



# Can flooding-induced greenhouse gas emissions be mitigated by trait-based plant species choice?

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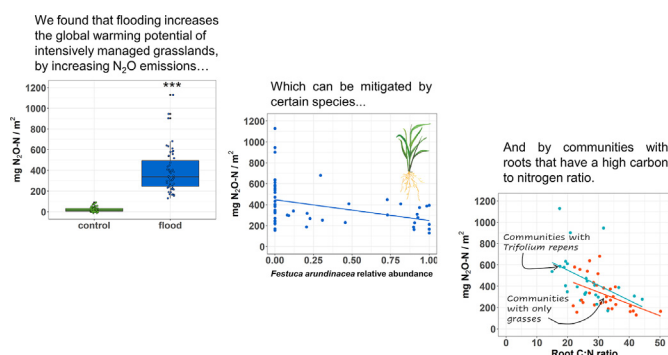
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## HIGHLIGHTS

- Flooding increases N<sub>2</sub>O from managed grasslands
- Plant community composition explains flood-induced N<sub>2</sub>O
- Festuca arundinacea* decreased, and *Trifolium repens* increased, flood-induced N<sub>2</sub>O
- Higher root C:N is related to lower flood-induced N<sub>2</sub>O

## GRAPHICAL ABSTRACT



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## ABSTRACT

Intensively managed grasslands are large sources of the potent greenhouse gas nitrous oxide (N<sub>2</sub>O) and important regulators of methane (CH<sub>4</sub>) consumption and production. The predicted increase in flooding frequency and severity due to climate change could increase N<sub>2</sub>O emissions and shift grasslands from a net CH<sub>4</sub> sink to a source. Therefore, effective management strategies are critical for mitigating greenhouse gas emissions from flood-prone grasslands. We tested how repeated flooding affected the N<sub>2</sub>O and CH<sub>4</sub> emissions from 11 different plant communities (*Festuca arundinacea*, *Lolium perenne*, *Poa trivialis*, and *Trifolium repens* in monoculture, 2- and 4-species mixtures), using intact soil cores from an 18-month old grassland field experiment in a 4-month greenhouse experiment. To elucidate potential underlying mechanisms, we related plant functional traits to cumulative N<sub>2</sub>O and CH<sub>4</sub> emissions. We hypothesized that traits related with fast nitrogen uptake and growth would lower N<sub>2</sub>O and CH<sub>4</sub> emissions in ambient (non-flooded) conditions, and that traits related to tissue toughness would lower N<sub>2</sub>O and CH<sub>4</sub> emissions in flooded conditions. We found that flooding increased cumulative N<sub>2</sub>O emissions by 97 fold and cumulative CH<sub>4</sub> emissions by 1.6 fold on average. Plant community composition mediated the flood-induced increase in N<sub>2</sub>O emissions. In flooded conditions, increasing abundance of the grass *F. arundinacea* was related with lower N<sub>2</sub>O emissions; whereas increases in abundance of the legume *T. repens* resulted in higher N<sub>2</sub>O emissions. In non-flooded conditions, N<sub>2</sub>O emissions were not clearly mediated by plant traits related with nitrogen uptake or biomass production. In flooded conditions, plant communities with

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high root carbon to nitrogen ratio were related with lower cumulative  $\text{N}_2\text{O}$  emissions, and a lower global warming potential ( $\text{CO}_2$  equivalent of  $\text{N}_2\text{O}$  and  $\text{CH}_4$ ). We conclude that plant functional traits related to slower decomposition and nitrogen mineralization could play a significant role in mitigating  $\text{N}_2\text{O}$  emissions in flooded grasslands.

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## 1. Introduction

Climate change is increasing the frequency and severity of extreme weather events, which will trigger pervasive changes in Earth's natural and managed ecosystems (Stocker et al., 2013; IPCC, 2018). Globally, the magnitude of extreme rainfall events is predicted to increase (Du et al., 2019), even in areas where the mean annual rainfall is predicted to decrease (Fowler et al., 2007; Kitoh and Endo, 2016), which increases flooding risk (Donat et al., 2016). The impact of flooding in managed grasslands is two-fold, as floods can compromise ecosystem functioning (e.g. primary productivity) and could exacerbate greenhouse gas emissions by disrupting nutrient and carbon cycling (Sánchez-Rodríguez et al., 2019a). The plant community has been shown to affect emissions of nitrous oxide ( $\text{N}_2\text{O}$ ) in non-flooded conditions (Abalos et al., 2014); and methane ( $\text{CH}_4$ ) emissions from flooded wetlands (Sutton-Grier and Megonigal, 2011). What is not known is how the plant community – via its composition or functional traits – could mitigate flood-induced increases in  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions from intensively managed grasslands.

Intensively managed grasslands in temperate climates are an important source of  $\text{N}_2\text{O}$  (Abalos et al., 2014; Hörtnagl et al., 2018), a potent greenhouse gas 265 times stronger than carbon dioxide ( $\text{CO}_2$ ) (IPCC, 2014). Flooding causes peaks in  $\text{N}_2\text{O}$  emissions at the onset and recession of the flood because nitrogen availability and  $\text{N}_2\text{O}$  emissions are highest in moderately anaerobic conditions (Firestone and Davidson, 1989; Butterbach-Bahl et al., 2013). This can lead to higher cumulative  $\text{N}_2\text{O}$  emissions over a flooding and drying cycle (Sánchez-Rodríguez et al., 2019a). While the absence of oxygen in saturated soil during flooding decreases  $\text{N}_2\text{O}$  emissions, it facilitates the production of  $\text{CH}_4$ , a greenhouse gas with a global warming potential 28 times higher than  $\text{CO}_2$  (IPCC, 2018). Grasslands are typically a  $\text{CH}_4$  sink (Hartmann et al., 2011), via oxidation of  $\text{CH}_4$  by methanotrophic bacteria in the soil (Dunfield, 2007). Flooding could shift grasslands from a  $\text{CH}_4$  sink to  $\text{CH}_4$  source (Sánchez-Rodríguez et al., 2019a), similar to rice paddies and wetlands (Kirschke et al., 2013). The increase in  $\text{N}_2\text{O}$  and  $\text{CH}_4$  at different points in the flooding and drying cycle could increase the overall global warming potential ( $\text{CO}_2$  equivalent emissions) of intensively managed grasslands (Sánchez-Rodríguez et al., 2019b). Greenhouse gas emissions could be mitigated by the specific composition of the plant community (Abalos et al., 2014) or the plant community's traits (Abalos et al., 2018), because of their interaction with the carbon and nitrogen cycles (Ström et al., 2005; Niklaus et al., 2006).

In non-flooded conditions, living plants can lower  $\text{N}_2\text{O}$  by taking up nitrogen, reducing soil mineral nitrogen, the substrate for  $\text{N}_2\text{O}$  producing processes (Abalos et al., 2014). Nitrogen uptake has been shown to be higher in plant communities with multiple species compared to monocultures of the same species (Finn et al., 2013). Therefore, mixtures of plant species could play a role in  $\text{N}_2\text{O}$  mitigation. Further, the combination of plant species (plant community composition) can also influence  $\text{N}_2\text{O}$  emissions. Legumes, which biologically fix nitrogen, can increase soil available nitrogen, contributing to higher  $\text{N}_2\text{O}$  emissions (Niklaus et al., 2006, 2016). Combining legumes with grasses could mitigate these emissions as excess nitrogen (from living or decaying legume roots) could be taken up by neighbouring grasses, increasing their nitrogen uptake and productivity (Nyfeler et al., 2011; Suter et al., 2015).

