

**Prolonged Flooding followed by drying increase greenhouse gas emissions differently
from soils under grassland and arable land uses**

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Highlights

1. Climate extreme impacts were investigated for arable and grassland soils
2. Arable soil emitted more N₂O under prolonged flooding/saturation
3. Grassland soil emitted more N₂O following drying
4. Overall, flooding-drying increased the GHG emissions from grassland compared to arable

Abstract

Under the predicted climate change scenarios, heavy precipitation could result in prolonged flooding (PF) and flooding-drying (FD) of soils under agriculture. The influence of PF and FD on soil greenhouse gas fluxes and NH_4^+ -N and NO_3^- -N dynamic of arable and grassland soils, which are the dominant land use types in UK soil are still unclear. A two months soil incubation experiments were conducted to find out the impact of PF and FD on soil nitrogen dynamic and greenhouse gas fluxes from arable and grassland soil. The result showed that there were less N_2O -N emissions in grassland and arable soil when soil moisture was higher than 100% water-holding capacity (WHC). Arable soil had more N_2O -N emissions when soil moisture was higher than 100% WHC compared to grassland soil due to a low pH. Grassland soil had more N_2O -N emissions when soil moisture was lower than 100% WHC compare to arable soil due to a high carbon and nitrogen. When soil moisture was greater than 100% WHC, the available NO_3^- -N in the soil controlled N_2O -N emissions of grassland more effectively. The N_2O -N emissions of grassland soil were more controlled by soil stable NH_4^+ -N and NO_3^- -N when soil moisture was lower than 100% WHC. The emissions of N_2O -N and CO_2 -C were increased with the time of FD. FD significantly increased N_2O -N, CO_2 -C, and CH_4 -C emissions in grassland soil compared to arable soil by 0.93, 2.15, and 37.29 times, respectively. The greenhouse gas (GHG) emissions need to be consider in the future when try to converting arable land use to grassland in order to increase the soil organic matter under climate change (heavy rain). Further research needs to be done to find out how to reduce the GHG emissions under climate change after transfer arable to grassland.

Keywords: prolonged flooding, flooding-drying, arable soil, grassland soil, greenhouse gas

1. Introduction

Carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) are the primary greenhouse gases (GHG) present in the Earth's atmosphere (IPCC, 2022). The atmospheric CO₂ concentration has increased to approximately 420 ppm and future rapid increase is expected to reach 550 ppm by mid-century and 1000 ppm by the end of this century (IPCC, 2022). N₂O is a long-lived GHG with a long-term global warming potential of 300 times greater than CO₂ (Carneiro et al., 2010) and is a major strong stratospheric ozone-depleting substance (Thompson et al., 2019). N₂O concentration in the atmosphere has also risen steadily since the mid-twentieth century (IPCC, 2022), from approximately 290 ppb in 1940 to 330 ppb in 2017 (Park et al., 2012). Atmospheric CH₄ continues to rise at a rate of approximately 22 Tg every year, as global sources are larger than sinks (IPCC, 2001). Agricultural soils are important source of GHG (Liu et al., 2019) which is the main source of non-CO₂ anthropogenic GHG and is responsible for 78.6 % of N₂O and 39.1 % of CH₄ emissions worldwide (IPCC, 2022).

Nitrogen (N) is a necessary nutrient in agricultural ecosystems (Fixon and West, 2002) for plant to use (Gilsanz et al., 2016), but the effectiveness of applied fertilizer N by plants is less than 40% (Chen et al., 2008). Most of applied fertilizer N is contribute to emitted N₂O. N₂O can be produced from several biological processes, including nitrification, denitrification, code nitrification, dissimilatory nitrate reduction to ammonia, nitrate assimilation, and chemo denitrification, which involve different microbial groups in soil (Harter et al., 2014). The two main microbial processes that produce N₂O are nitrification and denitrification (Hu et al., 2015; Sgouridis and Ullah, 2017). Nitrifying microbes' undertake biological oxidation of ammonium (NH₄⁺) to nitrite (NO₂⁻) and further to nitrate (NO₃⁻), and can produce N₂O by nitrification under aerobic soil conditions (Wrage et al. 2001). Denitrifying microbes reduce NO₃⁻ to NO₂⁻, nitric oxide (NO⁻), N₂O, and molecular nitrogen (N₂) by denitrification under anaerobic conditions (Köster et al., 2013). The majority of naturally occurring emissions of CH₄ can be

attributed to the biogenic processes of methanogens (methane-emitting microorganisms) as a final step in the anaerobic decomposition of organic matter (Gütlein et al., 2018) which are predominant in anaerobic areas rich in organic carbon (Conrad, 2009). CO₂ emissions come from the soil mineralization of soil organic carbon (Guo et al., 2019). In intact soil system where plants are present, CO₂ emissions originates both from microbial and plant respiration.

Soil CO₂, N₂O and CH₄ exchange are driven by aerobic and anaerobic microbial processes (Gütlein et al., 2018) which are influenced by soil properties and environmental factors such as temperature, precipitation, soil physical and chemical properties, texture, pH, oxygen concentration and nutrient availability (Miller et al., 2020). Soil pH manipulates the microbial community structure, and therefore, the decomposition or accumulation of soil organic carbon (SOC) (Malik et al., 2018) to influence the GHG emissions.

Land-use change affects soil GHG emissions due to changes in vegetation, soil hydrology and nutrient management (Gütlein et al., 2018). Grassland dominates the landscape in the United Kingdom (40%) (Caroline et al., 2017). 56506 km² of land is classed as arable and 96949 km² is classed as grasslands (improved, neutral, calcareous and acid) in the UK (Rowland et al., 2017). GHG emissions from soils are also linked to the hydrological conditions with deep water tables favouring CO₂ emissions, shallow water tables (less than ~20 cm) favouring CH₄ emissions, and fluctuating water tables potentially being conducive of N₂O emissions (Petersen et al., 2012; Poyda et al., 2016; Wilson et al., 2016).

Climate change is predicted to cause major changes in precipitation patterns with increased frequency and intensity of large rainfall events (IPCC, 2007). Flooding-drying cycle of soil may expose unavailable (physically protected) soil organic matter to microbes through breakdown of soil aggregates (Zhang et al., 2020) as well as changing the redox conditions in soils with implications for shifts in net N₂O, CH₄ and CO₂ emissions into the atmosphere.

Changes in flooding frequency of soils following future climate change will likely affect the timing and magnitude of N_2O emissions from the soil to the atmosphere (Jørgensen and Elberling, 2012). GHGs production and emissions may vary depending on soil physical properties, soil moisture status as influenced by precipitation under climate change extremes (Fay et al., 2010), and fertilization (Mazza et al., 2018). Therefore, changes in soil saturation following extreme precipitation events in future are likely to change the timing and extent of greenhouse gas emissions from soils and soil saturation is more likely to be severe in agricultural soil where precipitation interception is low compared to forest soils.

