Journal of Soils and Sediments (2022) 22:32–42
https://doi.org/10.1007/s11368-021-03051-5

SOILS, SEC 2 • GLOBAL CHANGE, ENVIRON RISK ASSESS, SUSTAINABLE LAND USE • RESEARCH ARTICLE

Short-term grazing rather than mowing stimulates N_2O production potential through enhancing the bacterial pathway in semiarid grasslands

Yingjin Song[1] · Jinlei Li[1] · Lei Zhong[1] · Jianming Xue[2] · Gaoyuan Li[1] · Jinwu Qing[1] · Yichao Rui[3] · Guanyi Chen[4,5] · Taogetao Baying[6] · Frank Yonghong Li[6]

Received: 5 May 2021 / Accepted: 6 August 2021 / Published online: 28 August 2021

Abstract

Purpose The study aimed to explore how grazing and mowing influence the archaeal, bacterial, and fungal nitrous oxide (N_2O) production potential and identify the key soil factors driving N_2O emissions from soil.

Methods Three treatments: fence (no grazing or mowing), grazing, and mowing were set in a field-scale experiment. Total (TNEA and TDEA), fungal (FNEA and FDEA), bacterial (BNEA and BDEA), and archaeal (ANEA and ADEA) nitrification and denitrification enzyme activities were measured to compare the effect of grazing and mowing on the potential of N_2O emission.

Results The TC varied from 16.7 to 19.0 g kg^{-1} and the TN content varied from 1.19 to 1.37 g kg^{-1} during the growing season. The TNEA, TDEA, BNEA, and BDEA were significantly higher in summer (July) ($p < 0.01$), and in grazing than other treatments ($p = 0.01$). Archaeal nitrification enzyme activity (ANEA) was significantly higher in July ($p < 0.01$), but showed no difference among control, grazing, and mowing. Fungal nitrification enzyme activity (FNEA) showed no difference among all treatments and seasons. Fungi made a greater contribution to TNEA and TDEA in all seasons except to TNEA in summer.

Conclusions Short-term grazing increased soil N_2O production potential through stimulating the bacterial nitrification and denitrification in summer which was the peak growth period of grassland. Mowing did not affect the N_2O production potential in all seasons, suggesting that the effect of grazing on the soil nitrogen cycle operated by microorganisms is more disturbance than mowing in this Inner Mongolia grassland.

Keywords Nitrification · Denitrification · Bacteria · Fungi · Archaea

1 Introduction

Nitrous oxide (N_2O), as a strong greenhouse gas highly capable of causing global warming, is produced by microorganisms in soil during the nitrification which converts the ammonium (NH_4) to nitrite (NO_2^-), and then to nitrate (NO_3^-), and by the denitrification that reduces nitrogen oxides such as NO_3^- to nitrogen gases (N_2O and N_2) (Zumft 1997; IPCC 2013). Normally, the nitrification and denitrification enzyme activities were used to indicate the ability of nitrifier and denitrifier (Zhong et al. 2017). The bacteria have been thought to be the dominant microbial functional groups in conventional nitrogen (N) cycle. This is partially ascribed to a series of studies in high fertility or relatively mesic ecosystems such as farmland or pasture with high bacterial abundance (Hayatsu et al. 2008; Klotz and Stein 2008;

Responsible editor: Dulce Flores-Rentería

✉ Lei Zhong
lei.zhong@tju.edu.cn

1 School of Environmental Science and Engineering, Tianjin University, Tianjin 300350, China

2 Riccarton, P.O. Box 29237, Christchurch 8440, New Zealand

3 Rodale Institute, Kutztown, PA 19530, USA

4 School of Mechanical Engineering, Tianjin University of Commerce, Tianjin 300134, China

5 School of Science, Tibet University, Lhasa 850012, China

6 Inner Mongolia Key Laboratory of Grassland Ecology, School of Ecology & Environment, Inner Mongolia University, Hohhot 010021, China

Di et al. 2009). According to recent reports, although the fungal nitrification process is still unclear, fungi are generally dominated in heterotrophic nitrification process in forested soils (Zhu et al. 2015). Fungi also plays an important role during the denitrification process in C- and N-rich soils or in arid lands (Marusenko et al. 2013; Chen et al. 2015; Zhong et al. 2018a, b). Moreover, although the ammoxidation archaea (*AOA*) have been proved very important in nitrification (Hayatsu et al. 2008), the archaeal denitrification can also be a significant source of N_2O production, but it is rarely studied (Offre et al. 2013). Therefore, further studies should be warranted to confirm whether different types of microorganisms are involved in microbial production of N_2O and the associated mechanisms.

Temperate grasslands are mostly used as grazing land for animal production and cover 11% of the earth's terrestrial surface (Sala 2001); it influences N_2O emissions and N_2O emissions estimated to be more than 10% of the global budget in grazed-grasslands (Oenema et al. 2007). Grazing and mowing are the major land-use types in the temperate grassland ecosystem, usually being assumed to have different effects on N_2O production in soil (Zhong et al. 2017). Mowing can significantly decrease N and C inputs belowground through reducing vegetation cover and biomass, leading to nutrient limitation to soil microorganisms (Wan and Luo 2003). Generally, mowing reduces the size of the plant root system and affects microbial N mineralization (Sørensen et al. 2008), nitrification, and denitrification and thus N_2O emissions (Pan et al. 2016). In contrast, grazing often alters the aeration or compaction of soils due to tramping (Oenema et al. 2007; Houlbrooke et al. 2008), changes plant biomass and composition through selective feeding (Leriche et al. 2001), and modifies the soil levels of N and organic matter qualitatively and quantitatively by returning dung and urine (Saggar et al. 2004). All these differences between mowing and grazing induced distinct effects on the N cycle and N_2O emission, e.g., replacement of free-grazing by mowing for 10 years increased the soil N storage by 10.2% in a temperate grassland (He et al. 2012). Recently, to better comprehend how grazing or mowing affects N_2O emission and its production process, the functions of bacterial nitrifiers and denitrifiers have been investigated quite extensively, but the studies of the fungal and archaeal N_2O production are little (Pan et al. 2016; Zhong et al. 2017; Zhang et al. 2018; Yin et al. 2020; Wang et al. 2021).

Previous studies are mainly focused on bacterial nitrifiers and denitrifiers or their activities, taking these to be the key factors on N_2O emission in grasslands. However, while many studies have explored N mineralization, nitrification, and even denitrification as well as bacterial nitrifiers and denitrifiers for better understanding of N_2O emission and ecosystem functioning by grazing or mowing (Pan et al. 2016; Zhong et al. 2017). However, there have been scarce reports as to how mowing and grazing affect the archaeal and fungal nitrification and denitrification and their contribution to N_2O production (Zhong et al. 2017, 2018a, b). Due to the optimum growing environments of bacteria, fungi, and archaea are different, their responses to environmental changes should be distinct. Compared with bacteria, archaea and fungi prefer those habits with higher soil organic C/N ratios, arid, and lower temperatures (Chen et al. 2015; Marusenko et al. 2013; Pietikäinen et al. 2005). Additionally, evidence showed that the abundance and activity of *AOA* is higher than ammonia-oxidizing bacteria (*AOB*) in temperate grassland (Chen et al. 2014; Zhong et al. 2017). Therefore, the lack of research concerning nitrification and denitrification by archaea and fungi will be a challenge for us to accurately predict the effect of grazing and mowing on N_2O production especially in a relatively arid environment such as temperate grassland.

