

Watering increased DOC concentration but decreased N₂O emission from a mixed grassland soil under different defoliation regimes

Yanjiang Cai^{1,2,3} · Scott X. Chang¹ · Bin Ma¹ · Edward W. Bork⁴

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Abstract Changes in grazing regime and climatic conditions (such as precipitation) may affect soil N₂O emissions; however, such effects have been inconsistent in grasslands. This study investigated the impact of simulated grazing, increased precipitation and N addition on soil N₂O emissions, as well as soil dissolved organic carbon (DOC) concentrations, from a mixed grassland site in southern Alberta, Canada. A batch incubation study was conducted on soils (0–10 cm) collected from long-term field plots exposed to defoliation at either high intensity at low frequency (HILF) or high intensity at high frequency (HIHF) or low intensity at high frequency (LIHF) throughout the growing season (May–August). A deferred control (DC) treatment was also included where defoliation only occurred once at year end. All defoliation treatments formed a full factorial experiment with the watering treatment (ambient moisture (AM) or watering (W)) in the field. Soil N₂O production was also quantified under an N addition treatment (no N and addition of 20 mg N kg⁻¹ (NH₄)₂SO₄) in the laboratory incubation experiment. Defoliation had no influence ($p > 0.05$) on soil DOC concentrations and cumulative

N₂O emissions. Watering increased DOC concentrations by 72–234 % but decreased N₂O emissions by 33–60 %. Increments in N₂O emissions after N addition were greater in the W (111–163 %) than the AM (64–67 %) treatment. Our results suggest that while defoliation regimes did not affect soil N₂O emissions, watering may induce greater N₂O loss in the more arid soil and N addition may further increase N₂O loss under high precipitation within soils of the dry mixed grassland ecosystem.

Keywords Defoliation · Climate change · Dissolved organic C · Dryland · Increased precipitation

Introduction

Grassland is one of the dominant land cover types in the world, covering about 40 % of global terrestrial ecosystems (Suttie et al. 2005). Grassland ecosystems play a key role in the regulation of global biogeochemical nitrogen (N) cycling, and managed grasslands are major contributors to the biosphere-atmosphere exchange of nitrous oxide (N₂O), a powerful greenhouse gas (Soussana and Lemaire 2014). The N₂O emission from agriculture (such as managed grassland and cropland) is estimated to be 4.1 Tg N year⁻¹ and contributes approximately 59 % of the total global anthropogenic N₂O emissions (IPCC 2013). Although the concentration of N₂O in the atmosphere (324 ppb in 2011) is relatively low (IPCC 2013), its global warming potential on a 100-year time horizon is approximately 300 times that of carbon (C) dioxide (CO₂) and it is also the dominant anthropogenic substance contributing to stratospheric ozone depletion (Ravishankara et al. 2009; IPCC 2013). Nitrification and denitrification by microbes are the main processes of N₂O production in soils. Within grassland ecosystems, N₂O fluxes are generally considered to be

✉ Scott X. Chang
scott.chang@ualberta.ca

¹ Department of Renewable Resources, University of Alberta, 442 Earth Sciences Building, Edmonton, Alberta T6G 2E3, Canada

² Key Laboratory of Mountain Environment Evolution and Regulation, Institute of Mountain Hazards and Environment, Chinese Academy of Sciences, Chengdu 610041, China

³ State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

⁴ Department of Agricultural, Food and Nutritional Science, University of Alberta, 410 Agriculture/Forestry Centre, Edmonton, Alberta T6G 2P5, Canada

closely linked to management practices, such as grazing-associated nutrient re-distribution or fertilization, and climatic conditions (Luo et al. 2013; Soussana and Lemaire 2014).

Defoliation by livestock grazing or by forage harvesting is one of the most important management practices in grasslands. However, no consensus exists regarding the effect of defoliation on grassland soil N₂O emissions. For example, Kammann et al. (1998) reported a reduction in N₂O fluxes after grass cutting and attributed this response to reduced NO₃⁻ concentration, which was caused by the greater ability of more frequently cut plants to compete for mineral N. In contrast, other studies have found that defoliation increased soil N₂O emissions (Nefel et al. 2000; Rafique et al. 2012), which was attributed to the enhancement of nitrification and denitrification as a result of increasing soil temperature, soil moisture, and soil N availability after forage removal. Working in an NH₄⁺-amended Bermuda grass pasture in the USA, Hutchinson and Brams (1992) did not find any effect of defoliation on soil N₂O emission but instead found that defoliation stimulated the emission of NO, indicating enhanced nitrification after defoliation. Another factor regulating grassland soil N₂O emissions after defoliation is the availability of soil labile organic C, which serves as the energy and C source for heterotrophic denitrifiers. However, defoliation has been found to either increase C exudation from roots to soil (Paterson et al. 2005; Hamilton et al. 2008) or reduce root mass and the allocation of C assimilates to roots (Holland and Detling 1990; Crawford et al. 2000; Guitian and Bardgett 2000) or not influence soil C availability (Bazot et al. 2005). Thus, the effect of defoliation on the dynamics of available soil C and associated N₂O emissions from grasslands remains unclear.

