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RESEARCH ARTICLE

Multi-cutting and sheep excrement influence plant growth and soil nitrogen mineralization in sown grassland

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Abstract
Purpose Multi-cutting and livestock excrement application are two key agricultural practices affecting nutrient cycling in the plant-soil system of sown grasslands. However, little is known about the combination of multi-cutting and livestock excrement application on nitrogen mineralization and utilization and their mechanisms regulating plant growth.
Methods The experiment was carried out in a salinized grassland at Linze Grassland Agricultural Station, Gansu Province, China. We tested the effects of plant multi-cutting (MC), sheep excrement (SE), multi-cutting and sheep excrement (MC + SE), and plant only (CK) on the soil nitrogen mineralization and plant growth of two forage plants, the spring wheat (*Triticum aestivum* L., cv. Yongliang 15) and common vetch (*Vicia sativa* L., cv. Lanjian 3). We measured the plant height, branch number, tiller number and crude protein content, and soil nitrification rate, ammonium rate, microbial nitrogen and microbial carbon, and abundance of soil ammonia-oxidizing bacteria and ammonia-oxidizing archaea.
Results MC and MC + SE significantly increased plant height, dry matter, and branch and tiller number of both forage crops. Although crude protein content and tiller number of spring wheat in SE decreased, these in MC + SE increased. SE and MC + SE significantly promoted nitrification rate in the soil by increasing the soil microbial nitrogen and soil ammonia-oxidizing bacteria abundance of the two forage crops.
Conclusions Combined application of both multi-cutting and sheep excrement could accelerate soil nitrogen mineralization and plant nitrogen uptake, which could be considered in pasture management to improve the sustainable productivity of grass-soil systems of sown grassland.

Keywords AOB abundance · *amoA* gene · Nitrogen mineralization · Microbial nitrogen

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Introduction

Soil nitrogen mineralization, an important performance of soil nitrogen supply capacity, is one of the most important internal ecosystem processes affecting the availability of available nitrogen in plants and microorganisms (Owen et al. 2003; Zhang et al. 2008). In grassland ecosystems, the level of nitrogen mineralization in grassland soil directly affects the

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productivity of forage grass (Hungate et al. 2003). Animal grazing, through removing aboveground biomass and depositing excrement, can alter the soil nitrogen functions and microbial community (Rossignol et al. 2011) and regulate the plant nitrogen cycle and productivity (Golodets et al. 2011). In arid grasslands, excess forage intake and excrement from overgrazing may lead to the failure of plants to absorb and utilize available nitrogen in time (Liu et al. 2015) or result in the loss of soil nitrogen, decreasing soil fertility and grassland productivity. However, the lack of knowledge of the effects of grazing on soil nitrification and ammonification is hampering the evaluation of soil fertility—grassland productivity feedbacks in arid regions. Therefore, it is of great significance to study the nitrogen transformation of forage crop-soil-microbial systems under different grazing management practices in arid regions.

Multi-cutting, the common agricultural practice and method often used to simulate foraging behavior of livestock during grazing, results in a denser root system by increasing the root-to-shoot-ratio (Keuter et al. 2013), and consequently promotes the nitrogen uptake of grassland plants and increase the nitrogen conversion rate of soil (Robson et al. 2010). Li et al. (2017) report that cutting once a year could even increase soil nitrogen storage and the net nitrogen mineralization rate. In contrast, other studies show that multi-cutting reduces the total mass of litter entering the soil, which leads to a long-term reduction in the soil nitrogen pool, reducing the gene abundance of ammonia oxidizing archaea (AOA), ammonia oxidizing bacteria (AOB) (Zhong et al. 2018), and the soil nitrogen conversion (Wang et al. 2011). Compared with multi-cutting that reduces the soil nitrogen cycle, nitrogen fertilizer application increases the nitrogen cycle and soil nitrogen mineralization (Shan et al. 2011; Wang et al. 2018a). Luo et al. (2019) report that application of both multi-cutting and nitrogen has no impact on the net nitrogen mineralization rate, whereas numerous studies show that application of both multi-cutting and nitrogen has additional effects on the net nitrogen mineralization rate and microbial nitrogen (Zhong et al. 2018). N application may compensate the negative effect of multi-cutting on soil mineralization (Lepš 2014; Robson et al. 2007). Correspondingly, multi-cutting inhibits the decrease of soil microbial activity and nitrogen leaching caused by nitrogen application through the enhancement of root system (Hoeft et al. 2014), and thus increases soil nitrogen retention (Maron and Jefferies 2001; Zhu et al. 2021). In addition, the combination of multi-cutting and nitrogen application will enhance vegetative growth by increasing the C/N ratio of plants, and increase the uptake of soil N by plants by driving regeneration (Kotas et al. 2017). In sown grassland systems, livestock excrement is often applied to the soil as a nitrogen fertilizer, returning a large amount of nitrogen to the soil (Cech et al. 2008; Jaramillo and Detling 1992) and establishing the nitrogen enrichment area (Tracy and Frank 1998). However, it is not clear whether livestock excrement has the combined effect with multi-cutting as N fertilizer in sown grassland. Therefore, we hypothesized that the combination of multi-cutting and sheep excrement application could significantly increase nitrogen mineralization of soils growing different forage crops by adjusting the soil microorganisms, and improve nitrogen utilization by increasing plant vegetative growth and plant nitrogen content (Fig. 1).