In this study, therefore, we conducted an incubation experiment to find out the effect of prolonged flooding/saturation and flooding-drying (extremely soil moisture condition) of soils on soil N_2O -N, CO_2 -C and CH_4 -C emissions and nitrogen dynamic under two grassland and arable land uses. We hypothesized that: (1) arable soil will lead to a high N_2O -N emission under prolonged flooding due to a high fertilization before soil sample; (2) flooding-drying will lead to a high N_2O -N emission in grassland and arable soil due to a mineralization of soil organic matter. The object of this study was to find out the influence of prolonged flooding and flooding-drying on soil N_2O -N, CO_2 -C and CH_4 -C emissions and ammonium (NH_4^+), nitrate (NO_3^-) and total dissolved nitrogen (TDN) under fertilized grassland and arable soil in UK.

2. Materials and methods

2.1. Study site description and soil collection

Soil samples were taken from a demonstration farm at Honeydale Farm ($51^\circ 51'\text{N}$, $1^\circ 35'\text{W}$) which is located in the Evenlode Valley, in the heart of the Cotswolds, OX7 6BJ, United Kingdom. Honeydale Farm is a 107-acre (43ha) farm in the Cotswolds. It is the home of FarmED. The nearest climate station is Little Rissington ($51^\circ 51'\text{N}$, $1^\circ 41'\text{W}$) which is 8 miles

away. The annual monthly maximum temperature (°C) is 13.41, monthly minimum temperature (°C) is 5.89. Days of air frost (days) is 46.14. Sunshine (hours) is 1631.53. Rainfall (mm) is 809.63. Monthly mean wind speed at 10 m (knots) is 10.30. General cropping is over chalk, spring and autumn cereals can be grown but the soils are especially vulnerable to nitrate leaching and attract stricter fertiliser limits. Suitable only for grassland where there is hard limestone. Lack of soil moisture is most likely limiting factor to yields (Cranfield soil and agrifood institute).

Grassland soil was restored for 4 years with 'HERBAL' Grazing Ley and used for grazed. Previous was used for arable cultivation (Wheat and Oats) over years before grass. This all-round mixture (20 species) provides wholesome and substantial forage for grazing and occasional cutting. It can provide grazing for early turnout and continues to produce forage right through the summer and autumn. Containing deep-rooting ingredients, this ley not only improves soil structure but also draws up essential vitamins and minerals for the ruminant animal (<https://www.cotswoldseeds.com/products/542/herbal-grazing-ley-four-year-drought-resistant-ley>). Arable soil was a control plot with winter wheat planted in October. It has been wheat or barley for thirty years. The soil parent material of grassland and arable soil is Oolitic limestone.

Soil were careful collected from 0-20 cm soil depth from the grassland and arable plots in May 18, 2022 (we were taking around 20 sample spots under both grassland and arable plots, and mix them as grassland and arable soil sample). Fresh soil samples were retained under a refrigerator of 4 °C after the removal of visible plant residues and stones and passed through a 2 mm sieve and homogenized. The soil properties are given in Table 1.

Table 1: Selected soil physical and chemical properties before experiment. TOC means total dissolved organic carbon. TDN means total dissolved nitrogen. TC means total carbon. TN means total nitrogen. WHC means water-holding capacity. (means with same index and indicated by same lower-case letter are not significantly different at $P \leq 0.05$ on the basis of Tukey HSD).

Soil type	NH ₄ ⁺ -N mg kg ⁻¹	NO ₃ ⁻ -N mg kg ⁻¹	TOC-C mg kg ⁻¹	TDN-N mg kg ⁻¹	TC g kg ⁻¹	TN g kg ⁻¹
Grassland	0.63a (0.07)	10.03a (0.76)	88.67b (1.10)	22.82a (0.26)	69.81b (0.95)	4.22b (0.13)
Arable	0.34a (0.06)	277.28b (4.65)	61.49a (1.76)	254.19b (4.24)	53.88a (0.58)	3.71a (0.21)
Soil type	Clay (%)	Silt (%)	Sand (%)	pH	WHC (%)	C/N
Grassland	46.54a (0.47)	33.23a (0.70)	20.23b (1.01)	7.36b (0.04)	72.42a (0.44)	16.66a (0.39)
Arable	46.12a (0.74)	39.45b (0.86)	14.43a (0.80)	6.90a (0.01)	70.52a (0.21)	14.95a (0.98)

Note: Average value (relative standard deviation), n=4.

2.2. Incubation experiment

Grassland soil and arable soil were packed into 1 L Mason Jar pot to achieve a bulk density of 0.9 g cm⁻³ (field bulk density) with a 10 cm high. Three replications were set for each land use soil type.

All pot was fertilized with 100 mg NH₄⁺-N kg⁻¹ soil (180 kg NH₄⁺-N ha⁻¹) using ammonium sulphate salt ((NH₄)₂SO₄) in order to find out the response of fertilized soils to soil saturation.

Denoised water were added to keep soil moisture as 110% water-holding capacity (WHC) and incubated for 4 weeks. Soil moisture were kept by weighting the pot for each of the day to simulate prolonged flooding (PF) (Figure 1).

All pots were fertilized with $100 \text{ mg NH}_4^+\text{-N kg}^{-1}$ soil again using ammonium sulphate salt $((\text{NH}_4)_2\text{SO}_4)$ after 4 weeks incubation of PF. Followed by adjustment of moisture 130% WHC for 4 weeks to mimic another intense precipitation event. Water loss through evaporation drawn down was not replaced over time to simulate drying following flooding (flooding-drying (FD) (Figure 1).

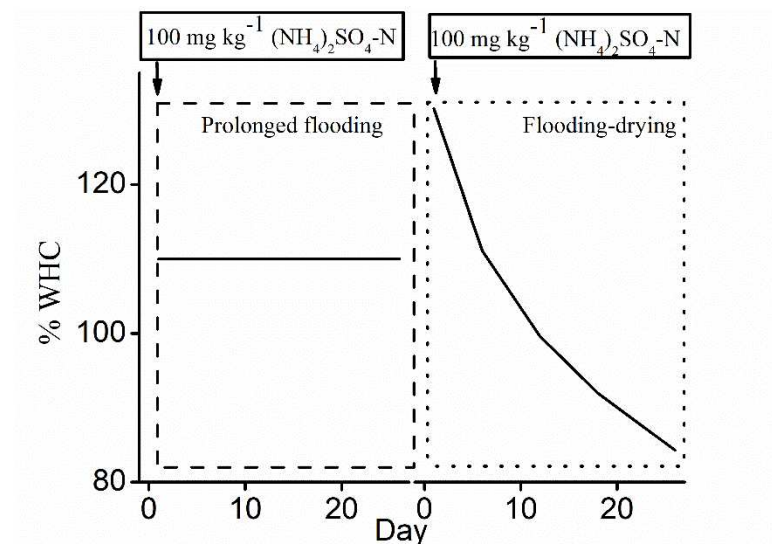


Figure 1. Soil water content of percentage water holding capacity (WHC) during prolonged flooding and flooding drying incubation.

