Analysis of soil microbial dynamics at a cropland-grassland interface in an agro-pastoral zone in a temperate steppe in northern China

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ABSTRACT

Grassland conversion is common around the world, and numerous studies have documented the effect of grassland conversion on biotic and abiotic soil conditions. Soil microbes are a key component of the underground ecosystem and are important in the circulation of materials and energy. In this study, we investigated the variations in the microbial community structures at a cropland-grassland interface. We used the transect line method and phospholipid fatty acid analysis in an agro-pastoral zone in northern China. The results show that significant differences occurred in the soil microbial community structures between grassland and cropland environments. These differences had significant seasonal variations. The variation pattern of soil microbial communities was very different from that of soil physical and chemical properties. Three main conclusions are presented: (1) Significant differences in soil properties, phospholipid fatty acid contents and microbial community structures occurred between the grassland and cropland environments after decades of reclamation. (2) At the grassland-cropland interface, we found a transition zone where the soil microbial community showed dramatic variations. (3) In the transition zone, soil microbial community structures were subjected to a stronger influence from farmland environments before sowing, but grassland played a major role after the crops were harvested.

1. Introduction

Global grassland conversion has been common in recent decades and continues today (Rounsevell et al., 2005; Stephens et al., 2008). From 1949 to 2000, converted grasslands accounted for 4.8% (approximately $1.9 \times 10^5 \, km^2$) of the total grasslands in China, and 18.2% of existing farmland originated from grassland conversion (Fan et al., 2000). Conversion has mostly occurred in the agro-pastoral zone in northern China (Wang, 2000b). Numerous studies have documented the effects of grassland conversion on soil conditions; these effects include soil organic carbon loss (Qiu et al., 2012), soil nutrient loss (Alem and Pavlis, 2014) and changes in soil physical properties (Guo et al., 2010). These changes in soil conditions eventually affect the soil microbial biomass (Pabst et al., 2013), diversity (Lienhard et al., 2013) and community structure (Cousins and Eriksson, 2008). Tillage affects soil microbes in several ways, and tillage-induced changes in the soil structure may be a feasible subject for studying the diffusional constraints that regulate aerobic and anaerobic microbial activity in the soil (Schjønning et al., 2011). Tillage activities lead to variations in plant litter and plant root systems that are attributed to significant changes in vegetation diversity and indirectly affect topsoil carbon and nitrogen, microbial activity and the composition and function of microbial communities (Yang et al., 2013). Some reports demonstrated that soil microbes are related to ecosystem stability; microbial communities with low catabolic diversity (such as crop fields) were less resistant to stress and disturbance than were microbial communities with high catabolic diversity (Torsvik and Ovreas, 2002). In agro-pastoral zones, the ecosystem is fragile and more easily disturbed by human activities (Liu et al., 2009a; Liu et al., 2009b). Soil microorganisms are an important part of the underground ecosystem. Clarifying the variations in soil microbial communities is helpful in understanding how grassland conversion affects underground ecosystems.

Soil microbes are a key component of the underground ecosystem and are critical for the circulation of materials and energy in the soil, including C and N cycling (Bardgett et al., 2008; Wieder, 2014). Soil microbes can be used as an a priori indicator of changes in the soil environment of grasslands due to their rapid responses to most environmental stressors (Kaur et al., 2005), such as temperature (Wang et al., 2018), heavy metals (Frostegard et al., 1993a; Olaniran et al., 2013), soil nutrient availability (Francioli et al., 2018), and low pH

(Zhalnina et al., 2015). These responses may reflect changes in the biomass and community structures of microorganisms (Kaur et al., 2005). In addition, soil microbial communities affect a wide range of soil functions and ecological services, including providing genetic resources and participating in geochemical circulation processes, such as carbon sequestration, waste decomposition and detoxification, soil formation and nutrient cycling (Smith et al., 2015). The validity of using soil microbial biomass, residual indices and microbial community structures as indicators of soil conditions has been confirmed by previous research (Frostegard et al., 1993b; Kaur et al., 2005; Murugan et al., 2014).

Various studies have addressed the variations in soil proprieties, microbial populations, metabolic activity, community structure and distribution characteristics that occur after grassland conversion in agro-pastoral zones (Jackson et al., 2003; Garcia and Nahas, 2012; Zhang et al., 2013). Previous studies demonstrated that agricultural practices have a strong impact on soil microbes. Tsiafouli et al. (2015) reported that agricultural practices have a significant effect on soil properties and biodiversity, and their research even demonstrated that intensive agriculture reduced soil biodiversity across Europe. A recent study suggested that different agricultural practices (conventional and organic cereal fields, leys and permanent pastures) have obviously different effects on Arbuscular mycorrhizal fungi (AMF) communities that are attributed to different management strategies (Manoharan et al., 2017). Some studies, using phospholipid fatty acid (PLFA) analysis, revealed that tillage resulted in an increased percentage of cyclopropyl (cy17:0 and cy19:0) fatty acids (Spedding et al., 2004), a decreased percentage of fungal PLFAs (18:2w6) (Spedding et al., 2004), and decreased gram-positive bacterial PLFAs (Denef et al., 2009).