Using common vetch and spring wheat as materials, we studied the effects of different action modes of grazing livestock, i.e., multi-cutting and sheep excrement, on the nitrogen conversion in the plant-soil system from different perspectives, which can provide references for rational grazing and health management of sown grassland.

Materials and methods

Site description

The present experiment was performed at the Linze Grassland Agriculture Station (LGAS) (100° 02'E, 39° 15'N; 1390 m a. s. l.), which is located in the core area of the Heihe Oasis in the arid inland Hexi Corridor, Gansu Province, China. It is adjacent to the Qilian Mountains to the south and the Alxa Desert to the north, belonging to the temperate continental secondary salinized meadow. The topography of the study area is flat, with an average vegetation coverage of 80%. *Plantago asiatica* L., *Agropyron cristatum* Gaertn., *Digitaria sanguinalis* Scop., *Chenopodium glaucum* L., *Portulaca oleracea* L. and *Setaria viridis* Beauv. are the dominant plant species growing in the study area. The main types of agricultural systems are specialized intensive cropping production systems

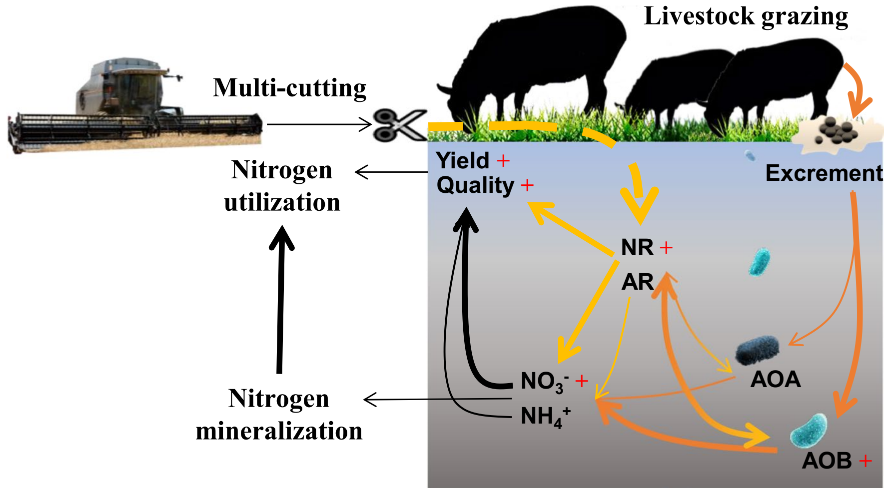

Fig. 1 A concept diagram shows the mechanisms of multi-cutting (or livestock grazing) and sheep excrement for nitrogen conversion in the plant-soil system. NR, nitrification rate; AR, ammonification rate; AOB, soil ammonia-oxidizing bacteria; AOA, ammonia-oxidizing archaea

(SICPs) and extensively integrated crop-livestock production systems (EICLs). The annual average precipitation is 118.4 mm, and the precipitation mostly occurs from May to September (Zhao et al. 2021). During this time, the precipitation accounts for more than 70% of the annual precipitation, and the evaporation is 1830.4 mm. The annual average temperature is approximately 7.7 °C, the annual sunshine duration is 3042 h, and the frost-free period is approximately 170 d. The annual cumulative temperature ≥ 0 °C is 3548 °C, that ≥ 10 °C is 3026 °C. In addition, effective accumulated temperature from May to September ≥ 0 °C is 1823.4 °C, and that from May to September ≥ 10 °C is 1213.4 °C. The soil physical and chemical properties at 30 cm soil depth were measured before experiment. Results suggest that the soil is categorized as Aquisalids according to USDA soil taxonomy (salt 0.7–0.9%), the texture is classified as sandy, soil bulk density is 0.93 g/cm^3, average soil organic carbon is 9.232 ± 0.081 g/kg, soil total nitrogen is 0.886 ± 0.010 g/kg, and the soil total phosphorus is 0.571 ± 0.010 g/kg.