2.3. Collection and measurement of emitted $\text{CO}_2\text{-C}$, $\text{N}_2\text{O-N}$ and $\text{CH}_4\text{-C}$

The $\text{CO}_2\text{-C}$, $\text{N}_2\text{O-N}$ and $\text{CH}_4\text{-C}$ emissions were calculated by measuring the concentration of these gases in the headspace of closed pots at different times. The $\text{CO}_2\text{-C}$, $\text{N}_2\text{O-N}$ and $\text{CH}_4\text{-C}$ samples were collected once a day during the first week, once per two days during the second week and one sampling per three days during the last 2 weeks (Guo et al., 2021a). The pots

were first closed by placing lids and gases were mixed and then samples were collected from the headspace every 30 min for one hour (i.e., at 0, 30 and 60 min) (Guo et al., 2021b). A 20-ml syringe and hypodermic needle was used to collect the gas samples and inject them into pre-evacuated 12-ml glass headspace exetainer vials fitted with a chloro-butyl rubber septum (Chromacol) (Guo et al., 2022a). Gas sampling was carried out between 09:00 and 10:00 a.m. The pots were then left open outside of the sampling periods. The concentrations of CO₂-C, N₂O-N and CH₄-C in the 12-ml glass exetainer vials were measured by gas chromatography (Agilent 7890A GC, Agilent, CA, USA). An electron capture detector (ECD) was used to detect the N₂O, with a temperature of 350 °C and an N₂ carrier gas. A flame ionization detector (FID) was used to detect the CH₄ and CO₂ concentration, with a temperature of 250 °C and an N₂ carrier gas. CH₄ is eluted and analysed first before the whole sample passes through a methanizer to convert the CO₂ to CH₄.

Total CO₂-C, N₂O-N and CH₄-C emissions during the experimental period were calculated from the daily emissions of the gases (Guo et al., 2022b). We used the fluxes of CO₂-C, N₂O-N and CH₄-C multiplied by the number of hours during sampling (24 hr in the first week, 48 hr in the second week and 72 hr in the last six weeks) and added all of them together to obtain the total emissions (mg kg⁻¹ or µg kg⁻¹). The global warming potential (GWP) was used to quantitatively assess the relative impacts of N₂O, CH₄ and CO₂ on climate change by summing the 100-year radiative forcing associated with each of the three measured GHG (Hawthorne et al., 2017). The conversion factors for the assessment of GWP for soil N₂O, CH₄ and CO₂ are 298, 25 and 1 respectively (IPCC, 2021). GWP was calculated by the following equation:

$$GWP = Total_{CO_2} \times 1 + Total_{CH_4} \times 25 + Total_{N_2O} \times 298 \quad (1)$$

Where $Total_{CO_2}$ means total emissions of CO₂, $Total_{CH_4}$ means total emissions of CH₄,

$Total_{N_2O}$ means total emissions of N₂O.

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197

198 2.4. NH_4^+ -N and NO_3^- -N measurement

199 2.4.1. Soil pore water collection

200 10 ml of soil pore water were collected once a day during the first week, once per two days
201 during the second week and one sampling per three days during the last 2 weeks under PF. 10
202 ml of soil pore water were collected once a day during the first week and once per two days
203 during the first 2 weeks under FD (the pore water was possible in the FD treatment when soil
204 moisture was higher than 100% WHC ($\text{SM} > 100\% \text{WHC}$). Pore water was collected via a fibre
205 microfluidic thread, which was set up in the soil.

206

207 2.4.2. Ion exchange resin membranes

208 Anion-exchange membranes sorb NO_3^- -N and cation-exchange membranes sorb NH_4^+ -N
209 from soil pore water through diffusion. These membranes were inserted into a 7.5 cm depth of
210 the soil for passive sensing of the mineral N during the course of the incubation. The cation
211 exchange membrane (CEM) and anion exchange membrane (AEM) (SNOWPURE, San
212 Clemente, CA, USA) were put in the deionized water for 48 hours at 90 °C, followed by drying
213 the membrane. The CEM was saturated with H^+ by leaving CEM strips overnight in 2 M HCl.
214 The AEM was saturated with HCO_3^- by leaving the AEM strips overnight in 1M NaHCO_3 . The
215 ion exchange resin membranes (IERM) are rinsed free of excess HCl and NaHCO_3 with
216 deionized water. A further 24 h equilibration and remove the chemical with deionized water is
217 required for the CEM and AEM. Prepared strips are kept moist in sealed plastic bags prior to
218 installation. CEM and AEM were inserted into soil and replace per each of week. Membranes

are carefully rinsed with deionized water until all traces of soil are removed. Desorption of ions is achieved by shaking each strip (cations and anions separated) for 2 hours in 17.5 ml 0.5 M HCl for 200 rpm shake.

2.4.3. Soil extraction

Subsoil samples were collected with a depth of 10 cm on days 5, 12, 19, and 26 of incubation. For soil mineral N analysis, 2 g fresh soil was extracted with 20 mL of 2 M KCl solution (1:10) for 1 h on a reciprocating shaker. The suspensions so obtained were centrifuged for 10 min, filtered through 0.45um Syringe Filter PES, and stored at 4 °C.

The concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in 0.5 M HCl (INH_4 and INO_3), pore water (WNH_4 and WNO_3) and 2 M KCl solution (SNH_4 and SNO_3) were measured by AQ400 Discrete Analyzer (SEAL Analytical Ltd, Wrexham, United Kingdom).

2.5. Soil total dissolved organic carbon, total dissolved nitrogen, total carbon, total nitrogen, pH and soil particle size measurement

10 g air dried soil was mixed with 25 mL deionized water (1:2.5) and shaken for 30 minutes in 200 rpm for pH measurement. The pH of the upper clear liquid was measured using a pH meter (Mettler Toledo, FiveEasyTM, FE20). Soil total dissolved organic carbon (TOC) and soil total dissolved nitrogen (TDN) were extracted with 2 M KCl solution (1:10) and measured by TOC-L CPH with ASI-L (Shimadzu, Kyoto, Japan). Soil particle size were measured by Mastersizer2000 particle analyzer (Malvern Panalytical, Malvern, United Kingdom). Soil total carbon (TC) and total nitrogen (TN) were measure by a Flash Smart elemental analyzer (Thermoscientific, Massachusetts, US).

242

243 2.6. Statistical analysis

244 The Tukey's HSD test was used to compare the means of soil total CO₂-C, N₂O-N, CH₄-C
245 emissions and soil GWP under different treatments if the treatment effects were significant at
246 the P = 0.05 level. Difference for soil initial index were compared between grassland soil and
247 arable soil under Tukey's HSD test.

248 All statistical analyses were performed using R statistical language (R 2.0.1, R Development
249 Core Team 2005). Correlation analysis were performed using the R statistical language.
250 Principal component analysis (PCA) and variance decomposition of CO₂-C, N₂O-N and CH₄-
251 C emissions were computed using the "RDA" and "VARPART" function of the "vegan"
252 library for R (Oksanen and O'Hara, 2005).

253

254 3. Results

255 3.1. Soil moisture changes with time during flooding-drying

256 Soil moisture were decreasing with time and decreasing to 100% WHC in the 10 days under
257 flooding-drying (Figure 2). Arable soil decreased faster compare with grassland soil (Figure
258 2).

