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Grazing Significantly Increases N_2O Emission Rates in Alpine Meadows of the Tibetan Plateau

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Alpine meadows are robust nitrous oxide (N_2O) sources that continually experience overgrazing on the Tibetan Plateau. However, the mechanisms underlying N_2O fluxes are poorly understood. The effects of grazing activity on grassland N_2O emission rates, soil and plant characteristics were investigated using a meta-analysis approach. This study revealed that the effect size of grazing was 0.31 ± 0.08 on N_2O emission rates ($p < 0.0001$), and N_2O fluxes increased by 36.27% than control. Light, moderate, and high grazing increased N_2O emission rates by 34.62, 19.48, and 62.16%, respectively. The effect size of moderate grazing was significantly lower than that of high grazing ($p < 0.05$). The effect size of grazing on pH was significant ($p < 0.05$), and pH increased by 6.51% compared with control. Both soil ammonia and nitrate levels increased by 12.24 and 8.60%, respectively. However, grazing decreased soil total carbon, total phosphorus, and available phosphorus by 14.4, 10.25, and 10.15%, respectively. Grazing significantly decreased plant diversity ($p < 0.05$), richness, and aboveground biomass by 15.16, 23.7, and 30.7% ($p < 0.01$), respectively ($p < 0.01$). Aboveground biomass significantly influenced effect size on N_2O emissions, explaining 13.36% of the variations. The direct coefficient of aboveground biomass on effect sizes was –0.631 based on the structural equation model. Although grazing significantly decreased aboveground biomass and diversity, moderate grazing is optimal for mitigating N_2O emissions on the Tibetan Plateau.

Keywords: Tibetan plateau, alpine meadow, effect size, structural equation model, grazing

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INTRODUCTION

Nitrous oxide (N_2O) contributes 8% to global greenhouse gas emissions, and its warming potential is 310 times higher than that of carbon dioxide (IPCC, 2022). Terrestrial ecosystems release 16.8 Tg $N_2O\ yr^{-1}$ globally, accounting for approximately 60% of the total source intensity (Feyissa et al., 2021; Ma L. et al., 2021). Alpine meadows, covering more than 35% of the Tibetan plateau area, are a representative vegetation type and an important source of N_2O (Gao et al., 2008; Wang et al., 2021).

Livestock grazing is a dominant economic activity on the Tibetan Plateau (Tang L. et al., 2019). Overgrazing has caused severe grassland degradation in more than 80% of the Tibetan Plateau over the last few decades and increased grassland N_2O emission rates (Zhang et al., 2014; Fu et al., 2021; Hargreaves et al., 2021). Light, moderate, highly degraded, and control grassland N_2O emission rates were 35.17, 53.40, 87.07, and 47.20 $\mu g\ m^{-2}\ h^{-1}$, respectively, in an alpine meadow of the northeast Tibetan Plateau (Guo et al., 2019). Light and intensive grazing treatments increased alpine grassland N_2O emissions by 27.5 and 68.1%,

