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Effects of yak excreta on soil organic carbon mineralization and microbial communities in alpine wetlands of southwest of China

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Abstract

Purpose Improving knowledge of how soil organic carbon (SOC) mineralization responds to excreta application is essential to better understand whether wetland carbon (C) pools will react to grazing. We investigated microbial activity and community structure in the different treatments of excreta addition experiments to examine how soil C mineralization responds to the excreta input in terms of microbial activities and compositions in wetland soils.

Materials and methods The microcosms of mineralization incubation of excreta addition were established. The structure of the microbial community was described by the fatty acid composition of the phospholipids (PLFA). The methylumbelliferyl-linked substrates (MUB) and l-dihydroxyphenylalanine (L-DOPA) substrates were used to investigate the activities of β-glucosidase (BG), N-acetyl-glucosaminidase (NAG), acid phosphatase (AP), cellobiohydrolase (CBH), and phenol oxidase (PO).

Results and discussion Excreta addition altered the cumulative C mineralization in swamp meadow (SM) and peatland (PL) soils, but SM was lower than PL. Excreta addition increased the biomass of individual PLFA and the fungi/bacteria ratio, suggesting that microbes are stimulated by nutrients and that the soil microbial community composition is modified by excreta inputs. The hydrolytic enzyme activities were higher in the PL soils than in the SM soils, but the trend was opposite for PO activity. The changes in pH, fungi, actinomycetes (ACT), AP, and CBH after yak fecal input significantly influenced the soil CO_2 efflux. Our findings suggest that yak grazing could influence the rate of C cycling in wetland soils by influencing microbial communities, enzyme activities, and soil pH.

Conclusions This study suggest that the yak excreta addition increased cumulative C mineralization in SM and PL soils, and the effect of dung addition was more significant than urine addition. The effect of yak excreta addition on SOC mineralization was related with the soil pH, microorganism structure, and enzyme activity which modified by the excreta addition. Soil pH, fungi, AP, and CBH were positively correlated with SOC mineralization, but ACT was negatively correlated with SOC mineralization. In addition, the changes in C and N sources with yak excreta addition play an important role in altering microbial enzyme activities. The input of yak feces into wetlands because of grazing could increase SOC mineralization and thereby promote C emission.

Keywords Alpine wetlands · Enzyme activity · Microbial community · SOC mineralization · Yak excreta

1 Introduction

It is estimated that 20–30% of the Earth's soil pool of 2500 Pg of C is stored in wetlands (Lal 2008). Because of the low decomposition rates of soil organic matter compared with production (Min et al. 2011), wetlands serve as a net C sink (Köchy et al. 2015). The factors that affect decomposition capacity include anaerobic conditions, low litter quality, and limited nutrient availability (Bragazza et al. 2006).

Many wetlands are used for livestock grazing (Middleton 2018). Grazing is a disturbance that can alter the successional dynamics of wetlands. Grazing directly affects plant community composition and indirectly affects the activity of decomposer organisms (Middleton 2018), thereby potentially altering ecosystem C fluxes (Mesa et al. 2015). Moreover, many studies have reported the effect of different grazing intensities and patterns on C emissions in wetlands (Ma et al. 2016), but the question of whether wetland SOC mineralization may be

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modified by yak excreta addition remains poorly explored, especially in the low-latitude alpine wetlands.

Grazing animals can give rise to "hot spots" by voiding excreta, which means high local additions of nitrogen (N) and readily available C that can stimulate soil emissions of CO_2 (Yu et al. 2016). Much organic matter is contained in waste, and some of the C in waste moves into the soil and promotes CO_2 emission (Laiho et al. 2017). In contrast, cattle manure applied in grassland can prevent C loss (Martínez et al. 2017). These opposing results demonstrate our poor understanding of soil C dynamics with excreta addition in soils. Moreover, most studies on CO_2 emissions from excreta input have been conducted in grasslands/pastures, with no data available from the effect of yak excreta addition on soil C decomposition in wetland.

Several studies have reported that the activities of extracellular enzymes secreted by microbes are positively related to organic matter turnover (Morrissey et al. 2014; Trivedi et al. 2016). Extracellular enzyme activities have been used to predict catabolic potential (Li et al. 2015). Low pH decreases microbial activities and decomposition of organic matter (Paz et al. 2016; Lu et al. 2017). Min et al. (2011) reported that soil CO_2 efflux was significantly influenced by changes in pH and enzyme activities after fertilization. Of the many factors influencing enzyme activities, soil pH can be highly influenced by excreta addition. Many studies have found that soil pH can be increased by excreta input due to the release of OH^- during urea hydrolysis in urine patches (Raiesi and Riahi 2014) and the alkaline nature of dung (Weeda 1967). However, Aarons et al. (2004) found that soil pH decreased under dung pads, and Whalen et al. (2000) also stated that soil pH increased after cattle manure input. Although we know that enzyme activities could be altered by dung and urine input, their responses to excreta input and to the consequent change in the soil pH in wetlands are insufficiently documented.