Experimental design

The study was carried out in pots with a diameter of 30 cm and a height of 20 cm. Two forage crops, *Triticum aestivum* L. (spring wheat, cv. Yongliang 15) and *Vicia sativa* L. (common vetch, cv. Lanjian 3), representing gramineous forage crops and leguminous forage crops, respectively, were selected for study. Before the experiment, seeds were immersed in water to promote germination. The germinated seeds were screened twice, and then the germinated seeds were transplanted into pots and planted separately according to different varieties. Each pot of common vetch had five plants and each pot of spring wheat had eight plants. Four treatments, i.e., plants with multi-cutting (MC), plants with sheep excrement application (SE), plants with multi-cutting and sheep excrement application (MC + SE), and plants only (CK, control), were established. There were eight replicates for each treatment, and each pot was arranged in a randomized design. The sheep excrement was a mixture of manure and urine, and the manure application amount was 4 g/pot, which was determined according to the forage yield and digestibility in the field experiment (Liu et al. 2015). The amount of urine applied was 25 mL/pot, which was determined according to the nitrogen content of forage grass, nitrogen deposition number of grazing sheep, and manure nitrogen content (Mikola et al. 2009). The urine pH of sheep was measured at approximately 8.5. The soil used in each basin was local farmland soil, the soil pH was 8.06 ± 0.09, and the soil organic carbon content was 8.06 ± 0.31 g/kg. Nitrogen and phosphorus were the main basal fertilizers applied in the experiment, and the dosages of the two were 22.5 g N /m^2 and 7.5 g P /m^2, respectively. The pots were watered regularly to keep the soil moisture at 50–60% of the field capacity. We cut the forage crops when they grew to approximately 25 cm and left stubble 5 cm for each cutting. Cutting and sheep excrement applications were simultaneously performed on 3rd June and 23rd June for spring wheat and on 10th June and 9th July for common vetch.

Sampling and analysis

The plant height and branch and tiller numbers of forage crops were measured weekly after emergence. The cut plants were placed in envelopes and taken back to the lab. After measuring the fresh weight using a weighing balance, the plants were dried at 105 °C for half an hour, baked to a constant weight at 65 °C and then crushed completely using a grinder. The crude protein content of forage crops was determined by using Auto Discrete Analyzers.

After the forage was harvested, soil was collected and sifted using 80-mesh screens (0.18 mm mesh diameter) to remove plant roots, sand and other impurities. Soil nitrate nitrogen (NO_3^--N) was determined by indophenol-blue colorimetry. Soil ammonium nitrogen (NH_4^+-N) was determined by ultraviolet spectrophotometry. The model of spectrophotometer was CARY60UV-VIS. Soil nitrification and ammonification rates were measured by laboratory culture methods. The contents of NO_3^--N and NH_4^+-N in one fresh soil were measured after being cultured at constant temperature and humidity (25 °C, 60% of the field water capacity of the soil) for one month. The nitrification rate and ammonium rate were calculated according to the change in soil available nitrogen content before and after culture for one month (Risk et al. 2013). In addition, soil microbial nitrogen (MBN) was determined by chloroform fumigation—potassium sulfate extraction—Kjeldahl nitrogen determination method, and soil microbial carbon (MBC) was determined by the chloroform fumigation—potassium sulfate extraction—potassium dichromate oxygen sulfate titration (Vance et al. 1987).

The gene copy numbers of soil ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) related to soil nitrification and denitrification were determined by real-time fluorescence quantitative PCR (qPCR). The specific operation steps were to weigh 500 mg of fresh soil first. Then, the Fast DNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA) was used to extract the total deoxyribonucleotide (DNA) in fresh soil, and the extracted total DNA was diluted to double and stored in a centrifuge tube in the refrigerator at -20 °C for later use. Then, the copy number of functional genes to be measured was analyzed by real-time fluorescence quantitative PCR. The abundance of AOA-*amoA* and AOB-*amoA* was determined by the number of gene copies per gram of dry soil (Banerjee et al. 2018).

Statistical analyses

IBM SPSS 22 was used for data analysis at a significant level set at $P < 0.05$. GraphPad Prism 9 was used for graphing. The effects of multi-cutting and sheep excrement on soil nitrogen mineralization were analysed by one-way ANOVA, with a Duncan's test for multiple comparisons. A logistic model was used to predict the final values of cumulative plant height, tiller or branch numbers, and the specific calculation formula is as follows:

$$y = \frac{t^S \times K}{t^S + {EC_{50}}^S}$$

where y is the cumulative plant height, tiller or branch densities, t is the time, S is the slope of curve, EC_{50} is the median effective concentration, and K is the final value of the upper limit of the cumulative plant height, tiller or branch densities of the logistic regression.

In addition, IBM SPSS AMOS 24 was used to construct the structural equation model (SEM), which investigated the path effects of multi-cutting and sheep excrement on the nitrogen mineralization and utilization of two forage crops. First, we constructed a priori hypothesis that includes all possible cascade paths (It was assumed that the combination of multi-cutting and sheep excrement application affected the soil nitrogen ammonification and nitrification by changing the ammonia-oxidizing archaea, soil ammonia-oxidizing bacteria, soil microbial carbon and soil microbial nitrogen of the two soils, respectively, and thus affected the dry matter and crude protein contents of the two plants). Secondly, the non-significant paths were repeatedly removed. Ultimately, only those variables with a variance inflation factor (VIF) of < 5 were incorporated into the final model.