How the plant community could mitigate  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions in flooded grasslands is not well known. In flooded conditions, decomposition may have a larger effect on  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions than nitrogen uptake and plant productivity, because of the increase in dead plant roots and shoots which introduce carbon and nitrogen into the soil. How decomposing plant litter affects greenhouse gas emissions is likely due to their quality and quantity. Nitrogen-rich litter from legumes decomposes faster than that from grasses (Roumet et al., 2016), and could increase  $\text{N}_2\text{O}$  emissions in flooded conditions. Plant communities with higher biomass, especially belowground, may increase  $\text{CH}_4$  emissions due to the increased availability of carbon sources (i.e. root litter) for  $\text{CH}_4$  production (Wang and Adachi, 2000).

Plant traits are the morphological, chemical, and physiological characteristics of a plant that are relevant to how a plant interacts with its environment and other organisms (Mariotte et al., 2017). Plant traits could underlie plant community effects on  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions, as they have been shown to explain cycling and losses of nitrogen (Grassein et al., 2015; de Vries and Bardgett, 2016) and carbon (De Deyn et al., 2008; Freschet et al., 2012). Plant communities with an 'acquisitive' resource uptake strategy, i.e. high specific leaf area, low leaf dry matter content, and high leaf nitrogen (Reich, 2014), can lower  $\text{N}_2\text{O}$  emissions in non-flooded conditions via efficient nitrogen uptake (Abalos et al., 2018). In flooded conditions, traits related to litter decomposition may play a larger role in mediating  $\text{N}_2\text{O}$  and  $\text{CH}_4$  than traits related to nutrient uptake. Plant species with a 'conservative' resource uptake strategy, i.e. with tougher, denser, nitrogen-poor tissues, could be related to lower  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions as they decompose slower than plants with opposite traits (Freschet et al., 2012). This lower litter decomposability could slow cycling of carbon and nitrogen, potentially reducing losses via  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions. In contrast, readily decomposable litter with more labile carbon compounds increases carbon supply to the microbial community, fuelling denitrification (McGill et al., 2010) and methanogenesis (Koelbener et al., 2010). Further, conservative species have been shown to be more prevalent in wet conditions (Zelnik and Čarni, 2008), suggesting that they can better tolerate saturated soil conditions (Moor et al., 2017), and may lower the amount of dead plant material (litter), reducing the sources for  $\text{N}_2\text{O}$  and  $\text{CH}_4$ .

Our objective was to test how the plant communities and their traits mediate flood-induced  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions. We used intact soil monoliths from an 18-month old field experiment with 11 different plant communities in a 4-month greenhouse experiment to determine how repeated flooding affects  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions from intensively managed grasslands. We hypothesize that:

- 1) Flooding will increase  $\text{N}_2\text{O}$  and  $\text{CH}_4$  emissions compared to ambient rainfall conditions.
- 2) Mixtures of two or four plant species will emit less  $\text{N}_2\text{O}$  compared to monocultures of the same species under both flooded and ambient conditions due to higher nitrogen uptake and biomass production compared to monocultures.
- 3) In flooded conditions, higher plant biomass at the onset of flooding will increase  $\text{CH}_4$  emissions due to increased carbon availability.
- 4) Plant communities with leaf and root traits related with an acquisitive strategy, according to the resource economic spectrum, will lower  $\text{N}_2\text{O}$  emissions under ambient rainfall (compared to conservative communities); communities with traits related to slower decomposition will lower  $\text{N}_2\text{O}$  emissions under flooded conditions (compared to communities with traits related to faster decomposition).

## 2. Methods

### 2.1. Experimental design

For our 4-month greenhouse experiment, we took intact monoliths (20 cm diameter  $\times$  25 cm depth) from an established field experiment in September 2017 (Wageningen, the Netherlands, 51.9884°N, 5.6616°E). This greenhouse experiment is also presented in another paper which focuses on the effects of flooding on plant community resilience (Oram et al., n.d.). The field experiment was established in March 2016 with three grass species (*Festuca arundinacea*, *Lolium perenne*, and *Poa trivialis*) and one legume (*Trifolium repens*) grown in monocultures, all two-species mixtures (6 combinations) and the mixture of all four species (1 combination). The plant species were chosen as they are frequently grown on nitrogen-rich soils and cover a relatively wide range of functional traits above- and belowground (Abalos et al., 2014; Mason et al., 2016). The field experiment was set up in a completely randomized block design with five replicates of each treatment (i.e. 55 field plots total). The soil is classified as a *typic endoaquoll* (Soil Survey Staff, 1999) with 75% sand, 23% silt and 2% clay. Physio-chemical characteristics of the 0–15 cm soil layer in March 2016 were: pH-CaCl<sub>2</sub>: 5.1; soil bulk density: 1.42 Mg/m<sup>3</sup>; organic matter: 2.2%; total N: 1.30 g/kg. The field plots were fertilized 3 times per year, each time with calcium ammonium nitrate at a rate of 50 kg N/ha (i.e. 150 kg N/ha/year).

For this greenhouse experiment, two monoliths were taken from each field plot by pressing a PVC pipe fit inside a metal frame into the ground with the digging bucket of a backhoe and pulling it out to extract the intact monolith (2 monoliths  $\times$  55 plots = 110 monoliths). The monoliths were taken from areas of the plot to ensure that there was similar plant cover between monoliths from the same plot and good representation of target species. The bottoms of all the PVC pipes containing the monoliths were then sealed (and were closed for the duration of the experiment) and transferred to the greenhouse (day:night regime of 16:8 h light:dark, 21:16 °C). Water holding capacity (WHC) was determined on a soil core taken in each field plot at the same time as the monolith was taken. The weight of the monolith at 60% WHC was calculated, this was maintained for all monoliths for a two-week adjustment period. Subsequently, monoliths in the control treatment (1 monolith from each plot) were maintained at 60% WHC throughout the experiment by weighing the monoliths and watering to the 60% WHC weight four times per week. Monoliths in the flood treatment (the other monolith from each plot) were subjected to two floods of two weeks, followed by a 5-week recovery period (in which these monoliths could dry to, and were then maintained at, 60% WHC by weighing). Each monolith in the flooded treatment experienced a mild flood, where the soil was completely waterlogged, but the plants were not submerged, equivalent to 45 mm rainfall (1.28 L water per monolith), and a severe flood, where the plants were initially submerged equivalent to 100 mm rainfall (2.84 L water per monolith). After the severe flood, the surface water was removed until the monoliths were at the 'waterlogged' weight (the weight of the first flood event), and then left to dry down to 60% WHC.