As a typical temperate grassland type, the grassland of Inner Mongolia is approximately 8.67×10^7 ha in area in the Eurasian steppe (Koechlin 1993). Increased intensities and frequencies of grazing and mowing have caused serious grassland degradation and modified the N cycle rate in recent decades (Akiyama and Kawamura 2007; Xu et al. 2008). However, the mechanism behind remains unknown. According to precedent reports for this region, grazing or mowing is influential to the productivity and diversity of plant species (Zhou et al. 2006; Gao et al. 2008), quality of soil (Han et al. 2008), soil microbial community (Zhou et al. 2010), and bacterial nitrifiers and denitrifiers (Zhong et al. 2017), and N_2O flux (Xu et al. 2008; Chen et al. 2019). Nonetheless, there has been no information regarding the archaeal and fungal nitrification and denitrification under grazing and mowing. Through incubation experimentation, the study aims to compare grazing with mowing effect on the possible N_2O production from archaeal, bacterial, and fungal nitrification and denitrification, in order to achieve contribution evaluation of the N_2O production capacity in a representative Inner Mongolian steppe. Based on the previous studies, we hypothesize that (1) soil fungi and archaea would make a greater contribution to N_2O production than soil bacteria because of the arid environment in this temperate grassland (Xu et al. 2008). (2) Grazing and mowing would decrease the N_2O production potential due to the grassland degradation by them (Zhong et al. 2017), and the bacterial, archaeal and fungal potential of N_2O production would respond differently to grazing and mowing. (3) The key driving factors would be markedly different since the archaeal, bacterial, and fungal populations prefer differing soil contexts (Chen et al. 2014; Zhong et al. 2018a, b).

2 Materials and methods

2.1 Study site description

The experimental site, the Grassland Ecosystem Research Station at the Inner Mongolia University (44°10' N, 116°28 E), is seated in Inner Mongolia's Xilin River Basin. It has a semiarid climate, with an annual mean temperature of 0 °C and an annual mean rainfall of 280 mm. Plants grow almost for 150 days yearly, spanning between early May and early September. For this region, winter is cold, dry, and long: extending for more than 6 months a year from October to March. Summer is warm wet and brief: lasting nearly 3 months from June to August, and it is an important season for grass growing due to more than 80% of the precipitation is distributed in this season. April/May is a short spring period and August/September constitutes autumn. Major plants *Leymus chinensis* and *Stipa krylovii* occupy 60–80% of the overground biomass in the grassland overall. According to identification, the experimental site soil is *dark chestnut* (Chernozems according to ISSS Working Group RB 1998).

The experimental grassland was on flat areas, and the enclosure was enclosed established in 2013 to investigate the effects of sheep grazing and autumn mowing (for hay) clipping on grassland ecosystems. The native experimental grassland was used with a very low stocking intensity by nomadic herders historically. None grassland plots of 33.3×33.3 m^2 were used to arrange the three grassland use treatments (grazing, mowing, and control with no grazing nor mowing), replicated three times in an incomplete randomized block design, which has three replicates — three fence plots, three grazing plots, and three mowing plots. Each plot was 33.3×33.3 m^2 in size. The grazing plots were grazed by a group of 6 sheep at a moderate grazing intensity, and the grazing intensity was controlled by setting on the sheep on the 20th of each calendar month from May to September, and the set off sheep treatment was conducted on the 21st until the grass height reached was approximately 6–7 cm from May to September; the stock rate was 6 adult sheep each plot. The mowing plots were mowed once one-cut haying in mid-August for hay every year by a lawn mower (also at a height of 6–7 cm above the ground).

2.2 Soil sampling and vegetation description

Sampling of soil was taken by triplicates in the year 2015: spring (27 May), summer (27 July), and autumn (27 September). For each sampling time, three soil cores (10 cm in diameter) were stochastically extracted from each plot's topsoil (depth 0–10 cm) and bulk mixed together as one sample, with 9 soil samples in total per time. After placing the sampled soils into plastic sealed bags, they were transported into laboratory and sieved to homogenize and remove the fine roots, then cooler-stored. All soil samples used for chemical analyses were air-dried, while those for enzyme analysis were assayed within 2 weeks after being 4 °C preserved. The plant standing biomass and diversity was only observed once on 27 July. After collection and 48 h of 65 °C oven-drying, the biomasses were weighed species-wise based on nine 1-m^2 quadrats per sampling plot (Nihlgard 1972).

2.3 Soil chemical analyses

An automated ion analyzer (Quickchem FIA Star 5010, LACHAT) was utilized for examining the soil levels of NO_3^-–N and NH_4^+–N in KCl extracts (2 mol). Soil moisture determination was performed following 24 h of 105 °C oven-drying. For soil level analysis of total carbon (TC) and total nitrogen (TN), the H_2SO_4–$K_2Cr_2O_7$ oxidation and the Kjeldahl acid digestion were respectively employed (Nelson and Sommers 1996).

2.4 Measurement of total, bacterial, fungal, and archaeal nitrification and denitrification enzyme activities

For measuring the total (TNEA), bacterial (BNEA), fungal (FNEA) and archaeal (ANEA) nitrification enzyme activities and using to indicate the N_2O production potential from total, fungal, and archaeal nitrification, which were measured following the protocol described by Dassonville et al. (2011) with some improvements. Each bottle (250 mL) was placed with fresh soil (equivalent to 10 g of dry soil), and then added with NH_4^+–N (50 μg N–$(NH_4)_2SO_4$ g^{-1} dry soil) solution (10 mL) and distilled water to make the final volume of 80 mL, followed by incubation for 48 h at 28 °C with oscillation (180 rpm) in a shaker. There were four chemical treatments: (1) streptomycin sulphate ($C_{42}H_{84}N_{14}O_{36}S_3$, a bactericide) at 3.0 mg g^{-1} in solution, (2) cycloheximide ($C_{15}H_{23}NO_4$, a fungicide) at 1.5 mg g^{-1} in solution (Castaldi and Smith 1998; Laughlin and Stevens 2002), (3) sterilized for 30 min by exposing soil slurry to a 0.3 MPa (pressure) and 121 °C (temperature) environment (abiotic reaction) (Heil et al. 2015), and (4) a no-inhibitor control. Sampling of the soil slurry (10 mL each) was accomplished at 0, 24, and 48 h and filtered during the incubation. Filtered samples were analyzed for $NO_3^- + NO_2^-$ density on an automated discrete analyzer (Smartchem 200, LACHAT). The nitrification enzymatic activity rates were measured based on the slope of time-dependent linear rate of the $NO_2^- + NO_3^-$ generation. TNEA = the rate of nitrification enzymatic activities from treatment (4). The abiotic reaction in nitrification incubation of soil was from treatment (3).

BNEA = the rate of nitrification enzymatic activities from treatment (4)–treatment (1). FNEA = the rate of nitrification enzymatic activities from treatment (4)–treatment (2). ANEA = the rate of nitrification enzymatic activities from treatment (4)–treatment (1)–treatment (2)–treatment (3).

