Effects of grassland converted to cropland on soil microbial biomass and community from agro-pastoral ecotone in northern China

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Abstract

Grassland converted to cropland affected soil physical and chemical properties and soil microbes. However, these parameters were often studied separately and their combined responses to grassland reclaim remain unclear. To evaluate the impacts of grassland cultivation on soil microbial communities (based on phospholipid fatty acids, PLFAs) and the links between soil microbes and physicochemical properties, we performed a paired field experiment following the conversion from native grasslands to 30-60 year-old cropland in the agro-pastoral ecotone of northern China. The concentrations of soil organic carbon (SOC), soil total nitrogen (STN) and the soil microbial biomass consistently decreased with grassland conversion to cropland. Grassland conversion also significantly decreased the relative abundance of bacterial and fungal PLFAs and the fungal to bacterial ratio (F:B) at 0-10cm soil layer, but those parameters remained unchanged below 10cm soil layers. Grassland conversion affected the microbial biomass mainly through soil C and N content rather than soil pH, moisture and aggregation. These findings revealed that cultivation-induced soil nutrient loss may enhance soil microbe depletion and affect microbial community assembly (shifts in fungi, AMF, Act, GP, and GN bacteria). This implies that conversion of grassland to cropland should be avoided because of the risk of degradation of soil nutrient and microbes.

1 Introduction

The conversion of grasslands to cultivated croplands is a common occurrence in the agro-pastoral ecotone of northern China, which covers 6.2×10^5 km^2 and is one of the major factors affecting the biodiversity and functioning of grassland ecosystems (Yang et al. 2015). Intensive farming have led to severe land degradation and have profound effects on soil physicochemical properties (Don et al. 2011; van der Gast et al. 2011; Wang et al. 2011; Kocyigit et al. 2012), nutrient turnover and microbial communities in soils (Lange et al. 2015), especially in topsoil. Most of previous studies investigating how grassland conversion affects soil properties change mainly focused on the top 30-cm depth (Poeplau et al. 2011), which is the depth recommended by the IPCC (2003). Nonetheless, these soil parameters were often studied separately and their combined effects to soil microbial community after grassland conversion remain unclear (Ying et al, 2013).

Soil microbes are essential to the maintenance of a large number of important ecosystem processes (Poeplau et al. 2011; Ying et al. 2013; Lange et al. 2015). Grassland cultivation replaces the original plant communities, which may exert great influence on soil microbes via regulating allocation of belowground photosynthates or root exudate (Ying et al. 2013). Plant harvest leads to lower input of above- and belowground biomass and plant cover (Wang et al. 2011; Poeplau et al. 2013) with vary in litter and their chemical composition (Hamer et al. 2008), which could decline carbon and nutrient accumulate in soil (Poeplau et al. 2011), change soil texture and increase wind and water erosion (Six et al. 2000). These changes in plant and soil will alter soil microbial growth, activity and community structure. Moreover, grassland cultivation influences soil physic-chemical properties (Jangid et al. 2011; Le Guillou et al. 2012; Baumann et al. 2013), leading to decreasing in content of soil nutrient and altering in soil pH (Don et al. 2011; van der Gast et al. 2011; Wang et al. 2011; Kocyigit et al. 2012). Consequently, microbial activities may be constrained because the reduced

availability of soil substrates and the changed pH in soils (Jangid et al. 2011; Ma et al. 2015). Furthermore, grassland cultivation results in the breakdown of soil aggregates, thereby exposing protected soil organic carbon (SOC) to microbial decomposition and altering soil moisture and aeration (Kocyigit et al. 2012), which may be indirect effected the soil microbial community (Le Guillou et al. 2012; Ma et al. 2015). All of these edaphic factors were altered after grassland converted to cropland, but it is unclear which factors have the dominant influence on soil microbial communities.

In this study, we investigated the effect of converting grassland to cropland (30-60 year conversion) on microbial community and biomass by using a paired design with a soil sampling depth of 0-30 cm in agro-pastoral ecotone of Northeast China. We addressed the following three questions: (i) Do microbial community and biomass vary at the topsoil after grassland conversion to cropland? (ii) Can we explain any variation in the soil microbes based on variations in the soil physicochemical properties? (iii) What is the determine factors in affecting the soil microbial community?