Both rainfall events are realistic within climate change scenarios (Stocker et al., 2013; KNMI, 2018). Further, the peak water volume in rivers is increasing, which will increase the frequency and extent of flooding in grasslands bordering rivers (Hirabayashi et al., 2013). Monoliths were fertilized in line with management practices in intensively managed grasslands in temperate climate (Suter et al., 2015; Sutton et al., 2011). Granular calcium ammonium nitrate was added in two applications of 50 kg N/ha one week prior to the first and the second flood (thus, 100 kg N/ha were added in total).

### 2.2. Greenhouse gas emissions

Nitrous oxide and CH<sub>4</sub> emissions were measured 42 times over the 4-month (113 day) experiment using the closed flux chamber

technique. Flux measurements were taken daily for four days after fertilizer addition and flooding, and then two to three times per week for the remainder of the experiment, in line with previous studies (Abalos et al., 2014; Lubbers et al., 2011). Polypropylene flux chambers (20 cm diameter  $\times$  14 cm height) fitted with Teflon tubes were placed on the monoliths for approximately 30 min. Gas measurements of the headspace were taken with an Innova 1312 photo-acoustic infrared gas analyser (LumaSense Technologies A/S, Ballerup, Denmark) fitted with Teflon tubes which connected to the flux chambers. This method measures the N<sub>2</sub>O and CH<sub>4</sub> fluxes originating from the soil, as well as from the plants themselves, as plants can act as 'chimneys', transferring CH<sub>4</sub> emissions from an anaerobic environment to the atmosphere (e.g. Bhullar et al., 2013), and can release N<sub>2</sub>O (Lenhart et al., 2019).

Cumulative emissions were calculated assuming linear changes in gas concentration between measurements (Abalos et al., 2014). The global warming potential was calculated as the CO<sub>2</sub> equivalent of cumulative N<sub>2</sub>O and CH<sub>4</sub> over the four month experiment using the IPCC values for a 100-year timeframe: N<sub>2</sub>O and CH<sub>4</sub> have CO<sub>2</sub> equivalents of 265 and 28, respectively (IPCC, 2014). It should be noted that extrapolation of the global warming potential calculated based on a 4-month greenhouse experiment to a larger scale should be done with caution. Here, we focus on the relative differences between plant communities in control and flood conditions and emphasise that upscaling the absolute values to field scale should be avoided.

### 2.3. Soil analysis

Soil samples were taken from the monoliths after the recovery from the first flood and at the final harvest. One soil core (1 cm diameter  $\times$  25 cm depth) per monolith was taken. After the first soil sampling (after the recovery from the first flood), quartz sand was used to refill the soil core hole to minimize changes in gas diffusion characteristics. Soil was dried at 40 °C for 24 h and sieved to 2 mm. Soil mineral nitrogen and pH were determined by extracting dry soil in a 1:10 (w/v) suspension with 0.01 M CaCl<sub>2</sub> (Houba et al., 2000). Soil and CaCl<sub>2</sub> were shaken for 2 h, and a subsample was taken to measure pH. The remainder of the suspension was centrifuged and filtered (45  $\mu$ m). Extracts were analysed for total dissolved N (N-Nts), nitrate-N (N-NO<sub>3</sub><sup>-</sup> + NO<sub>2</sub><sup>-</sup>) and ammonium-N (N-NH<sub>4</sub><sup>+</sup>) with segmented flow analysis (SFA; Skalar 6 channel SFA analyser, Skalar, Netherlands, 2003).

### 2.4. Plant biomass

Aboveground biomass was harvested to 2 cm after the 2-week adjustment period to confirm that the initial aboveground biomass of the monoliths was initially similar before the flood treatment began (Fig. S1), after each flood, and after each recovery period (i.e. five times in total). Aboveground biomass was sorted per species, dried at 70 °C for 72 h, and weighed. For all analysis, aboveground biomass is considered as the cumulative biomass (sum of five harvests) per species for species-level analyses or per monolith for community-level analyses. After the final harvest, belowground biomass was collected by washing the monoliths over a 0.5 mm sieve and collecting the roots which were then dried at 70 °C for 96 h and weighed.

### 2.5. Plant traits

Aboveground, specific leaf area (SLA; leaf area/leaf dry weight), leaf dry matter content (LDMC; leaf saturated weight/leaf dry weight), leaf nitrogen (mg N/g leaf biomass), and leaf carbon (mg C/g leaf biomass) concentrations were measured at the final harvest. SLA and LDMC were measured according to Cornelissen et al. (2003). The youngest fully expanded leaf was taken from five individuals of each species in each monolith. Saturated weight was determined by placing the leaves in Petri dishes with moist paper towel, storing at 4 °C overnight, blotting dry, and weighing. Leaf area of the saturated leaves was determined

using a LI-3100C Area Meter (Li-Cor Inc., NE, USA). Leaves were then dried at 70 °C for 48 h and weighed to determine dry weight. Nitrogen and carbon contents were determined by ball-milling dried leaf tissue into a fine powder, weighing approximately 4 mg into tin cups, which was analysed at the UC Davis Stable Isotope Facility (California, USA) using an elemental analyser (PDZ Europa ANCA-GSL, Sercon Ltd., Cheshire, UK). Nitrogen uptake (mg N) is the nitrogen concentration (mg N/g biomass dry weight) in leaves multiplied by the aboveground biomass (g biomass).

Belowground, we measured specific root length (SRL; root length/root dry weight), root tissue density (RTD; root dry weight/root volume), root nitrogen (mg N / mg root biomass) and root carbon (mg C / mg root biomass) concentrations. At the final harvest, roots from the entire monolith were first carefully washed and a representative subsample was taken (~0.5 g fresh weight). Subsamples were stored in 70% ethanol at 4 °C until staining with neutral red for 24 h and scanning with an Epson Perfection V700/750 scanner. Images were analysed with WinRhizo (Regent Instruments Inc., Quebec City, QC, Canada) to estimate root length, diameter, and volume. Scanned roots were dried for 48 h at 70 °C, and SRL and RTD were calculated. Root nitrogen and carbon concentrations was determined in the same way as leaf nitrogen and carbon (see above).

Leaf traits are expressed as the community weighted mean (CWM) (Garnier et al., 2004).

$$CWM = \sum_{i=1}^s w_i \cdot x_i$$

Where  $w$  is the relative abundance of species  $i$ , and  $x$  is the trait value of species  $i$ . Root traits are expressed on the community level.

## 2.6. Statistical analysis

The effect of plant community composition (11 communities) or culture (monoculture or mixture), rainfall treatment (control or repeated flooding), and the interaction between plant community composition or culture and rainfall treatment on greenhouse gas emissions were tested with linear mixed effects models using the function `lme` {nlme} (Pinheiro et al., 2016), fitted with a type III SS ANOVA, `anova` {base}. Field plot nested within block was included as a random factor (`random = ~1|block/field plot`) to account for non-independence of the pairs of monoliths (control and flood) taken from the same field plot. When residual variances were heterogeneous a variance structure was included in the model using the function `varIdent`{nlme}. Models with and without the variance structure were compared using the function `anova`{base}, and the model with significantly lower AIC was retained. When considering only flooded monoliths, cumulative N<sub>2</sub>O was log<sub>10</sub> transformed to improve model residual distribution. Differences between levels of a factor were determined using a Tukey

pairwise posthoc test with the function `emmeans`{emmeans} (Lenth, 2018). The Tukey test was run on the significant interaction of the ANOVA model, and if the interaction (e.g. between flood and plant community composition), was not significant, it was run on the significant main effects. Compact letter display (CLD) from `emmeans` was used to determine which treatments were significantly different from each other.