Together with defoliation, changes in precipitation and atmospheric N deposition are considered key drivers of changes in grassland ecosystems worldwide (Fay et al. 2003; Stevens et al. 2010; Bell et al. 2014; Basto et al. 2015). Emissions of N₂O from grassland ecosystems are also sensitive to changes in precipitation and N deposition. Changes in moisture availability, both positive and negative, and N deposition have occurred due to global climate change and industrial development, respectively (Galloway et al. 2004; IPCC 2007). Many studies have shown that increased precipitation (or irrigation) elevated N₂O emissions from grassland soils owing to (1) an increase in soil moisture content that creates anaerobic conditions for denitrification and (2) accelerated soil organic matter decomposition that enhancing the supply of N and C substrates for nitrification and denitrification (Du et al. 2006; Chen et al. 2013; Liu et al. 2015). In contrast, others have found lower N₂O fluxes under watering (Gu and Riley 2010; Sainju et al. 2012), a response attributed to the complete denitrification of N₂O to N₂ under very low gas diffusion rates post-watering. In addition, both positive and indifferent influences of N addition (including atmospheric N deposition)

have been reported on soil N₂O emissions (Mosier et al. 2003; Ambus and Robertson 2006; Chen et al. 2013), with the effect of water addition on N₂O emission either greater (Liu et al. 2015) or lower (Chen et al. 2013) when accompanying N addition. As a result, no consensus currently exists regarding the effect of defoliation, altered precipitation, and N addition on soil N₂O emissions from grassland ecosystems.

Grasslands are extensively distributed in the mixedgrass prairie region of Western Canada (Adams et al. 2005). In the Brooks plain of the mixedgrass prairie region in SE Alberta, May–August precipitation increased by 14 % from 1901 to 2002 (Shen et al. 2005). Human activities may also substantially elevated the reactive N deposition in North American prairie regions (Galloway et al. 2004; Templer et al. 2012). However, the response of soil N₂O emissions to defoliation frequency and intensity, changes in summer precipitation, and exogenous N additions have never been simultaneously studied in northern temperate grasslands. Such research will provide critical information for understanding soil N₂O emissions from these agro-ecosystems. We conducted field sampling and laboratory incubations to examine the effects of different defoliation treatments, increased precipitation, and N addition on soil N₂O emissions in northern mixedgrass prairie. The main objectives of this study were (1) to compare soil N₂O emissions from long-term defoliation and watering treatments with or without N addition and (2) to determine the relationship between soil N₂O emissions and key environmental factors.

Materials and methods

Site description and soil sampling

Sampling occurred at the Mattheis Research Ranch in the dry mixedgrass prairie region of Alberta, Canada. The region has a mean annual precipitation of 354 mm and a mean daily temperature of 4.2 °C from 1971 to 2000 (Adams et al. 2005). The site (N 50° 53' 40.2", W 111° 52' 26.3") was a mesic, lowland range site with an Orthic Brown Chernozemic soil (SCWG 1998) with sandy loam texture. The soil had a soil organic matter content of 2.5 %, pH of 6.3, and EC of 3.7 ds m⁻¹. The primary forage species were *Pascopyrum smithii*, with *Koeleria macrantha* (Ledeb.) J.A. Schultes and *Hesperostipa comata* as sub-dominants. The study area is a native grassland, receives no fertilization and has had long-term grazing by beef cattle in a rotational system involving 1–2 pulses of exposure to cattle annually (where each bout is 1–2 weeks in duration), for a moderate stocking rate (~0.6 animal unit months per hectare) each year. In 2010, an experimental area was fenced to initiate more tightly controlled defoliation treatments to further understand the tradeoff between different simulated herbage use patterns and levels based on grazing systems.

The experiment used a fully randomized factorial design and included four different simulated grazing (i.e., defoliation) treatments and two altered precipitation (watering) levels, with six replicated plots (1 × 1 m) per treatment combination and a 0.5-m buffer between plots. Defoliation treatments included a control where a single defoliation occurred annually that was deferred to the end of the growing season (deferred control (DC)) or defoliation from the end of May through late August at either high intensity at low frequency (HILF) or high intensity at high frequency (HIHF) or low intensity at high frequency (LIHF). All herbage were manually cut at 2 cm stubble height in late August for the DC treatment; for the HILF and HIHF treatments, they were clipped at 2 cm stubble height every 6 and 3 weeks, respectively; while herbage was clipped at 5 cm height every 3 weeks in the LIHF treatment. The DC treatment represented the commonly held notion that native grasslands are best grazed by postponing defoliation until the end of the growing season, after plants had senesced and therefore become more defoliation tolerant. All the other three treatments represented various patterns of use during the growing season, which is more likely to occur in reality given that livestock grazing commonly occurs during summer. Given this, the HIHF was designed to mimic heavy, frequent grazing, as one would find in a heavy continuous pattern of livestock use where cattle have constant access to the same patch of ground, and therefore graze and regrow the same areas over time. In contrast, the HILF and LIHF patterns were designed to quantify the relative tradeoffs of either going to a lower frequency of intense use during the sensitive growing season, such as you find with pulse, or mob-style, grazing, where cattle graze for short but intense periods of time, following by a long recovery period, or a lower intensity of use where cattle are moved frequently between pastures and grazing periods are kept short in duration, thereby minimizing stress on vegetation. A low intensity and low frequency (LILF) treatment was not assessed because it is not a system likely to be used by livestock producers. Watering treatments included ambient moisture (AM) and watering (W). Water was added to maintain an equivalent of 150 mm of monthly precipitation throughout the growing season. This level is twice the average precipitation in June, the month with the greatest rainfall, and was intended to ensure water availability did not limit plant growth. Watering was conducted at approximately 10-day intervals. All treatments commenced in late May and finished in late August from 2010 through 2015.