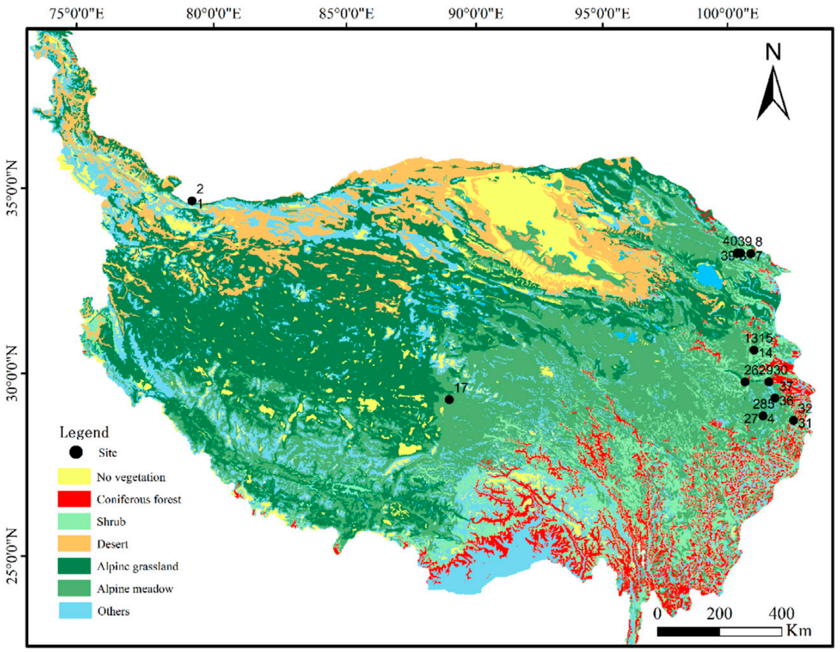

FIGURE 1 | Main vegetation types and 40 research sites on the Tibetan plateau.

respectively, on the northwest Tibetan Plateau (Yin et al., 2020). Moderate grazing increased the annual average N_2O emissions of alpine meadows by 62 and 65.79% on the northeast and southwest Tibetan Plateau, respectively (Zhu et al., 2015; Zhan et al., 2021). Moderate grazing and warming with grazing significantly increased the average annual N_2O flux by 57.8 and 31.0%, respectively, on the northeast Tibetan Plateau (Hu et al., 2010). Thus, the effects of grazing on N_2O emission rates showed robust spatial heterogeneity across the Tibetan Plateau.

Microbial communities were altered in post-grazing grassland soils and linked to soil biogeochemical processes, such as nitrification and denitrification, and N_2O generation processes (Zhang et al., 2014; Wang et al., 2016). The grassland N_2O emission rate is significantly negatively correlated with biomass, soil water-filled pore space, organic carbon, and soil available phosphorus (Du et al., 2019; Zhan et al., 2021). Heavy grazing reduces N_2O emissions by nearly 40% because of reduction in soil moisture and substrate availability, such as soil dissolved organic carbon and inorganic N levels (Tang S. et al., 2019; Yao et al., 2019). However, no relationship was observed between the soil N_2O emission rate and temperature or rainfall on the northeast Tibetan Plateau (Li et al., 2018).

The Tibetan Plateau covers approximately 25% of the total area in China, and it is very sensitive to anthropogenic perturbation (Du et al., 2019). Establishing a proper grazing intensity is urgently needed to reduce N_2O emissions from degraded grasslands while maintaining livestock productivity. In this study, we have addressed the following hypotheses: First, grazing increases grassland N_2O emission rates, especially at high grazing intensities. Second, the effect size of grazing on N_2O emission rates is dominated by soil available nitrogen content and altitude.

MATERIALS AND METHODS

Data Compilation

Published papers were collected using keywords "grazing" and "nitrous oxide or N_2O" and "Tibet*" in Web of Science from January 1990 to April 2022. Furthermore, 49 articles were selected and conserved in the Endnote library (Endnote X9). Most full-version papers and doi numbers were automatically added by the function "find full text." Others were captured through doi by using Google Scholar.

Then, complete articles were screened based on the following criteria: 1) all studies were conducted, including control and its grazing activity (light, moderate and high grazing). 2) N_2O concentrations were analyzed using the static chamber method and chromatographic concentration analysis. Some data were extracted from published paper figures by using WebPlotDigitizer software. Here, we collated 21 published studies, including 40 field experiment results (**Figure 1**). Soil and plant characteristics were also recorded.

We calculated the log response ratios (*RR*, hereafter response ratios) as a measure of effect size. The 95% confidence intervals (CIs) were calculated. A random-effect-model meta-analysis was performed, and the data were analyzed with R statistical software by using the Meta package.

$$\ln R = Ln\frac{x_e}{x_c} = \ln(x_e) - \ln(x_c)$$

where X_e and X_c are the mean values of each individual trait measure in the treatment and control groups, respectively. In addition, lnR <0 indicated a decrease in trait response to grazing activity; otherwise, it indicated an increasing effect. The variance in lnR was calculated as follows:

$$V_{\ln R} = \frac{S_e^2}{N_e x_e^2} + \frac{S_c^2}{N_c x_c^2}$$

where S_e, N_e, x_e, S_c, N_c, and x_c are standard deviations, sample sizes, and mean values of the grazing treatments and control, respectively.