For measuring the total (TDEA), bacterial (BDEA), fungal (FDEA) and archaeal (ADEA) denitrification enzyme activities and using to indicate the N_2O production potential from total, fungal, and archaeal denitrification, which were measured as per the procedure elaborated in Marusenko et al. (2013) and Patra et al. (2006) with some improvements. Each bottle (250 mL) was placed with fresh soil (equivalent to 10 g of dry soil), and then added with a 6-mL solution filled with KNO_3 (50 μg NO_3-N g^{-1} dry soil), glutamic acid (0.5 mg C g^{-1} dry soil), and glucose (0.5 mg C g^{-1} dry soil). The next step was adding distilled water to the mark, which helped in offering optimum denitrification conditions. There were four chemical treatments: (1) 3.0 mg g^{-1} solution of streptomycin sulphate bactericide ($C_{42}H_{84}N_{14}O_{36}S_3$), (2) 1.5 mg g^{-1} solution of cycloheximide fungicide ($C_{15}H_{23}NO_4$) (Castaldi & Smith 1998; Laughlin & Stevens 2002), (3) sterilized for 30 min by exposing soil slurry to a temperature of 121 °C and a pressure of 0.3 MPa (Heil et al. 2015), and (4) a no-inhibitor control. To suppress the N_2O-to-N_2 reduction and maintain an anaerobic denitrification, the N gas and 10% C_2H_2 (v/v) were used as the air at the bottle headspaces. Then, the soil slurry–containing bottles were subjected to 48 h of 28 °C incubation while shaking at 180 rpm. In the course of incubation, gas samples (10 mL each) were collected using syringes at 0, 24, and 48 h and analyzed the N_2O concentration via gas chromatography. The denitrification enzymatic activity was determined based on the regression line slopes using the 0-, 24-, and 48-h values of N_2O content following incubation. TDEA = the rate of denitrification enzymatic activities from treatment (4). The abiotic reaction in denitrification incubation of soil was from treatment (3). BDEA = the rate of denitrification enzymatic activities from treatment (4)–treatment (1). FDEA = the rate of denitrification enzymatic activities from treatment (4)–treatment (2). ADEA = the rate of denitrification enzymatic activities from treatment (4)–treatment (1)–treatment (2)–treatment (3).

The fungal, bacterial, and archaeal contribution to soil TNEA was predicted by BNEA, FNEA, or ANEA as a percentage of BNEA + FNEA + ANEA. The fungal, bacterial, and archaeal contribution to soil total denitrification enzyme activity was estimated by the ratio of BDEA, FDEA, or ADEA to BDEA + FDEA + ADEA.

2.5 Statistical analysis

The influences of treatments (control, grazing, and mowing), seasons (Spring, Summer, and Autumn), and their interplays on the soil trait measurements were examined for statistical significance via repeated-measures ANOVA using SAA version 9 (SAS Institute, USA). Significant differences among three treatments or three seasons were further examined at a 0.05 level through Duncan's multi-range test. Within every plot, the structure of correlation along seasons was compound symmetrical. All data were tested to ensure that they satisfy the requirement for normality before the statistical analysis.

Aided by IBM® SPSS® Amos™ 20, structural equation modeling (SEM) was used to explore the effect paths among chemical, physical, and biological traits of soil, as well as the N_2O generation potentials. Variables like soil moisture (SM), TN, TC, NH_4^+-N and NO_3^--N; FNEA, BNEA, ANEA and TNEA; and FDEA, BDEA, ADEA, and TDEA were taken into consideration in the SEM model. Given the small sample number for the variable number per modeling ($n = 27$), the estimates were likely conservative and fit (Shipley 2000; Kang and Shipley 2009). We performed SPSS 18-aided principal component analysis (PCA) prior to the SEM procedure to represent NH_4^+, NO_3^-, TC, and TN by "SC", thereby reducing the model variable number to attain a better model fit to data. The degree of fitting between the model-implied covariance structures and the actual data covariance structures was determined through χ^2 test, and the p value > 0.05 from the χ^2 test indicates adequate model fit. The estimated standardized coefficients were examined by analyzing correlation matrices and were considered significant when $p < 0.05$ (Petersen et al. 2012).

3 Results

3.1 Soil analysis

As shown in Fig. 1a, during growing period, the SM content varied between 9.7 and 15.0%, with significantly higher summertime value than in the rest of the seasons ($p < 0.01$). Compared to the control group, the treatment groups (grazing and mowing) exhibited evidently lower SM values ($p = 0.02$). No significant interplay was noted between treatments and seasons on the SM variable.

The TC varied from 16.7 to 19.0 g kg^{-1}, and the TN content varied from 1.19 to 1.37 g kg^{-1} during the growing season, and both showed no significant difference across treatments or seasons (data not shown). The NH_4^+-N concentration revealed that seasons interacted prominently with treatments ($p < 0.01$), with the grazing group exhibiting significantly stronger interaction ($p < 0.01$) than the rest of the treatments during summertime (Fig. 1b). According to the data, the summertime and autumn levels of NO_3^--N were prominently higher than the springtime level ($p < 0.01$) (Fig. 1c). The measured mean standing biomass of plants on 27 July, where the biomass values peaked, was 56.00 g, 5.49 g, and 26.49 g DM m^{-2} in control, grazing, and mowing treatments, respectively.

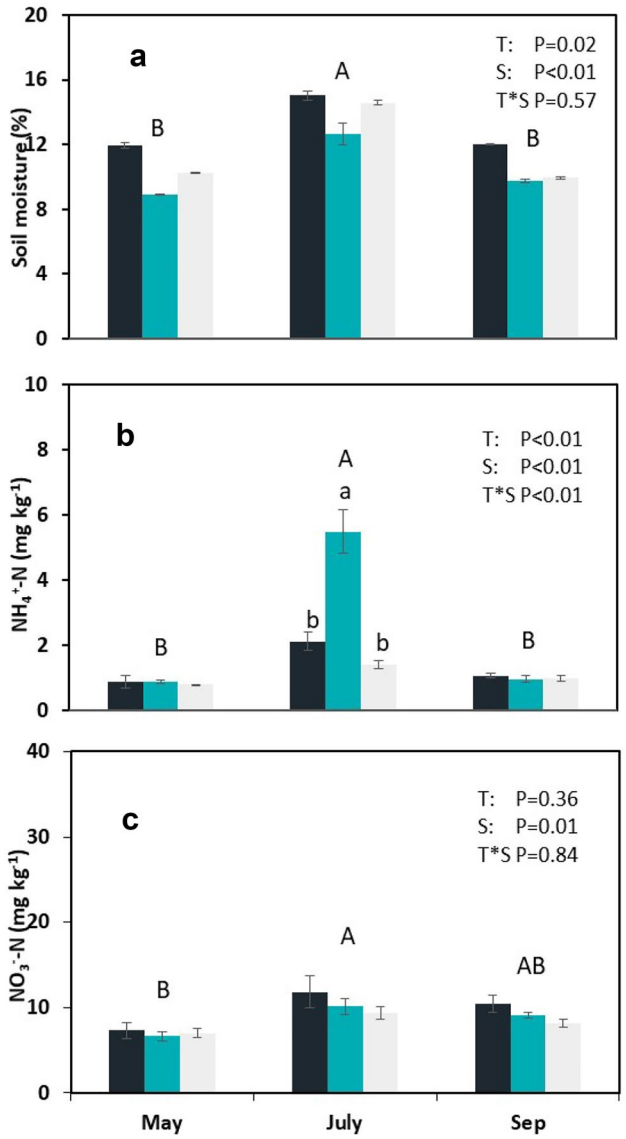

Fig. 1 Effects of various treatments on the SM (Soil moisture) content (**a**), NH_4^+-N (**b**), and NO_3^--N (**c**) levels over different seasons (May (spring), July (summer) and Sep (autumn)). Black square means control, teal square means grazing, white square means mowing; T means different treatments, S means different seasons. Data are expressed as means ± 1 s.e.m ($n = 3$), whereas different capital and lowercase letters indicate significant treatment and season differences, respectively ($p < 0.05$)

3.2 Nitrification and denitrification enzyme activities of bacteria (BNEA, BDEA), fungi (FNEA, FDEA), and archaea (ANEA, ADEA)

The TNEA was considerably higher in summer (July) than in other seasons ($p < 0.01$) and in the grazing treatment than in other treatments in summer ($p < 0.01$) (Fig. 2a). No meaningful differences in FNEA were found among the treatments and seasons (Fig. 2b). Although the ANEA at the summer time was markedly higher than the rest of the seasons ($p < 0.01$), the ANEA values had no difference among the treatments. Markedly higher BNEA was found in the control group during spring period ($p < 0.01$) and in the grazing group during summer period ($p < 0.01$). Significant interactions of treatment by season ($p < 0.01$) were found for TNEA and BNEA (Fig. 2c, d).