Results

Effects of sheep excrement and multi-cutting on the growth of two forage crops

Compared with CK, the final value of cumulative plant height of common vetch increased by 75.87% and 70.97% for treatments MC+SE and MC respectively (Fig. 2A), while that of spring wheat increased by 89.73% and 90.57%, respectively (Fig. 2B). Compared with that in MC and SE, the final value of cumulative branch number of common vetches in MC+SE increased by 11.02% and 24.37%; however, MC increased in final value by 12.02%

compared with SE (Fig. 2C). The final value of cumulative tiller number of spring wheat in MC+SE and MC were significantly higher than that in SE and CK (Fig. 2D).

The dry matter of common vetch in MC and MC+SE was respectively 53.35% and 56.42% higher than that in CK, and 45.42% and 48.33% higher than that in SE ($P<0.05$) (Fig. 3A). While the dry matter of spring wheat in MC and MC+SE was respectively 50.01% and 70.24% higher than that in CK, and 35.54% and 53.83% higher than that in SE ($P<0.05$) (Fig. 3B).

The crude protein content of common vetch in MC+SE and MC was respectively increased by 91.43% and 84.98% compared with that in CK ($P<0.001$) (Fig. 3C). In addition, the crude protein content of spring wheat in MC was 139.48% higher than that in SE ($P<0.05$). While that of spring wheat in MC+SE significantly increased by 3.21% and 147.17%, respectively, compared with that in treatments MC and SE ($P<0.05$) (Fig. 3D).

Effects of sheep excrement and multi-cutting on the soil nitrogen mineralization rate

The nitrification rate in SE was significantly higher for spring wheat soil than that in the other three treatments (Fig. 4). There was no significant difference in nitrification rate for common vetch soil in CK and MC+SE. However, those were both significantly higher than that in MC and CK, respectively. There was no significant change in the ammonification rate between the common vetch and spring wheat soils in the four treatments ($P>0.05$) (Fig. 4).

Effects of sheep excrement and multi-cutting on the microbial nitrogen (MBN) and microbial carbon (MBC) in soil

There was no significant difference in MBC content among the four treatments, regardless of the common vetch soil or spring wheat soil ($P>0.05$) (Fig. 5). The application of sheep excrement resulted in significant differences in MBN content in the two types of soil ($P<0.05$), showing that in MC+SE and SE were all significantly higher than that in CK and MC. Furthermore, the MBN contents in common vetch soil and spring wheat soil in MC+SE were respectively 68.13% and 72.09% higher than that in MC. There was no significant difference in MBN content between SE and MC+SE or between CK and MC in both forage crops ($P>0.05$) (Fig. 5).

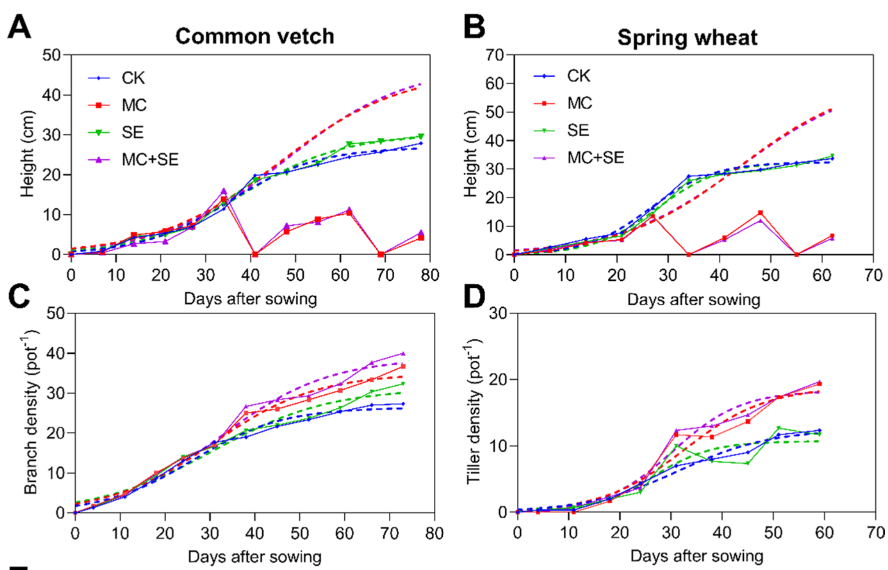

Fig. 2 Cumulative plant height and branch/tiller densities of common vetch (left) and spring wheat (right) affected by multi-cutting and sheep excrement. The parameter K is the final value of the upper limit of the cumulative plant height, branch/tiller densities of the logistic regression

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Parameter	Common vetch		Spring wheat	
K	Height(cm)	Branch density(pot^{-1})	Height(cm)	Tiller density(pot^{-1})
CK	26.90	26.39	32.44	12.50
MC	45.99	34.94	61.82	18.91
SE	30.11	31.19	32.51	10.70
MC+SE	47.31	38.79	61.55	18.34