The effect size of grazing on N_2O emission rates, soil and plant characteristics, and CI were calculated based on the random-effect model:

$$\text{Weight of an individual study } w_i^* = 1/\left(v_i + \tau^2\right)$$

where v_i represents the intra-study variance, and τ^2 represents the inter-study variance.

$$\text{Average effect size } \bar{y} = \frac{\sum_{i=1}^{k} w_i^* y_i}{\sum_{i=1}^{k} w_i^*}$$

$$\text{Standard error } SE = \sqrt{\frac{1}{\sum_{\mathrm{i}=1}^{\mathrm{k}} w_i^*}}$$

95% CI of average effect value: $CI = \bar{y} \pm 1.96\ SE$, and y_i refers to the single study effect value.

Statistical Analysis

Meta-statistical analyses were performed using R 3.6.2, and a random-effect model of the meta-analysis was run in *metafor*1.9-8 (Benítez-López et al., 2017). The random-effect models were used to analyze the estimated values and standard errors (rma). Then, mixed-effect models (mods) were used to explain significant residual heterogeneity with different moderators, including categorical and continuous variables. The explained moderator heterogeneity statistic (Qm) was also calculated to test for significance in single covariate meta-regressions.

Drive factors of climate factors and soil characteristics for effect sizes were analyzed with the structural equation model using "piecewiseSEM" package. All figures were drawn using SigmaPlot 10.0 and R statistics.

Publication Bias

Biases against publishing negative results may exist in research fields. In this study, a regression test for funnel plot asymmetry of publication bias was performed using a mixed-effect meta-regression model (funnel and Egger's test, rma).

RESULTS

Responses of Alpine Meadow N_2O Emission Rates, Soil, and Plant Characteristics to Grazing Activity

Grazing activities had a robust and positive effect on N_2O emission rates ($p < 0.0001$, **Table 1**), with an estimated value of 0.31 ± 0.08 effect size (95% CI: 0.15–0.47). This indicated that grazing disturbance increased alpine meadow N_2O emission rates by 36.27% on the Tibetan Plateau. Furthermore, high grazing activity significantly increased N_2O emission rates by approximately 62.16% ($p < 0.0001$), and it was significantly higher than that of moderate grazing ($p < 0.05$, **Table 1**). Light and moderate grazing increased by 34.62 and 19.48%, respectively. Thus, moderate grazing is optimal for mitigating N_2O emissions on the Tibetan Plateau.

Grazing activity increased grassland soil ammonia and nitrate levels, pH, temperature, dissolved organic carbon, and moisture by 12.24, 8.60, 6.51, 5.33, 3.56, and 1.16%, respectively (**Figure 2**). The effect size of grazing on pH was significant ($p < 0.05$). However, grazing decreased soil total carbon, total phosphorus, available phosphorus, soil organic carbon, available potassium, total nitrogen, and bulk soil by 14.4, 10.25, 10.15, 6.57, 5.85, 4.3, and 1.97%, respectively. In addition, the effect size of available potassium was significant ($p < 0.01$, **Figure 2**). Grazing significantly decreased plant diversity ($p < 0.05$), richness, and aboveground biomass ($p < 0.01$) by 15.16, 23.7, and 30.7%, respectively (**Figure 2**).

Effects of Factors on Grassland N_2O Emission Rate Based on Explained Moderator Heterogeneity Statistic

Aboveground biomass significantly influenced the effect size based on test for moderators ($p < 0.05$, **Table 2**). The mixed-effect model results indicated that aboveground biomass could explain 13.36% of the variations in effect size, and the effect size decreased as the aboveground biomass increased. Furthermore, nitrate content and precipitation were the main factors that affected the effect sizes by approximately 4.49 and 3.18%, respectively (**Table 2**).