The TDEA, BDEA, FDEA, and ADEA all were evidently higher during summertime as compared to the rest of the season (Fig. 3).

3.3 The contribution of bacteria, fungi, and archaea to the total nitrification and denitrification enzyme activities

BNEA, FNEA, and ANEA contributed varyingly to TNEA, ranging from 15 ± 4 to 44 ± 1%, from 25 ± 3 to 58 ± 1%, and from 20 ± 1 to 33 ± 1%, respectively. FNEA contributed more noticeably to TNEA during the spring and autumn periods ($p < 0.01$). Significant interactions of seasons with treatments were noted for this variable ($p < 0.01$), with the grazing group exhibiting evidently stronger interaction during autumn period ($p < 0.01$). The contribution of BNEA and ANEA to TNEA showed no difference among the treatments in spring and autumn periods (Fig. 4a).

BDEA, FDEA, and ADEA also contributed varyingly to TDEA, ranging from 25 ± 1 to 38 ± 2%, from 47 ± 2 to 61 ± 2%, and from 10 ± 2 to 17 ± 1%, respectively. The contribution of FDEA and ADEA to TDEA was significantly lower in spring ($p < 0.01$) and autumn ($p < 0.01$), respectively (Fig. 4b).

3.4 Factors controlling the total nitrification and denitrification potential in the soils

The soil factors (SC) were the data of TN, TC, NH_4^+-N, and NO_3^--N by principal component analysis, and its result is shown in Fig. S1. The first principal components were capable of interpreting the soil factor variations and explained 58.2% of the total variance. After the TN, TC, NH_4^+-N, and NO_3^--N were replaced by SC, the SEM results demonstrated fitting of the conceptual models for both TNEA ($\chi^2 = 4.375$, $d.f. = 6$, RMSEA = 0.0421, CFI = 0.95, GFI = 0.96, NFI = 0.91 $p = 0.598$) and TDEA ($\chi^2 = 6.283$, $d.f. = 7$, RMSEA = 0.0309, CFI = 0.92, GFI = 0.97, NFI = 0.90, $p = 0.198$) to the observation data in the conventional system, as shown in Fig. 5.

To TNEA, the foremost factor was FNEA, followed by factors like ANEA and BENA (Fig. 5a). For FNEA, BNEA, and ANEA, SM was counted as the foremost factor to FNEA, as well as to ANEA; however, SC was the most significant for BNEA. Analogous to TNEA, for TDEA, the FDEA was the foremost factor as well, which was followed

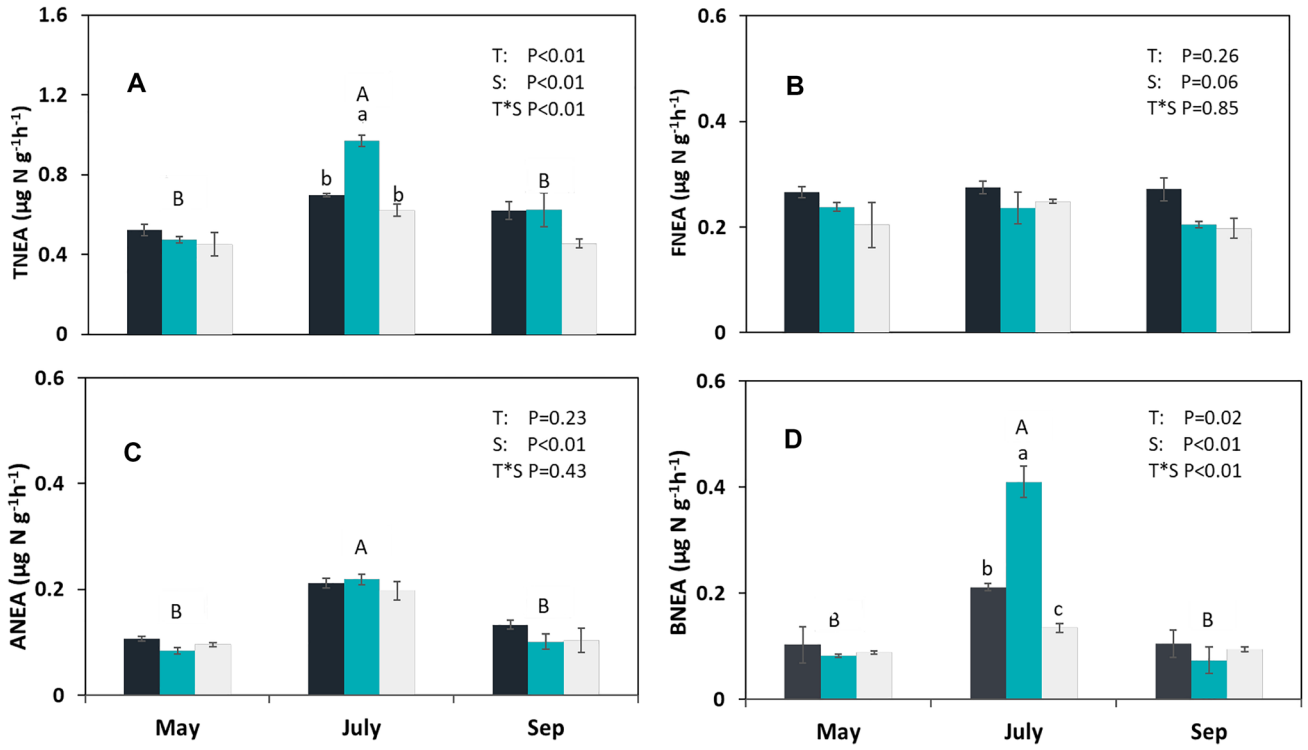

Fig. 2 Effect of treatments on soil total nitrification enzyme activity (TNEA) (**a**), fungal nitrification enzyme activity (FNEA) (**b**), archaeal nitrification enzyme activity (ANEA) (**c**), and bacterial nitrification enzyme activity (BNEA) (**d**) in different seasons (May (spring), July (summer) and Sep (autumn)). Black square means control, teal square means grazing, white square means mowing; T means different treatments, S means the different seasons. Data are expressed as means ± 1 s.e.m ($n = 3$), whereas different capital and lowercase letters indicate significant treatment and season differences, respectively ($p < 0.05$)

by BNEA and AENA, as shown in Fig. 5b. For FDEA, BDEA, and ADEA, SM was also counted as the foremost to FDEA, as well as to ADEA; however, SC was the most important for BDEA.

4 Discussion

N_2O emission is attributed primarily to the nitrification and denitrification of microbes, but the role of fungi especially the archaea in N_2O production processes remains underexplored. Our results show that fungi play a major role in nitrification and denitrification processes in the studied semi-arid steppe grassland; the contribution of FNEA to TNEA varies from 25 ± 1 to 58 ± 1%, and the contribution of FDEA to TDEA varies from 47 ± 2 to 62 ± 2%, across the seasons (except for the nitrification process in July) (Fig. 4). This finding is in accordance with the suggestions that fungi play an important role in N cycle process (McLain and Martens 2005, 2006; Collins et al. 2008). In contrast to previous studies that only focused on the merits of bacteria and fungi on the generation of N_2O, it revealed that the fungi contribution to the nitrification and denitrification potential varied from 25 to 59% (Fig. 4) which was lower than the previously reported values, which were commonly greater than 50% contribution in temperate grasslands (Zhong et al. 2018b), meadow grassland (Zhong et al. 2018a), and farms (Herold et al. 2012). The fungi overestimated contribution in previous studies could be due to the limitation of their methodology that did not exclude the contribution of archaea in nitrification and denitrification. On the other hand, the contribution of BNEA to TNEA was 44 ± 3% in July, which showed a different trend compared with other treatments and seasons in our site; it might be due to the urine and dung return (Zhong et al. 2014). The urine and dung return significantly increased the soil NH_4^+-N content then increased the bacterial nitrification enzyme activity because the bacteria favor the inorganic nutrients compared with the fungi and archaea in the summer (Chen et al. 2015).