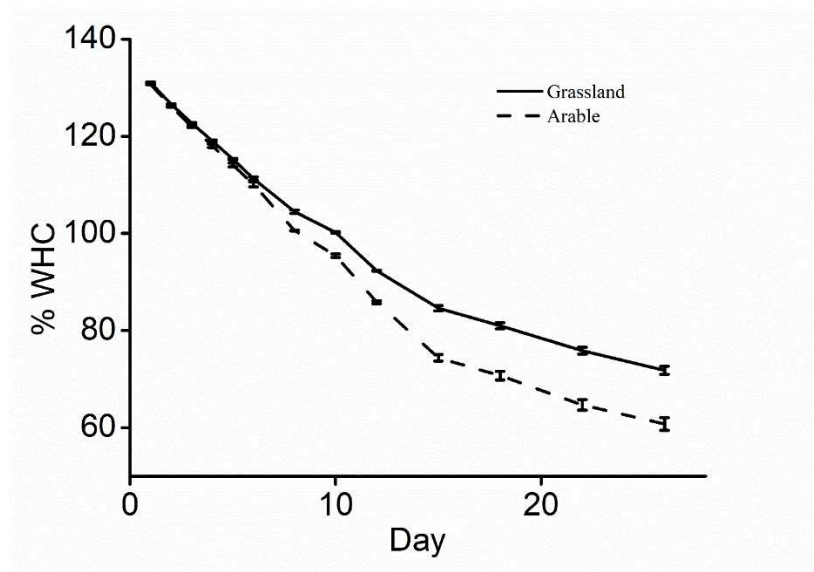


Figure 2. Soil moisture changes with time under grassland and arable soil during flooding-drying treatment. The data values are means of three independent pot replicates and error bars represent the standard error of the means (n=3)

3.2. Soil CO₂-C, N₂O-N and CH₄-C emissions

3.2.1. Rate of CO₂-C, N₂O-N and CH₄-C emissions

Rate of N₂O-N, CO₂-C and CH₄-C emissions in grassland and arable soils did not change with time under PF, N₂O-N and CH₄-C emissions in arable soil did not change with time under FD (Figure 3). Arable soil had a higher rate of N₂O-N emissions than grassland soil under PF (Figure 3).

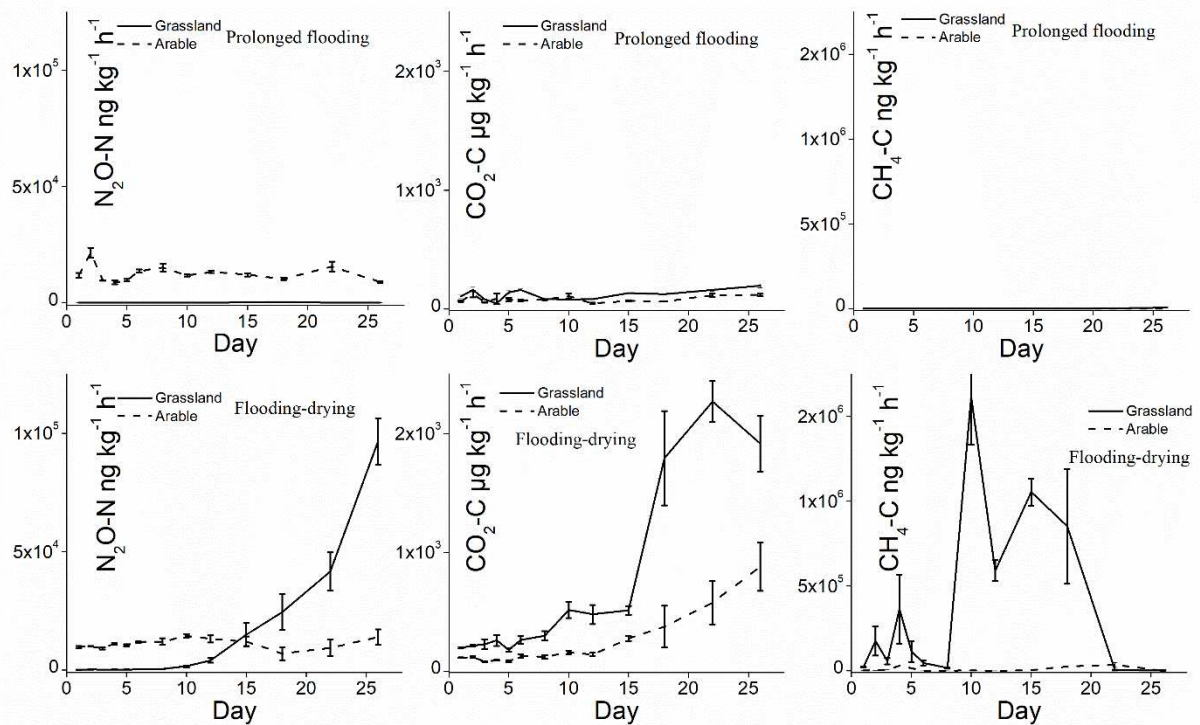


Figure 3. Rate of soil $\text{CO}_2\text{-C}$, $\text{N}_2\text{O-N}$ and $\text{CH}_4\text{-C}$ emissions with time under grassland and arable soil. The data values are means of three independent pot replicates and error bars represent the standard error of the means (n=3)

Rate of $\text{N}_2\text{O-N}$ emissions in grassland soil under FD were increasing with time, especially after 10 days when moisture level started to drop below 100% WHC. Arable soil had a higher rate of $\text{N}_2\text{O-N}$ emissions than grassland soil at the first 15 days under saturated conditions, but arable soil had a lower rate of $\text{N}_2\text{O-N}$ emissions than grassland soil after 15 days when moisture started to fall below 80% WHC at day 15 (Figure 3). Thus, arable sustained high N_2O emission under saturation and grassland enhanced N_2O production following a decline in soil moisture below 80% WHC at day 15 onwards. Rate of $\text{CO}_2\text{-C}$ emissions in arable and grassland soil under FD were increasing with time (Figure 3). Grassland soil had a higher rate of $\text{CO}_2\text{-C}$ emissions than arable soil under FD (Figure 3). Rate of $\text{CH}_4\text{-C}$ emissions in grassland soil

under FD were increasing and then decreasing with time (Figure 3). Arable soil had a lower rate of CH₄-C emissions than grassland soil under FD (Figure 3).

3.2.2. Total CO₂-C, N₂O-N and CH₄-C emissions

The grassland soil under FD had the significantly highest total N₂O-N, CO₂-C and CH₄-C emissions (Table 2). The grassland soil under PF had the significantly lowest total N₂O-N emissions (Table 2). The grassland and arable soil under PF had the significantly lowest total CO₂-C emissions (Table 2). The arable soil under FD, grassland and arable soil under PF had the significantly lowest total CH₄-C emissions (Table 2).

Table2: Soil total CO₂-C, N₂O-N and CH₄-C emissions. (means with same gas and indicated by same lower-case letter are not significantly different at $P \leq 0.05$ on the basis of Tukey HSD).

	Soil type	N ₂ O-N mg kg ⁻¹	CO ₂ -C mg kg ⁻¹	CH ₄ -C mg kg ⁻¹
Prolonged	Grassland	0.08(0.02) a	79.50(1.50) a	0.70(0.05) a
flooding	Arable	7.82(0.21) b	53.72(2.82) a	0.02(0.01) a
Flooding-	Grassland	13.31(0.72) c	623.40(30.01) c	278.02(43.91) b
drying	Arable	6.89(0.76) b	197.89(21.91) b	7.26(1.24) a

Note: Average value (relative standard deviation), n=3.

3.2.3. Global warming potential

Grassland soil had significantly highest GWP under FD (Table 3). Grassland soil had significantly lowest GWP under PF (Table 3). Arable soil had middle GWP under both PF and FD (Table 3).