The relations between N<sub>2</sub>O or CH<sub>4</sub> emissions and above- and below-ground biomass, soil mineral nitrogen, and plant traits were tested using linear mixed effects models (`lme`), and type III SS ANOVA (`anova`). To understand if there were species-specific effects on N<sub>2</sub>O and CH<sub>4</sub> across the communities, we tested how cumulative N<sub>2</sub>O and CH<sub>4</sub> emissions related to the relative abundance of each species. Relative abundance of a species is the cumulative aboveground biomass of *species<sub>i</sub>* / cumulative aboveground biomass of *community<sub>i</sub>*,  $i$  being a specific species in a specific community. Cumulative aboveground biomass is the sum of aboveground biomass from all harvests.

All statistical analyses were performed in R version 3.5.0. (R Core Team, 2018). Figures were made using the packages `ggplot2` (Wickham, 2016) and `cowplot` (Wilke, 2019).

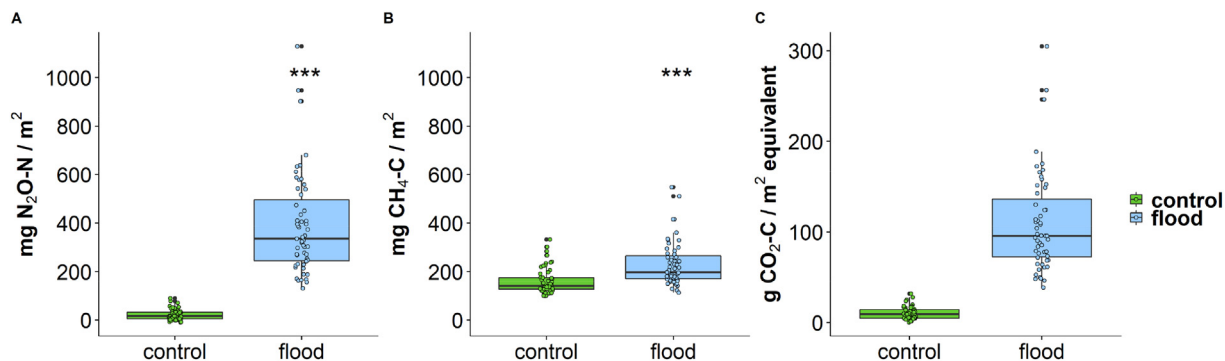
## 3. Results

### 3.1. Flood effect on cumulative N<sub>2</sub>O and CH<sub>4</sub> emissions and the global warming potential

Flooding increased cumulative N<sub>2</sub>O emissions by 97 fold ( $F_{1,54} = 234.97$ ,  $P < 0.001$ ) and CH<sub>4</sub> emissions by 1.6 fold ( $F_{1,54} = 46.26$ ,  $P < 0.001$ ) on average (Fig. 1 A, B). This led to a significantly higher global warming potential (mg CO<sub>2</sub>-C m<sup>-2</sup> equivalent of N<sub>2</sub>O and CH<sub>4</sub>) of flooded compared to non-flooded monoliths ( $F_{1,54} = 433.86$ ,  $P < 0.001$ , Fig. 1 C), which was mainly due to the increase in N<sub>2</sub>O emissions. The mean ratio ± standard error between the global warming potentials of N<sub>2</sub>O:CH<sub>4</sub> was  $1.15 \pm 0.15$  in non-flooded and  $18.15 \pm 1.56$  in flooded conditions. There was a drastic increase in N<sub>2</sub>O fluxes at the onset of flooding, especially the first, mild flood where the soil was waterlogged, compared to the second, severe flood where there was a layer of water over the soil surface (Fig. 2 A). Nitrous oxide emissions produced during the first and second flood accounted for approximately 20% and 0.97%, respectively, of total cumulative N<sub>2</sub>O emissions (Fig. 2 A).

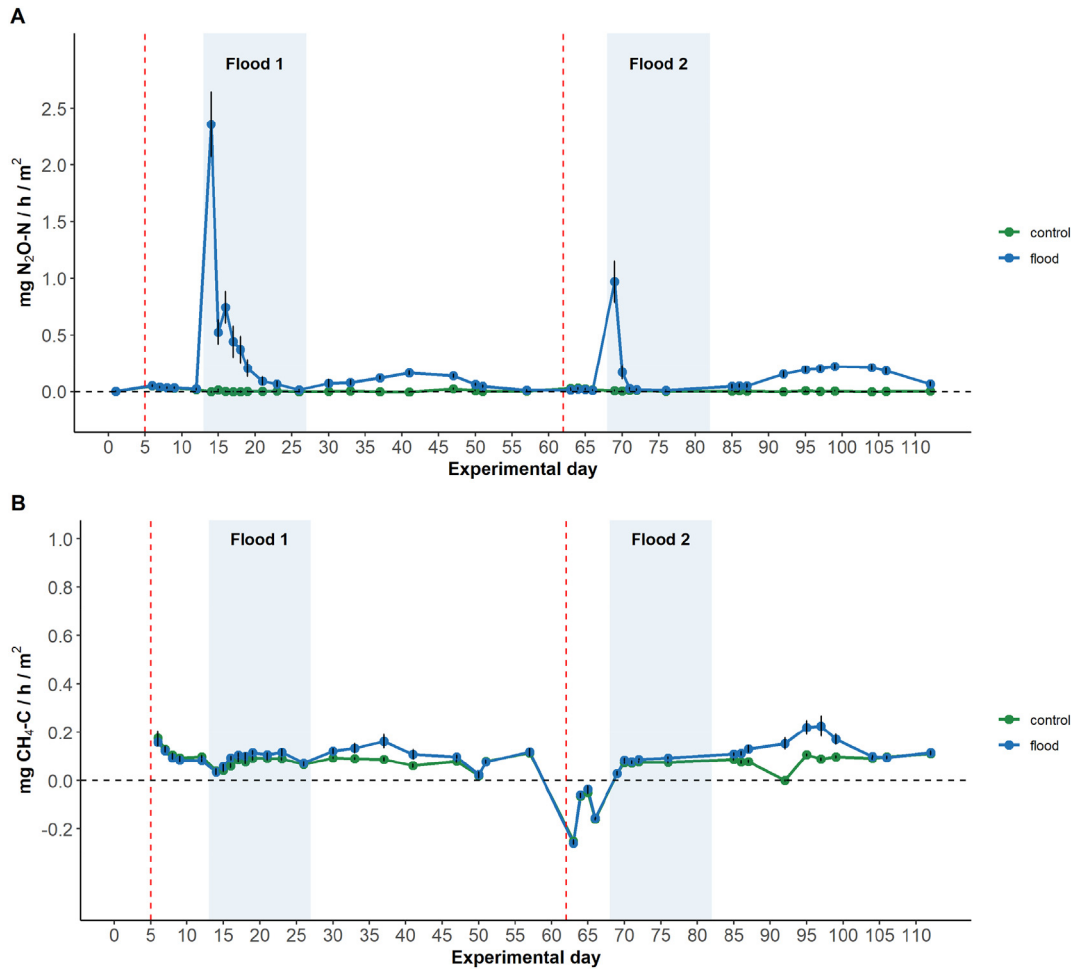
### 3.2. Plant community effects on N<sub>2</sub>O and CH<sub>4</sub> emissions and the global warming potential

