The effect of drainage on CO₂, CH₄ and N₂O emissions in the Zoige peatland: a 40-month in situ study

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SUMMARY

The Zoige peatland on the Qinghai-Tibet Plateau, the largest alpine peatland in the world, is currently experiencing unprecedented water stress due to climate change and human activities. However, the consequences for emissions of greenhouse gases have not been well studied. We conducted a 40-month in situ field experiment (including deep, shallow and control water table treatments) to examine the effect of drainage on greenhouse gas emissions (CO₂, CH₄ and N₂O). The results showed that drainage significantly increased the emission rates and the total cumulative emissions of CO₂ (i.e., ecosystem respiration) and N₂O, and reduced CH₄ emissions. However, under drained conditions, the cumulative emissions of CO₂ and N₂O increased significantly during the growing season (May to September) only, whereas the cumulative emissions of CH₄ decreased significantly during both the growing season and the non-growing season (October to April). In addition, drainage significantly increased the biomass of aerobic bacteria and methanotrophs. These results indicate that emissions of greenhouse gases in the Zoige peatland are sensitive to short-term drainage and that future studies should consider the response of greenhouse gas fluxes to environmental changes in the non-growing season.

KEY WORDS: greenhouse gases, PLFA biomass, Qinghai-Tibet Plateau, soil microbes, water table

INTRODUCTION

Carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) are important atmospheric greenhouse gases (IPCC 2014). Peatlands are particularly active in mediating fluxes of these three gases between the soil and the atmosphere (Martikainen et al. 1993). Although covering only 3 % of the global land area, peatlands store about 30 % of the global soil carbon pool (Gorham 1991, Grasset et al. 2016) and release 9 % of global CH₄ emissions (Harriss et al. 1993). Emissions of N₂O from peatlands to the atmosphere are relatively low but the global warming potential of N₂O is 265 times higher than that of CO₂ (IPCC 2014). The production of these gases is highly dependent on oxygen availability, which is closely associated with water table height in peatlands (Aerts & Ludwig 1997, Veber et al. 2018). Specifically, the production of CO₂ is favoured under aerobic conditions whereas CH₄ is produced under strictly anaerobic conditions only, and N₂O is released from both nitrification that requires aerobic conditions and denitrification that requires anaerobic conditions (Olefeldt et al. 2017). Therefore, the position of the water table is crucial to CO₂, CH₄ and N₂O dynamics because water table fluctuations will alter the availability of oxygen in the peat layers.

The Zoige peatland in the eastern part of the Qinghai-Tibet Plateau is the largest high-altitude peatland in the world (Xiang et al. 2009). Soil organic carbon storage in the Zoige peatland is estimated to be 0.48 Pg (Chen et al. 2014), corresponding to...
approximately 6.2% of the soil organic carbon storage in China (Cui et al. 2015, Wu et al. 2017). The Zoige peatland is similar to many other peatlands globally in that it is currently experiencing unprecedented water stress as a result of both climate change and direct disturbance by humans. For example, the average temperature in the area has increased by 0.4°C per decade since 1970 (Yang et al. 2014), the associated potential evapotranspiration has increased by 8 mm per decade (Li et al. 2014) and annual precipitation has decreased by an average 22–28 mm per decade (Yang et al. 2014). Moreover, this peatland has experienced intensive human activities, especially drainage for pasture expansion since the 1960s. Nearly 1000 artificial drainage channels with a total length of 2864 km have drained nearly 41% of the total area of the Zoige peatland (Dong et al. 2010). Thus, climate change and intensive human activities have resulted in reduced water input to, and increased water output from the Zoige peatland. This caused lowering of the water table by 5–15 cm from the 1970s to the 2000s (Xiang et al. 2009).