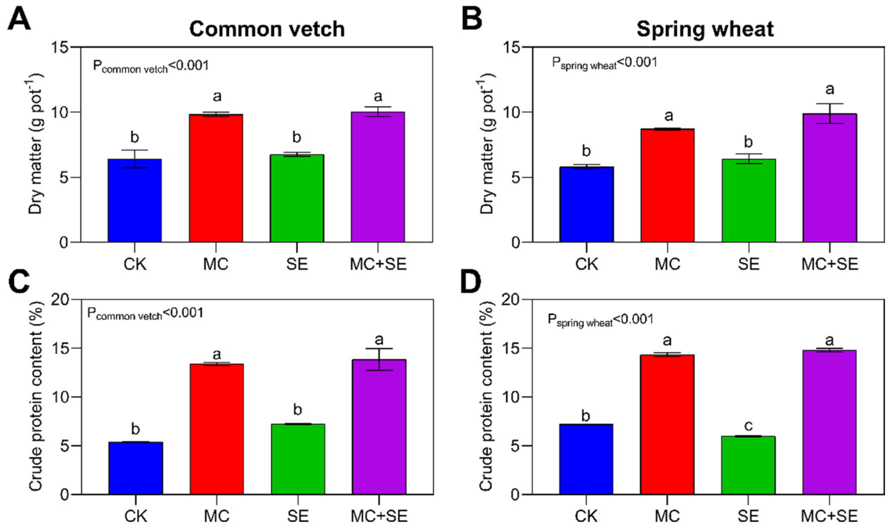

Fig. 3 Dry matter (DM) and crude protein content (CPC) of common vetch (left) and spring wheat (right) affected by multi-cutting and sheep excrement. Columns with the same letters are not significantly different ($P > 0.05$)

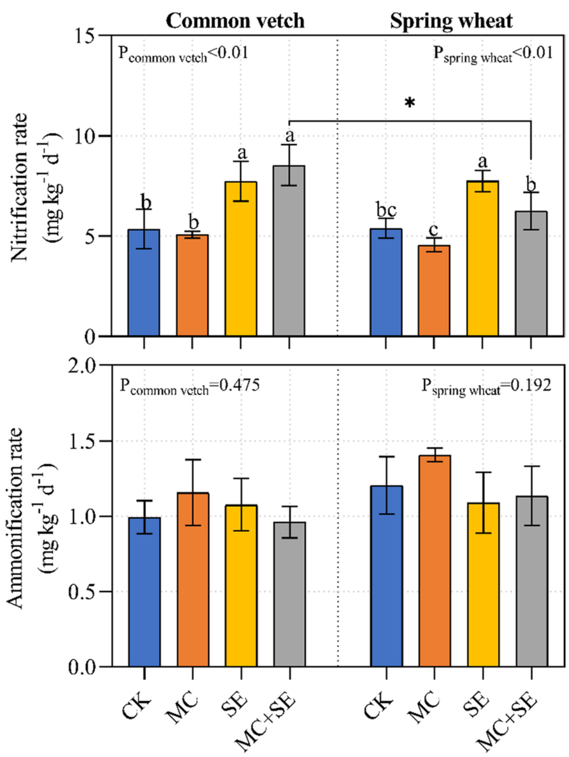

Fig. 4 Effects of multi-cutting and sheep excrement on the nitrification rate (NR) and ammonification rate (AR) of common vetch (left) and spring wheat (right). Columns with the same letters are not significantly different ($P > 0.5$). * Spearman correlation: $|r| > 0.70$, $P < 0.05$

Effects of sheep excrements and multi-cutting on the gene abundance of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) in soil

The abundance of AOB in common vetch and spring wheat soils was 13.47% and 26.54% higher than that of AOA in CK, respectively ($P < 0.05$) (Fig. 6). The abundance of AOB in common vetch and spring wheat soils was respectively 15.53% and 36.45% higher than that of AOA. Therefore, the abundance of AOB in the soil of both forage crops was superior to that of AOA.

For the two forage crops, treatment had no significant effect on the abundance of AOA in the soil ($P > 0.05$). Compared with SE, MC significantly decreased the abundance of AOB in common vetch soil ($P < 0.05$). The abundance of AOB in spring wheat soil in SE and MC+SE was significantly higher than that in CK and MC ($P < 0.05$). The abundance of AOB in spring wheat soil significantly increased by 7.73% in MC+SE compared with that in CK. There was no significant difference in the abundance of AOB between SE and MC+SE or CK and MC in spring wheat soils (Fig. 6).

Structural equation model (SEM) and correlation

There was a significant positive correlation between crude protein content (CPC) and dry matter (DM) of both forage crops ($r \geq 0.8$) and between soil microbial nitrogen (MBN) and the abundance of soil ammonia-oxidizing bacteria (AOB) in spring wheat and between MBN and nitrification rate (NR) in common vetch. ($r \geq 0.7$). However, the NR and ammonification rate (AR) in spring wheat shows a significant negative correlation ($r \leq 0.7$) (Fig. 7).