In control conditions, mixtures of two or four species emitted less cumulative N<sub>2</sub>O emissions than monocultures ( $F_{1,49} = 4.05$ ,  $P < 0.05$ ), but did not differ in their cumulative CH<sub>4</sub> emissions, and tended to have a lower global warming potential, than monocultures ( $F_{1,49} =$



**Fig. 1.** Flooding significantly increased (A) cumulative N<sub>2</sub>O emissions ( $F_{1,54} = 234.97$ ,  $P < .001$ ), (B) cumulative CH<sub>4</sub> emissions ( $F_{1,54} = 46.26$ ,  $P < .001$ ), and led to a significant increase in (C) the global warming potential, CO<sub>2</sub>-C equivalent of N<sub>2</sub>O and CH<sub>4</sub> emissions, over the experimental period ( $F_{1,54} = 433.86$ ,  $P < .001$ ). Note that the y axis scale of (C) differs from that of (A) and (B). Points are jittered for visualization purposes.



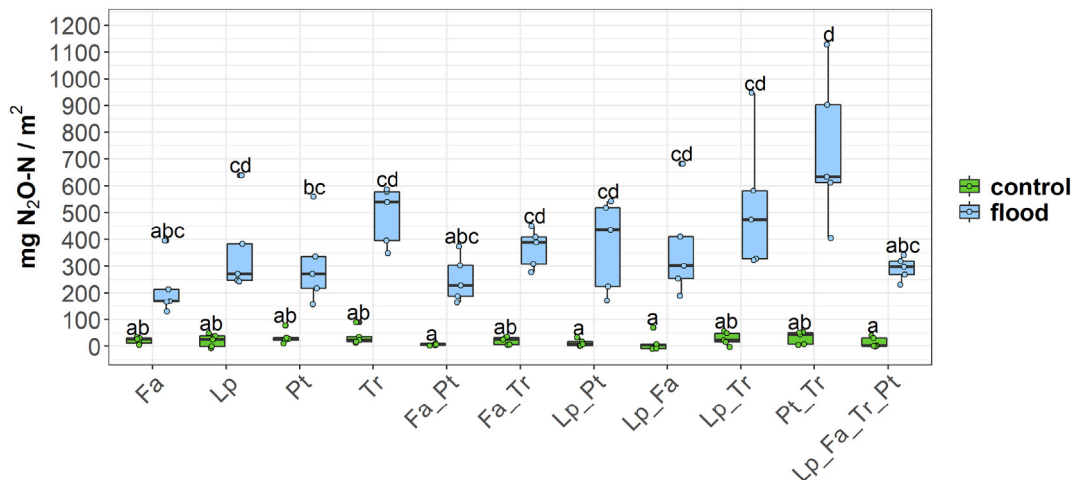


**Fig. 2.** (A) Daily  $\text{N}_2\text{O}$  and (B)  $\text{CH}_4$  fluxes from flooded and control monoliths. The first mild flood occurred on days 13–27, the second severe flood on days 68–82 (blue shaded areas). Vertical red dashed lines indicate nitrogen fertilization, points indicate mean  $\pm$  standard error. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.46,  $P = 0.069$ ). In flooded conditions, mixtures and monocultures did not differ in their cumulative  $\text{N}_2\text{O}$  emissions or global warming potential (NS), while mixtures emitted more  $\text{CH}_4$  ( $F_{1,49} = 6.28$ ,  $P < 0.05$ ).

We found that an interaction between plant community composition and flooding affected cumulative  $\text{N}_2\text{O}$  emissions ( $F_{10,44} = 3.57$ ,  $P < 0.01$ , Fig. 3). In flooded conditions, cumulative  $\text{N}_2\text{O}$

emissions differed between plant communities ( $F_{10,40} = 6.39$ ,  $P < 0.001$ ) but did not differ in control conditions (NS). Flooding significantly increased cumulative  $\text{N}_2\text{O}$  emissions (compared to the control) from most plant communities, but not in the *F. arundinacea* and *P. trivialis* monocultures, the *F. arundinacea* + *P. trivialis* mixture, and the 4-species mixture (Fig. 3). Plant community



**Fig. 3.** Cumulative  $\text{N}_2\text{O}$  emissions from plant communities in flooded or control (non-flooded) monoliths. Letters indicate differences based on a Tukey posthoc test. *Festuca arundinacea* (Fa), *Lolium perenne* (Lp), *Poa trivialis* (Pt), *Trifolium repens* (Tr).

composition affected cumulative CH<sub>4</sub> emissions in control ( $F_{10,40} = 5.57$ ,  $P < 0.001$ ), but not in flooded conditions (NS, Fig. S2). The effects of the plant community on the global warming potential largely mirrored the effects on cumulative N<sub>2</sub>O emissions. Like N<sub>2</sub>O, plant community composition effects on the global warming potential depended on flooding (flooding: plant community composition interaction,  $F_{10,44} = 3.61$ ,  $P < 0.01$ , Fig. S3). Plant community composition tended to affect the global warming potential in control conditions ( $F_{1,40} = 2.03$ ,  $P = 0.055$ ), and significantly affected the global warming potential in flooded conditions ( $F_{1,40} = 5.73$ ,  $P < 0.001$ ).