Our study also revealed the important role of archaea in the soil nitrification and denitrification, where the archaea contributed more prominently to these processes as compared to the bacteria (Fig. 4). This result was in line with the previous observation that the *AOA* is more important than *AOB* for nitrification in temperate grassland (Di et al. 2009). Fungi

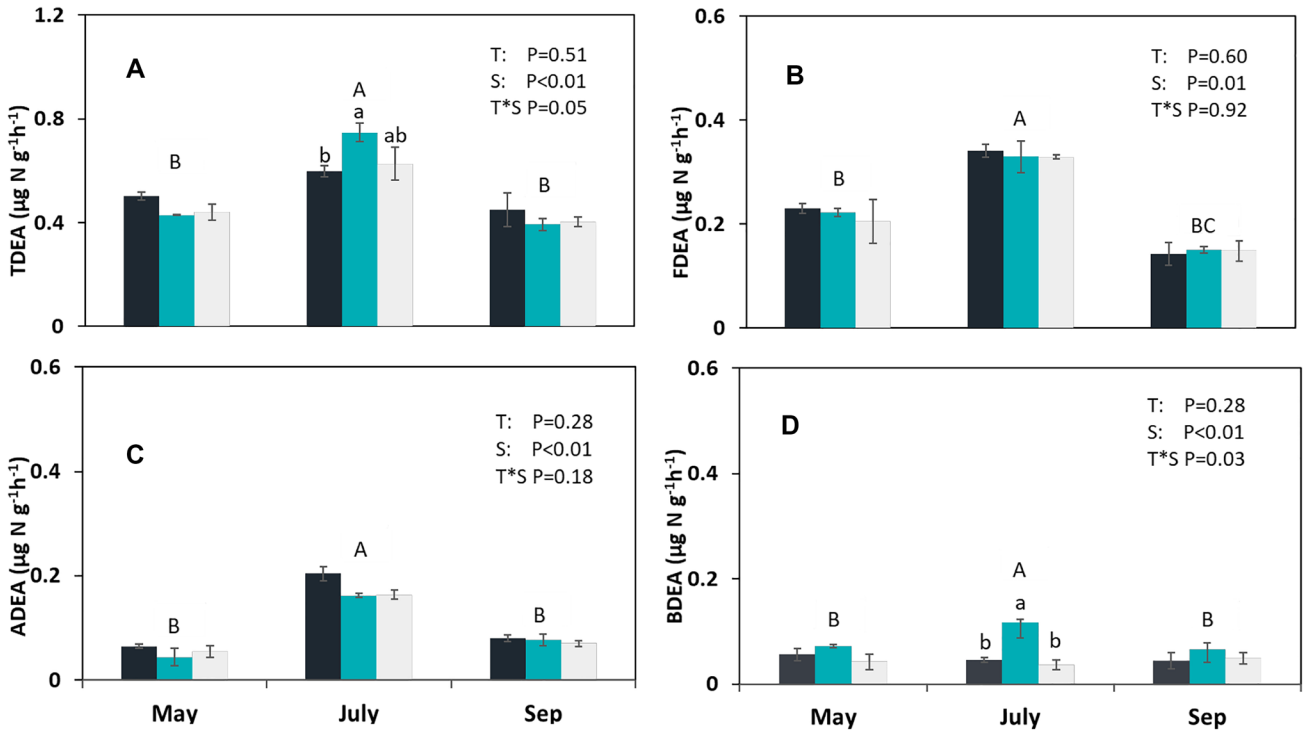

Fig. 3 Effect of treatments on soil total denitrification enzyme activity (TDEA) (**a**), fungal denitrification enzyme activity (FDEA) (**b**), archaeal denitrification enzyme activity (ADEA) (**c**) and bacterial denitrification enzyme activity (BDEA) (**d**) in different seasons (May (spring), July (summer) and Sep (autumn)). Black square means control, teal square means grazing, white square means mowing; T means different treatments, S means the different seasons. Data are expressed as means ± 1 s.e.m ($n = 3$), whereas different capital and lowercase letters indicate significant treatment and season differences, respectively ($p < 0.05$)

and archaea prefer the arid, low substrate conditions (Prosser and Nicol 2008; Jia and Conrad 2009). The studied grassland has a low soil moisture of only 8–15%, and low contents of soil nutrients, much lower than those managed and mesic ecosystems such as agricultural or forest ecosystems (Huang et al. 2017; Zhou et al. 2017). The studied semi-arid steppe grassland provides a favorable environment for fungi and archaea (Bai et al. 2010); thus, fungi or archaea play a more important role than bacteria in the potential N_2O production.

According to our findings, the second assumption was partly supported by the results. Our study finds that 3 years of grazing and mowing did not reduce the potential N_2O production in all seasons (Figs. 2 and 3); moreover, grazing significantly increased the TNEA and TDEA in July. It showed that short time grazing and mowing would increase the N cycle rates in the early grazing (Risser and Parton 1982). Our results supported the second assumption that the bacterial, archaeal, and fungal potential of N_2O production would respond differently to grazing. Although the fungal and archaeal processes of N_2O production played a major role but was not changed by grazing or mowing in the Inner Mongolia grassland, grazing significantly increased bacterial nitrification and denitrification enzyme activities in July, and it might be the major reason for the increase of the TNEA and TDEA (Figs. 3 and 4). Previous studies mainly explained the N_2O production process based on the soil moisture variations, as well as the changes of C and N concentrations. Grazing would stimulate N cycling rate by decoupling N and C return through excreta (Phetteplace et al. 2001) or the negative effect through reducing soil moisture in this semiarid environment (Wang et al. 2006; Xu et al. 2008; Chamindu Deepagoda et al. 2019). Our result is not in conflict with those studies and showed that the soil moisture was significantly decreased (Fig. 1), but the TC and TN contents were quite unaffected by grazing treatment (data unlisted). Coinciding with the increased soil NH_4^+-N concentration by grazing in July (Fig. 1), the greater TNEA and TDEA in summer indicated that the positive effect of available N return through excreta was greater than the negative effect of reduced soil moisture by grazing in this season. Our study further revealed that through regulating microbial pathway of N_2O production, short-term grazing in summer stimulated the potential of N_2O production in this temperate grassland mainly due to the induced bacterial nitrification and denitrification rates. The structural equation modeling regarding the correlation of soil environment variables with the N_2O production potential demonstrated that the soil condition (nutrition) was a more important factor than soil