2 Materials and methods

2.1 Site descriptions

This study was conducted in the three counties of Guyuan (41deg39' N, 115°46' E), Kangbao (41°84/ N, 114°33/ E), and Huade (41°55'N, 113°58'E), which encompass the majority of the distribution of the agro-pastoral ecotone in Northeast China. The conversion of steppe to agricultural land started in the early 20th century due to population growth. These native grasslands have been used for grazing in history and one adjacent sites that have been cultivate about 30-60 year. The land use history was the same for both grassland and cropland sites at the same region. Before the grassland conversion, grazing (at an intensity of 3-5 sheep ha^{-1}) had been implemented for more than 10 years. Native grasslands received no fertilizer, croplands received approximately 25 kg N $ha^{-1}year^{-1}$ as urea once a year in the middle of May. The cropland was tilled for conventional tillage practices and no irrigation every year. Dominant plants in grassland ecosystems were *L. chinensis* , *A. cristatum* and *S. krylovii* . Over the past several decades, a large area of grassland has been converted to croplands with annual crop rotation between naked oat (*Avena nuda*), corn (*Zea mays*), potato (*Solanum tuberosum*), buckwheat (*Fagopyrum sagittatum*), or flax (*Linum usitatissimum*). The cultivated management regime has been lasted for 30-60 years. The information of study sites are described in detail in (Figure S1, Table 1).

2.2 Soil sampling and analysis

In each county, all of the sites with the natural vegetation is*Leymus chinensis* (Trin.) Tzvel. dominated steppe, which originally covered the entire study area. After examining the land-use history, 2-4 locations with the same grassland and cropland characteristics and similar topography were chosen at each site. Guyuan, Huade and Kangbao were assigned four, three and two locations, respectively, and pairs of sampling points (grassland vs. cropland) were identified at each location. The number of locations differed among the sites because some sites were not large enough to accommodate four pairs of sampling points. The distance was [?] 2 km between every two locations in the same county. The distances were more than 100 m between adjacent pairs and less than 50 m between grassland and adjacent cropland points. Ten soil cores were sampled randomly using a 3.5-cm diameter stainless steel auger and were combined to create replicates for the 0–10-, 10–20-, and 20–30-cm layers from each sampling point. The soil samples were sealed in plastic bags and transported to the laboratory.

After the visible plant residues and stones were removed, the sampled soil was passed through a 2-mm mesh and divided into two subsamples. One subsample was stored at -20°C and was used to measure the soil microbial communities and soil moisture, soil NH_4^+-N and NO_3^--N, while the other subsample was air-dried to analyze pH, soil aggregate, SOC, soil total nitrogen (STN) and soil total phosphorus (STP) concentrations. A 10 g soil from each composite subsample was dried at 105°C for soil moisture measurement. Soil pH was measured in a 1:5 soil:water suspension (v/v) with a digital pH electrode (Shanghai, China). Soil aggregates were obtained by wet sieving and further fractionated five size classes (0.053–2mm) following the method described by Elliott (1986). The 100g air-dried soil samples was submerged in deionized water for a duration

of 5 minutes on a set of sieves with 1-, 0.5-, 0.25-, 0.106- and 0.053-mm meshes and then shaken with an amplitude of 3cm and a frequency of 50 min^{-1}. After wet sieving, all aggregates were oven-dried at 65 °C until weighted and then contained in a shallow pan. The SOC concentration was determined following the Walkley-Black dichromate oxidation procedure (Bao, 2000), and STN was measured by Kjeldahl digestion in a 2300 Auto Kjeltec Analyzer Unit (FOSS, Sweden). For STP concentration analysis, the dry samples were digested with H_2SO_4 and H_2O_2 at 380°C for approximately 3h, and then analyzed by the vanado-molybdate method with a spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan). Soil NH_4^+-N and NO_3^--N content were analyzed by extracting inorganic nitrogen at 120 rpm for 2.5 h with 60 ml of 2 mol/L KCl from 10 g fresh subsamples, and determined by a Flow-Solution analyzer (Flowsys, Ecotech, Germany).