For this study, four of the six replicates of each treatment were randomly selected for sampling during the growing season on the 12th of June and 28th of July 2015, 6 years after the initiation of treatments. Within each plot, four soil cores (3.5 cm diameter and 10 cm deep) were randomly collected and thoroughly mixed to form a composite sample. Immediately after removal, soil samples were put in

polyethylene bags, placed in a cooler with ice packs, and transported to the laboratory.

Incubation experiment

Soils were promptly sieved (2 mm) to remove gravel and plant residue. For each incubation, 25.0 g (on an oven-dry basis) of soil was weighed and placed in a 250-mL flask. Soil moisture content for the no N addition treatments was adjusted to 60 % water holding capacity (WHC) by directly adding deionized water. For the N addition treatments, each soil received 2 mL (NH₄)₂SO₄ solution (0.25 mg N mL⁻¹) by pipetting solutions uniformly over the soil surface, resulting in the addition of 20 mg N kg soil⁻¹. This amount of N is equivalent to 20 g N m⁻² year⁻¹, or 20 kg N ha⁻¹ year⁻¹ in the field, in which ammonium might be the dominant form of N (Pearson and Stewart 1993). The final moisture content of each soil was subsequently adjusted to 60 % WHC by adding deionized water.

Flasks were incubated in the dark for 20 days at 20 °C. To prevent water loss, each flask was covered with aluminum foil. Flasks were weighed every 2–3 days to determine water loss and were supplemented with distilled water to maintain constant soil moisture. Gas samples were taken from the headspace of flasks on days 1, 2, 3, 5, 8, 11, 14, 17, and 20. At each gas sampling, flasks were sealed using an airtight rubber stopper for 24 h. At 0 and 24 h, gas samples from the headspace of each flask were collected using a 20-mL airtight syringe injected into evacuated 12-mL vials for N₂O and CO₂ analysis on a gas chromatograph (GC, Varian CP-3800, Mississauga, Canada) by the electron capture detector and thermal conductivity detector, respectively.

Analysis of soil properties

Soil moisture content was measured by oven-drying at 105 °C to a constant mass. The soil WHC was determined as the gravimetric water content of soil when saturated and allowed to drain over 6 h (Fierer and Jackson 2006). Soil pH was determined in soil-water suspensions (1:5 soil/water ratio, w/v) using a digital pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, MA, USA). Concentrations of exchangeable NH₄⁺ and NO₃⁻ were analyzed via steam distillation using magnesium oxide and Devarda's alloy in sequence after extraction with 2 M KCl (1:10 soil/water ratio, w/v) (Kwak et al. 2016). To quantify dissolved organic C (DOC) and total dissolved N (TDN), 5 g of fresh soil were extracted with 50 mL deionized water, filtered through a 0.45-μm membrane filter, and analyzed using a TOC-VCSN analyzer (Shimadzu, Kyoto, Japan).

Data analysis and statistics

Data were statistically analyzed using the SPSS software package for Windows (version 17.0, SPSS Inc., Chicago, IL,

Table 1 Field moisture content and pH for soils sampled on June and July 2015 and the effects of defoliation [deferred control (DC), low intensity at high frequency (LIHF), high intensity at low frequency (HILF), and high intensity at high frequency (HIHF)] and watering [ambient moisture (AM) or watering (W)] on soil moisture content (SMC) and pH

Treatment		June		July	
		SMC (% WHC)	pH	SMC (% WHC)	pH
AM	DC	11.7 ± 1.2	5.67 ± 0.04 b	35.8 ± 0.6	5.80 ± 0.05 b
	LIHF	10.1 ± 0.2	5.79 ± 0.04 ab	33.5 ± 1.9	5.74 ± 0.04 b
	HILF	8.09 ± 0.48	5.85 ± 0.13 ab	30.6 ± 1.7	6.25 ± 0.05 a
	HIHF	9.96 ± 0.86	6.00 ± 0.05 a	34.3 ± 0.8	5.69 ± 0.06 b
W	DC	15.2 ± 0.5	7.97 ± 0.09 ab	37.7 ± 2.5	8.56 ± 0.04 a
	LIHF	13.9 ± 0.5	8.14 ± 0.15 a	41.5 ± 2.3	8.42 ± 0.14 a
	HILF	15.9 ± 0.5	7.56 ± 0.09 b	41.1 ± 0.8	8.41 ± 0.06 a
	HIHF	18.3 ± 2.6	7.90 ± 0.03 ab	39.7 ± 1.7	8.16 ± 0.14 a
Two-way ANOVA					
Defoliation (D)		ns	*	ns	***
Watering (W)		***	***	***	***
D × W		ns	**	ns	**

Values are means with standard error ($n = 4$). Due to a significant interaction between defoliation and watering on field soil pH, a one-way ANOVA with defoliation as a fixed factor was run separately for each watering treatment. The different lowercase letters within the same column for each watering treatment indicate differences among the four defoliation treatments at $p < 0.05$

ns not significant

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

USA). Following the assessment of normality, the pH, moisture content, and concentrations of DOC, TDN, NH_4^+ , and NO_3^- in the fresh soil as well as the cumulative soil N_2O emission were first analyzed with a repeated measures two-way ANOVA, using defoliation and watering as fixed factors. As the interaction was significant ($p < 0.01$), a one-way ANOVA including defoliation (DC, LIHF, HILF, and HIHF) was run separately for each watering treatment. To quantify background N_2O emissions, linear regression was used to analyze the relationship between cumulative soil N_2O emission and soil CO_2 emission or individual soil property or the change in DOC, TDN, exchangeable NH_4^+ , or NO_3^- concentrations during incubation without N addition. Additionally, the relationship between cumulative soil N_2O emission and the change in DOC, TDN, exchangeable NH_4^+ , or NO_3^- concentrations during incubation for all treatments was quantified to assess the effects of changing soil conditions on soil N_2O emissions.