Structural Equation Model of Direct and Indirect Effects on Grasslands Effect Sizes Among Different Factors

The effect size of grazing activity on N_2O emission rates was mainly controlled by grassland aboveground biomass with a structural equation model. The direct coefficient of aboveground biomass on effect size was −0.631 ($p < 0.05$, **Figure 3**). The direct coefficients of temperature and precipitation on the effect sizes were 0.579 and −0.456, respectively. The indirect coefficients of precipitation, air temperature, and nitrate were −0.365, −0.311, and 0.153, respectively.

Publication Bias Analysis of Effect Sizes on Grassland N_2O Emissions

Egger's regression test for funnel plot asymmetry indicated that this result was not significantly affected by publication bias by using meta-analysis models (z = 0.0862, $p = 0.9313$, **Figure 4**).

TABLE 1 | Effect sizes of grazing activities and intensities on grasslands N_2O rates on Tibetan Plateau.

Items	Effect Sizes	Increase Range (%)	95% Confidence Interval	P	Df
Grazing activity	0.31 ± 0.08	36.27	0.15–0.47	<0.001***	39
Light grazing	0.29 ± 0.21 ab	34.62	–0.12 – 0.71	0.711	5
Moderate grazing	0.18 ± 0.12 b	19.48	–0.05 – 0.41	0.410	18
High grazing	0.48 ± 0.13 a	62.16	0.22–0.75	<0.001***	14

Note: effect sizes meant average ± stand error. Same letters meant no significant differences in the same column.

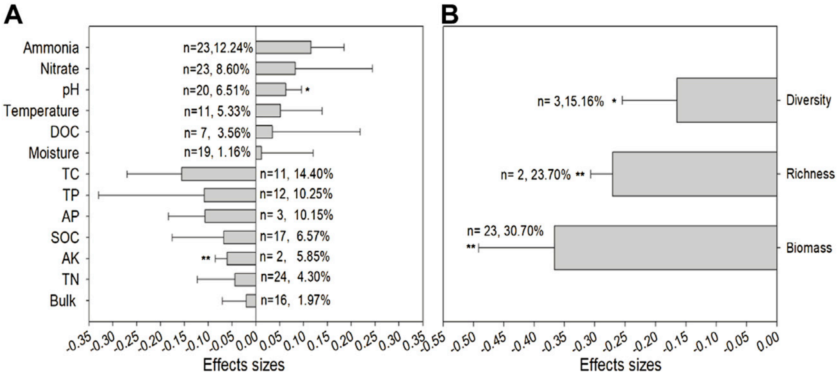

FIGURE 2 | Effect sizes of grazing on soil **(A)** and plant **(B)** characteristic and ranges of increase and decrease on the Tibetan. Note: ** and * meant $p < 0.01$ and $p < 0.05$. DOC, TC, TP, AP, SOC, AK, TN meant dissolved organic carbon, total carbon, total phosphorus, available phosphorus, soil organic carbon, available kalium, and total nitrogen.

TABLE 2 | Analysis of air temperature and altitude and other factors on effect size.

Moderators	Test of Moderators (QM)	P	Model	R^2 (%)
Biomass	5.36	0.02*	Y = 0.7374–0.0013 x	13.36
Nitrate	2.06	0.145	Y = 0.2031 + 0.0135 x	4.49
Precipitation	2.12	0.151	Y = 0.0009 x—0.2111	3.18
SOC	1.12	0.29	Y = 0.4880–0.0072 x	0.86
Bulk	0.66	0.42	Y = 0.8278–0.5230 x	0.01
pH	0.31	0.58	Y = 0.7629–0.0750 x	0.01
Temperature	0.06	0.81	Y = 0.3124–0.0068 x	0.01

Note: Y means effect size. SOC, meant soil organic carbon.