Microbial activity has a strong impact on soil C cycling. Many studies have focused on microbial structure after fertilization. C and N additions could change the soil nutrient supply and then influence decomposition via microbial changes in both function and structure (Su et al. 2017). The microbial groups have a preference for utilization of C with different forms of organic C (Kramer and Gleixner 2008; Wang et al. 2014). Therefore, microbial groups may be altered with C and N sources changed by excreta input. Nitrate additions bring about a microbial shift from fungal to bacterial dominance, with the corresponding decline in the decomposition rate (Wang et al. 2014). Moreno-Cornejo et al. (2015) found that changes in microbial biomass after fertilization had a positive correlation with those of soil CO_2 release. In contrast, the change in the microbial biomass did not correspond to the soil C mineralization (Min et al. 2011; Dai et al. 2017).

In this study, we incubated two different types of wetland soils with excreta addition. We investigated microbial activity and community structure in the different treatments of excreta addition experiments to examine how soil C mineralization responds to the excreta input in terms of microbial activities and compositions. Our hypotheses were as follows: (1) excreta input will increase SOC mineralization by influencing soil nutrient content; (2) SOC mineralization in respond to soil microbial community structure is modified by the excreta addition; and (3) excreta input promotes the increase of soil enzyme activity and affects SOC mineralization.

2 Materials and methods

2.1 Sit description

We conducted our field research in the internationally important Bitahai Wetland (27° 46′~27° 57′ N, 99° 54′~100° 08′ E; elevation 3512 m above sea level) in southwest China. The site is located where the Tibet Plateau zone, subtropical monsoon climate zone, and Indo-China Peninsula monsoon climate zone meet; summer is short, warm, and rainy, while winter is long and cold. The annual average temperature is 5.4 °C, and the valid cumulative temperature is approximately 1392.8 °C. The annual average precipitation is approximately 617.6 mm, and approximately 76% of it falls from June to September. The Bitahai Wetland is a typical swamp meadow and peatland. The dominant vegetation includes *Blysmus sinocompressus*, *Deschampsia caespitosa*, *Sanguisorba filiformis*, *Carex lehmannii*, and *Carex nubigena*.

2.2 Methods

2.2.1 Soil sampling

For our research, the typical SM and PL located along the topographical gradients from lowland to highland were selected. Three uniform plots of 10 × 10 m were chosen from each site (SM, PL) for the sample collection, and the distance between plots was greater than 10 m. From each plot, at least 10 soil cores (5 cm in diameter) were randomly collected from 0 to 10 cm in SM and PL. Samples from each plot were mixed thoroughly to form one composite sample, and three samples were obtained for the respective SM and PL. Soil samples were kept in a cooler at 4 °C for laboratory incubation and analysis. The physical and chemical properties of the soils are shown in Table 1. The total C and N concentrations were analyzed using a TOC elemental analyzer (vario TOC, Elementar, Germany) and continuous-flow analyzer (AA3, SEAL, Germany), respectively. Soil pH was determined in a soil/water (1:5, *w*/*w*) slurry.

2.2.2 Incubation experiment

Yaks were enclosed at night, and fresh dung and urine samples were collected from the camping area the next morning. These

Table 1 Physical and chemical characteristics of SM and PL soils

Soil type	Bulk density (g cm^{-3})	SOC (g kg^{-1})	TN (g kg^{-1})	pH	C/N	Water content (%)
SM	0.52 ± 0.03	200.59 ± 2.0a	11.13 ± 4.06a	5.46 ± 0.03	19.22 ± 5.05	46.65 ± 0.05
PL	0.48 ± 0.05	374.45 ± 5.94b	22.67 ± 4.10b	5.03 ± 0.01	16.70 ± 2.60	45.58 ± 0.01

Values are expressed as the mean ± SE. Lowercase letters indicate significant differences in the different soil types

samples were kept frozen in a freezer and carefully mixed before samples were applied to the soils. The C content of dung and urine were 483.57 ± 5.15 and 170.17 ± 4.65 g kg^{-1}, respectively. The N content of dung and urine were 24.10 ± 2.07 and 8.76 ± 1.30 g kg^{-1}, respectively. The pH of dung and urine were 7.99 ± 0.01 and 8.67 ± 0.31, respectively. The dung moisture content was 80.53%.