Results

Field soil pH and water content

Soil moisture content was significantly affected by watering but not by defoliation or their interaction (Table 1). Soil moisture content was significantly ($p < 0.05$) higher in the W than the AM treatment for each defoliation treatment. The average soil moisture content in the AM treatment was 9.96 and 33.6 %

WHC in the June and July samplings, respectively, with 15.8 and 40.0 % WHC, respectively, in the W treatment.

Soil pH was significantly affected by defoliation, watering, and their interaction (Table 1). Soil pH in the HILF treatment exceeded that of the DC treatment under AM, though significant ($p < 0.001$) differences were limited to the July sampling. Similarly, HIHF defoliation led to greater soil pH compared to the DC under AM, a response that was significant ($p < 0.05$) only in the June sampling. In contrast, no differences in pH were found at any time between the DC and any of the other three defoliation treatments under W. Soil pH in the AM treatment was consistently lower than that in the W treatment within each defoliation treatment. Mean soil pH in the AM and W treatments was 5.83 and 7.89, respectively, in the June sampling, and 5.87 and 8.39, respectively, in the July sampling.

Field soil DOC, TDN, and inorganic N concentrations

Both DOC and TDN concentrations in the freshly collected soil were significantly affected by watering, but not by defoliation or their interaction (Table 2). Soil DOC and TDN concentrations were significantly ($p < 0.05$) greater in the W than the AM treatment of each defoliation treatment in both the June and July samplings. The DOC concentrations in the AM and W treatments were 122 and 243 mg C kg^{-1} , respectively, in the June sampling, and 123 and 371 mg C kg^{-1} , respectively, in the July sampling. Average TDN values in the AM and W treatments were 20.5 and 34.6 mg N kg^{-1} ,

Table 2 Field concentrations of soil DOC, TDN, NH₄⁺-N, and NO₃⁻-N for soils sampled on June and July 2015 and the effects of defoliation [deferred control (DC), low intensity at high frequency (LIHF), high intensity at low frequency (HILF), and high intensity at high frequency (HIHF)] and watering [ambient moisture (AM) or watering (W)] on the above four soil properties

Sampling time	Treatment	DOC (mg C kg ⁻¹)		TDN (mg N kg ⁻¹)		NH ₄ ⁺ -N (mg N kg ⁻¹)		NO ₃ ⁻ -N (mg N kg ⁻¹)	
		June	July	June	July	June	July	June	July
AM	DC	113 ± 4	121 ± 7	19.6 ± 1.2	23.4 ± 0.9	6.43 ± 0.37	7.98 ± 0.09	13.2 ± 0.1 a	8.19 ± 0.62
	LIHF	120 ± 6	110 ± 3	20.8 ± 1.3	25.7 ± 2.6	8.20 ± 0.90	8.83 ± 0.95	14.2 ± 0.5 a	5.23 ± 0.82
	HILF	123 ± 8	128 ± 1	20.6 ± 0.7	23.6 ± 1.1	6.97 ± 0.64	7.59 ± 0.06	14.4 ± 0.3 a	6.90 ± 0.88
	HIHF	133 ± 14	131 ± 6	21.2 ± 1.8	25.0 ± 1.5	7.19 ± 1.12	7.52 ± 0.18	13.0 ± 0.3 a	8.23 ± 0.83
W	DC	258 ± 11	353 ± 13	34.8 ± 1.1	36.8 ± 1.8	4.70 ± 0.42	8.25 ± 0.31	13.6 ± 0.3 b	1.29 ± 0.18
	LIHF	240 ± 8	368 ± 5	35.0 ± 0.9	40.9 ± 0.6	5.22 ± 0.51	8.21 ± 0.20	13.9 ± 0.3 b	2.07 ± 0.71
	HILF	245 ± 6	379 ± 16	33.3 ± 1.0	40.3 ± 1.8	6.34 ± 0.77	8.21 ± 0.24	14.1 ± 0.1 b	1.03 ± 0.06
	HIHF	229 ± 5	383 ± 19	35.3 ± 3.0	45.7 ± 3.0	6.14 ± 0.67	8.10 ± 0.14	16.2 ± 0.7 a	5.38 ± 2.34
Two-way ANOVA									
Defoliation (D)		ns	ns	ns	ns	ns	ns	*	*
Watering (W)		***	***	***	***	**	ns	*	***
D × W		ns	ns	ns	ns	ns	ns	***	ns

Values are means with standard error (n = 4). Due to a significant interaction between defoliation and watering on NO₃⁻-N, a one-way ANOVA with defoliation as a fixed factor was run separately for each watering treatment. The different lowercase letters within the same column for each watering treatment indicate differences among the four defoliation treatments at p < 0.05

ns not significant

*p < 0.05; **p < 0.01; ***p < 0.001

respectively, in the June sampling, and 24.4 and 40.9 mg N kg⁻¹, respectively, in the July sampling.