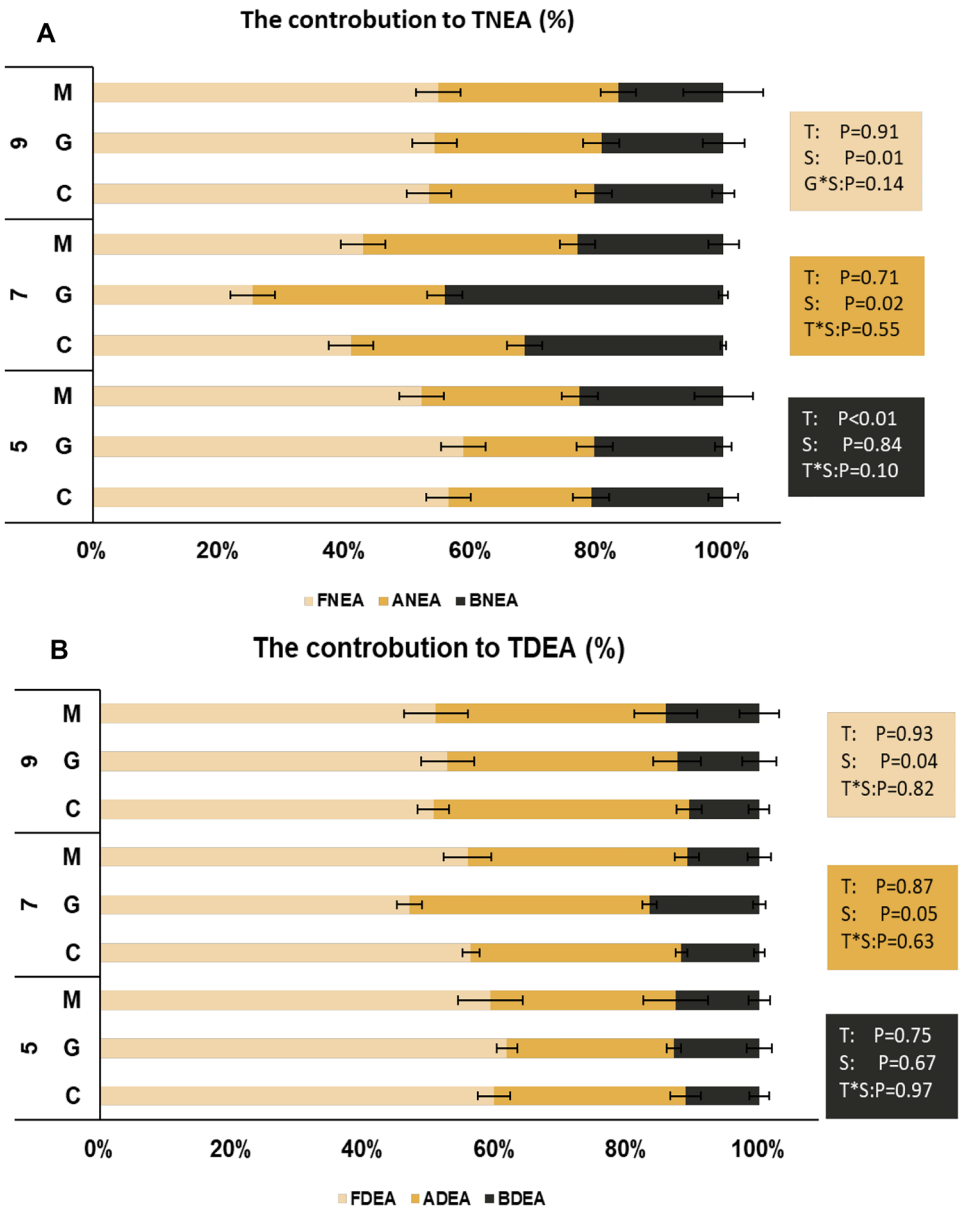

Fig. 4 Fungal, bacterial and archaeal contributions to the total nitrification enzyme activity (TNEA) (**a**) and total denitrification enzyme activity (TDEA) (**b**). C means control; G means grazing; M means mowing. Values are means ± 1 s.e.m ($n = 3$), whereas different capital and lowercase letters indicate significant treatment and season differences, respectively ($p < 0.05$)

to control the BNEA and BDEA (Fig. 5). Grazing did not change the bacterial NEA and DEA in spring (May) and autumn (September) (Figs. 3 and 4) due to the limited available N (NH_4^+-N and NO_3^--N) (Fig. 1). This indicates that although there were manure returns, the low-temperature environment limited the decomposition of animal manure, as a result of the low release of available nutrients (Zhong et al. 2018b). In this study, no significant changes of the archaeal and fungal NEA and DEA by grazing or mowing with the seasons imply that the fungi and archaea were resistant to environmental changes (Marusenko et al. 2013; Offre et al. 2013), or the changes by treatments were insufficient to cause any difference in archaeal or fungal NEA or DEA that was noticeable.

In this study, 3 years of mowing did not affect the N_2O production in all seasons (Figs. 3 and 4). It has been reported that mowing can affect the soil N cycle by regulating plant species diversity, root biomass and exudates (Bardgett et al. 1998), and nutrient deposits belowground, then resulting in the change of substrates to soil N cycle (Wan and Luo 2003). In our study site, however, soil TN, NH_4^+-N, and NO_3^--N were all not changed by mowing across the seasons (Fig. 1). Although the aboveground plant biomass was significantly reduced by mowing after mid-August, the plant diversity was not changed ($p = 0.72$, data not shown). Therefore, the root biomass and exudates and nutrient deposition belowground might not be changed by mowing too. In contrast, grazing could quickly change the soil N cycle process by regulating the

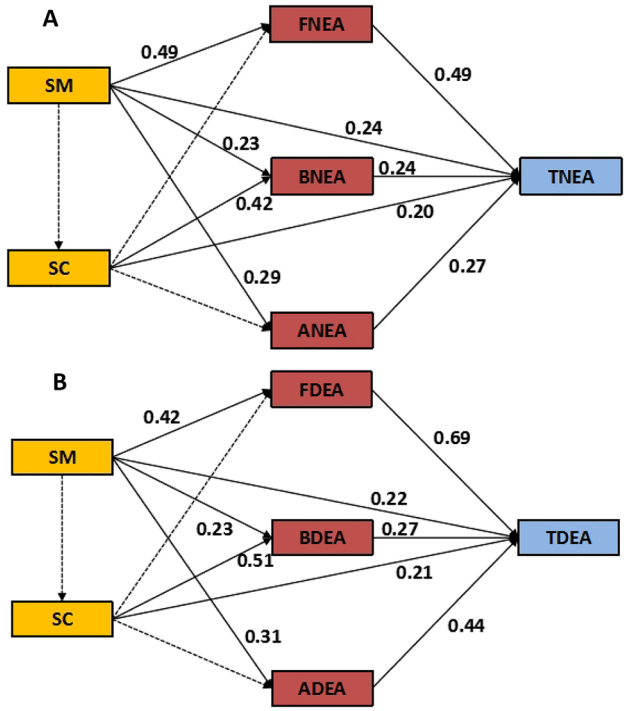

Fig. 5 Path plot of the final model, which depicts the pattern observations in both total nitrification enzyme activity (TNEA) (**a**) and total denitrification enzyme activity (TDEA) (**b**). The coefficients related with arrows represent the coefficients for multiple linear regressions. In the model, SM denotes soil moisture, and SC denotes the soil factors used to replace TN, TC, NH_4^+-N, and NO_3^--N through PCA. Dashed arrows indicate the insignificant directions and effects ($p > 0.05$), while solid arrows indicate the significant directions and effects ($p < 0.05$). The number of the pathway is the coefficient

plant diversity and urine and dung return to soil (Saggar et al. 2004; Keil et al. 2011). Mowing reduced the soil in all seasons (Fig. 1); however, the reduction of soil moisture content by mowing was much smaller than grazing (Fig. 1a) and insufficient to result in TNEA or TDEA shifts. As a consequence, the impact of mowing on the soil nitrogen cycle process was less than that of grazing in a semiarid grassland.