SEM shows that common vetch and spring wheat had similar determinants (Fig. 8). MC+SE had significant

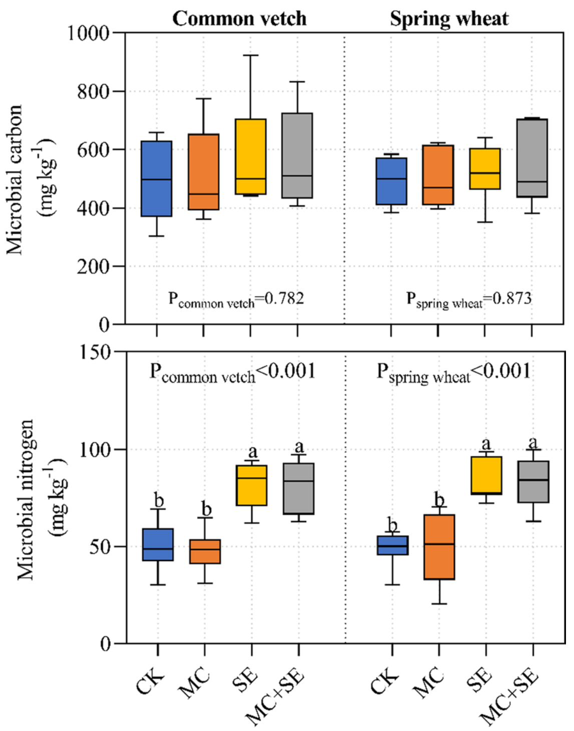

Fig. 5 Effects of multi-cutting and sheep excrement on the soil microbial carbon (MBC) and soil microbial nitrogen (MBN) of common vetch (left) and spring wheat (right). Columns with the same letters are not significantly different ($P > 0.5$)

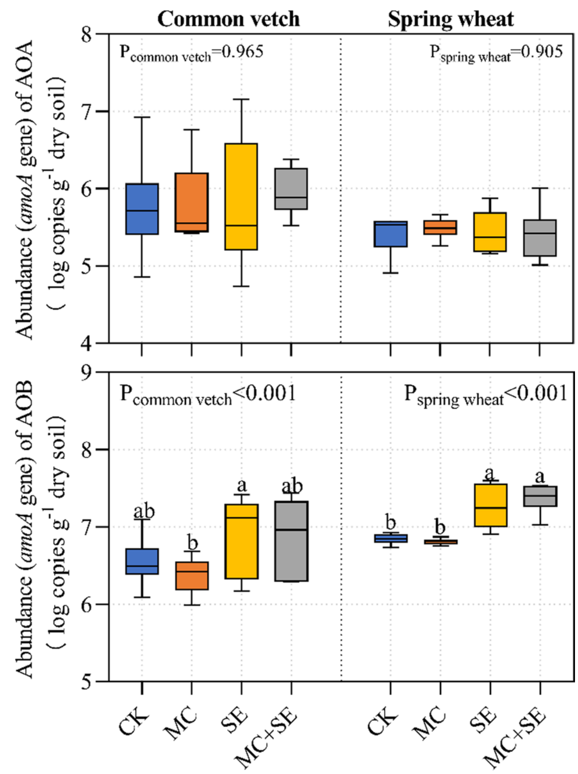

Fig. 6 Effects of multi-cutting and sheep excrement on the abundance of soil ammonia-oxidizing archaea (AOA) and soil ammonia-oxidizing bacteria (AOB) of common vetch (left) and spring wheat (right). Columns with the same letters are not significantly different ($P > 0.5$)

direct effects on the crude protein contents of common vetch and spring wheat. Furthermore, MC + SE had significant effects on the abundance of AOB and the rate of net nitrogen mineralization, which significantly affected the content of NO_3^--N in the two soils, so that more inorganic nitrogen in soil was absorbed and utilized by the common vetch and spring wheat. Ultimately, the crude protein contents of both forage crops increased. Meanwhile, MC + SE were the main factors determining the soil MBN content increase, which led to increases in the CPC of both forage crops.

Discussion

Growth of forage crops

Our study demonstrates that the growth of leguminous (common vetch) and gramineous forage (spring wheat) differed in response to multi-cutting and excrement. Sheep excrement increased the soil nitrification rate of gramineous and legumes forage, but decreased the tiller numbers, plant height and crude protein content of gramineous but not the leguminous forage. This may be because the sheep manure, a type of fertilizer without fermentation, can induce a temperature (> 50 °C) higher than that in the stacking process (Martínez-Avalos et al. 1998). At the same time fibrous bacteria in sheep manure can promote cellulose decomposition (Xie et al. 2018; Zhang et al. 2018). Therefore, the accumulation of sheep excrement near the rhizosphere may result in a significantly lower growth rate of gramineous forages in the combined treatment of multi-cutting and excrement than that in multi-cutting only (Huo et al. 2017). However, the addition of manure and urine, which are exogenous nitrogen fertilizers, did not affect root protein due to the inherent biological nitrogen fixation effect of common vetch.