There were no significant differences in exchangeable NH₄⁺ or NO₃⁻ concentrations of the freshly collected soil among the four defoliation treatments, with the exception of the W treatment in the June sampling where HIHF defoliation had a significantly greater (p < 0.05) soil NO₃⁻ concentration (16.2 mg N kg⁻¹) than the other three defoliation treatments (Table 2). Soil NO₃⁻ concentrations were significantly affected by watering and defoliation in both the June and July sampling periods, while the significant interaction between watering and defoliation was only found in the June sampling. Soil

exchangeable NH₄⁺ concentrations were significantly lower (p < 0.01) in the W than the AM treatment in the June sampling, but no difference existed between these two treatments in the July sampling. Similarly, soil NO₃⁻ concentrations were significantly lower (p < 0.001) in the W than the AM treatment, but only in the July sampling.

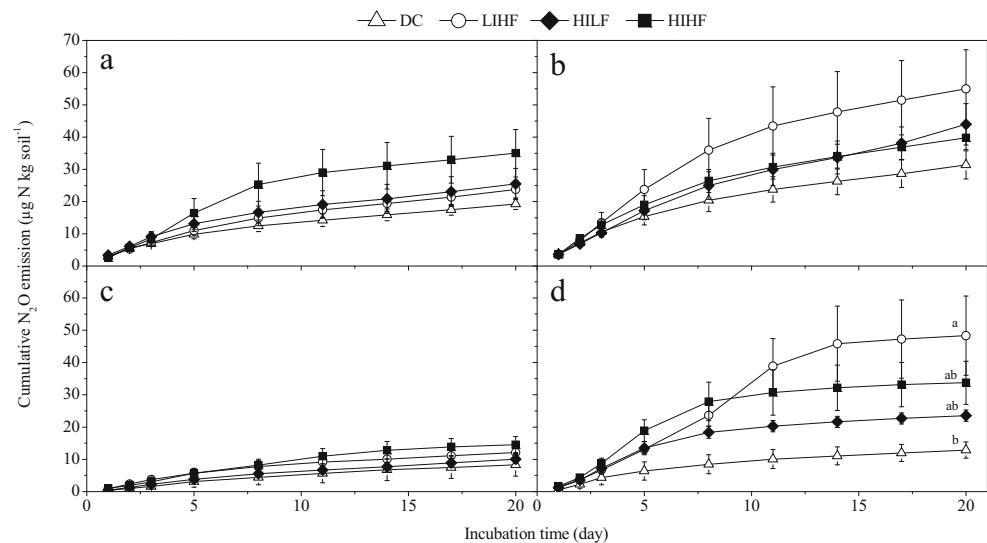
Soil N₂O emissions

Soil N₂O emissions under no N addition were only affected significantly by watering but not by defoliation or their interaction in either the June or July sampling (Table 3). Assessed across all treatments with and without N addition, soil N₂O emissions were significantly influenced by N addition, watering, and their interaction. Cumulative emissions of N₂O from soils taken from the DC treatment in the June sampling (ranging from 8.3 to 31.4 μg N kg⁻¹) were slightly lower than those (10.1–55.0 μg N kg⁻¹) from the other three defoliation treatments, but these differences generally remained non-significant (Fig. 1). The only exception to this was between the DC and HILF treatments under watering and subsequent N addition. No differences in cumulative N₂O emissions were found among the four defoliation treatments in the July sampling (Fig. 2), and N₂O emissions in the DC treatment did not consistently differ from the other three defoliation treatments.

Table 3 ANOVA df, F, and p values for the cumulative N₂O emission relative to the fixed effects of defoliation, moisture, and N addition for each soil sampling time

	June			July		
	df	F value	p value	df	F value	p value
Defoliation (D)	3	2.98	0.051	3	0.71	0.558
Watering (W)	1	30.2	<0.001	1	23.4	<0.001
D × W	3	0.68	0.575	3	0.35	0.791
N addition (N)	1	36.6	<0.001	1	60.9	<0.001
N × D	2	5.04	<0.001	2	0.47	0.830
N × W	6	11.3	<0.001	6	15.9	<0.001
N × D × W	6	0.57	0.751	6	1.54	0.184

Fig. 1 Cumulative soil N₂O emissions from the deferred control (DC), low intensity at high frequency (LIHF), high intensity at low frequency (HILF), and high intensity at high frequency (HIHF) defoliation treatments during the 20-day incubation. Data are shown for soils sampled on June 2015 from the treatments of **a** ambient moisture without N addition, **b** ambient moisture with N addition, **c** watered without N addition, and **d** watered with N addition. Vertical bars are standard errors of the mean ($n = 4$)



Cumulative N₂O emissions were significantly lower in soil from the W than the AM treatment, regardless of sampling time (Figs. 1 and 2, Table 4). The addition of N enhanced cumulative N₂O emissions in each defoliation treatment, with increments from the AM treatment in the June sampling ranging from 14 to 132 % (averaging 64.4 %), which was lower than that observed in the W treatment that ranged from 56 to 299 % (averaging 163 %) (Fig. 1, Table 4). Increments of N₂O from the soil removed in the AM and W treatments in the July sampling ranged from 47.4 to 80.9 % (average = 66.8 %) and from 56.5 to 164 % (average = 111 %), respectively (Fig. 2, Table 4).