Fresh soil samples (80 g dried weight) were placed in 750-mL glass jars. The treatments included soil with urine addition (UI_1 in SM and UI_2 in PL), soil with dung addition (DI_1 in SM and DI_2 in PL), and soil without addition (CK_1 in SM and CK_2 in PL). Fresh dung and urine were separately applied to the soils. According to the method of Lovell and Jarvis (1996), field investigation shows that the input volume of yak dung is 46.6 kg m^{-2}. The dung application rate was 0.49 g g^{-1} soil of SM and 0.52 g g^{-1} soil (wet weight) of PL, respectively. Urine was added in accordance with the average urine volume of 4 L m^{-2} reported by Van Groenigen et al. (2005) in field grazing. The urine application rate was 0.042 mL g^{-1} soil of SM and 0.045 mL g^{-1} soil (wet weight) of PL, respectively. Three replicate microcosms for each treatment were established, with a total of 18 microcosms.

The microcosms were preincubated in an electroheating standing-temperature cultivator for 7 days and then shifted to mineralization incubation. The experiment is an aerobic experiment. During mineralized culture, the cap of culture bottle will be opened at each titration, and the small beaker containing sodium hydroxide solution will be taken out. During the long incubation interval, the culture bottle will be opened every 2 days for 20 min, allowing the air in the bottle to fully exchange the air in the bottle with the external world. The released CO_2 was measured using alkali-trapping techniques (Wang et al. 2014) at 2, 4, 6, 8, 11, 14, 20, 27, 34, 43, 50, and 57 days after incubation. Briefly, a glass vial containing 40 mL of 1 M NaOH solution was placed in each glass jar to trap evolved CO_2 from the soil. All glass jars with soil were incubated in the dark for 57 days at 25 °C. Three additional glass jars with a beaker containing 20 mL of 1 M NaOH were sealed, serving as controls to account for CO_2 trapped from the air. At different measuring events, the vials containing NaOH were removed and titrated with 0.5 M HCl in the presence of $BaCl_2$. The evolved CO_2 from the soil sample was calculated from the difference in the value of evolved CO_2 in the glass jars with and without soil. At 6, 14, 27, 34, and 50 days, the glass jars were weighed, and the water content of the mesocosms was adjusted to maintain moisture content. The pH of the soils at the end of incubation is shown in Table 2.

2.2.3 Microbial community and enzyme activities

At the end of incubation, soil was removed from the glass jars and analyzed for enzyme activity, microbial biomass, and community structure. The structure of the microbial community was described by the PLFAs in the soil. PLFAs were determined following the method described by Ameloot et al. (2014). Approximately 6.0 g of soil was weighed into 50-mL sterilized tubes, and exact weights were recorded. Bligh-Dyer solutions ($MeOH/CHCl_3$/citrate buffer = 2:1:8) were used to extract fatty acids from soil samples. After extracting the supernatant, citrate buffer and $CHCl_3$ were added in a wash step. Then, nonpolar phases were transferred and evaporated by N_2, leaving behind only fatty acid. Then, PLFAs were separated from neutral lipids and glycolipids with a silicic acid-bonded solid-phase extraction column, and samples were dried with N_2. Dried lipids were saponified and methylated to fatty acid methyl esters (FAMEs). FAMEs were resuspended in hexane, 10 mL of nonadecanoic acid methyl ester (0.1 μg μL^{-1}) was added as an internal standard, and then the mixture was dried with N_2. Individual FAMEs were identified using the MIDI Sherlock Microbial Identification System (MIDI, Newark, DE, USA). Individual biomarkers were assessed according to Wang et al. (2016). Biomarkers representing GP were i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0, and those representing GN were 16:1ω7c, 18:1ω7c, 18:1ω5c, and cy17:0. The sum of 10Me16:0, 10Me16:0, and 10Me18:0 was used to quantify ACT. The total bacterial community was assumed to be represented by the sum of the biomarker PLFAs for Gram-positive and Gram-negative bacteria. PLFA 18:2ω6, 9c was considered as an indicator for fungi.

MUB were used to investigate the activities of BG, NAG, AP, and CBH. BG is responsible for the decomposition of cellulose, NAG for chitin, AP for phosphate groups in soils, and CBH for labile litter. One and a half grams of fresh soil was treated with 100 mL MUB-substrate solution and then incubated for 4 h. After centrifugation at 6000 rpm for 5 min, 300 mL of the supernatant was transferred to a black microplate, with the fluorescence determined at emission and excitation wavelengths of 450 and 330 nm, respectively

Table 2 pH of SM and PL soils at end of incubation

Characteristics	SM			PL		
	CK_1	DI_1	UI_1	CK_2	DI_2	UI_2
pH	5.65 ± 0.31Aa	6.79 ± 0.08Ab	6.18 ± 0.07Ac	5.43 ± 0.06Aa	6.88 ± 0.01Bb	6.27 ± 0.21Bc

Values are expressed as the mean ± SE. Lowercase letters indicate significant differences among treatments on the same soil type, while capital letters represent significant differences among different soil type for the same treatment

(Synergy H4, BioTek, USA). The PO activity was determined using L-DOPA as a substrate (Pind et al. 1994). One and a half grams of soil was treated with 10 mL of 10 mM L-DOPA (Sigma). After 15 min of shaking, the reaction liquid was centrifuged at 5000 rpm for 5 min. An L-DOPA standard series was measured together with the filtrates at 460 mm using a spectrophotometer (Cary 60, Agilent, USA).