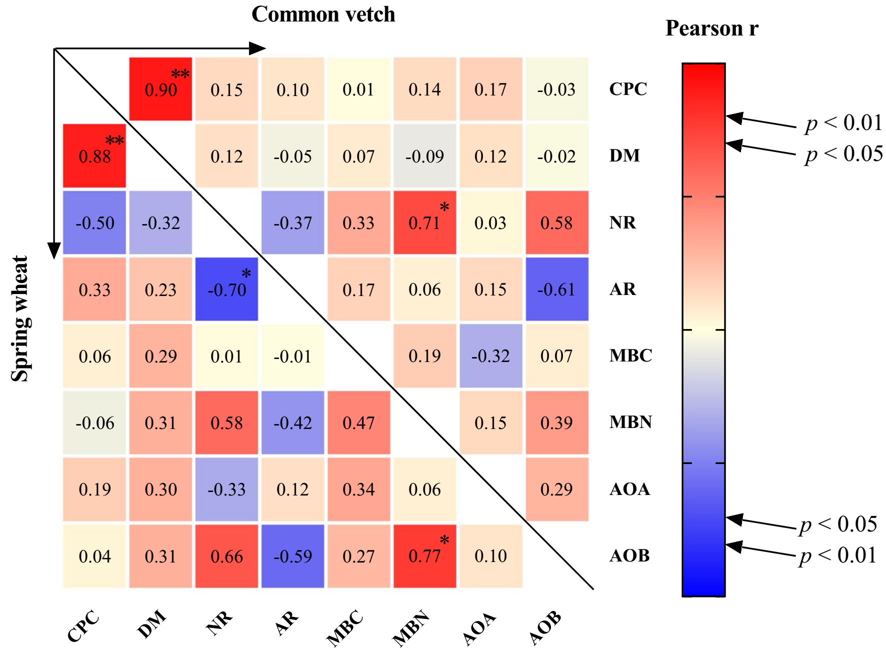

Fig. 7 Matrix of Pearson correlation coefficients between crude protein content (CPC), dry matter (DM), nitrification rate (NR), ammonification rate (AR), soil microbial carbon (MBC), soil microbial nitrogen (MBN), ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) of common vetch (left) and spring wheat (right). *(|r|≥0.7 and $p<0.05$) indicate significant correlation; **(|r|≥0.8 and $p<0.01$) indicate a very significant correlation

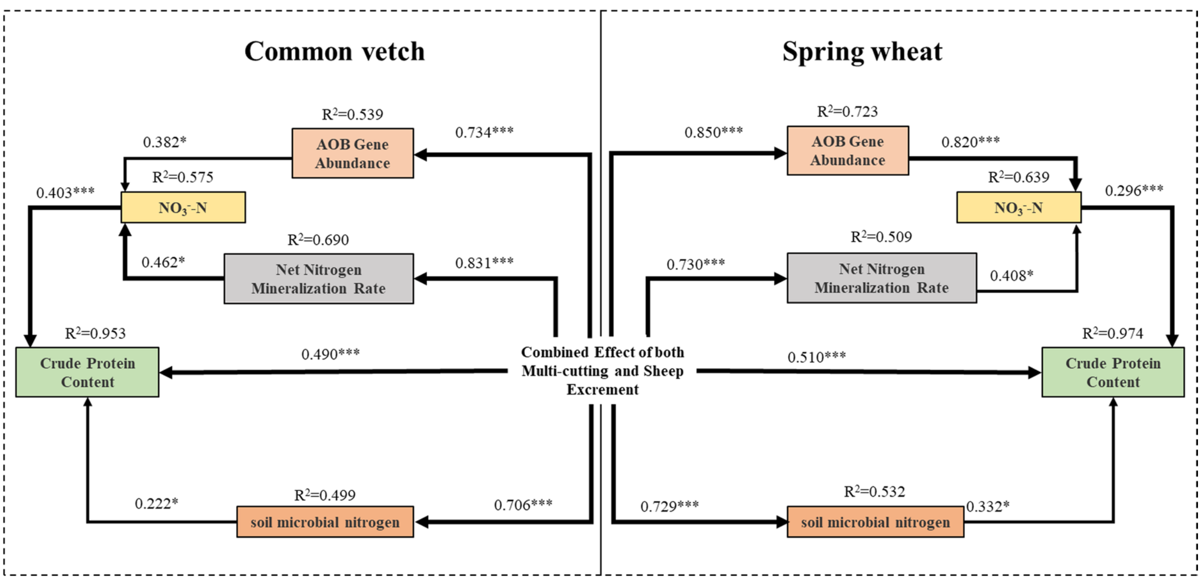

Fig. 8 Pathway maps of the interaction between multi-cutting and sheep excrement of both forage crops. The left panel shows the pathway map of common vetch ($\chi^2=10.744$; degrees of freedom (df) = 7; $p=0.150$), and the right panel shows the pathway map of spring wheat ($\chi^2=9.295$; degrees of freedom (df) = 7; $p=0.201$)

Grazing removing the aboveground biomass of forage crops will reduce interspecific competition (Brede and Duich 1984; Lee et al. 2011; Shao et al. 2012). After which the forage crops regenerate, i.e., reconstruction of stems, leaves, and other tissues and organs (Moinardeau et al. 2019; Zhao et al. 2020). This is a compensatory growth of forage, which accelerates the absorption of nutrient elements (Zhang et al.

