of bacterial diversity in Chinese forest soils (Wu et al., 2010; Wei et al., 2008). However, many scientific questions remain unresolved due to shortcomings in traditional technologies. Massive DNA sequencing technologies developed in recent years allow us to study microbial ecology at a higher level of resolution and give us the opportunity to deepen our understanding of microbial community structures. The generated sequencing data is of great utility for both scientists and administrative decision makers.

In this study we focused on the environmental effects of historical land use in Wuying, China, where a typical farm to forest restoration project began early in the 1990s. The study aimed to characterize soil chemical properties and the composition of soil bacterial communities and to assess their variation in three types of land use (primary forest, secondary forest and farmland). We determined soil pH, organic matter contents, soil nutrients and atrazine residual levels. The bacterial community was analyzed by 454-sequencing of 16s rRNA gene sequences. We hypothesized that 20 years (i) has not been sufficient for the remediation of atrazine contamination by natural attenuation in restored secondary forest soil; (ii) reforestation activities result in a shift of the soil bacterial community composition towards that of a natural forest environment.

2. Materials and methods

2.1. Study sites

The study sites are located on a forested plain in Wuying, Northeastern China (129°11′16.42″E, 48°09′47.24″N), along the Tangwang River, within the Fenglin National Forest Reserve. The altitude ranges from 400 to 800 m above sea level with gentle slopes of 3–7°. Timber harvesting in Wuying was active from the 1950s to the 1990s. Part of the forested land was clear-cut and converted to agriculture for the cultivation of mainly soybeans (*Glycine max*), corn (*Zea mays*) and spring wheat (*Triticum aestivum*). Beginning in the 1970s, chemical fertilizers, pesticides and herbicides were utilized to increase grain production, and the application of these agricultural chemicals continues to this day. The gradual conversion of low-yield farmland into secondary forest began in Wuying in the 1990s.

Three land use types were selected for sampling (Fig. 1): (i) the conifer and broad leaf mixed primary forest (PF) consisting of approximately 75% Korean pine (*Pinus koraiensis*), 10% birch (*Betula platyphylla*) and 15% other tree species, e.g., *Phellodendron amurense*,*Quercus mongolica*, *Pinus sylvestris*, *Larix gmelinii*; (ii) the secondary forest (SF) which was reforested from agricultural field with mainly Korean Pine (*P. koraiensis*) in the early 1990s; and (iii) farmland (FL) which was mainly used for cultivation of corn (in some years soybeans were cultivated as well). In 2010 corn (*Z. mays*) was cultivated in all FL sampling sites. Henceforth, the abbreviations in the parentheses will be used when referring to the sampling sites in different land use types. The dominant understory vegetation species in PF and SF are *Acanthopanax senticosus*, *Athyrium spinulosum*, *Corylus mandshurica*, *Leymus secalinus*, *Parasenecio hastatus*, *Ribes burejense*, *Thalictrum fructumcornu*, *Tilia mandshurica* and *Urtica fissa*, with SF containing more grasses than PF.

2.2. Soil sampling and analyses

For each land use type, eight sampling sites were chosen (24 sites in total). In FL eight sampling sites were selected from eight independent farms belonging to different owners (Fig. 1). PF and SF sampling sites were chosen randomly near Korean pine trees. To minimize temporal confounding effects, we sampled all sites in the same land use type in a single day. As a result, all soil samples were collected in three days in the middle of July 2010. From each sampling site, one soil sample was collected using a stainless steel corer (5 cm diameter, depth 0–15 cm) at a point three meters from the trunk of a tree in PF and SF and from a random spot in FL. The samples were stored in plastic bags on ice until frozen at −20 °C in the laboratory. The samples were thawed at room temperature and sieved to remove stones, roots and large particles.

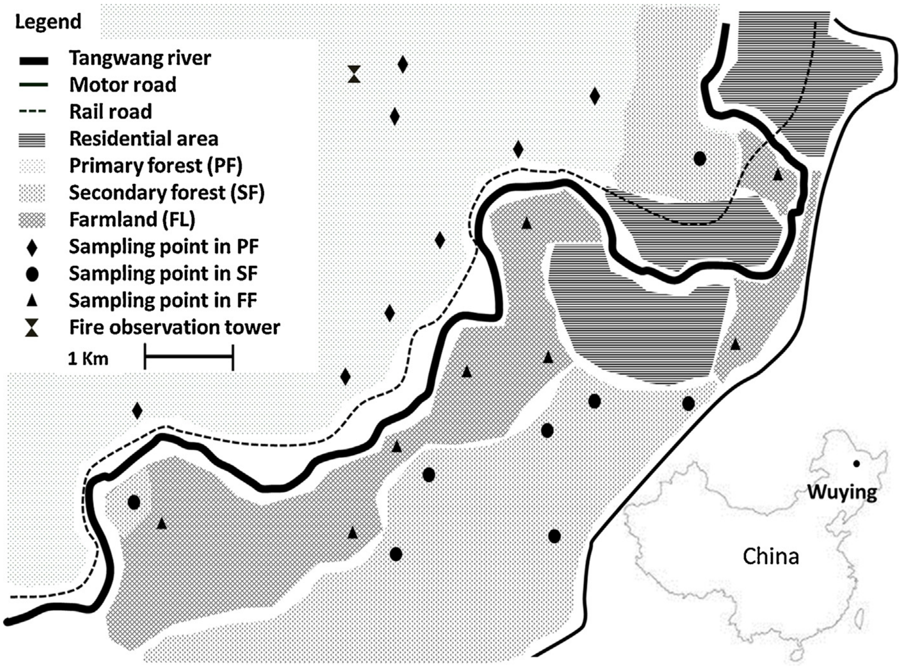

Fig. 1. Location of study area in China and distribution of sampling sites.