Unlike N₂O, the addition of N decreased cumulative CO₂ emissions in the AM treatment (a reduction of 56 mg C kg⁻¹ in June and 31 mg C kg⁻¹ in July) but increased CO₂ emissions in the W treatment (an increase of 13 mg C kg⁻¹ in June and 41 mg C kg⁻¹ in July) (Table 4). Cumulative N₂O emissions

increased linearly with cumulative CO₂ emission in both the no N addition ($R^2 = 0.60$, $p < 0.001$; Table 5) and N addition ($R^2 = 0.47$, $p < 0.01$; data not shown) treatments.

Relationships between soil N₂O emissions and soil properties

In the absence of N addition, cumulative N₂O emissions increased linearly with both field soil NO₃⁻ concentration ($R^2 = 0.28$, $p < 0.05$; Table 5) and the change in soil TDN concentrations during incubation ($R^2 = 0.49$, $p < 0.01$). Cumulative N₂O emissions also decreased linearly with field soil pH ($R^2 = 0.45$, $p < 0.01$), soil moisture content ($R^2 = 0.46$, $p < 0.01$), field soil DOC ($R^2 = 0.46$, $p < 0.01$), and TDN concentrations ($R^2 = 0.58$, $p < 0.01$) as well as the change in concentrations of soil exchangeable NH₄⁺ ($R^2 = 0.43$, $p < 0.01$) and NO₃⁻ ($R^2 = 0.54$, $p < 0.01$).

Fig. 2 Cumulative soil N₂O emissions from the deferred control (DC), low intensity at high frequency (LIHF), high intensity at low frequency (HILF), and high intensity at high frequency (HIHF) defoliation treatments during the 20-day incubation. Data are shown for soils sampled on July 2015 from the treatments of **a** ambient moisture without N addition, **b** ambient moisture with N addition, **c** watered without N addition, and **d** watered with N addition. Vertical bars are standard errors of the mean ($n = 4$)

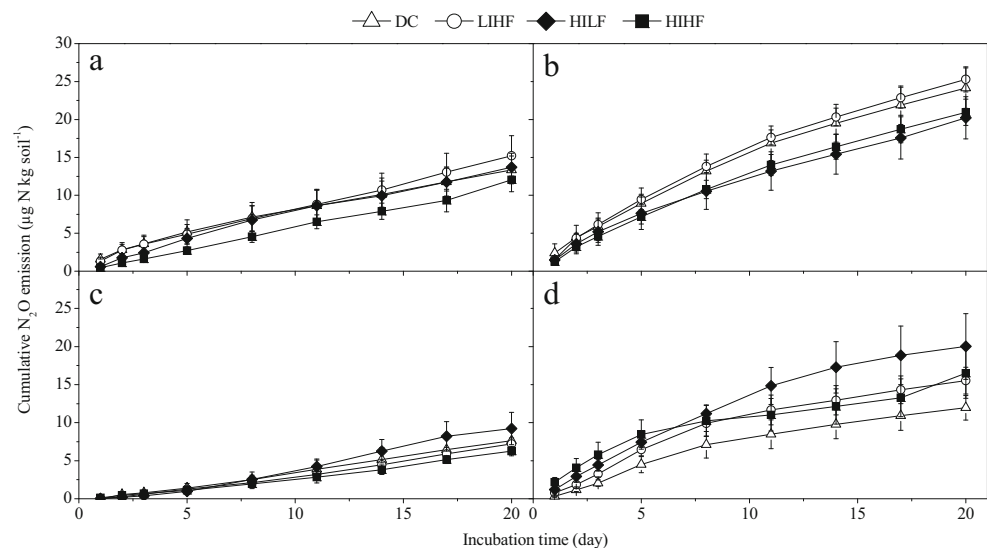


Table 4 Cumulative N₂O and CO₂ emissions and variation in soil DOC, TDN, NH₄⁺-N, and NO₃⁻-N concentrations during the 20-day incubation period, obtained by subtracting the field values from the final values

Factor	Treatment	June		July	
		Without N addition	With N addition	Without N addition	With N addition
N ₂ O (μg N kg ⁻¹)	AM	25.9 ± 2.6 b *	42.5 ± 4.0 a *	13.6 ± 0.9 b *	22.6 ± 1.2 a *
	W	11.3 ± 1.3 b	29.6 ± 4.6 a	7.59 ± 0.70 b	16.0 ± 1.5 a
CO ₂ (mg C kg ⁻¹)	AM	409 ± 13 a *	353 ± 9 b	291 ± 8 a *	260 ± 9 b
	W	322 ± 12 b	365 ± 15 a	228 ± 6 b	269 ± 7 a
ΔDOC (mg C kg ⁻¹)	AM	-63.4 ± 4.0 a	-80.5 ± 3.5 b	-23.9 ± 1.8 a *	-25.3 ± 2.9 a *
	W	-52.2 ± 4.3 a *	-73.8 ± 4.5 b	-71.7 ± 9.7 a	-113 ± 6.8 b
ΔTDN (mg N kg ⁻¹)	AM	21.5 ± 1.3 b *	36.4 ± 1.4 a *	16.0 ± 0.9 b *	33.3 ± 1.0 a *
	W	13.4 ± 1.7 b	29.1 ± 2.0 a	12.3 ± 1.2 b	26.5 ± 1.1 a
ΔNH ₄ ⁺ -N (mg N kg ⁻¹)	AM	-5.19 ± 0.42 a	-24.9 ± 0.5 b	3.03 ± 0.30 a	-14.5 ± 0.9 b
	W	-3.75 ± 0.42 a *	-16.0 ± 0.4 b *	5.27 ± 0.57 a *	-15.6 ± 0.2 b
ΔNO ₃ ⁻ -N (mg N kg ⁻¹)	AM	-11.3 ± 0.2 b	-10.4 ± 0.2 a	30.3 ± 1.6 b	52.9 ± 1.7 a
	W	19.2 ± 2.2 b *	35.3 ± 2.1 a *	28.8 ± 2.2 b	54.8 ± 1.7 a