DISCUSSION

Effects of Grazing on Grassland N_2O Emission Rates on the Tibetan Plateau

The average global temperature in 2021 was approximately 1.11°C above pre-industrial levels, and atmospheric N_2O was 333.2 ppb, approximately 123% of pre-industrial levels (WMO, 2021). Grassland N_2O emission rates increased by 5.05% because of nitrogen excreted by grazing animals in Europe (Oenema et al., 1997). In South Brazil, grazing increased grassland N_2O emission rates from 5.54 to 15.83 μg m^{-2} h^{-1} (Schirmann et al., 2019). Across the Tibetan Plateau, grazing treatments increased N_2O emissions from 5.42 to 266.71% (Lin et al., 2009; Ma S. et al., 2021). Light grazing increased N_2O emission rates by 5.40–63.91% (Guo et al., 2019; Luo et al., 2020), and extreme grazing decreased N_2O emission rates by 28.57% on the central Tibetan Plateau (Wei et al., 2012). The opposite effect and strong heterogeneity across different studies were because of different aboveground biomass, soil organic carbon content, air temperature and precipitation (Yao et al., 2019; Feyissa et al., 2021). Grazing increased soil N_2O emissions by regulating *nirK* and *nosZ* denitrifiers in alpine meadows (Zhang et al., 2021).

In the present study, we found that grazing increased alpine meadow emission rates by 36.27% on the Tibetan Plateau. As the grazing intensity increased, the influence on extent of emission first decreased from 34.62% (light) to 19.48% (moderate) and then increased by approximately 62.16% (high grazing). The effect was the lowest for moderate grazing, which was revealed to significantly increase grassland biomass and diversity on the northeast and southeast Tibetan Plateau (Zhu et al., 2015; Hu et al., 2017; Ma L. et al., 2021).

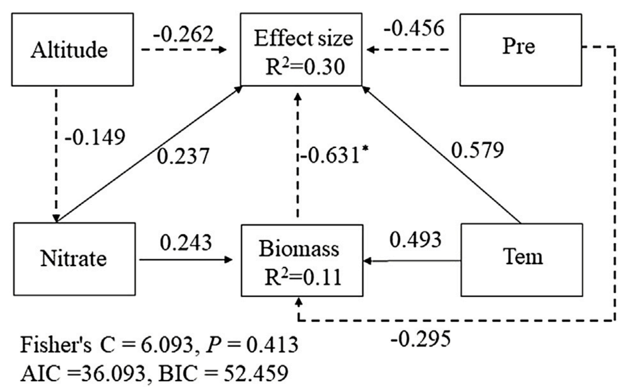

FIGURE 3 | Driven factors on effect size and biomass and nitrate through structural equation model. Note: Tem and Pre meant air temperature and precipitation. Solid and dashed lines meant positive and negative effect. * meant $p < 0.05$.

There are three reasons for this increased effect of grazing on N_2O emission rates on the Tibetan Plateau. First, grazing activities increased available nitrate and pH in grassland soils, providing adequate substrate and suitable environments for N_2O production. In this study, we also found that grazing increased soil ammonia and nitrate by 12.24 and 8.60%, respectively. Second, grazing livestock provide a lot of dung and urine to grassland soils. Ruminants may excrete 75–95% of the consumed nitrogen to grasslands (Feyissa et al., 2021). The feeding and trampling behaviors of grazing animals and their excreta can directly or indirectly increase soil emissions of N_2O (Ma S. et al., 2021). Cumulative N_2O emissions for both urine and dung patches were 1.8–3.7 times greater than those of control plots in alpine meadows (Lin et al., 2009). Third, grazing significantly decreased grassland biomass and richness by 30.70 and 23.70%, respectively, on the Tibetan Plateau. This reduction in aboveground biomass and richness increased the competition priority of microorganisms. Subsequently, N_2O emission rates significantly increased on the Tibetan Plateau.