5 Conclusions

We conclude that fungi play a dominant role in the N_2O production process in the semiarid Inner Mongolian grassland except for nitrification process, followed by archaea and bacteria, and that grazing alters the microbial pathway of N cycle process, increasing the role of bacteria and enhancing the soil N_2O production potential in the growth season. It suggested that precise forecast for N_2O emission with land use will benefit from the distinguishing bacterial, fungal, and archaeal N cycle process in grasslands. Mowing does not affect the N_2O production processes across all the seasons, indicating a less effect of mowing than grazing on the soil N cycle processes. We propose that mowing can be better to balance the negative effects of continuous grazing on soil N cycling and availability.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11368-021-03051-5.

Funding This work was supported by the Tianjin Science and Technology Committee (Grant No. 19JCQNJC13900), the National Key Research and Development Program of China (2020yfd1100300), and the Department of Science and Technology of Inner Mongolia Autonomous Region (Grant for the Research on Grassland Functional Processes and Ecosystem Services).

Declarations

Conflict of interest The authors declare no competing interests.

References

Akiyama T, Kawamura K (2007) Grassland degradation in China: methods of monitoring, management and restoration. Grassl Sci 53:1–17. https://doi.org/10.1111/j.1744-697x.2007.00073.x

Bai YF, Wu JG, Clark CM, Naeem S, Pan QM, Huang JH, Zhang LX, Han XG (2010) Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands. Glob Chang Biol 16:358–372. https://doi.org/10.1111/j.1365-2486.2009.01950.x

Bardgett RD, Wardle DA, Yeates GW (1998) Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biol Biochem 30:1867–1878. https://doi.org/10.1016/S0038-0717(98)00069-8

Castaldi S, Smith KA (1998) Effect of cycloheximide on N_2O and NO_3^- production in a forest and an agricultural soil. Biol Fertil Soils 27:27–34. https://doi.org/10.1007/s003740050395

Chamindu Deepagoda T, Jayarathne J et al (2019) Soil-gas diffusivity and soil-moisture effects on N_2O emissions from intact pasture soils. Soil Sci Soc Am J 83:1032–1043. https://doi.org/10.2136/sssaj2018.10.0405

Chen H, Mothapo NV, Shi W (2015) Fungal and bacterial N_2O production regulated by soil amendments of simple and complex substrates. Soil Biol Biochem 84:116–126. https://doi.org/10.1016/j.soilbio.2015.02.018

Chen W, Zheng XH, Wolf B et al (2019) Long-term grazing effects on soil-atmosphere exchanges of CO_2, CH_4, and N_2O at different grasslands in Inner Mongolia: a soil core study. Ecol Indic 105:316–328. https://doi.org/10.1016/j.ecolind.2017.09.035

Chen YL, Hu HW, Han HY et al (2014) Abundance and community structure of ammonia-oxidizing archaea and bacteria in response to fertilization and mowing in a temperate steppe in Inner Mongolia. FEMS Microbiol Ecol 89:67–79. https://doi.org/10.1111/1574-6941.12336

Collins SL, Sinsabaugh RL, Crenshaw C et al (2008) Pulse dynamics and microbial processes in aridland ecosystems. J Ecol 96:413–420. https://doi.org/10.1111/j.1365-2745.2008.01362.x

Dassonville N, Guillaumaud N, Piola F et al (2011) Niche construction by the invasive Asian knotweeds (species complex Fallopia): impact on activity, abundance and community structure of denitrifiers and nitrifiers. Biol Invasions 13:1115–1133. https://doi.org/10.1007/s10530-011-9954-5

Di HJ, Cameron KC, Shen JP et al (2009) Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. Nat Geosci 2:621–624. https://doi.org/10.1038/ngeo613

Gao YZ, Giese M, Lin S et al (2008) Belowground net primary productivity and biomass allocation of a grassland in Inner Mongolia is affected by grazing intensity. Plant Soil 307:41–50. https://doi.org/10.1007/s11104-008-9579-3

Han G, Hao X, Zhao M et al (2008) Effect of grazing intensity on carbon and nitrogen in soil and vegetation in a meadow steppe in Inner Mongolia. Agric Ecosyst Environ 125:21–32. https://doi.org/10.1016/j.agee.2007.11.009

Hayatsu M, Tago K, Saito M (2008) Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. Soil Sci Plant Nutr 54:33–45. https://doi.org/10.1111/j.1747-0765.2007.00195.x

He N, Zhang Y, Dai J et al (2012) Land-use impact on soil carbon and nitrogen sequestration in typical steppe ecosystems, Inner Mongolia. J Geogr Sci 22:859–873. https://doi.org/10.1007/s11442-012-0968-4

Heil J, Liu S, Vereecken H et al (2015) Abiotic nitrous oxide production from hydroxylamine in soils and their dependence on soil properties. Soil Biol Biochem 84:107–115. https://doi.org/10.1016/j.soilbio.2015.02.022

Herold MB, Baggs EM, Daniell TJ (2012) Fungal and bacterial denitrification are differently affected by long-term pH amendment and cultivation of arable soil. Soil Biol Biochem 54:25–35. https://doi.org/10.1016/j.soilbio.2012.04.031

Houlbrooke DJ, Littlejohn RP, Morton JD et al (2008) Effect of irrigation and grazing animals on soil quality measurements in the North Otago Rolling Downlands of New Zealand. Soil Use Manag 24:416–423. https://doi.org/10.1111/j.1475-2743.2008.00183.x

Huang Y, Xiao X, Long X (2017) Fungal denitrification contributes significantly to N_2O production in a highly acidic tea soil. J Soils Sediments 17:1599–1606. https://doi.org/10.1007/s11368-017-1655-y

IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge (United Kingdom) and New York

ISSS Working Group RB (1998) World reference base for soil resources. In: Schulte A, Ruhiyat D (eds) Soils of tropical forest ecosystems. Springer, Berlin, Heidelberg

Jia Z, Conrad R (2009) Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. Environ Microbiol 11:1658–1671. https://doi.org/10.1111/j.1462-2920.2009.01891.x

Kang CS, Shipley B (2009) A correction note on "a new inferential test for path models based on directed acyclic graphs." Struct Equ Model 16:537–538. https://doi.org/10.1080/10705510903008279

Keil D, Meyer A, Berner D et al (2011) Influence of land-use intensityon the spatial distribution of N-cyclingmicroorganisms in grassland soils. FEMS Microbiol Ecol 77:95–106. https://doi.org/10.1111/j.1574-6941.2011.01091.x

Klotz MG, Stein LY (2008) Nitrifier genomics and evolution of the nitrogen cycle. FEMS Microbiol Lett 278:146–156. https://doi.org/10.1111/j.1574-6968.2007.00970.x

Koechlin J (1993) Natural grasslands: eastern hemisphere and resume. Coupl RT 496:291–301

Laughlin RJ, Stevens RJ (2002) Evidence for fungal dominance of denitrification and codenitrification in a grassland soil. Soil Sci Soc Am J 66:1540–1548. https://doi.org/10.2136/sssaj2002.1540

Leriche H, LeRoux X, Gignoux J et al (2001) Which functional processes control the short-term effect of grazing on net primary production in grasslands? Oecologia 129:114–124. https://doi.org/10.1007/s004420100697

Marusenko Y, Huber DP, Hall SJ (2013) Fungi mediate nitrous oxide production but not ammonia oxidation in aridland soils of the southwestern US. Soil Biol Biochem 63:24–36. https://doi.org/10.1016/j.soilbio.2013.03.018

McLain JET, Martens DA (2005) Nitrous oxide flux from soil amino acid mineralization. Soil Biol Biochem 37:289–299. https://doi.org/10.1016/j.soilbio.2004.03.013