Mean ± standard error (n = 16). The different lowercase letters within the same row for each sampling time indicate differences between treatments with and without N addition for each watering treatment at p < 0.05

AM ambient moisture, W watering

*p < 0.05, significant differences between AM and W treatments for each parameter of each N addition in a sampling time

For all treatments under AM conditions, cumulative N₂O emissions declined linearly with a greater change in soil DOC (R² = 0.67, p < 0.001), exchangeable NH₄⁺ (R² = 0.52, p < 0.01), and NO₃⁻ (R² = 0.67, p < 0.001) concentrations. Within the W treatment, a negative linear relationship was also found between cumulative N₂O emissions and the change in

concentration of soil exchangeable NH₄⁺ across all treatments (R² = 0.40, p < 0.01).

Discussion

Effects of defoliation on N₂O emissions

The lack of defoliation effects on soil N₂O emissions in each sampling (Figs. 1 and 2, Table 3) is consistent with Braun et al. (2013), in which grasses were cut to 7.5 cm height in another mixed grassland in Western Canada. The lack of defoliation effects was likely due to similar soil moisture content and inorganic N concentrations between the defoliation and control treatments. Soil moisture and N availability, together with soil available C, are important factors regulating nitrification and denitrification as well as changes in N₂O emission after defoliation (e.g., Neftel et al. 2000; Rafique et al. 2012; Jackson et al. 2015). In this study, fresh soils often had similar water content among the different defoliation treatments (Table 1) and were used for laboratory incubation by adjusting to a constant soil-water content. In addition, although we found that HIF defoliation could elevate field soil NO₃⁻ concentration under W, our overall analysis indicated that defoliation did not alter TDN, exchangeable NH₄⁺, or NO₃⁻ concentrations in soil (Table 2), and no differences in soil DOC concentrations were found among any defoliation treatments.

Our findings are consistent with prior studies on soil exchangeable NH₄⁺ and NO₃⁻ concentrations (Jackson et al.

Table 5 Relationships between cumulative N₂O emission and other parameters from the laboratory incubation experiments without N addition

Parameter	Equation	R ²	p
CO ₂	y = -12.4 + 0.09x	0.60	<0.001
pH ^a	y = 44.9 - 4.34x	0.45	0.004
Soil moisture ^a	y = 24.8 - 0.41x	0.46	0.004
Required water addition for incubation	y = 0.14 + 0.41x	0.46	0.004
DOC ^a	y = 25.3 - 0.05x	0.46	0.004
TDN ^a	y = 35.6 - 0.70	0.58	0.001
NH ₄ ⁺ -N ^a	-	0.00	0.970
NO ₃ ⁻ -N ^a	y = 7.19 + 0.78x	0.28	0.036
ΔDOC ^b	-	0.01	0.710
ΔTDN ^b	y = -2.10 + 1.05x	0.49	0.003
ΔNH ₄ ⁺ -N ^b	y = 14.4 - 1.09x	0.43	0.006
ΔNO ₃ ⁻ -N ^b	y = 18.6 - 0.44x	0.54	0.001

^a Field soil properties

^b Delta (Δ) = changes in concentrations during the 20-day incubation (calculated by subtracting the initial values from the final values after the 20-day incubation)

2015) as well as those on soil-soluble C concentrations (Bazot et al. 2005). In contrast, some previous studies have demonstrated that defoliation increases root exudation and rhizospheric microbial biomass shortly after (i.e., from several hours to several days) grass cutting, which may enhance soil C and N availability during this period (Murray et al. 2004; Hamilton et al. 2008). Unfortunately, we did not collect soil samples immediately after grass cutting in June (the previous cutting event was on the 27th of May 2015) and July (8th of July 2015) sampling, within the LIHF, HILF, and HIHF treatments, and thus may have missed the opportunity to detect the immediate effect of defoliation on C and N availability. While herbage yield in the LIHF treatment was lower ($p < 0.05$) than that in the DC treatment (Bork et al., submitted), this difference may not be sufficient to affect DOC or inorganic N concentrations. Therefore, we suggest that the insignificant impact of defoliation on soil N₂O emissions was likely due to the undifferentiated levels of soil C and N availability for nitrifiers and denitrifiers.