Consistent with findings in many other peatlands (Aerts & Ludwig 1997, Hargreaves & Fowler 1998, Regina et al. 1999, Blodau & Moore 2003, Strack et al. 2006, Dinsmore et al. 2009, Järveoja et al. 2016), drainage has been found to increase the emissions of CO₂ and N₂O but reduce the emissions of CH₄ in the Zoige peatland (Yang et al. 2014, Zeng & Gao 2016, Yang et al. 2017). The drainage effect on gas emissions from the peat soils has been examined using peat cores in which a persistent lowering of the water level has been artificially sustained. This might have consequences for interpretation and possibilities for extrapolation of the results, because the water table cannot remain at a constant level in the field. In addition, there are few available data on the response of greenhouse gas emissions in the Zoige peatland to drainage during the non-growing season. Therefore, in situ field experiments that cover both the growing season and the non-growing season are needed.

The objective of the present study was to examine the influence of relatively long-term (i.e., several years) drainage on the emissions of CO₂, CH₄ and N₂O in the Zoige peatland. In particular, we aimed to determine whether the drainage effect differed between the growing season and the non-growing season. We conducted an in situ field experiment that consisted of three treatments: deep, shallow and control water tables, which were achieved by experimental drainage with ditches 50 cm, 20 cm, and 0 cm (intact) deep, respectively. We monitored the position of the water table, soil moisture, soil temperature and gas flux rate for 40 months across four years. We also quantified the biomass of microbes, i.e., aerobic bacteria and methanotrophs, to further investigate the mechanisms behind greenhouse gas emissions.

METHODS

Site description
This study was conducted on the Zoige peatland, at a site 10 km from Hongyuan County town (32° 48' N, 102° 33' E) in Sichuan Province (Figure 1). The altitude is approximately 3500 m above sea level. The climate is cold and continental, characterised by a short summer and a long winter. There is no evident spring or autumn. According to data collected from 1970 to 2016 at the Hongyuan County Climate Station (located 3 km from the study site and at the same altitude), the annual mean temperature was 1.7°C, with maximum and minimum monthly means of 11.1°C and -9.4°C observed in July and January, respectively. The annual mean precipitation is 756 mm, of which 80% occurs between May and August and less than 5% is in the form of snow. The area of peatland in Hongyuan County is approximately 492 km², equal to approximately 11% of the total area of the Zoige peatland. Peat thickness in this area ranges from 0.3 to 10 m with a mean dry mass accumulation rate of 0.03 g m⁻² yr⁻¹, an average pH of 6.6–7.0, an average soil organic carbon content of 350 g kg⁻¹ and a soil total nitrogen content of 15 g kg⁻¹ (Wu et al. 2017). Vegetation cover in the study site is over 90% and consists mostly of sedge species including Scirpus pumilus, Blysmus sinocompressus, Carex multiensis, Kobresia humilis.
and *Kobresia setchwanensis*. Rushes (*Juncus leucanthus* and *Juncus allioides*) are common but at relatively low abundance. Grasses such as *Poa pratensis*, *Deschampsia caespitosa* and *Agrostis matsumurae*, and forb species such as *Chamaesium paradoxum* and *Anemone trullifolia var. linearis*, are also abundant. Bryophytes are not apparent in our study site (Cao et al. 2017). In addition, this peatland has been under intensive grazing for decades. Yaks are the main grazing livestock (Xiang et al. 2009).

**Experimental set-up**
The field experiment was set up as a single-factor three-level block design, and each block involved deep and shallow water table treatments as well as a control. Each treatment was replicated six times (six blocks), resulting in a total of 18 plots.

The water table level was manipulated by ditching at a location with homogeneous peatland. In April 2013, a 240 m long, 0.5 m wide and 1 m deep drainage ditch (hereafter called the major ditch) was dug in a fenced, flat 2 ha area of peatland. The major ditch was connected to a small river. Eighteen 6 × 6 m plots were established (at regular intervals with 30 m between adjacent plots) approximately 30 m away from the major ditch, with nine plots distributed along each side of the major ditch. Six plots were drained by 50 cm deep ditches and six plots by 20 cm deep ditches, which served as deep and shallow water table treatments, respectively, and the remaining six plots were kept intact, serving as controls (see Figure 2).

**Sampling and measurement**
We installed perforated polyvinyl chloride pipes (1.5 m in length and 5 cm in diameter) at the centre of each plot to record the height of the water table. The height of the water table in each pipe was measured using a 1.5 m long ruler every three to ten days during the study period, except in winter when the soil was frozen (from November to March).