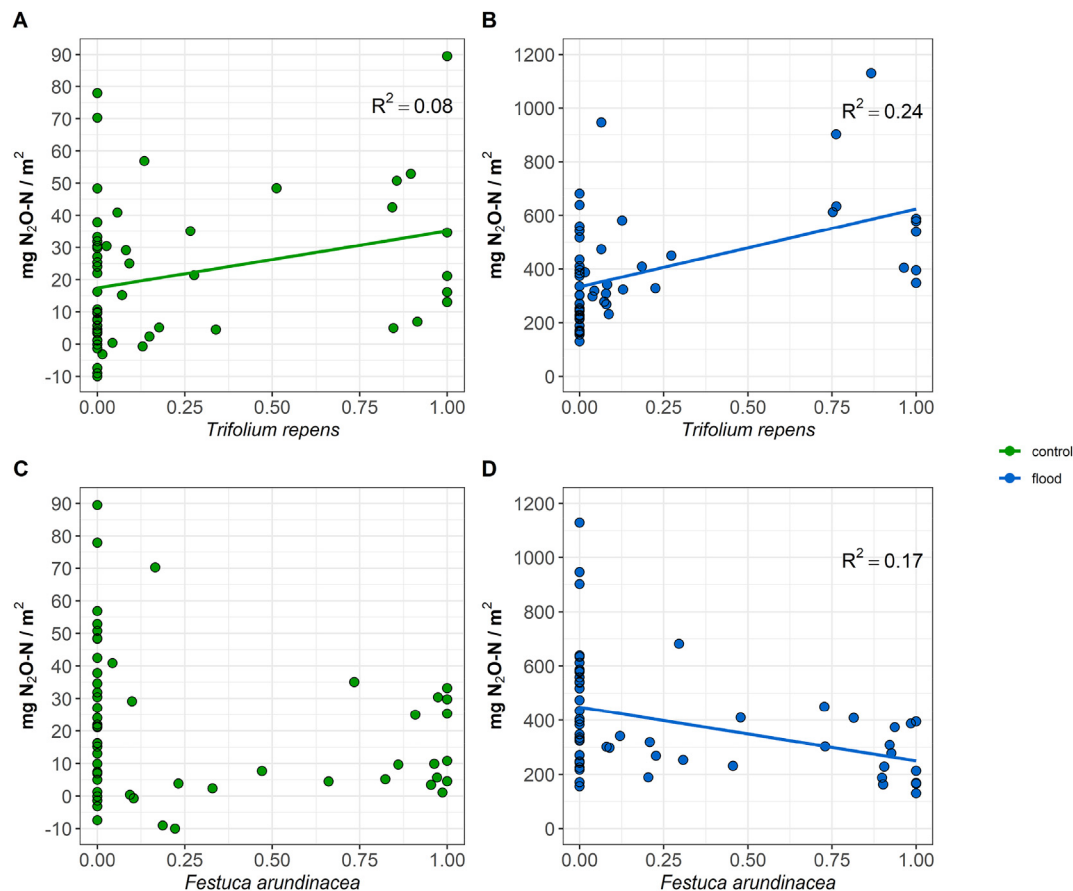
An increase in the relative abundance of aboveground biomass of *T. repens* was related with an increase in cumulative N<sub>2</sub>O emissions from control ( $F_{1,48} = 8.44$ ,  $P < 0.01$ , Fig. 4 A), and flooded monoliths ( $F_{1,49} = 19.72$ ,  $P < 0.001$ , Fig. 4 B). In flooded conditions, an increase in *T. repens* relative abundance was also related with an increase in soil nitrate (NO<sub>3</sub><sup>-</sup>) after the first flood event and recovery ( $F_{1,49} = 4.72$ ,  $P < 0.05$ ,  $R^2 = 0.07$ ), and after the second flood and recovery ( $F_{1,49} = 8.77$ ,  $P < 0.01$ ,  $R^2 = 0.10$ ). The relative abundance of *T. repens* did not affect NO<sub>3</sub><sup>-</sup> in control conditions. In contrast, an increase in the relative abundance of *F. arundinacea* was related to lower cumulative N<sub>2</sub>O emissions from flooded monoliths ( $F_{1,49} = 12.72$ ,  $P < 0.001$ , Fig. 4 D), but did not relate to N<sub>2</sub>O emissions from control monoliths. The relative abundance of *L. perenne* or *P. trivialis* did not affect cumulative N<sub>2</sub>O emissions in control or flooded conditions. Cumulative CH<sub>4</sub> emissions were only positively related to the relative abundance of *T. repens* in control conditions ( $F_{1,49} = 22.41$ ,  $P < 0.001$ ), and not to the relative abundance of other species in control or flooded conditions (NS).

### 3.3. Relationships between plant community biomass and nitrogen uptake with N<sub>2</sub>O and CH<sub>4</sub> emissions

Aboveground biomass differed between plant communities in control ( $F_{10,40} = 8.93$ ,  $P < 0.001$ ) and flooded conditions ( $F_{10,40} = 7.70$ ,  $P < 0.001$ ), Fig. S4 A. Belowground biomass differed between plant communities regardless of flooding (plant community composition,  $F_{10,40} = 83.91$ ,  $P < 0.001$ , Fig. S4 B). Overall, mixtures and monocultures did not differ in terms of aboveground biomass, but produced more belowground biomass in flooded conditions ( $F_{1,49} = 4.31$ ,  $P < 0.05$ ).

Cumulative N<sub>2</sub>O emissions decreased with increasing belowground biomass in control monoliths and flooded monoliths, and did not relate with aboveground biomass (Table 1). The relation between N<sub>2</sub>O emissions and belowground biomass was driven by the presence of *T. repens*, and was not related in communities with only grasses (NS). Cumulative CH<sub>4</sub> emissions were positively related with aboveground biomass in flooded conditions ( $F_{1,49} = 10.37$ ,  $P < 0.01$ ,  $R^2 = 0.12$ ), and negatively related to belowground biomass in control conditions ( $F_{1,49} = 10.11$ ,  $P < 0.01$ ). However, both relations were driven by the presence of *T. repens*; plant biomass did not affect cumulative CH<sub>4</sub> in grass-only communities.

In control conditions, cumulative N<sub>2</sub>O emissions were not related to leaf nitrogen content (total nitrogen in leaves). However, in flooded conditions, N<sub>2</sub>O significantly increased with increasing leaf nitrogen content, which was driven by *T. repens*, and not related in communities with only grasses (Table 1). Cumulative N<sub>2</sub>O emissions were negatively related with root nitrogen content in flooded conditions when the legume was present (Table 1). Soil mineral nitrogen (NO<sub>3</sub><sup>-</sup> or ammonium, NH<sub>4</sub><sup>+</sup>) did not consistently explain cumulative N<sub>2</sub>O emissions (Table 1).



**Fig. 4.** Relation between *T. repens* relative abundance (in terms of aboveground biomass) and the increase in cumulative N<sub>2</sub>O emissions in (A) control monoliths and (B) flooded monoliths. Relation between *F. arundinacea* relative abundance and the decrease in cumulative N<sub>2</sub>O emissions in (C) control monoliths and (D) flooded monoliths. Solid lines indicate significant relationship ( $P < .05$ ). Nitrous oxide emissions in flooded conditions (B, D) were log transformed.

**Table 1**

The relations between cumulative N<sub>2</sub>O emissions and soil nitrogen, plant biomass, leaf nitrogen (N) uptake and root N uptake. A positive or negative sign indicates the direction of the relationship, 'grasses only' indicates that all communities with *T. repens* have been excluded. *n* indicates the number of data points in the analysis. Bold F values indicate statistical significance.