Effects of watering on N₂O emissions from soils without N addition

Lower N₂O emissions (Figs. 1 and 2) but greater initial soil DOC and TDN concentrations (Table 2) under watering contradict findings from previous studies that show positive effects of increased precipitation (or irrigation) on soil N₂O emissions and DOC concentrations, along with enhanced anaerobic denitrification under elevated soil moisture content (e.g., Du et al. 2006; Chen et al. 2013; Liu et al. 2015). In our study, the positive linear relationships between emissions of N₂O and CO₂ and between N₂O emissions and the depletion of NO₃⁻ in the soil (Table 5) suggest that denitrification could be an important mechanism for N₂O production. In the absence of an external NO₃⁻ source, soil NO₃⁻ is mainly produced from nitrification of NH₄⁺, and the depletion of exchangeable NH₄⁺ positively influenced the consumption of NO₃⁻ and associated N₂O emissions in our study (Table 5), further suggesting a potential joint role of nitrification/denitrification in regulating N₂O emissions.

As expected, watering increased soil moisture content relative to the AM treatment (Table 1) and a greater amount of water was required for the AM treatment to adjust soil moisture content to 60 % WHC for incubation (Table 5). The latter may induce a greater activation of soil microbes and hence increase mineralization of soil organic matter (Orchard and Cook 1983). In our study, the higher rate of soil CO₂ emission in the AM treatment (Table 4) confirms this. This finding is consistent with Harrison-Kirk et al. (2013), who observed higher C mineralization and N₂O flushes following rewetting in a very dry than a moderately dry silt loam or clay loam soil. Our result suggests that N₂O loss immediately after rainfall would be greater in the AM than in the W treatment in the field

owing to the higher stimulating effect caused by rewetting in the drier soil (in the AM treatment).

Aside from the direct effects of elevated moisture availability from watering, we also found that watering increased soil pH (Table 1). Greater soil pH in the W treatment is likely due to the decreased soil oxygen content associated with moist conditions, and which might lead to more hydrogen ion (H⁺) consumption by denitrification compared to the AM treatment (Zhang et al. 2014). However, field soil pH was also negatively related to soil N₂O emission (Table 5). It has been suggested that N₂O reductase can be inhibited by a low pH level and the N₂O/(N₂O + N₂) ratio can increase with greater soil pH, and N₂ may be preferentially produced over N₂O in alkaline soils (Simek and Cooper 2002; Cuhel et al. 2010). Therefore, greater soil pH (i.e., above 7) within the W treatment could cause more complete denitrification and hence lead to more N₂O consumption relative to the AM treatment.

Influences of N addition on soil N₂O emission

The greater rate of N₂O emissions after the addition of 20 kg N ha⁻¹ of (NH₄)₂SO₄ (Table 4) is consistent with Hutchinson and Brams (1992) who applied 52 kg N ha⁻¹ as (NH₄)₂SO₄. Greater nitrification generally occurs in N fertilized soils compared to non-fertilized soils (Hutchinson and Brams 1992; Li and Lang 2014; Tian et al. 2014). In the current study, increased depletion of exchangeable NH₄⁺ and greater accumulation of NO₃⁻ were often accompanied by higher N₂O emissions following N addition (Table 4), indicating that increased nitrification may be the main mechanism responsible for increasing N₂O emissions. Additionally, although N₂O emissions were typically greater in the AM than the W treatment, increments in N₂O emissions under N addition were always greater in the W (111–163 %) than the AM (64–67 %) treatment. It is worth noting that N addition elevated CO₂ emissions in the W treatment but reduced CO₂ emissions in the AM treatment (Table 4); the latter is consistent with Mo et al. (2008) who evaluated N impacts on an acidic forest soil.

Decreases in CO₂ emissions are likely due to the negative effect of N addition on C mineralization, and this effect may be significant in low-pH soils that have low microbial activities (Fu et al. 1987). Relative rates of consumption of DOC after N addition were lower in the AM (1–17 %) than in the W (22–41 %) treatment (Table 4), implying that greater heterotrophic activity occurred in the W treatment, and the positive effect of N addition on CO₂ emissions in the W treatment may therefore indicate that the enhancement of heterotrophic denitrification could be responsible for increasing N₂O emissions after N addition. As a result, we postulate that the elevated N₂O emissions after N addition were caused mainly by increased nitrification within the ambient moisture treatment, together with increased nitrification and denitrification in plots receiving watering. Further research is needed to

identify the specific N transformation processes and related microbial activities within soil containing different moisture levels.

Conclusions

We conclude that different defoliation intensities and frequencies did not influence field soil C and N availability or soil N₂O emissions in a mixed grassland of Western Canada. In contrast, relative to ambient moisture conditions, watering and associated increases in soil-water content led to greater pH, as well as higher soil DOC and TDN concentrations, but lower N₂O emissions. The addition of N under laboratory conditions induced greater N₂O emissions and increased consumption of soil DOC but led to lower CO₂ emissions in soils from the ambient moisture treatment. Enhancement ratios of N₂O emissions affected by N addition were greater in the watering than the ambient moisture treatment. The results of this study have implications for evaluating the potential impact of increased precipitation and N deposition under climate change on grassland soil properties and associated N₂O emissions. Further research using the ¹⁵N-tracer technique and molecular techniques should be conducted to investigate the relative contributions of nitrification and denitrification to N₂O evolution and to establish possible links between nitrifier and denitrifier communities and N₂O production. In addition, nitrite (NO₂⁻) accumulation, especially after the application of ammonium-based nitrogen fertilizers (Ma et al. 2015), is a key factor regulating N₂O production processes, and the dynamics in soil NO₂⁻ concentrations should be addressed in future studies.

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