2.3 Soil microbial community analysis

The soil microbial community was determined by analyzing the phospholipid fatty acids (PLFAs), which were extracted and quantified from 8.0 g of dry soils using a procedure described by (Bossio et al. 1998). A 19:0 methyl ester internal standard was used to calculate the PLFA concentrations. The a13:0, i14:0, i15:0, i16:0, i17:0 and a17:0 fatty acids were chosen to represent PLFAs from gram-positive (Gram+) bacteria (Frostegard et al. 1996), and the 16:1ω7c, 17:1ω8c, 18:1ω5c, 18:1ω9t, 17:0cy and 19:0cy fatty acids were chosen to represent PLFAs from gram-negative (Gram-) bacteria (Frostegard et al. 1996). The 18:2ω6c and 18:2ω9c fatty acid was chosen to represent the fungal biomass PLFA (Frostegard et al. 1993; Kaur et al. 2005), and 16:1ω5c was used as an indicator of arbuscular mycorrhizal fungi (AMF) (Olsson 1999). The 10 Methyl 16:0, 10 Methyl 17:0 and 10 Methyl 18:0 fatty acids were chosen to represent actinomycete (Act) (Frostegard et al. 1996). The PLFA fungal to bacterial ratio (F:B ratio) and Gram positive bacteria to Gram negative bacteria ratio (Gp:Gn ratio) was calculated to estimate the relative abundance of these two groups (Frostegard et al. 1996), and the total fatty acid percentages for each microbial group described above were calculated to represent their relative contributions to the total microbial biomass.

2.4 Statistical analysis

Statistical analyses were performed using the R i386 3.3.1 (R Development Core Team) software package. The t-tests were used to compare grassland and cropland at same soil layer in all sites on SOC, STN, STP, C:N ratio, NH_4^+_N and NO_3^-_N, soil aggregations, soil microbial biomass, microbial relative abundance and F:B ratio. Three-way ANOVA was used to test for significant differences among site, land use, soil depth and their interactions on the soil microbial biomass. Correlation analysis were conducted to identify the soil parameters accounting for the soil microbial biomass.

3 Results

3.1 Effects of grassland conversion on soil physicochemical properties

Grassland cultivation significantly decreased SOC, STN, soil moisture and pH, but did not change the C:N ratio (Figure 1, Table S1). We also observed significant decreases in SOC, STN and STP concentrations at increasing soil depths in grassland across all three sites (Table S1). However, the SOC, STN and STP concentrations in cropland did not differ significantly among different soil layers (Table S1). Moreover, we found significant decreases in >1mm soil aggregation and increases in <1mm soil aggregations (Figure S2) in the cropland sites compared with the reference grasslands. Furthermore, grassland conversion to cropland significantly declined the soil NH_4^+-N concentration, but increased the NO_3^--N concentration (Figure S3).

3.2 Effects of grassland conversion on soil microbes

Conversion of grassland to cropland significantly decreased the soil total microbial biomass by 14%, Gram+ bacteria biomass by 19%, Gram- bacteria biomass by 22%, fungal biomass by 24%, actinomycete biomass by 17% and AMF biomass by 34%. Grassland conversion also significantly reduced the relative abundance of bacterial and fungal FLFAs and F:B ratio at surface 0-10cm soil (Figure 2A, B, C). In terms of soil functional composition, Gram+ and Gram- bacteria are the most abundant groups (Figure 2D).

The difference in soil microbial biomass between grassland and cropland varied with soil depth (Table 2); in

the 0-10-cm and 10–20-cm soil layers, the cropland had lower soil microbial biomass at the various sites (P <0.01, Table 2) but had higher soil microbial biomass than grassland in the 20-30-cm soil layer (P <0.05, Table 2). For each soil layer, the differences in Gram+, Gram-, Fungi, Act and AMF between the cropland and grassland showed patterns similar to that of total microbial biomass (Table 2). The site (S), land use (LU) and soil depth (SD) significantly affected the soil microbial biomass (Table 3). There was no statistically significant interactions between S and the others factors (LU and SD) (P > 0.05), while LU and SD exist significant effect on soil microbial biomass (Table 3, P <0.05). Moreover, the correlation analysis showed that soil C and N were positively correlated with biomass of most lipid categories associated with bacteria, fungi and actinomycetes, while other soil parameters including pH, SM, NH_4^+, NO_3^- and aggregations had no effects on those variables (Table 4).