2.3 Statistical analysis

ANOVA was performed to examine differences in all variables between and within groups of SM soil and PL soil. Two-way ANOVA was used to analyze the effect of excreta addition on the average soil CO_2 efflux, enzyme activities, and microbial community structure. Tukey's honestly significant difference test was performed as a post hoc test to separate the means when the differences were significant. The relationship between soil CO_2 efflux and soil pH, microbial community structure, and enzyme activities were determined by Pearson's linear correlation coefficient. Linear regression analysis was used to investigate the effect of each variable on the CO_2 efflux. Stepwise regression analysis was performed for all enzymes to determine the most powerful predictors for soil CO_2 efflux. All effects noted were significant at the $P < 0.05$ level, and statistical analyses were performed using SPSS 19.0 for Windows.

3 Results

3.1 SOC mineralization

Excreta addition significantly increased cumulative C mineralization in SM and PL soils ($P < 0.001$) (Fig. 1). During the whole culture period, the cumulative C mineralization amount of soils with different treatments showed as $DI_2 > DI_1 > UI_2 > UI_1 > CK_1 > CK_2$. In SM, the amount of cumulative C mineralization in the dung addition treatment was 1.03 and 0.97 times higher than that in the control ($P < 0.001$) and urine addition treatment ($P < 0.001$), respectively, but there was no significant difference between the control and urine addition treatment ($P = 0.964$) at the end of incubation. In PL, the amount of cumulative C mineralization in the dung addition treatment was 1.74 and 0.91 times higher than the control ($P < 0.001$) and the urine addition treatment ($P < 0.001$), respectively, and the urine addition treatment was 0.44 times higher than the control ($P = 0.001$) at the end of incubation. There were significant differences in cumulative C mineralization between SM and PL soils under the same treatments ($P < 0.05$).

Soil pH was measured at the end of incubation (Table 2). Both the dung and urine input treatments increased the pH in the SM and PL soils ($P < 0.05$). The difference in the pH values between the control and excreta addition treatment groups (ΔpH) was positively related to the differences in the soil CO_2 efflux (ΔCO_2) ($\Delta CO_2 = 643.49e^{\Delta pH\ 0.5839}$, $R^2 = 0.799$, $P < 0.01$, $n = 18$).

3.2 Soil microbial community structures

The soil microbial biomass was significantly higher in SM soils than in PL soils ($P < 0.001$), except for fungi biomass (Table 3). Excreta addition significantly enhanced the biomass of total PLFA, bacteria, and fungi ($P < 0.01$) but significantly decreased the ACT ($P < 0.05$) in SM soils (Table 4). Dung addition enhanced the biomass of total PLFA, bacteria, and fungi but significantly decreased the ACT ($P < 0.05$) in PL soils. However, urine additions decreased the biomass of total PLFA, bacteria, fungi, and ACT in PL soils. The ratio of fungi to bacteria was increased by excreta addition in peatland soils ($P < 0.001$) but did not significantly change in SM soils. The increase in GN biomass with dung addition led to a decreased ratio of GP to GN. However, the decrease in GP biomass with urine additions led to a lower ratio of GP to GN.

The cumulative C mineralization was positively related to fungi ($P < 0.01$), fungi/bacteria ($P < 0.01$), and GP ($P < 0.01$), but negatively related to ACT ($P < 0.01$) in SM soils (Table 5). In contrast, the cumulative C mineralization was positively related to bacteria ($P < 0.01$), fungi ($P < 0.01$), fungi/bacteria ($P < 0.01$), and GN ($P < 0.01$), but negatively related to ACT ($P < 0.01$) in PL soils. The difference in the fungi values between the control and excreta addition treatment groups (Δfungi) was positively related to the differences in the soil CO_2 efflux (ΔCO_2) ($\Delta CO_2 = 9.098\Delta fungi - 18.326$, $R^2 = 0.815$, $P < 0.01$, $n = 18$). However, the difference in the ACT values between the control and excreta addition treatment