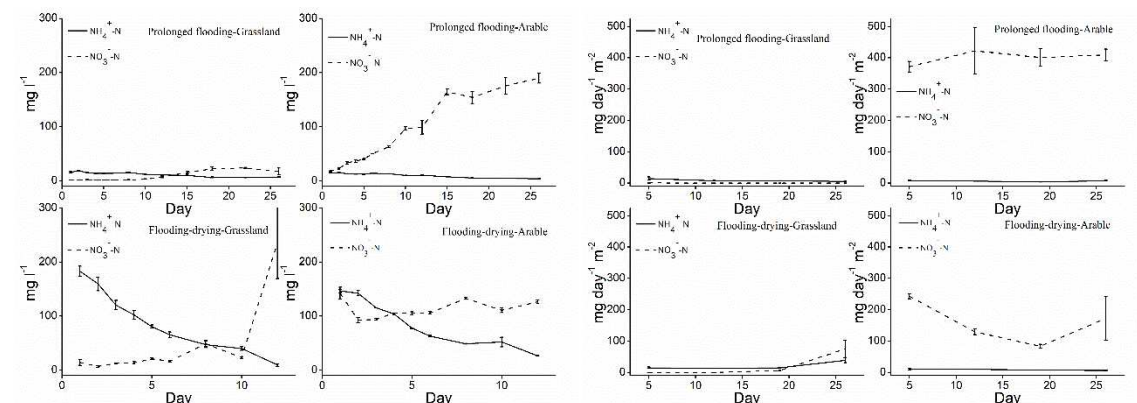
Table 3: Global warming potential. (means indicated by same lower-case letter are not significantly different at $P \leq 0.05$ on the basis of Tukey HSD).

GWP g CO ₂ kg ⁻¹	Prolonged flooding	Flooding-drying
Grassland	0.39(0.02) a	24.02(2.25) c
Arable	7.52(0.21) b	7.42(0.84) b

Note: Average value (relative standard deviation), n=3.

3.3. Soil NH₄⁺-N and NO₃⁻-N concentrations dynamic

The WNH₄ of grassland was decreasing and WNO₃ of grassland were increasing with time under PF and FD (Figure 4 a). WNH₄ of grassland were decreasing heavily under FD (Figure 4 a). The WNH₄ of arable were decreasing and WNO₃ of arable were increasing with time heavily under PF (Figure 4 a). The WNH₄ of arable were decreasing heavily and WNO₃ of arable were increasing slightly with time under FD (Figure 4 a).



a

b

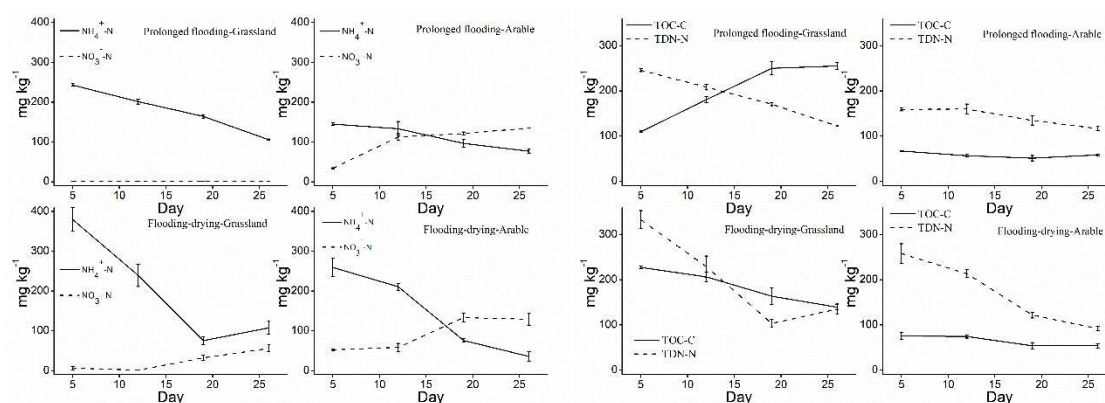


Figure 4. Soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations from soil pore water (a), ion exchange resin membranes (b), soil extraction by KCl (c) and extracted total dissolved organic carbon and total dissolved nitrogen concentrations (d) with time under grassland and arable soil. The data values are means of three independent pot replicates and error bars represent the standard error of the means ($n=3$)

The NH_4^+ and NO_3^- of grassland, and NH_4^+ of arable were very small and not changed with time under PF and FD (Figure 4 b). The NO_3^- of arable soils were not changing with time under PF, and NO_3^- of arable were highest under PF compare with other soil and treatment (Figure 4 b). NO_3^- of arable were decreasing in the first 20 incubation day and then increasing after 20 day with time under FD (Figure 4 b).

The NH_4^+ of grassland were decreasing and NO_3^- of grassland were stable with time under PF (Figure 4 c). The NH_4^+ of grassland were decreasing heavily and NO_3^- of grassland were increasing slightly with time under FD (Figure 4 c). The NH_4^+ of arable were decreasing slightly and NO_3^- of arable were increasing slightly with time under PF (Figure 4 c). The NH_4^+ of arable were decreasing heavily and NO_3^- of arable were increasing slightly with time under FD (Figure 4 c).

The TDN-N of grassland were decreasing and TOC-C of grassland were increasing with time under PF (Figure 4 d). The TDN-N and TOC-C of grassland were decreasing with time under FD (Figure 4 d). The TDN-N of arable were decreasing and TOC-C of arable were keeping stable with time under PF and FD (Figure 4 d).

3.4. Principal component analysis (PCA)

Principal component 1 (PC1) had explained 37.2% of our factors. Principal component 2 (PC2) had explained 26.3% of our data (Figure 5). Samples of Grassland and Arable soil which SM>100%WHC, and Grassland and Arable soil with SM<100%WHC were clearly separated from each other (Figure 5). Grassland and arable soils, with soil moisture SM>100%WHC had small correlation with soil N₂O-N emissions rate. Grassland or Arable soil which SM<100%WHC had a big correlation with N₂O-N emissions rate, which varied overall and peaked on different days in the two land use types (Figure 5). WHC had a big and negative correlation with soil N₂O-N emissions rate (increase % of WHC would decrease soil N₂O-N emissions rate). Soil CO₂-C emissions rate had a positive and big correlation with soil N₂O-N emissions rate (Figure 5).