4 Discussion

Despite some fertilizer inputs to the cropland in this study, significant losses of SOC and STN occurred in cropland compared with grassland, which suggested that the nutrient pools may be vulnerable to grassland conversion over time. This was consistent with previous studies that demonstrated that the conversion of grassland to cropland usually induces SOC and STN loss (Don et al. 2011; Wang et al. 2011; Ding et al. 2013). It is mainly because the removal of plant biomass reduces nutrient cumulate in soil, which in turn decreases the microbial biomass because less energy is available from soil organic matter decomposition (Schnitzer et al. 2011). Moreover, the breakdown by tillage results in large aggregates transforming into fine aggregates (Figure S2), which makes them more susceptible to erosion by wind and water that will lead to soil nutrient loss (Six et al. 2000). However, soil C:N ratios were significantly different between grassland and cropland sites. This indicates that the decrease in nitrogen could keep up with the pace of soil carbon loss after grassland cultivation.

In this study, grassland conversion consistently reduced the total soil microbial biomass, Gram+, Gram-, Fungi, AMF and Act. The greater microbial biomass under grassland soil has been previously reported (Jangid et al. 2011) as there are more favorable soil environmental conditions for microbes under grassland resulting in larger microbial biomass. The decreasing soil microbial biomass induced by the grassland conversion probably resulted from the declines in the concentrations of SOC and STN, which provide energy sources for microbe turnover. Crop growth during growing season (August is the mid-growing season in this ecotone ecosystem) significantly influences soil microbial biomass because crops compete with microorganisms for substrates (Zhang et al. 2012), and the higher vegetation biomass in cropland soils than in grassland soils during the growing season suggests that crops require more available nutrients, which might lead to the rapid depletion of labile substrates without inputs from plant residues and rhizodeposition (Bever et al. 2010; Zhang et al. 2012). In addition, the soil microclimate is cooler and moister under grassland soils compared to the drier and warmer in cropland soils. Loss of soil nutrients and water has been linked to increased susceptibility to other stresses (soil pH and soil aggregates) (Bever et al. 2010).

We found that neither F:B ratio nor relative abundance of bacteria and fungi changed with grassland conversion. This indicated that grassland conversion not only reduced the soil microbial activity but also altered the soil microbial community structure. One possible interpretation might be that grassland conversion affect microbial composition primarily by altering the soil nutrient (soil organic carbon, total nitrogen), as indicated by the correlation analysis. Soil microbial community may acclimate grassland environments and sustained a relatively stable structure. A higher F:B ratio in topsoil of grassland is associated with higher decomposition efficiency and greater carbon storage potential in soil (Ding et al. 2013). Moreover, this indicated soil fungi is likely to more sensitive and easily degradation to grassland reclaim (Poeplau et al. 2011), implying that the turnover rate may increase in whole microbial communities (Six et al. 2000). Previous studies have suggested that soil fungi often dominates the decomposition of soil organic matter because the lower nutrient demands and metabolic activities than bacteria in a low nutrient content (Jangid et al. 2011; Ma et al. 2015; Moon et al. 2016). Furthermore, the translocation of nutrients can be promoted by the hyphae of soil fungi (Klein et al. 2004), which would decrease significantly in response to physical disturbances (Helgason et al. 2008; Drenovsky et al. 2010), such as plowing under cultivation, so grassland

soils are more favorable for the formation of fungal hyphal networks that play an significant role in the cycling of soil carbon and nitrogen (Hu et al. 2014).

The site (S), land use (LU) and soil depth (SD) have significantly effect on soil microbial biomass (Table 3). However, there was no statistically significant interactions between site (S) and the others factors (LU and SD). It indicated that decrease in soil microbial biomass after grassland cultivation is a prevailing phenomenon among different site. Moreover, soil depth has been observed to be a determinant of SOC and STN concentrations and soil microbial community composition in grassland soils. Soil depth provided varied environmental conditions as reflected by changes in soil characteristics and PLFA biomass, and the decreases in soil nutrient with increasing soil depth may be a major reason for the pronounced decrease in soil microbial biomass in lower grassland soil layers (Guo et al. 2002; Ding et al. 2013; Moon et al. 2016). In contrast, the decreases in soil microbial biomass, SOC and STN were less pronounced in cropland because tillage practices homogenize the soil substrates and resources across the plow layer (Drenovsky et al. 2010), thus leading to higher soil microbial biomass, SOC and STN in 0-30 cm soil layer in cropland.