McLain JET, Martens DA (2006) N_2O production by heterotrophic N transformations in a semiarid soil. Appl Soil Ecol 32:253–263. https://doi.org/10.1016/j.apsoil.2005.06.005

Nelson DW, Sommers LE (1996) Total carbon, organic carbon, and organic matter. In: Sparks D, Page A, Helmke P et al (eds) Methods of Soil Analysis Part 3: Chemical Methods. Soil Science Society of America, Inc., Madison, WI, USA, pp 961–1010. https://doi.org/10.2136/sssabookser5.3.c34

Nihlgard B (1972) Plant biomass, primary production and distribution of chemical elements in a beech and a planted spruce forest in south Sweden. Oikos 23:69–81. https://doi.org/10.2307/3543928

Oenema O, Oudendag D, Velthof GL (2007) Nutrient losses from manure management in the European Union. Livest Sci 112:261–272. https://doi.org/10.1016/j.livsci.2007.09.007

Offre P, Spang A, Schleper C (2013) Archaea in biogeochemical cycles. Annu Rev Microbiol 67:437–457. https://doi.org/10.1146/annurev-micro-092412-155614

Pan H, Li Y, Guan X et al (2016) Management practices have a major impact on nitrifier and denitrifier communities in a semiarid grassland ecosystem. J Soils Sediments 16:896–908. https://doi.org/10.1007/s11368-015-1321-1

Patra AK, Abbadie L, Clays-Josserand A et al (2006) Effects of management regime and plant species on the enzyme activity and genetic structure of N-fixing, denitrifying and nitrifying bacterial communities in grassland soils. Environ Microbiol 8:1005–1016. https://doi.org/10.1111/j.1462-2920.2006.00992.x

Petersen DG, Blazewicz SJ, Firestone M et al (2012) Abundance of microbial genes associated with nitrogen cycling as indices of biogeochemical process rates across a vegetation gradient in Alaska. Environ Microbiol 14:993–1008. https://doi.org/10.1111/j.1462-2920.2011.02679.x

Phetteplace HW, Johnson DE, Seidl AF (2001) Greenhouse gas emissions from simulated beef and dairy livestock systems in the United States. Nutr Cycl Agroecosystems 60:99–102. https://doi.org/10.1023/A:1012657230589

Pietikäinen J, Pettersson M, Bååth E (2005) Comparison of temperature effects on soil respiration and bacterial and fungal growth rates. FEMS Microbiol Ecol 52:49–58. https://doi.org/10.1016/j.femsec.2004.10.002

Prosser JI, Nicol GW (2008) Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. Environ Microbiol 10:2931–2941. https://doi.org/10.1111/j.1462-2920.2008.01775.x

Risser PG, Parton WJ (1982) Ecosystem analysis of the tallgrass prairie: nitrogen cycle (Oklahoma). Ecology 63:1342–1351. https://doi.org/10.2307/1938862

Sala OE (2001) Temperate Grasslands. In: Chapin FS, Sala OE, Huber-Sannwald E (eds) Global Biodiversity in a Changing Environment. Ecological Studies (Analysis and Synthesis). Springer, New York, pp 121–137

Saggar S, Bolan NS, Bhandral R et al (2004) A review of emissions of methane, ammonia, and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. New Zeal J Agric Res 47:513–544. https://doi.org/10.1080/00288233.2004.9513618

Shipley B (2000) A correction note on a new inferential test for path models based on directed acyclic graphs. Struct Equ Model 16:537–538. https://doi.org/10.1080/10705510903008279

Sørensen LI, Kytöviita MM, Olofsson J et al (2008) Soil feedback on plant growth in a sub-arctic grassland as a result of repeated defoliation. Soil Biol Biochem 40:2891–2897. https://doi.org/10.1016/j.soilbio.2008.08.009

Wan S, Luo Y (2003) Substrate regulation of soil respiration in a tallgrass prairie: results of a clipping and shading experiment. Global Biogeochem Cycles 17:1–12. https://doi.org/10.1029/2002gb001971

Wang C, Wan S, Xing X et al (2006) Temperature and soil moisture interactively affected soil net N mineralization in temperate grassland in Northern China. Soil Biol Biochem 38:1101–1110. https://doi.org/10.1016/j.soilbio.2005.09.009

Wang J, Luo Y, Quan Q et al (2021) Effects of warming and clipping on CH_4 and N_2O fluxes in an alpine meadow. Agric for Meteorol 297:108278. https://doi.org/10.1016/j.agrformet.2020.108278

Xu Y, Wan S, Cheng W et al (2008) Impacts of grazing intensity on denitrification and N_2O production in a semiarid grassland ecosystem. Biogeochemistry 88:103–115. https://doi.org/10.1007/s10533-008-9197-4

Yin M, Gao X, Tenuta M et al (2020) Enhancement of N_2O emissions by grazing is related to soil physicochemical characteristics rather than nitrifier and denitrifier abundances in alpine grassland. Geoderma 375:114511. https://doi.org/10.1016/j.geoderma.2020.114511

Zhang CJ, Yang ZL, Shen JP et al (2018) Impacts of long-term nitrogen addition, watering and mowing on ammonia oxidizers, denitrifiers and plant communities in a temperate steppe. Appl Soil Ecol 130:241–250. https://doi.org/10.1016/j.apsoil.2018.06.017

Zhong L, Du R, Ding K et al (2014) Effects of grazing on N_2O production potential and abundance of nitrifying and denitrifying microbial communities in meadow-steppe grassland in northern China. Soil Biol Biochem 69:1–10. https://doi.org/10.1016/j.soilbio.2013.10.028

Zhong L, Bowatte S, Newton PCD et al (2018a) An increased ratio of fungi to bacteria indicates greater potential for N_2O production in a grazed grassland exposed to elevated CO_2. Agric Ecosyst Environ 254:111–116. https://doi.org/10.1016/j.agee.2017.11.027

Zhong L, Wang S, Xu X et al (2018b) Fungi regulate the response of the N_2O production process to warming and grazing in a Tibetan grassland. Biogeosciences 15:4447–4457. https://doi.org/10.5194/bg-15-4447-2018

Zhong L, Zhou X, Wang Y et al (2017) Mixed grazing and clipping is beneficial to ecosystem recovery but may increase potential N_2O emissions in a semiarid grassland. Soil Biol Biochem 114:42–51. https://doi.org/10.1016/j.soilbio.2017.07.002

Zhou X, Guo Z, Chen C et al (2017) Soil microbial community structure and diversity are largely influenced by soil pH and nutrient quality in 78-year-old tree plantations. Biogeosciences 14:2101–2111. https://doi.org/10.5194/bg-14-2101-2017

Zhou X, Wang J, Hao Y et al (2010) Intermediate grazing intensities by sheep increase soil bacterial diversities in an Inner Mongolian steppe. Biol Fertil Soils 46:817–824. https://doi.org/10.1007/s00374-010-0487-3

Zhou Z, Sun OJ, Huang J et al (2006) Land use affects the relationship between species diversity and productivity at the local scale in a semiarid steppe ecosystem. Funct Ecol 20:753–762. https://doi.org/10.1111/j.1365-2435.2006.01175.x

Zhu T, Meng T, Zhang J et al (2015) Fungi-dominant heterotrophic nitrification in a subtropical forest soil of China. J Soils Sediments 15:705–709. https://doi.org/10.1007/s11368-014-1048-4

Zumft WG (1997) Cell biology and molecular basis of denitrification. Microbiol Mol Biol Rev 61:533–616. https://doi.org/10.1128/mmbr.61.4.533-616.1997