Influence Mechanism of Grazing on Grassland N_2O Emission and Mitigation on the Tibetan Plateau

Limiting warming to around 1.5°C in the Paris Agreement requires global greenhouse gas emissions to be reduced by 43% by 2030 (WMO, 2021), and N_2O should be reduced by 20% (IPCC, 2022).

Grazing activity has significant effects on plant communities and soil properties (Feyissa et al., 2021). Heavy grazing intensity accelerated N cycling rates and increased plant-soil system N in the alpine meadow (Zhong et al., 2017). In this study, we revealed that the effect size of grazing on N_2O fluxes was driven by aboveground biomass, with a direct coefficient of −0.631 based on the structural equation model. Furthermore, aboveground biomass could explain 13.36% of the variations in effect size by using the mixed-effect model. Thus, an increase in grassland aboveground biomass would help to significantly mitigate N_2O emission rates on the Tibetan Plateau.

Different vegetation types affected grassland N_2O emission rates; emission rates in *Kobresia humilis* and *Potentilla fruticosa* meadows were 47.8 and 60.6 μg m^{-2} h^{-1}, respectively (Du et al., 2008). N_2O is mainly driven by the simultaneous effects of grassland biomass and soil temperature on the Tibetan Plateau (Lin et al., 2009). N_2O fluxes were significantly positively correlated to soil nitrate content, organic carbon, and biomass (Yao et al., 2019). Soil temperature and biomass are the major contributors to alpine meadow N_2O fluxes on the northwest Tibetan Plateau (Yin et al., 2020). Grassland denitrification genes increased when biomass was higher, which was concomitant with increased N_2O emissions (Wang et al., 2016; Tang L. et al., 2019).

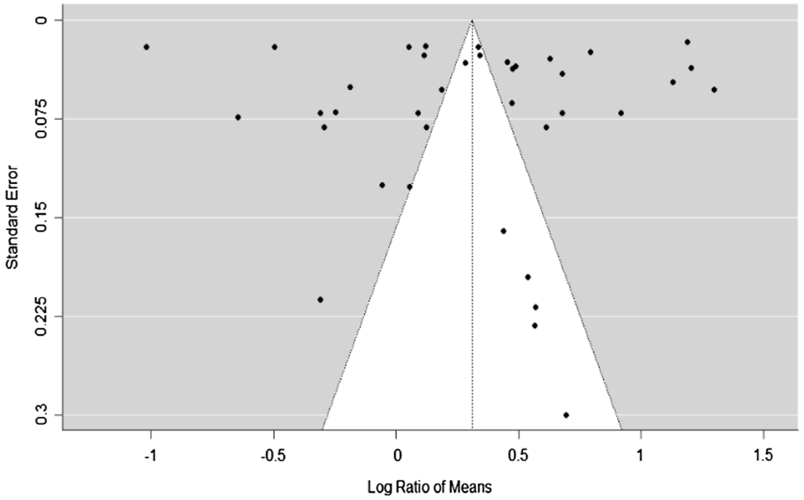

FIGURE 4 | Funnel plot of effect sizes of grazing on grassland N_2O emission rates. Note: Egger's regression test for funnel plot asymmetry ($z = 0.0862$, $p = 0.9313$).

CONCLUSION

In summary, alpine meadows are atmospheric N_2O sources on the Tibetan Plateau. Grazing activity significantly increased alpine meadow N_2O emission rates. Increased range of effect size on N_2O emission rates was high grazing, light grazing, and moderate grazing in sequence. Moderate grazing was found to be optimal for mitigating N_2O emissions. Grazing significantly increased soil pH but decreased soil available potassium. Grazing significantly decreased grassland coverage, richness, and biodiversity. The effect size of grazing on N_2O emission was driven by aboveground biomass. The increase in aboveground grassland biomass is beneficial for mitigating N_2O emissions on the Tibetan Plateau.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

WL: conceptualization, writing—original draft preparation, and project administration. QX: methodology and resources, and validation. HZ: formal analysis, investigation, and data curation. YD: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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