2020) and increases the crude protein content of forages (Cui et al. 2020; Martin et al. 2020). The compensatory growth could also enhance the branching and tillering ability of forages and thus effectively promote the reconstruction and enable forages to maintain their basic photosynthetic leaf area, which is an adaptive mechanism of forage to cutting (Howieson and Christians 2008; Lu et al. 2020). However, there was no significant difference in tillering number between gramineous forages in combined treatment of multi-cutting and excrement and multi-cutting only because of the decreased permeability of soil during the decay process of unfermented manure (Hossain et al. 2005; Miller et al. 2002; Pascual et al. 1997). The decreased permeability of soil would also slow down the growth of gramineous forages in a short period and lead to a significant decrease in crude protein content (Kang et al. 2019; Meng et al. 2014; Zaller and Köpke 2004). The different performances of leguminous forage may be due to the presence of rhizobia, which regulate and stabilize the nitrogen use of leguminous forage crops (Jensen et al. 2012).

Soil microorganisms

Nitrification and ammonification are key soil microbial processes that affect the nitrogen utilization of forage crops in the soil-grass system (Wang et al. 2018b). Nitrification and ammonification had no significant effect on soil microbial carbon content, this may be due to the more pronounced response of soil microbial carbon to the underground biomass of forage grass (Liu et al. 2012), which is strongly correlated with soil carbon transfer (Yang et al. 2019). The application of manure and urine increased the soil microbial nitrogen content, because the active substances contained in manure may stimulate rhizosphere microbial activities, provide nutrients for the survival of microbes and accelerate the circulation of nutrient elements in the soil (Zhang et al. 2019). The addition of manure and urine increased the gene abundance of ammonia-oxidizing bacteria in soil, which may be because the urine addition increases the soil pH and thus changes the structure of ammonia-oxidizing bacteria flora, resulting in increased nitrification activity. The addition of excrement could also accelerate the conversion of nitrogen and affect the microbial communities involved in the process. However, we show that the application of manure and urine had no significant effect on the abundance of ammonia-oxidizing archaea. This may be attributed to two reasons: (1) ammonia-oxidizing bacteria gene copies are more dominant than ammonia-oxidizing archaea in the tested soil, and (2) ammonia-oxidizing bacteria plays a major role in the nitrification and denitrification of the tested soil. Therefore, sheep excrement addition influenced the abundance of ammonia-oxidizing bacteria only. Contrary to other reports (e.g., Du et al. 2019; Wang et al. 2018a), results of our study may be relevant to soil texture and plant type tested.

N mineralization

As an important process of the nitrogen cycle, the mineralization of soil can convert organic nitrogen into inorganic nitrogen (i.e., available nitrogen), which can be absorbed and utilized by forage crops (Chen et al. 2019). The manure of livestock mainly includes undigested forage grass, water, urea, metabolites of livestock, and microbes, in which nitrogen mainly exists in manure in the forms of organic nitrogen, NO_3^--N and NH_4^+-N (Eriksen-Hamel and Whalen 2006). In the process of excrement degradation, organic nitrogen is decomposed by microbes and oxidized into NO_3^--N under the action of related microbes and enzymes, then enters the soil and becomes an important part of soil available nitrogen (Zhou et al. 2017). The urine of grazing livestock is alkaline, which could change soil pH in a short period of time and accelerate the decomposition of soil organic matter to a certain extent. Our results show that the soil ammonification rates of the two forage crops did not significantly change in each treatment. This is probably because alkaline soil environments stimulated oxidation and conversion of NH_4^+-N to some extent. The response of nitrification rates to multi-cutting in both forage crops was significantly lower than that to sheep excrement, which may be because sheep excrement directly provides nutrients, while multi-cutting affects interspecific competition (Zhou et al. 2017).

This study provides experimental evidence that the combination of multi-cutting and sheep excrement application could promote soil nitrogen mineralization and plant nitrogen utilization in sown grassland, which could be considered in pasture management to improve the sustainable productivity of grass-soil systems. However, our study also found that the combined effect on plant nitrogen utilization was affected unilaterally by multi-cutting, while soil nitrogen mineralization was strongly controlled by sheep excrement. This may be related to the amount of sheep manure and the frequency of cutting. Future work will determine the effects of different frequencies of cutting and different gradients of sheep manure application, as well as their combined effects on nutrient cycling in the grass-soil system.

Conclusions

Multi-cutting significantly increased the branch number of common vetch and the tiller number of spring wheat, which promoted their compensatory growth and increased the yield and crude protein content of forage crops compared to sheep excrement addition only. Sheep excrement addition significantly increased the content of soil NO_3^+-N by increasing the AOB abundance in the soil of the two forage crops, and thus improved the soil nitrification rate. Application of both multi-cutting and sheep excrement could accelerate soil nitrogen mineralization and plant nitrogen uptake, promoting nitrogen utilization in the soil-grass system of sown grassland.

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Author's contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Lina Shi, Shenghua Chang, Cheng Zhang and Wuchen Du. The first draft of the manuscript was written by Xinzhou Zhao. All authors commented on previous versions of the manuscript and read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

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