Emissions of CO₂, CH₄ and N₂O were sampled using the static chamber method (Yang et al. 2014).
Figure 2. Diagram of the experimental design showing how the drainage treatments were deployed.

Once every month during the growing season (May to September) and once every two months in the non-growing season (October to April) from August 2013 to November 2016 (i.e., 40 months). The bottomless chambers (50 cm in length, width and height) were made of stainless steel and were covered with polystyrene (Styrofoam) insulation to prevent heat exchange during sampling. At the top surface of the chamber, a pipe (5 mm diameter) connected the chamber headspace to the atmosphere, with additional piping located inside the chamber to maintain the air-pressure balance. During each flux measurement, the chambers were placed on base collars made of stainless steel that had been inserted to a depth of 10 cm in the centre of each field plot. All base collars were left in place throughout the experimental period. For the determination of CO₂, CH₄ and N₂O, air samples were collected between
09:00 and 11:00 hrs on each sampling date, when soil temperature (4.3 °C) was similar to the daily mean soil temperature (4.7 °C). Before sampling, the chambers were closed for ten minutes to establish an equilibrium state (Zeng & Gao 2016). Then, 100 mL headspace samples were extracted into airtight, pre-evacuated vacuum bags at ten-minute intervals (i.e., 10, 20, 30 and 40 minutes after chamber closure). When sampling was finished, the chamber was immediately removed from the base collar to minimise any effects on soil conditions and plant growth, and the gas samples were brought to the laboratory. CO₂, CH₄ and N₂O concentrations were then determined by gas chromatography (Agilent 7890A) (Wang et al. 2017). Finally, the emission rate $F$ of CH₄, CO₂ and N₂O was calculated as:

$$F = \frac{dc}{dt} \cdot \frac{M \cdot P \cdot T}{V_0 \cdot P_0 \cdot T} \cdot H$$  \hspace{1cm} [1]

where $dc/dt$ is the rate of gas concentration change (the sample sets were rejected unless they yielded a linear regression $R^2$ value greater than 0.9); $M$ is the molar mass ($g\,mol^{-1}$) of each gas; $P$ is the atmospheric pressure at the sampling site; $T$ is the absolute temperature at the time of sampling; $V_0$, $P_0$, and $T_0$ are the molar volume, atmospheric pressure and absolute temperature under standard conditions ($V_0 = 22.4\,L\,mol^{-1}$, $P_0 = 101\,kPa$, $T_0 = 273.15\,K$); and $H$ is the chamber height (50 cm) above soil surface defining the actual gas volume in the chamber (Yang et al. 2014). On each sample date, air temperature and soil temperature at 5 cm depth were measured in each plot using a digital thermometer (JM624, Tianjin Jinming Instrument Co. Ltd., Tianjin, China). Soil moisture content at 5 cm depth was measured in each plot using a TDR 300 soil moisture meter (Spectrum Technologies, Inc., USA).

In order to explore the microbial mechanisms underlining the drainage effect on gas emissions, five soil cores were collected from the 0–20 cm layer using a cylindrical soil auger (20 cm long and 5 cm in diameter) from each plot in July 2016. The soil samples were mixed and then stored at -20 °C prior to phospholipid fatty acids (PLFA) analyses by gas chromatography (Bossio & Scow 1998, Frostegård et al. 2011, Shao et al. 2016). Lipids were extracted from 5 g of dry-weight-equivalent fresh soil using a chloroform:methanol:phosphate buffer (1:2:0.8). Phospholipids were then separated into neutral, glyco- and phospholipids using solid-phase extraction columns by eluting with chloroform (CHCl₃), acetone and methanol, respectively. Subsequently, the phospholipids were subjected to a mild-alkali methanolysis to recover fatty acid methyl esters. Samples were then redissolved in 200 ml of hexane containing nonadecanoic acid methyl ester (19:0) as an internal standard and were analysed using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an Ultra 2-methylpolysiloxane column. A 2-μl injection with a 1:50 split was analysed at an oven temperature of 260 °C, a flame ionisation detector temperature of 300 °C and pressure 107 PSI at a constant flow rate of 0.4 mL min⁻¹. Peaks were identified using bacterial fatty acid standards and MIDI peak identification software (MIDI Inc., Newark, DE, USA). Concentrations of each PLFA were standardised using a 19:0 internal standard. The biomass of aerobic bacteria was estimated by summing fatty acids, 16:0, 17:0, 15:0 2OH, 15:0 anteiso, 15:0 iso, 15:0 3OH, 15:0 anteiso, 16:0 iso, 16:1 iso G, 16:1 iso H, 17:0 2OH, 17:0 anteiso and 17:0 iso (Jain et al. 1997, Makula 1978, Tunlid 1992, Vestal & White 1989). The biomass of methanotrophs was determined by 16:1 ω5c (Hill et al. 2000).