Our results revealed that grassland cultivation affected microbial biomass mainly through enhanced soil nutrient resources rather than and soil pH, moisture and aggregation. This finding is consistent with recent studies reporting that resource availability controlled the responses of the plant soil system to land use change (Lange et al. 2015).These findings suggest that soil microbes are highly vulnerable to grassland cultivation and that this vulnerability is determined by the disruption of feedback processes between soil nutrient properties and soil microbes due to grassland conversion. It is essential to effectively evaluate soil properties before grasslands are converted to cropland (Jangid et al. 2011), especially in agro-pastoral ecosystems that are particularly sensitive to environmental changes and are difficult to restore.

5 Conclusions

Cultivation-induced loss of soil nutrients may enhance soil microbe depletion in semi-arid agro-pastoral ecotone ecosystems over the long term. These findings suggested that soil microbial biomass and community may be degraded under scenarios of grassland conversion into cropland. This implies that conversion of grassland to cropland should be avoided because of the risk of loss of soil ecosystem functions.

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DATA AVAILABILITY STAEMENT

The data are available in the Dryad Data Repository (https://doi.org/10.5061/dryad.tb2rbnzzg).

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Table 1 Experimental sites information

Sampling Sites	Latitude	Longitude	Altitude (m)	MAT (°C)	MAP (mm)	Grassland characteristics	Grassland c
						Dominant species	Managemen
Guyuan	41º39/	115º46/	1383	1.0	400	*L. chinensis*	grazing
Kangbao	41º84/	114º33/	1460.8	1.2	354	*L. chinensis*	grazing
Huade	41º55/	113º58/	1456	1.6	330	*L. chinensis*	grazing

Table 2. Soil microbial biomass PLFA (nmol g^{-1} soil) at different soil depth under different land-use types

Site	Land Use	Depth	Gram+	Gram-	Fungi	Act	AMF	Others	Total
Guyuan	Grassland	0-10cm	8.55Aa	9.91Aa	3.31Aa	4.74Aa	1.67Aa	18.03Aa	46.45Aa
		10-20cm	6.55Ab	7.18Ab	2.06Ab	3.09Ab	1.11Ab	16.08Ab	36.48Ab
		20-30cm	4.81Bc	4.17Ac	1.32Ac	2.12Ac	0.69Ac	12.89Bc	26.21Ac
	Cropland	0-10cm	5.16Bc	5.40Ba	1.63Bb	2.68Ba	0.73Bb	16.55Ba	32.54Ba
		10-20cm	6.02Aa	5.37Bb	1.72Ba	2.76Ba	0.73Ba	13.74Bb	30.48Bb
		20-30cm	5.37Ac	4.62Ac	1.48Ab	2.46Ab	0.67Aa	13.46Ab	28.33Ac
Kangbao	Grassland	0-10cm	7.30Aa	6.61Aa	2.61Aa	2.97Aa	1.21Aa	16.96Aa	38.41Aa
		10-20cm	7.24Ab	5.98Ab	1.91Ab	2.56Ab	0.92Ab	15.45Ab	34.97Ab
		20-30cm	3.86Bc	2.80Bc	0.77Bc	1.29Ac	0.42Ac	11.11Bc	20.60Bc
	Cropland	0-10cm	4.65Ba	3.99Bb	1.40Bb	1.85Ba	0.53Bb	12.45Ba	25.46Bab
		10-20cm	4.65Bb	4.59Ba	1.57Ba	1.97Ba	0.73Ba	12.74Ba	26.93Ba
		20-30cm	4.41Ac	3.55Ab	1.10Ac	1.57Ab	0.42Ac	12.59Aa	24.29Ab
Huade	Grassland	0-10cm	5.88Aa	4.62Aa	2.05Aa	3.04Aa	0.74Aa	14.36Aa	30.83Aa
		10-20cm	4.89Ab	3.86Ab	1.32Ab	2.44Ab	0.45Ab	12.02Ab	25.13Ab
		20-30cm	3.33Bc	2.04Bc	0.76 Bc	1.53Bc	0.27Bc	11.16Bc	18.35Bc
	Cropland	0-10cm	3.35Bb	2.80Bc	1.01Bb	1.69Bb	0.34Bb	11.24Bb	20.41Bc
		10-20cm	4.18Ba	3.37Ab	1.27Aa	2.20Aa	0.44Aa	12.06Aab	23.50Bb
		20-30cm	4.20Aa	3.60Aa	1.17Aa	2.31Aa	0.45Aa	13.39Aa	25.11Aa