	Cumulative N <sub>2</sub> O emissions							
	Control				Flood			
	<i>F</i>	<i>P</i>	—	<i>n</i>	<i>F</i>	<i>P</i>	—	<i>n</i>
Soil nitrogen (T2)								
N-NH <sub>4</sub> <sup>+</sup> (mg N-NH <sub>4</sub> <sup>+</sup> kg soil <sup>-1</sup> )	F <sub>1,49</sub> = 2.81	‡		55	F <sub>1,49</sub> = 1.12	NS		55
N-NO <sub>3</sub> <sup>-</sup> + N-NO <sub>2</sub> <sup>-</sup> (mg N-NO <sub>3</sub> <sup>-</sup> + N-NO <sub>2</sub> <sup>-</sup> kg soil <sup>-1</sup> )	F <sub>1,48</sub> = 1.39	NS		54	F <sub>1,49</sub> = 2.61	NS		55
Total soil N (mg N kg soil <sup>-1</sup> )	F <sub>1,48</sub> = 0.13	NS		54	<b>F<sub>1,49</sub> = 4.37</b>	*	+	55
N-DON (mg N-DON kg soil <sup>-1</sup> )	F <sub>1,49</sub> = 2.49	NS		55	F <sub>1,49</sub> = 2.93	‡	+	55
pH	F <sub>1,49</sub> = 2.16	NS		55	F <sub>1,49</sub> = 2.62	NS		55
Plant biomass								
Aboveground biomass	F <sub>1,49</sub> = 0.03	NS		55	F <sub>1,49</sub> = 0.24	NS		55
Aboveground biomass (grasses only)	F <sub>1,24</sub> = 1.61	NS		30	F <sub>1,24</sub> = 0.43	NS		30
Belowground biomass	<b>F<sub>1,49</sub> = 4.18</b>	***	—	55	<b>F<sub>1,49</sub> = 14.5</b>	***	—	55
Belowground biomass (grasses only)	F <sub>1,24</sub> = 1.74	NS		30	F <sub>1,24</sub> = 0.23	NS		30
Leaf N uptake								
Leaf N uptake (g N in leaf biomass)	F <sub>1,49</sub> = 3.21	‡	+	55	<b>F<sub>1,49</sub> = 8.58</b>	**	+	55
Leaf N uptake (grasses only)	F <sub>1,24</sub> = 0.32	NS		30	F <sub>1,24</sub> = 0.30	NS		30
Root N uptake								
Root N uptake (g N in root biomass)	F <sub>1,49</sub> = 1.82	NS		55	<b>F<sub>1,49</sub> = 4.63</b>	*	—	55
Root N uptake (grasses only)	F <sub>1,24</sub> = 0.58	NS		30	F <sub>1,24</sub> = 1.21	NS		30

P < 0.001 \*\*\*, P < 0.01 \*, P < 0.05 †, P < 0.10 ‡, P > 0.10 NS.

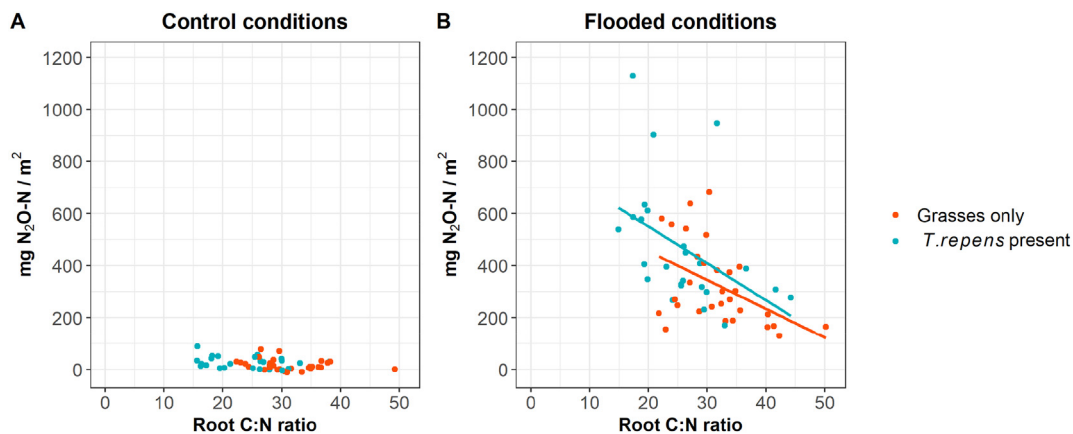
### 3.4. Relationships between plant traits and N<sub>2</sub>O and CH<sub>4</sub> emissions

In control conditions, cumulative N<sub>2</sub>O emissions were positively related with SLA (F<sub>1,49</sub> = 12.69, P < 0.001, R<sup>2</sup> = 0.12) and root nitrogen concentration (F<sub>1,49</sub> = 9.77, P < 0.05), and were negatively related with root C:N (F<sub>1,49</sub> = 12.54, P < 0.001, R<sup>2</sup> = 0.11) and RTD (F<sub>1,49</sub> = 5.90, P < 0.05, R<sup>2</sup> = 0.06). However, these relations driven by the presence of *T. repens*, which had distinct trait values from the grass species (Fig. S5), and these relations were no longer significant when considering communities with only grasses. In flooded conditions, we found similar relations; cumulative N<sub>2</sub>O emissions positively related with SLA (F<sub>1,49</sub> = 10.52, P < 0.01, R<sup>2</sup> = 0.15) and negatively related to RTD (F<sub>1,49</sub> = 7.68, P < 0.01, R<sup>2</sup> = 0.12). Again, these relations were driven by the presence of *T. repens* and were not significant in communities with only grasses. However, in flooded conditions, cumulative N<sub>2</sub>O emissions were negatively related to the plant community's

root C:N ratio (F<sub>1,49</sub> = 27.20, P < 0.001, R<sup>2</sup> = 0.32), and this relation was significant for communities with *T. repens* (F<sub>1,19</sub> = 8.80, P < 0.01, R<sup>2</sup> = 0.26) and with only grasses (F<sub>1,24</sub> = 9.63, P < 0.01, R<sup>2</sup> = 0.22) (Fig. 5 B). In line with this, root nitrogen concentration was positively related with cumulative N<sub>2</sub>O emissions in flooded conditions overall (F<sub>1,49</sub> = 30.87, P < 0.001), in communities with *T. repens* (F<sub>1,18</sub> = 14.96, P < 0.01), and in communities with only grasses (F<sub>1,18</sub> = 4.94, P < 0.05).

Cumulative N<sub>2</sub>O emissions were not related to LDMC, leaf N concentration (mg N/g aboveground biomass), or leaf C:N, in control or flooded conditions. Cumulative N<sub>2</sub>O emissions were significantly positively related with SRL in control conditions in communities with only grasses (F<sub>1,24</sub> = 6.11, P < 0.05, R<sup>2</sup> = 0.12). However, this relation was driven by very high SRL values of *P. trivialis* (Fig. S5).

There were no relations between the plant traits we considered and CH<sub>4</sub> emissions.



**Fig. 5.** Root C:N and cumulative N<sub>2</sub>O emissions in control conditions (A), and in flooded conditions (B). In control conditions, the negative relation was driven by the presence of *T. repens* (i.e. was only significant when legumes were present). In flooded conditions, cumulative N<sub>2</sub>O emissions were significantly related to root C:N ratio overall (F<sub>1,49</sub> = 27.20, P < .001, R<sup>2</sup> = 0.32), in communities with only grasses (orange line, F<sub>1,24</sub> = 9.63, P < .01, R<sup>2</sup> = 0.22) and in communities that included *T. repens* (blue line, F<sub>1,19</sub> = 8.80, P < .01, R<sup>2</sup> = 0.26). Nitrous oxide emissions in flooded conditions were log transformed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

## 4. Discussion

### 4.1. Flooding increases the global warming potential via increases in N<sub>2</sub>O and CH<sub>4</sub> emissions

Flooding increased the global warming potential of our intensively managed grassland communities from 101 kg/ha in control communities to 1098 kg/ha CO<sub>2</sub>-C in flooded communities over the 113 day experiment. Flooding has been shown to increase the global warming potential of grasslands from approximately 21 to 2790 kg CO<sub>2</sub>-C equivalent/ha (Sánchez-Rodríguez et al., 2019a). This highlights that the predicted increase in flooding (IPCC, 2018) will likely increase the global warming potential of intensively managed grasslands (Li et al., 2019).