**Data analysis**

We calculated monthly mean values for May to September (5 months) as growing season mean values and monthly means for January, March and November (3 months) as non-growing season mean values, and further averaged the mean values for growing season and non-growing season (over 4 years) as 40-month mean values. Cumulative emissions of CO₂, CH₄ and N₂O were calculated following the protocols of Ma et al. (2009), whereby:

$$\text{Cumulative emissions} = \sum_{i=1}^{n} F_i \cdot D_i$$  \hspace{1cm} [2]
where $D_i$ indicates the number of days, $F_i$ is the emission rate measured on the $i^{th}$ sampling date, and $n$ is the number of sampling intervals. A linear mixed model (LMM with drainage and date (or season) as fixed factors, block as random factor), was used to determine the effects of drainage, measurement date (or season) and their interaction (drainage × date or drainage × season) on gas emissions (or cumulative emissions) over the experimental period. LMM with drainage as a fixed factor and block as random factor was also used to examine the effect of drainage on the cumulative emissions of each gas, PLFA biomass of aerobic bacteria, and PLFA biomass of methanotrophs. Normality of the LMM residuals was tested by the Shapiro-Wilk normality test. Once a significant drainage effect was detected, post hoc Tukey’s HSD tests were used to further determine the differences among the treatments. All statistical analyses were performed in R 3.3.1 (R Core Team 2017).

**RESULTS**

**Water table height, soil moisture and temperature**

The height of the water table (relative to the soil surface) fluctuated greatly during the study period, ranging from -90 cm to -16 cm, -93 cm to -18 cm, and -93 cm to 0.45 cm (i.e., above the soil surface) in the deep, shallow and control water table treatments, respectively (Figure 3a). The difference in water table level between the deep treatment and the control ranged from -3 to 33 cm, and between the shallow treatment and the control from -2 to 24 cm. The mean water table height was on average 12 cm and 15 cm lower in the shallow and deep treatment, respectively, than in the control plots (Figure 3a).

Corresponding to the variations in water table height, the mean soil moisture content 5 cm below the ground surface over the 40 months was 11 % and 14 % lower in the shallow and deep water table treatments, respectively, than in the control plot.
(Figure 3b). Soil moisture content was on average 8% and 14% (for the growing season) and 13% and 15% (for the non-growing season) lower in the shallow and deep water table treatments, respectively, than in the control (Figure 3b). Mean soil temperature 5 cm below the ground surface was on average 14 °C lower during non-growing seasons than during growing seasons (Figure 3c), but there were no significant differences in soil temperature among the different treatments (Table A1, Figure 3c).

**CO₂ emissions**

The average CO₂ emission rate was 372 mg m⁻² h⁻¹, 338 mg m⁻² h⁻¹ and 310 mg m⁻² h⁻¹ in the deep, shallow and control treatments, respectively, over 40 months (Figure 4a). Drainage generally increased the CO₂ emission rate but the drainage effect varied from month to month, as indicated by the significant interaction between drainage treatment and month (Table A2). CO₂ emission rate was significantly higher in drained plots than in the control in May 2014, June 2014, September 2014 and September 2015 (Figure 4a). Drainage significantly increased the cumulative emission of CO₂ during the growing season but not during the non-growing season (Figure 5a). Specifically, the cumulative CO₂ emission was 24% higher in the deep treatment than that in the control. Drainage significantly increased the total cumulative CO₂ emission over the study period ($F_{2,10} = 4.67$, $P = 0.037$), with emissions in the deep and shallow water table treatments 20% and 9%, respectively, higher than in the control (Figure 5a).