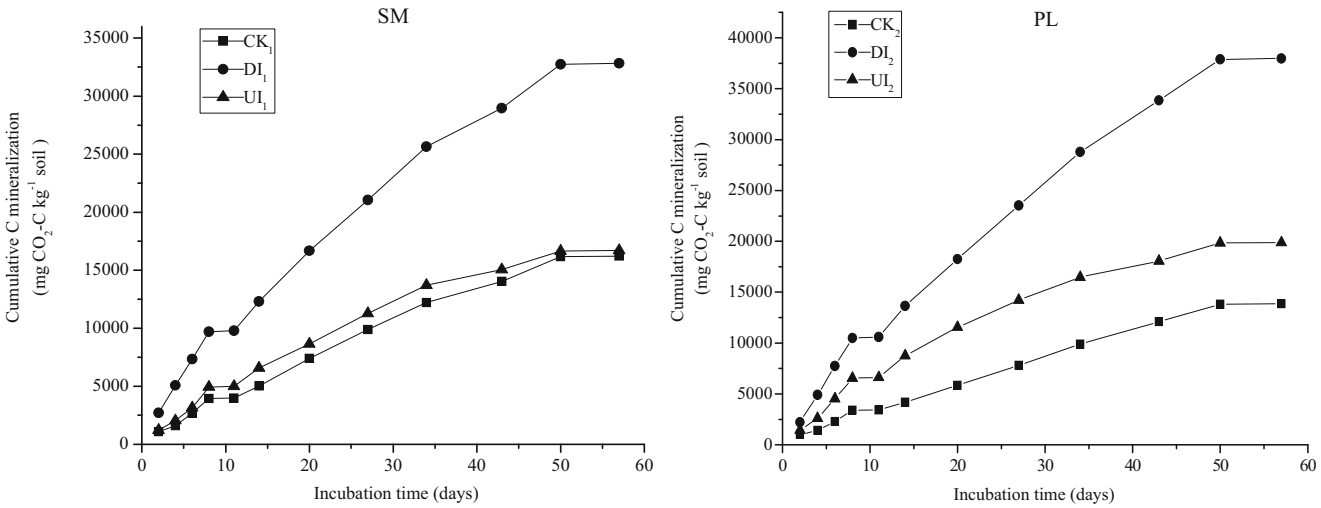

Fig. 1 Effects of excreta addition on cumulative SOC mineralization in the SM and PL soils. The presented values are the means ($n = 3$)

groups (ΔACT) was negatively related to the differences in the soil CO_2 efflux (ΔCO_2) ($\Delta CO_2 = -0.5082\Delta ACT + 40.860$, $R^2 = 0.270$, $P < 0.05$, $n = 18$).

3.3 Enzyme activities

The effects of excreta addition on the enzyme activities differed between SM and PL soils. The AP, NAG, CBH, and BG activities were higher in the PL soils than in the SM soils, but the trend was opposite for PO activity (Fig. 2). Excreta addition promoted the increase of AP and CBH activity, inhibited the activity of NAG and PO both in SM and PL soils. In contrast, dung input significantly promoted the activity of BG, but urine input inhibited the activity of BG in both PL and SM soils.

Compared with soil type, the enzyme activities were heavily influenced by excreta addition (Table 4). Across all treatments and soils, enzyme activities were a strong indicator of CO_2 efflux ($R^2 = 0.790$, $P = 0.047$, $n = 48$). The cumulative C mineralization was positively related to AP ($P < 0.01$) and CBH in SM and PL soils (Table 5). The difference in the AP and CHB values between the control and excreta addition treatment groups (ΔAP) was positively related to the differences in the soil CO_2 efflux (ΔCO_2) ($\Delta CO_2 = 0.147\Delta AP + 5.054$, $R^2 = 0.795$, $P < 0.01$, $n = 18$; $\Delta CO_2 = 2.703\Delta CBH + 11.28$, $R^2 = 0.543$, $P < 0.01$, $n = 18$).

4 Discussion

4.1 Effects of excreta addition on SOC mineralization

This study showed that excreta addition increased rates of CO_2 production across the two different types of wetland soils, although there was different accumulating C mineralization. The difference in cumulative mineralization also suggests that the increase in soil CO_2 efflux induced by the addition of yak dung was greater than of urine input.