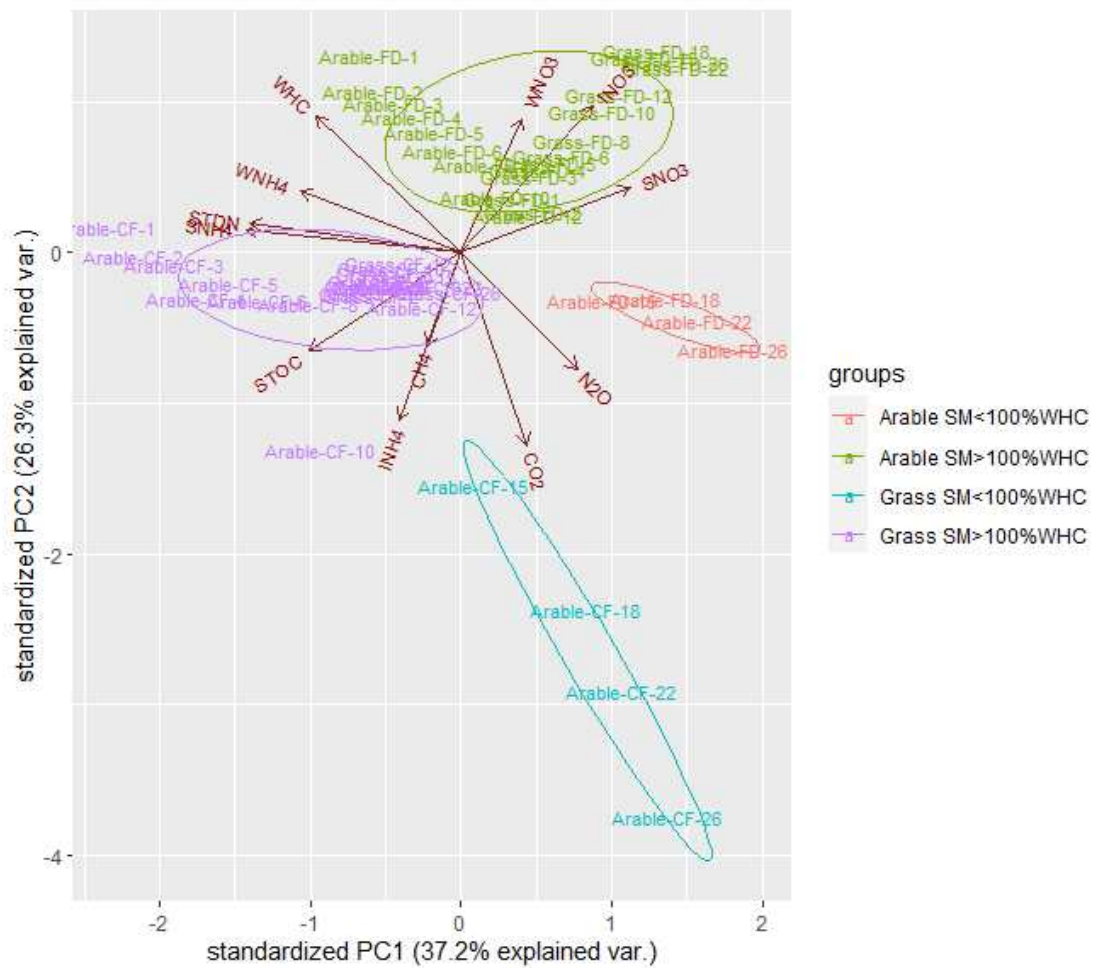


Figure 5. Principal component analysis. N₂O, rate of soil N₂O-N emissions; CO₂, rate of soil CO₂-C emissions; CH₄, rate of soil CH₄-C emissions; WHC, % of soil water holding capacity; INH₄, soil NH₄⁺-N concentrations from ion exchange resin membranes; INO₃, soil NO₃⁻-N concentrations from ion exchange resin membranes; WNO₃, soil NO₃⁻-N concentrations from soil pore water; WNO₃, soil NO₃⁻-N concentrations from soil pore water; SNH₄, soil NH₄⁺-N concentrations from soil extraction by KCl; SNO₃, soil NO₃⁻-N concentrations from soil extraction by KCl; STOC, soil total dissolved organic carbon-carbon; STDN, soil total dissolved nitrogen-nitrogen; SM, soil moisture.

3.5. Correlation analysis

Rate of soil N₂O-N emissions in grassland soils under SM>100%WHC showed significant correlation with % of soil WHC and WNO₃ (Table 4). While rate of soil N₂O-N emissions from grassland soils under SM<100%WHC had significant correlation with % of soil WHC, INH₄, INO₃ and SNO₃ (Table 4).

Table 4: Correlation analysis. N₂O-N, rate of soil N₂O-N emissions; CO₂-C, rate of soil CO₂-C emissions; CH₄-C, rate of soil CH₄-C emissions; WHC, % of soil water holding capacity; INH₄, soil NH₄⁺-N concentrations from ion exchange resin membranes; INO₃, soil NO₃⁻-N concentrations from ion exchange resin membranes; WNH₄, soil NH₄⁺-N concentrations from soil pore water; WNO₃, soil NO₃⁻-N concentrations from soil pore water; SNH₄, soil NH₄⁺-N concentrations from soil extraction by KCl; SNO₃, soil NO₃⁻-N concentrations from soil extraction by KCl; TOC, soil total dissolved organic carbon; TDN, soil total dissolved nitrogen; SM, soil moisture.

Soil Type		SM>100%WHC			SM<100%WHC		
		N ₂ O-N	CO ₂ -C	CH ₄ -C	N ₂ O-N	CO ₂ -C	CH ₄ -C
Grassland	N ₂ O-N	1	0.78**	0.59**	1	0.65	-0.73
	CO ₂ -C	0.78**	1	0.81**	0.65	1	-0.69
	CH ₄ -C	0.59**	0.81**	1	-0.73	-0.69	1
	WHC	-0.59**	-0.36	-0.37	-0.89*	-0.86	0.66
	INH4	0.01	0.13	0.1	0.97**	0.67	-0.86
	INO3	-0.39	-0.54**	-0.34	0.97**	0.68	-0.86
	WNH4	-0.09	0.22	0.07			
	WNO3	0.95**	0.64**	0.32			
	SNH4	-0.02	0.26	0.12	-0.52	-0.86	0.28
	SNO3	-0.07	0.27	0.07	0.89*	0.89*	-0.69
	TOC	0.22	0.43*	0.2	-0.86	-0.91*	0.65
	TDN	-0.05	0.21	0.09	-0.47	-0.84	0.23
Arable	N ₂ O-N	1	0.29	-0.17	1	0.15	-0.84
	CO ₂ -C	0.29	1	-0.02	0.15	1	0.1
	CH ₄ -C	-0.17	-0.02	1	-0.84	0.1	1
	WHC	-0.34	-0.31	0.3	0.16	-0.93*	-0.36
	INH4	-0.14	0.43*	0.41	0.12	-0.96**	-0.33
	INO3	0.01	-0.61**	-0.32	0.77	0.68	-0.43
	WNH4	-0.31	0.26	0.52*			
	WNO3	-0.18	0.31	0.07			
	SNH4	-0.21	0.34	0.51*	0.32	-0.88*	-0.47
	SNO3	-0.09	0	-0.1	-0.53	0.74	0.6

TOC	-0.09	0.39	0.38	0.5	-0.77	-0.58
TDN	-0.24	0.34	0.53*	0.31	-0.89*	-0.46

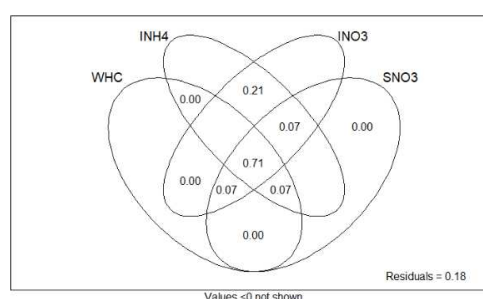
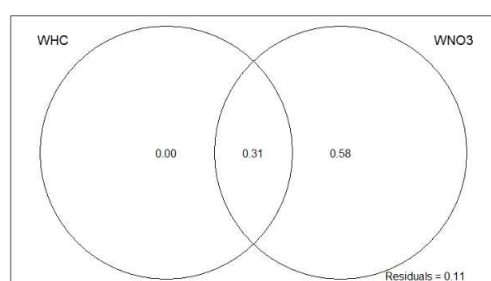
* Significant at $p < 0.05$.