Different lowercase letters in the same land use indicate significant differences at 0.05 level. Different uppercase letters between the land-uses in the same soil layer indicate significant differences at 0.05 level

Table 3. F ratios resulting from the repeated-measures ANOVA testing the effects of site (S), land use (LU) and soil depth (SD) treatments on the soil microbial biomass.

Item	Gram+	Gram-	Fungi	Act	AMF	Total
Site	**8.40****	**11.13*****	**8.36****	**9.66*****	**12.81*****	**12.62*****
Land use	**8.67****	**5.52***	**9.88****	**5.87***	**12.05****	**7.343***
Soil depth	**5.73****	**6.72****	**13.69*****	**8.44*****	**7.31****	**9.62*****
S*LU	0.28	1.08	0.76	0.41	1.90	0.685
S*SD	0.32	0.78	0.64	1.07	0.77	0.975
LU*SD	**7.58****	**5.38****	**11.72*****	**8.43*****	**6.96****	**8.28****
S*LU*SD	0.37	0.12	0.11	0.11	0.15	0.045

Notes: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 4. Pearson's correlation coefficients for parameters of soil with microbial biomass in cropland.

	Total	Bacteria	Fungi	Gram+	Gram-	Act	AMF
SOC	**0.86***	**0.84***	**0.81***	**0.78***	**0.85***	**0.84***	**0.78***
STN	**0.81***	**0.80***	**0.75***	**0.74***	**0.80***	**0.79***	**0.72***
STP	0.45	0.41	0.40	0.38	0.42	0.41	0.39
C:N ratio	0.45	0.44	0.48	0.39	0.44	0.44	0.49
NH_4^+	0.07	0.10	0.11	0.06	0.06	0.04	0.04
NO_3^-	0.07	-0.03	-0.02	-0.05	-0.05	0.04	0.04
SM	0.35	0.42	0.33	0.37	0.43	0.31	0.45
pH	-0.62	-0.62	-0.52	-0.60	-0.62	-0.57	-0.61
>1mm	0.06	0.09	0.07	0.10	0.10	0.09	0.04
0.5-1mm	0.11	0.07	0.09	0.09	0.09	0.09	0.12
0.25-0.5mm	-0.15	-0.14	-0.16	-0.10	-0.18	-0.18	-0.14
0.106-0.25mm	-0.12	-0.11	-0.06	-0.09	-0.12	-0.08	-0.07
0.053-0.106mm	-0.15	-0.17	-0.17	-0.16	-0.16	-0.13	-0.18

***** represent significant relationships ($P < 0.05$).

Note: SOC: soil organic carbon content; STN: soil total nitrogen content; STP: soil total phosphorus content; NH_4^+: soil NH_4^+ content; NO_3^-: soil NO_3^- content; pH: soil pH value; SM: soil moisture; Soil aggregate includes >1 mm, 0.5mm-1mm, 0.25mm-0.5mm, 0.106mm-0.25mm, 0.053mm-0.106mm aggregate.

Figure Legends

Figure 1 Soil organic carbon, total nitrogen, total phosphorus and C:N ratio in grassland and cropland. The different lower letter represent a significant differences between grassland and cropland at same soil layers.

Figure 2 Relative abundance of bacteria (A) and fungi (B), F:B ratio (C) and relative abundances of soil dominant PLFA types (D) after grassland converted to cropland. $*P < 0.05$; ** P <0.01; *** P< 0.001; n.s.: not significant ($P > 0.05$)