Yak manure contains much organic matter. Fecal addition is equivalent to fertilization to provide nutrients to the soil to promote enhanced soil CO_2 emissions (Zhang et al. 2007). Excreta addition obviously improved the availability of soil

Table 3 Changes in concentrations (μmol g^{-1} soil) of PLFAs and two PLFA ratios in SM and PL soils at the end of incubation

	Total biomass	Bacteria	Fungi	ACT	Fungi/bacteria	GP	GN	GP/GN
CK_1	35.43 ± 1.0Aa	27.36 ± 0.92Aa	3.72 ± 0.28Aa	4.34 ± 0.26Aa	0.14 ± 0.01Aa	12.63 ± 0.95Aa	14.74 ± 0.46Aa	0.86 ± 0.08Aa
DI_1	41.63 ± 0.62Ab	32.54 ± 0.92Ab	5.68 ± 0.34Ab	3.41 ± 0.02Ab	0.17 ± 0.01Aa	14.63 ± 0.40Ab	17.91 ± 0.73Ab	0.82 ± 0.06Aa
UI_1	38.72 ± 0.54Bc	30.60 ± 0.87Ac	4.17 ± 0.39Ba	3.94 ± 0.10Ac	0.14 ± 0.02Aa	12.07 ± 0.61Ba	18.54 ± 0.28Ab	0.65 ± 0.02Ab
CK_2	17.75 ± 0.11Ba	10.57 ± 0.33Ba	4.57 ± 0.26Ba	2.61 ± 0.22Ba	0.43 ± 0.04Ba	6.73 ± 0.03Ba	3.84 ± 0.30Ba	1.76 ± 0.13Ba
DI_2	18.77 ± 0.82Ba	10.87 ± 0.46Ba	6.49 ± 0.32Bb	1.42 ± 0.15Bb	0.60 ± 0.03Bb	6.13 ± 0.26Ba	4.73 ± 0.26Bb	1.30 ± 0.06Bb
UI_2	15.47 ± 0.60Bb	8.84 ± 0.80Bb	4.33 ± 0.18Ba	2.30 ± 0.26Ba	0.49 ± 0.07Bc	5.01 ± 0.61Bb	3.83 ± 0.21Ba	1.31 ± 0.10Bc

Values expressed as the mean ± SE. Data are reported for different taxa and two PLFA ratios (GP/GN and fungi/bacteria) under control, dung addition, and urine addition. Lowercase letters indicate significant differences among treatments of the same soil type, while capital letters represent significant differences among different soil types for the same treatment

Table 4 Effects of excreta addition and soil type on the soil microbial community and enzyme activity

	Bacteria	Fungi	ACT	BG	NAG	AP	CBH	PO
Soil	< 0.01	0.001**	< 0.01	< 0.001**	0.306	0.009**	0.003**	0.056
Excreta	< 0.01	< 0.01	< 0.01	0.002**	< 0.001**	< 0.001**	0.003**	0.016*
Soil × excreta	< 0.01	0.112	0.309	0.541	0.168	0.13	0.134	0.353

*P value is significant at < 0.05; **P value is significant at < 0.01

N, stimulated the microbial activity in the soil and then accelerated the SOC mineralization (Ameloot et al. 2014), making the soil C mineralization rate higher in the excreta addition treatments than the CK. Therefore, grazing increased microbial biomass and activity by increasing the quantity and quality of resources to the microbial community (Toal et al. 2000). The microbial activity of the feces itself also plays an important role in the decomposition of feces and soil organic matter. The input of yak urine increases the N content in the soil (Chen et al. 2015) and then increases the demand for C from soil microbes and promotes the decomposition of SOC (Huang et al. 2011).

In this study, it was found that the pH significantly increased in both soils at the end of incubation in the excreta addition treatments to varying degrees (Table 2); this was positively related to the differences in the soil CO_2 efflux, which may be due to the alkaline nature of excreta (During and Weeda 1973). Whalen et al. (2000) found that cattle manure amendments could increase the pH of acid soils, and Shang et al. (2013) stated that the increase in soil organic matter content due to yak excrement addition could increase the soil pH. Ye et al. (2012) found that more C substrates were made available to microbial decomposers through increased CO_2 when potential low pH limitation in PL soils was removed.

The cumulative mineralization of UI_2 was significantly higher than that of CK_2 in PL soils, but there was no significant difference between UI_1 and CK_1 in SM soils. Soil C/N ratio was the best predictor in explaining C mineralization (Paul 2007). Soil C/N ratio plays an important role in determining microbial community structure and significantly related to fungal lipid biomarkers (Wan et al. 2015). Paul (2007) have stated that fungi having a higher C use efficiency when C/N range from 10:1 to 15:1. In our study, the soil C/N ratio of PL is relatively more conducive to the growth of fungal microorganisms and thus promotes the decomposition of organic matter by fungi. In addition, soil microorganisms are direct indicators of decomposition because they excrete the extracellular enzymes required in decomposing complex high molecular weight compounds (Weand et al. 2010). With the addition of urine, microbes in the SM soil do not require more decomposition of organic matter to obtain N nutrients, thereby reducing C mineralization (Wang et al. 2013). However, even if the soil C/N ratio is lower in PL soils than in SM soils, because its soil organic matter content is much higher than that of SM soils, the urine input to promote increased soil N nutrients will further stimulate the soil enzyme activity to improve the decomposition of organic matter, increasing C mineralization.