** significant at $p < 0.01$.

Rate of soil CO₂-C emissions in grassland soil under SM>100%WHC showed significant correlation with INO₃, WNO₃ and TOC (Table 4). While rate of soil CO₂-C emissions from grassland soil under SM<100%WHC showed significantly correlation with SNO₃ and TOC (Table 4). Rate of soil CO₂-C emissions in arable soil under SM>100%WHC showed significantly correlation with INH₄ and INO₃ (Table 4). While rate of soil CO₂-C emissions from arable soil under SM<100%WHC showed significantly correlation with % of soil WHC, INH₄, SNH₄ and TDN (Table 4). Rate of soil CH₄-C emissions in arable soil under SM>100%WHC showed significantly correlation with WNH₄, SNH₄ and TDN (Table 4).

3.6. Variation partitioning

WNO₃ of grassland soil had the highest contribution to N₂O-N emissions (58%) when SM>100%WHC (Figure 6 a). The combine effect of SNO₃ and INH₄ had the highest contribution to N₂O-N emissions (71%) when SM<100%WHC in grassland soil (Figure 6 b).



(a)

(b)

Figure 6. Variation partitioning of N_2O -N emissions for soil moisture larger than 100% water-holding capacity (a) and soil moisture lower than 100% water-holding capacity (b) in grassland soil. WHC, % of soil water holding capacity; INH_4 , soil NH_4^+ -N concentrations from ion exchange resin membranes; INO_3 , soil NO_3^- -N concentrations from ion exchange resin membranes; WNO_3 , soil NO_3^- -N concentrations from soil pore water; SNO_3 , soil NO_3^- -N concentrations from soil extraction by KCl.

4. Discussion

4.1. The GHG emissions and NH_4^+ -N and NO_3^- -N dynamic under prolonged flooding

The SNH_4 of grassland and arable were decreasing and SNO_3 of grassland and arable were stable or increasing slightly with time under PF while the WNH_4 of grassland and arable was decreasing and WNO_3 of grassland and arable were increasing with time under PF (Figure 4). The possible reason is that a small amount of NH_4^+ -N that were absorbed by soil particle (stable) slowly released to soil pore water (available) with time, the available NH_4^+ -N and TDN -N seem to have been key substrates for nitrification for NO_3^- -N production in our grassland and arable soil. Combine with the lower emission of N_2O and GWP under PF (Figure 3 and Table 3). There for, we think a small amount of NO_3^- -N were denitrified to N_2O -N and most of NO_3^- -N were denitrification to N_2 under 110%WHC of PF. Same with Wu et al. (2017) that excessive moisture condition can suppress emissions of N_2O through its reduction to N_2 because of complete denitrification. Previous studies reporting low N_2O emissions from flooded fields (Shaaban et al., 2018; Song et al., 2021; Xu et al., 2022), which is commensurate with our findings that PD has lower N_2O emissions compare with FD. Flooding conditions generally induce soil anaerobic conditions, which consequently limiting mineralization of organic C and N, ultimately resulting in less substrates for N_2O production via nitrification and

denitrification (Li et al., 2022; Neubauer and Megonigal, 2021) and favourable for complete denitrification producing N_2 rather than N_2O , and therefore low N_2O emissions (Cai et al., 2013; Mazza et al., 2018). N_2O emissions decreased during continuous flooding soil is also more rapid NO_3^- depletion and entrapment within the micropores, possibly limiting further denitrification to produce N_2O and N_2 gases (McNicol and Silver 2014).

Arable soil had a higher rate of N_2O -N emissions than grassland soil under PF (Figure 3), and the arable soil had significantly higher total N_2O -N emissions and GWP than grassland soil under PF (Table 2 and table 3) with a higher INO_3 concentration (Figure 4 b) and decomposition of TDN-N (Figure 4 d). The possible reason is that arable soil had a smaller pH compare with grassland soil and arable soil had more nitrate compared with grassland soil from the beginning (Table 1). Čuhel et al. (2010) found that the relative importance of N_2O as product of denitrification is higher at low pH. The functionality of *nosZ* gene for synthesizing N_2O -reducing enzyme (N_2O reductase) is limited under low pH indicating higher N_2O emissions (Shaaban et al. 2018). High soil pH values also typically shift the denitrification end product ratio towards N_2 instead of N_2O (Šimek et al. 2002) and also lower N_2O formation during nitrification (Mørkved et al. 2007). Bell et al., (2015) also found that the annual emissions factor for the Scottish arable soil was 3 to 5 times higher than grassland sites elsewhere in a field trials of UK, which is commensurate with our findings that arable soil had more emissions than grassland.

There was only a small emission for CO_2 and CH_4 in both grassland and arable soil under PF (Figure 3 and Table 2). The highly possible reason is that the high-water content limits the decomposition of soil organic matter and dissolved the CH_4 . Provided by Devèvre and Horwáth (2000) that mineralization of soil organic carbon was restrained under the waterlogged conditions by restricted microbial growth and activities, and thereby impeded CO_2 production

(Khalid et al., 2019). Another reason is that permanent flooding also favoured the amount of dissolved CH_4 (Mazza et al., 2018).

4.2. The GHG emissions and NH_4^+ -N and NO_3^- -N dynamic under flooding-drying

The WNH_4 of grassland and arable was decreasing and WNO_3 of grassland and arable were increasing with time under FD while the SNH_4 of grassland and arable were decreasing heavily and SNO_3 of grassland and arable were increasing slightly with time under FD (Figure 4 c). When $\text{SM} > 100\% \text{WHC}$, less emissions of N_2O were detected under FD in grassland and arable soil (Figure 3). Same condition with the PD (described before). When $\text{SM} < 100\% \text{WHC}$, there were a lot of emissions of N_2O -N under FD in grassland soil and N_2O -N emissions was increasing with the decreasing of soil moisture to end of the day (Figure 3). The mostly reason for that is the nitrification-denitrification and denitrification were increasing with time in grassland soil. Same with Freibauer et al. (2004) and Goldberg et al. (2010) that degradation of soil organic matter as a result of drainage and cultivation will stimulate net N mineralization and N transformations via nitrification and denitrification which can then lead to N_2O production. At the capillary fringe above the water level, the soil was characterised by having close to sub-saturated soil moisture degrees and mixed aerobic/anaerobic conditions, promoting the environmental conditions favourable for both N_2O production via denitrification and NO_3^- reduction via DNRA (Megonigal et al., 2003). A lot of researcher (Congreves et al., 2019; Miller et al., 2020) found that the product of N_2O by denitrification increasing with decreasing water-filled pore space from 90% to 60% and peak at optimum water filled pore space of 65%–70% (Pärn et al. 2018; van Lent et al., 2015; Werner et al., 2007). At higher water contents, denitrification becomes more prevalent leading to maximum emissions at around 80% WFPS (Shepherd, 2009). In agricultural soils, the total N_2O emissions in a season

are strongly related to the episodes of large N₂O pulses observed after irrigation and rainfall events which are primarily derived from denitrification (Trost et al. 2013). Consistency with our result that N₂O-N emissions was increasing with the decreasing of soil moisture to end of the day (which soil moisture still higher than 60% WHC).