![Figure 4](image-url)  
**Figure 4.** Monthly emission rates of (a) carbon dioxide (CO₂), (b) methane (CH₄), and (c) nitrous oxide (N₂O) for the control, shallow and deep water table treatments during the experiment. Error bars represent ±1 Standard Error.
CH₄ emissions

The average CH₄ emission rate was 0.05 mg m⁻² h⁻¹, 0.05 mg m⁻² h⁻¹ and 0.14 mg m⁻² h⁻¹ in the deep, shallow and control treatments, respectively, over 40 months (Figure 3b). In contrast to CO₂ emissions, drainage generally reduced CH₄ emission rates during the study period (Figure 4b). Drainage significantly reduced CH₄ emissions during both the non-growing season and the growing season (Table A3, Figure 5b). Over 40 months, drainage significantly (F₂,10 = 8.86, P = 0.006) reduced the total cumulative emission of CH₄ by 64 % and 63 % in the deep and shallow treatments, respectively, relative to the control (Figure 5b).

Figure 5. Cumulative emissions of (a) carbon dioxide (CO₂), (b) methane (CH₄), and (c) nitrous oxide (N₂O) during the growing seasons, the non-growing seasons and over 40 months, for the control, shallow and deep water table treatments. Different letters indicate a significant difference among the control, shallow and deep water table treatments (P < 0.05). Error bars represent ±1 Standard Error.
**N₂O emissions**

The average N₂O emission rate was 11.8 µg m⁻² h⁻¹, 13.4 µg m⁻² h⁻¹ and 6.0 µg m⁻² h⁻¹ in the deep, shallow and control treatments, respectively. Drainage significantly increased the N₂O emission rate (Figure 4c), the cumulative emission during the growing season (Figure 5c) and the total cumulative emission over the study period (F₂,₁₀ = 7.61, P = 0.010, Table A3). Specifically, the cumulative emission of N₂O during the growing season was 198 % higher in the shallow treatment than in the control. The total cumulative emission of N₂O over 40 months was 108 % and 139 % higher in the deep and shallow treatments, respectively, than in the control (Figure 5c).

**PLFA biomass of microbes**

Drainage significantly increased the biomass of aerobic bacteria (F₂,₁₀ = 6.44, P = 0.016) and methanotrophs (F₂,₁₀ = 26.33, P < 0.001) (Figure 6). Aerobic bacterial biomass was 12 % and 4 % higher in the deep and shallow water table treatments, respectively, than in the control (17.3 nmol g⁻¹) (Figure 6a). Methanotroph biomass was 33 % and 18 % higher in the deep and shallow water table treatments, respectively, than in the control (Figure 6b).

**DISCUSSION**

We found that drainage increased CO₂ and N₂O emissions, which is consistent with the results of many other studies (e.g., Martikainen et al. 1993, Regina et al. 1999, von Arnold et al. 2005, Dinsmore et al. 2009, Yang et al. 2014, Yang et al. 2017, Wang et al. 2017). In the present study, about 20 cm of water table decline increased growing-season CO₂ emissions by 24 %, which is comparable to results from a recent study conducted in the same peatland employing soil cores (Yang et al. 2017), where the CO₂ emission rate increased by 23 % when the water table height decreased from 0 cm to -20 cm. The N₂O emissions in our study are comparable to those observed by Gao et al. (2014) for several reasons. First, drainage reduced soil moisture and, thus, enhanced soil aeration and oxygen concentrations. This may facilitate the growth and activity of aerobic microbes which further stimulate soil organic carbon decomposition (Hribljan et al. 2014, Wang et al. 2017) and nitrification (Aerts & Ludwig 1997, Gao et al. 2014, Liimatainen et al. 2018), thereby increasing the emissions of CO₂ and N₂O. Secondly, drainage also changed the species composition and structure of the plant community. In a previous study conducted at the same experimental site, Cao et al. (2017) demonstrated that drainage increased above-ground plant biomass. The changes in the plant community increased CO₂ emissions by altering CO₂ production (Xia et al. 2009). Thirdly, drainage increased the abundance of soil animals such as earthworms, soil mites, springtails and nematodes (Wu et al. 2017, Wei et al. 2018), which may contribute part of the increase in CO₂ emissions.
However, the significant effect of drainage on CO₂ and N₂O emissions was observed only in the growing season. In the non-growing season, low temperatures may limit the activity of microbes and the decomposition of organic matter (Updegraff et al. 2001) in both drained and undrained plots. During our experimental period, mean monthly temperature in the non-growing season (October to April) was 14 °C lower than in the growing season (May to September). Consequently, only 22 % of the CO₂ was released in the non-growing season.