4.2 Effects of excreta addition on soil microbial community

Excreta addition had a direct effect on the microbial biomass by temporarily increasing available soil C and N nutrients. Gomez et al. (2006) found that organic matter amendments increased substrate utilization and altered the soil biota composition. Excreta addition increased the ratio of fungi to bacteria, especially in dung input treatments, demonstrating that fungi are major sinks for newly added C (Wang et al. 2014), as fungi are regarded as the main decomposers of recalcitrant polymeric compounds (Esperschütz et al. 2011). Fanin et al. (2015) stated that the fungi/bacteria ratio increased in N-fertilized plots. Zhang et al. (2012) had stated that addition of organic manure significantly improved the soil fertility status which is more beneficial to the fungi. The positive effect of the fungi/bacteria ratio in the excreta input suggests that fungi grow better than bacteria as C and N availability increases (Högberg et al. 2010; Wang et al. 2013).

Table 5 Pearson's correlation analysis of cumulative SOC mineralization, pH, microbial community and enzyme activity in SM and PL soils

	pH	Bacteria	Fungi	ACT	Fungi/bacteria	GP	GN	GP:GN	BG	NAG	AP	CBH	PO
CO_2-C (SM)	0.836**	0.546	0.936**	− 0.840**	0.895**	0.844**	0.333	0.292	0.306	0.246	0.951**	0.689*	− 0.487
CO_2-C (PL)	0.713*	0.844**	0.914**	− 0.952**	0.814**	0.011	0.881**	− 0.596	0.588	− 0.392	0.897**	0.870**	− 0.218