Previous research has focused on either croplands or grasslands; studies of the interface between these two land use types are rare. The interface between croplands and grasslands can be regarded as an ecotone or a community edge, which frequently occurs in agro-pastoral zones. Ecotones are sensitive to environmental stress and are often ecologically fragile areas (Wielgolaski et al., 2017). The effects of changes in land use, which are usually adverse to the natural environment, most likely begin at the interface between grasslands and croplands (Joly and Myers, 2001). At present, the properties and community structures of soil microbes at the cropland-grassland boundary in agro-pastoral zones are not well known. Therefore, studies of the interface between croplands and grasslands are important for grassland management, and the associated variations may partly explain how cultivation affects the grassland.

In our previous research, we found that there were some significant transition zones for the variations in vegetation communities, soil moisture, soil temperature, soil organic matter and soil available nitrogen between grassland and cropland in this sample area (Wang, 2000a; Liu et al., 2010; Wang et al., 2010). And, we find that the quantity of microbes in the 5 years old *Leymus chinense* grassland was significantly higher than that in the farm land and alfalfa grassland (Zhen et al., 2012). Measurements of ground vegetation and soil properties usually vary greatly in transition zones. Some studies have also shown that vegetation, soil properties and the soil microbial community exhibit dramatic shifts in performance at the ecological boundary (Köchy and Wilson, 2005; Buxbaum and Vanderbilt, 2007). For example, Shen et al. (2016) reported significant shifts in functional gene diversity, which were attributed to soil pH and organic carbon variation at the treeline ecotone. In this study, we investigated the variations in microbial community structures at a cropland-grassland interface. We used the line transect method and phospholipid fatty acid analysis, which can effectively reflect variations in the structure of soil microbial communities (Wielgolaski et al., 2017). In addition, total PLFAs can generally be used to measure the soil microbial biomass, while high-throughput sequencing cannot generate these data. The aim of our study was to analysis spatial variabilities in soil properties and PLFA indicators at the cropland-grassland interface. Specifically, we

posed the following questions: (1) Is there a transitional zone for the variation in soil microbial communities between cropland and grassland? In this zone, do soil microbial measurements vary greatly or soil microbial community structures differ from those of grassland and cropland? (2) If this region exists, then what are the properties of the soil microbes in this region, and what causes these conditions?

2. Materials and methods

2.1. Site description

This study was conducted at a national field station in a temperate steppe ecosystem in Guyuan County in Hebei Province (115°40′E and 41°46′N). This station is located in a semi-arid continental monsoonal climate zone at an altitude of 1460 m. The annual average temperature is 1 °C. The average annual rainfall is approximately 430 mm; precipitation mostly occurs in July, August and September. To evaluate the effect of the cropland-grassland interface on microbial traits, two connected sites were selected. One sample site was cropland, where *Avena chinensis* had been cultivated for the 30 previous years. Another sample site was natural temperate grassland, where *Leymus chinensis* and *Stipa krylovii* were the dominant species. Accompanying species included *Agropyron cristatum, Salsola collina, Cleistogenes squarrosa, Potentilla acaulis, Potentilla anserine, Lespedeza bicolor,* and *Sphallerocarpus gracilis.* The sampling area for soil properties and vegetation is relatively stable based on our years of monitoring.

The soil in study area is a haplic calcisol classified as a calcisols according to the FAO/Unesco system of soil classification. In the 0–20 cm soil layer, sand grains are dominant in soil particle composition, accounting for 55–60% in zonal grassland soil, and up to 75% in farmland. The average bulk density of 0–20 cm soil layer in grassland soil is 1.28 g/cm³, and farmland is about 1.42 g/cm³.

2.2. Setting the transect line and sampling the soil

A transect line was established perpendicular to the interface between the cropland and grassland. The interface was designated by a fence, which was defined as the midpoint of the transect line or point 0. From this midpoint, the transect line extended outward into the cropland and grassland (Fig. 1). The following 14 sampling points were selected in each land use type along the transect line, starting from the midpoint: 1 m, 2 m, 3 m, 4 m, 5 m, 6 m, 7 m, 8 m, 9 m, 10 m, 20 m, 30 m, 40 m, and 50 m. In total, 29 sampling points were used on one transect line (i.e., 14 points in the grassland, 14 points in the cropland, and 1 midpoint). At each sampling point, a quadrat (1 m × 1 m) was set to collect soil samples. The midpoint of each quadrat location was used to calculate the distance between the sample sites and point 0. Sequential samples were collected from adjacent regions of same quadrat. Soil samples were collected in May (before the cropland was sown) and in August and October (after the cropland was harvested) in 2012 using a soil corer with a diameter of 5 cm. The quadrats were divided into four equal small squares with a cross shape. Then, coring at a depth of 0–20 cm was performed at the center of each small quadrat. The soil
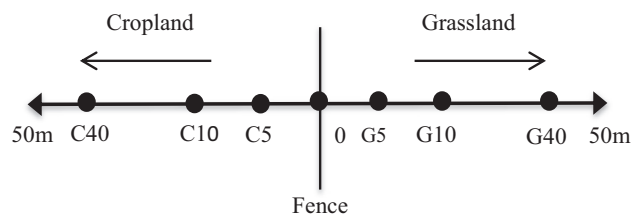

Fig. 1. Schematic diagram of the transect line. Soil samples were tagged with the letter C (for cropland) or the letter G (for grassland). Numbers represent the distance of quadrat center and the midpoint (point 0) of transect line.