We also found that drainage significantly reduced CH₄ emissions, which is consistent with the results of many other studies (e.g., Laine et al. 2009, Couwenberg et al. 2011, Yrjälä et al. 2011, Yang et al. 2014, Zeng & Gao 2016, Wang et al. 2017, Yang et al. 2017). A decline in water table height of approximately 20 cm reduced growing-season CH₄ emissions by 70 %, which is a greater reduction than was observed in previous studies in the Tibetan peatlands employing soil cores (47 % in Yang et al. 2014, 57 % in Wang et al. 2017). The underlying mechanisms were: 1) a decrease in soil moisture and an associated increase in aerobic conditions that suppressed populations of methanogens that need anaerobic conditions (Dijkstra et al. 2012) but might facilitate the growth and activity of methanotrophs that consume CH₄ (Roulet et al. 1993); 2) a shift of the plant community from sedges to forbs resulting from drainage (Cao et al. 2017) may limit the transport of CH₄ from the soil to the atmosphere (Laine et al. 2009, Yrjälä et al. 2011). The fact that drainage significantly reduced CH₄ emissions in the non-growing season may be due to the thinner frozen layer in the drained plots (Dörsch et al. 2004), although we did not investigate changes in the frozen layer within the present study.

CONCLUSION

This study demonstrated that in situ drainage treatments increased CO₂ and N₂O emissions, and reduced CH₄ emissions. This finding is consistent with the results of many other studies, although the sampling frequency in our study was relatively low (every one or two months) during the 40-month period. Importantly, the significant drainage effect on CH₄ emissions indicates that greenhouse gas fluxes respond to climate change and anthropic disturbance during the non-growing season. This should be considered within future studies in the Zoige peatland.

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Appendix

Table A1. Results (Degrees of freedom (df), Sum of squares (SS), F values and P values) of the Linear Mixed Model (LMM) showing the effects of drainage treatment (Drainage), monitoring month (Month) and their interactions on water table height, soil moisture content and soil temperature during the monitoring years. Values are considered significant if P < 0.05 (in bold type).

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Table A2. Results (Degrees of freedom (df), Sum of squares (SS), F values and $P$ values) of the Linear Mixed Model (LMM) showing the effects of drainage treatment (Drainage), monitoring month (Month) and their interactions on emission rates of carbon dioxide (CO$_2$), methane (CH$_4$), and nitrous oxide (N$_2$O) during the monitoring years. Values are considered significant if $P<0.05$ (in **bold** type).

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<tr>
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Table A3. Results (Degrees of freedom (df), Sum of squares (SS), F values and $P$ values) of the Linear Mixed Model (LMM) showing the effects of drainage treatment (Drainage), season and their interactions on the cumulative emissions of carbon dioxide (CO$_2$), methane (CH$_4$), and nitrous oxide (N$_2$O) during the monitoring years. Values are considered significant if $P<0.05$ (in **bold** type).

<table>
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<th>Cumulative CH$_4$</th>
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<th>Cumulative N$_2$O</th>
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<td>Drainage</td>
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<td>5.53</td>
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<td>15.07</td>
<td>19.24</td>
<td><strong>&lt;0.001</strong></td>
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<tr>
<td>Season</td>
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<td>2.70E+08</td>
<td>600</td>
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<td>2.65</td>
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<td>Drainage $\times$ Season</td>
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