*P value is significant at < 0.05; **P value is significant at < 0.01

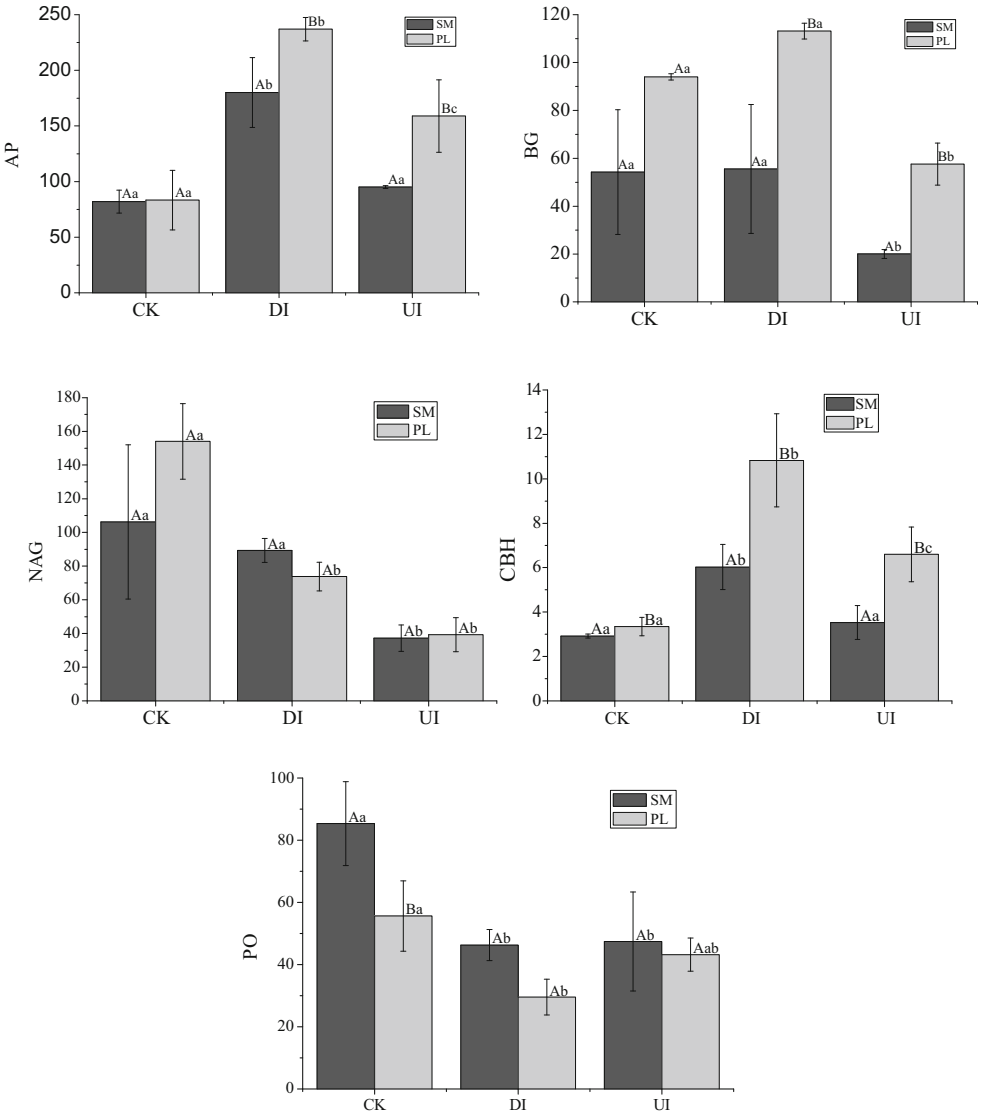

Fig. 2 The activities of BG, NAG, AP, CBH, and PO were determined at the end of the incubation. Data were pooled across the addition rates. Means are shown, with ± SD (AP, BG, NAG, and CBH, $n = 24$, PO, $n = 3$). Lowercase letters indicate significant differences among treatments on the same soil type, while capital letters represent significant differences among different soil types for the same treatment. The units of the AP, BG, NAG, and CBH are μmol g^{-1} dry soil h^{-1}, and those of the PO are μmol diqc g^{-1} dry soil min^{-1}

In addition, GN is also an important group of bacteria involved in C turnover (Elfstrand et al. 2008; Kramer and Gleixner 2008; Esperschütz et al. 2011). C availability altered by C input manipulation favored the growth of some microbial groups over others, resulting in shifts in the microbial community (Cederlund et al. 2014). A lower ratio of GP to GN suggests that excreta addition modified the bacterial community composition because of the benefit from the increases in the availability of organic substrates (Peacock et al. 2001). The difference in the fungal/bacteria and GN/GP ratios among the treatments also supports the hypothesis that the yak fecal input altered the soil microbial community composition.

4.3 Effects of excreta addition on enzyme activity

Different enzymes played different but essential roles in decomposition. An increasing effect of excreta addition on microbial decomposition manifested itself in increased extracellular enzyme activities. In accordance with the soil CO_2 efflux, the enzyme activity increased in response to excreta addition in the SM and PL soils, although the NAG and PO enzyme activities decreased with the excrement input. The lower soil hydrolytic enzyme activity in SM suggested that decomposition occurred at a slower rate than PL.

The AP activity was increased with excreta addition in this study. This might be because the excreta addition increased the available C and then promoted the activity of AP. The greater the supply of C was, the more P was needed because C is one of the most important nutrients for microbes (Min et al. 2011). Keuskamp et al. (2015) also stated that ammonia application significantly promoted phosphatase activity. BG activity is induced by the presence of the substrate (Lynd et al. 2002). Debosz et al. (1999) also noted that BG activity is higher in high organic matter input treatments. Livestock feces and urine are sources of soil C and N (Liang et al. 2018). In the present study, the BG activity increased in DI but

decrease in UI might be due to the higher supply of C and N in dung treatments than urine treatments.

Sinsabaugh et al. (1993) assumed NAG activity to be induced by low N conditions, whereas at high N concentrations, noncompetitive inhibition could occur. N-acquiring enzymes have been found to decrease under high N availability in many studies (Moorhead and Sinsabaugh 2006; Min et al. 2011). The decreased NAG activity observed in this study may indicate an alleviated microbial need for N sources. In our study, the CBH increased with excreta addition, which is consistent with the result obtained from arable soil, where CBH activity was enhanced by long-term application of the same type of fertilizer (Ai et al. 2012). The changes in soil cumulative mineralization are strongly associated with CBH activity. This means that cellulose hydrolase plays an important role in improving soil C mineralization by promoting cellulose degradation in soil. This is consistent with previous studies on similar findings that CBH increased in soils where respiration was promoted by manure addition (Fan et al. 2012). The activity of PO was reduced after excreta input in both soils. Cusack et al. (2010) revealed that N addition decreased oxidative enzymes but increased hydrolytic enzymes. This view is reflected in our findings.

5 Conclusions

This study suggest that the yak excreta addition increased cumulative C mineralization in SM and PL soils, and the effect of dung addition was more significant than urine addition. The effect of yak excreta addition on SOC mineralization was related with the soil pH, microorganism structure, and enzyme activity which modified by the excreta addition. Soil pH, fungi, AP, and CBH were positively correlated with SOC mineralization, but ACT was negatively correlated with SOC mineralization. In addition, the changes in C and N sources with yak excreta addition play an important role in altering microbial enzyme activities. The input of yak feces into wetlands because of grazing could increase SOC mineralization and thereby promote C emission.

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