The TDN-N and TOC-C of grassland were decreasing with time under FD (Figure 4 d). but, the TDN-N of arable were decreasing and TOC-C of arable were keeping stable with time under FD (Figure 4 d). Arable soil also had a lower N₂O-N emissions rate than grassland soil when soil moisture smaller than 80% WHC under FD (Figure 3) and the grassland soil had a higher total N₂O-N emission and GWP than arable soil under FD (Table 3 and table 4). The possible reason is that the nitrogen, carbon and microbial in arable soil limited the nitrification and denitrification, and therefore lower the production of N₂O-N under SM<100%WHC. C-substrate availability and N₂O emissions from denitrification and nitrification are always positively related to each other (Li et al., 2005; Wan et al., 2009) due to nitrification being primarily an autotrophic process and heterotrophic nitrification only accounting for 20% or less mostly under low pH conditions (Liu et al., 2015). Gütlein et al. (2018) also found that N₂O emissions correlated positively with soil moisture and total soil nitrogen content. Short and temporary drying-rewetting frequency enhanced denitrifier activity by availing physically protected organic matter (Fierer and Schimel, 2002). N₂O derived from soil organic matter decomposition dominate overall fluxes (Maljanen et al., 2010). Abbasi et al. (2011) also found that the process of denitrification and production of N₂O be smaller in arable soil deficient in organic matter compared to grassland soil due to less availability of organic C. Another reason is that Grassland soil has a higher pH (Table 1), and then lead to a bigger N₂O-N emission (Figure 3 and Table 2) when SM<100%WHC. Fan et al. (2018) reported significantly higher N₂O emissions rates from three alkaline soils (pH 7.6–8.2) as compared to an acidic soil with a pH of 5.6. Oxidation of NH₄⁺ is completely inhibited at pH 5 and increases with a higher pH

(Wang et al., 2018) through a pH-driven shift in the microbial community structure and/or microbial activities (Ottosen et al., 2009).

The soil total carbon and TOC were mainly anaerobic digestion to CH₄ before 15 days (especially when soil moisture between 110%WHC and 80%WHC) (Figure 3), and were mainly aerobic digestion to CO₂ after 15 days in grassland soil under flooding-drying when soil moisture lower than 80%WHC (Figure 3). CH₄ production is expected to mainly occur below the groundwater table (Segers, 1998). Anaerobic conditions at flooding stage of the experiment promoted methanogenic activities while suppressed methanotrophic activities leading to CH₄ production (Shaaban et al., 2022). Drainage will limit the production of CH₄ due to highest oxidation potentials near the oxic/anoxic interface (Petersen et al., 2012), but also increase the potential for CH₄ oxidation during passage through the unsaturated zone to the atmosphere (Petersen et al., 2012). CO₂ fluxes were dominant at drained sites (Kandel et al., 2018) and showed a decreasing trend following an increase of soil water content as commonly observed in previous studies (Smith et al. 2003; McNicol and Silver 2014) due to a lower O₂ availability and the consequent inhibition of aerobic respiration when a large proportion of pores are saturated under flooding (Mazza et al., 2018). CO₂ emissions were significantly larger in the flooding converted to wet soil treatment as compared with the continuous flooding and wet soil treatments owing to increased mineralization and C contents (Khalid et al., 2019). The emissions of CH₄ after 100% WHC (Figure 3) maybe were due to the gas entrapment into microaggregates and a delayed release (either as emissions or leaching) (Mazza et al., 2018). CH₄ emissions increased during the 2nd flooding event (FD) instead of PF at the first time (Figure 3) maybe was caused by a possible mechanism of adaptation of microbial communities (Lagomarsino et al., 2016).

The grassland soil had a higher rate of CO₂-C and CH₄-C emissions than arable soil under FD (Figure 3). The grassland soil under FD had the significantly higher total CO₂-C and CH₄-

C emissions and GWP than arable soil (Table 3 and 4). The organic matter and total N contents are higher in grassland soils than in arable soils (Table 1) which caused the potential for mineralisation is higher after disturbances (drainage) (Eickenscheidt et al., 2014). (Volpi et al. (2017) found that CO₂ emissions is positively linked to soil organic carbon content and denitrification activity which increased denitrification activity also increases CO₂ efflux (Groffman and Crawford, 2003). Therefore, grassland soil under FD had the significantly higher total CO₂-C and CH₄-C emissions and GWP than arable soil. Thomson et al. (2010) detected significantly higher respiration rates in dried and rewetted microcosms. When soil moisture content changed from flooding to 60% WFPS, CH₄ emissions decreased, but N₂O and CO₂ substantially increased due to flooded soil released N and C and triggered C and N cycling (Shaaban et al., 2022). Same with us that higher GHG emissions and GWP under FD than PF were found in grassland soil (Table 2 and 3).

4.3. The influence of different type of soil NH₄⁺-N and NO₃⁻-N on GHG emissions

NH₄⁺-N and NO₃⁻-N in pore water (WNH₄ and WNO₃) are available for soil microbial to use since they are existing in the pore water. NH₄⁺-N and NO₃⁻-N in 0.5 M HCl from IERM (INH₄ and INO₃) are labile for soil microbial to use. NH₄⁺-N and NO₃⁻-N in 2 M KCl from soil extraction (SNH₄ and SNO₃) are stable for soil microbial to use.

Grassland and arable soil sample of N₂O-N emissions were clearly separate between SM>100%WHC and SM<100%WHC (Figure 5). Senbayram et al. (2009) found that the availability of a high level of NO₃⁻ as a result of nitrification together with labile C under anaerobic soil conditions serves as a driving force for N₂O emissions. Same with our result that the N₂O-N and CO₂-C emissions was more controlled by soil available NO₃⁻-N when soil moisture higher than 100% WHC while N₂O-N emissions was more controlled by soil stable

$\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ when soil moisture lower than 100% WHC in grassland soil (Figure 6 a and b).

5. Conclusion

More $\text{N}_2\text{O-N}$ emissions and large GWP were found in arable soil under PF. But more $\text{N}_2\text{O-N}$, $\text{CO}_2\text{-C}$, $\text{CH}_4\text{-C}$ emissions and large GWP were found in grassland soil under FD. The GHG emissions of grassland soil were more controlled by soil available $\text{NO}_3^-\text{-N}$ when soil moisture was higher than 100% WHC. The $\text{N}_2\text{O-N}$, $\text{CO}_2\text{-C}$ and $\text{CH}_4\text{-C}$ emissions of grassland soil were more controlled by soil stable $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and TOC when soil moisture was lower than 100% WHC.

The GHG emissions also need to be consider when we try to converting arable land use to grassland, although grassland sequester more soil C than arable and thus the balance between GHG emission and C capture need consideration in these critical land use changes under future climate. Further research needs to forces on how to reduce the GHG emissions under climate change (heavy rain) after convert arable to grassland to meet human need.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

555

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