We found that N<sub>2</sub>O contributed to most of the global warming potential, especially in flooded conditions. Flooding increased N<sub>2</sub>O emissions from 0.21 kg N<sub>2</sub>O-N/ha in control conditions to 3.90 kg N<sub>2</sub>O-N/ha in flooded conditions, within the range reported for fertilized grasslands in ambient (e.g. 0.21–0.24 kg N<sub>2</sub>O N/ha in Shi et al., 2019), and flooded conditions (e.g. 1.0–14.2 kg N<sub>2</sub>O-N/ha in Sánchez-Rodríguez et al., 2019b). Our findings are in line with previous research showing that higher precipitation increases N<sub>2</sub>O emissions (Brown et al., 2012; Liu et al., 2015).

The flood-induced increase in cumulative CH<sub>4</sub> emissions, from 1.60 kg CH<sub>4</sub>-C/ha in control conditions to 2.31 kg CH<sub>4</sub>-C/ha in flooded conditions, was minor because the 2-week flooding periods was likely too short to lower the redox potential to a level where CH<sub>4</sub> is produced. Longer floods, e.g. the 8-week flood in the study of Sánchez-Rodríguez et al. (2019b), have been shown to result in greater increases in CH<sub>4</sub> than N<sub>2</sub>O (Sánchez-Rodríguez et al., 2018; Sánchez-Rodríguez et al., 2019a).

### 4.2. Plant community effects on N<sub>2</sub>O and CH<sub>4</sub> emissions

In control conditions, mixtures of plant species emitted less N<sub>2</sub>O emissions than monocultures (0.18 kg N<sub>2</sub>O-N/ha/year compared to 0.27 kg N<sub>2</sub>O-N/ha/year equivalent on average). Mixtures of plant species have been predicted to emit less N<sub>2</sub>O than monocultures (Abalos et al., 2014), because of higher nitrogen uptake (Fornara and Tilman, 2008; Finn et al., 2013), which reduces nitrogen available for nitrification and denitrification processes. In unfertilized grasslands, mixtures can mitigate N<sub>2</sub>O emissions (Niklaus et al., 2016). However, in the same study, addition of nitrogen fertilizer (at the same rate used in our study, 100 kg N/ha) removed this effect. Here, we found that in our fertilized, non-flooded grassland communities, mixtures of two or four species lowered cumulative N<sub>2</sub>O emissions in control, but not flooded conditions.

In flooded conditions, N<sub>2</sub>O depended on the plant community composition, with four communities able to mitigate the flood-induced increase in N<sub>2</sub>O emissions. Three of these communities contained the grass, *F. arundinacea*, and an increase in the relative abundance of *F. arundinacea* was related to lower N<sub>2</sub>O emissions. This is in agreement with previous studies that show that *F. arundinacea* can mitigate N<sub>2</sub>O emissions under ambient rainfall conditions (Abalos et al., 2014). Here, we show its potential to also mitigate flood-induced increases in cumulative N<sub>2</sub>O emissions.

In control and flooded conditions, the relative abundance of *T. repens* was positively related to cumulative N<sub>2</sub>O emissions, in line with studies that find that legumes contribute to an increase in soil nitrogen (e.g. Thilakarathna et al., 2016) and N<sub>2</sub>O emissions in ambient rainfall conditions in non-fertilized (e.g. Niklaus et al., 2006; Niklaus et al., 2016) and fertilized grasslands (Bowatte et al., 2018). We show that in an intensively managed system, *T. repens* was related with increases in N<sub>2</sub>O emissions in both flooded and control conditions. The N<sub>2</sub>O emissions associated with legumes could be mitigated by reducing mineral fertilizer applied to grasslands with legumes (Hauggaard-Nielsen et al., 2016), as has been shown in ambient conditions (Jensen et al., 2012; Fuchs et al.,

2018). However, the practice of fertilizing grasslands with legumes remains common, as yield benefits are apparent at fertilization rates of 50 to 150 kg N/ha/year (Nyfeler et al., 2011). In flooded conditions, we show that the positive relation between N<sub>2</sub>O and *T. repens* strengthens, which could be due to plant mortality and decomposition of nitrogen-rich tissue (discussed below). In flooded conditions, pairing *T. repens* with grasses that can efficiently take up the nitrogen provided (via symbiotic fixation and decomposition of nitrogen-rich tissues) may lower N<sub>2</sub>O emissions. However, in our study, cumulative N<sub>2</sub>O emissions were not lower when *T. repens* was combined with any of the three grass species (compared to the *T. repens* monoculture).

### 4.3. Plant nitrogen uptake and inputs and N<sub>2</sub>O emissions

Under ambient rainfall conditions, nitrogen uptake and biomass production are key mechanisms by which grass species mediate N<sub>2</sub>O emissions (Abalos et al., 2014, 2018). However, we did not find the expected negative relation between nitrogen uptake or aboveground biomass and N<sub>2</sub>O emissions. It could be that in flooded conditions, decomposition may have a larger influence on N<sub>2</sub>O emissions than nitrogen uptake. This could explain the positive relation between nitrogen uptake of communities with *T. repens* and cumulative N<sub>2</sub>O emissions. More nitrogen-rich plant litter could lead to higher cumulative N<sub>2</sub>O emissions via faster decomposition. Thus, the effects of *T. repens* and *F. arundinacea* on cumulative N<sub>2</sub>O emissions may be in part due to the quality and quantity of their leaf and root biomass that turns over during and after flood stress.

The lower C:N ratio of *T. repens* leaves ( $10.22 \pm 0.26$ ) and roots ( $17.54 \pm 0.78$ ), compared to *F. arundinacea* (leaves:  $14.72 \pm 0.86$ , roots:  $39.22 \pm 1.47$ ), could result in faster decomposition and nitrogen mineralization. Legume litter generally decompose faster than grass litter (e.g. Roumet et al., 2016). Decomposition of *T. repens* root litter may have contributed to the higher available nitrogen that was found in flooded *T. repens* soil, leading to higher N<sub>2</sub>O emissions. Further, *F. arundinacea* produced considerably more root biomass than *T. repens* (in monoculture). On the one hand, this could increase interception and uptake of nitrogen, reducing mineral nitrogen available to be converted to N<sub>2</sub>O. However, since we did not find a strong relation between nitrogen uptake and N<sub>2</sub>O emissions, a more likely explanation may be the influence of *F. arundinacea* on the denitrifier community. The quality of the carbon inputs, which relates to root C:N (McGill et al., 2010), has been shown to mediate denitrifier community activity (Bremer et al., 2009). Higher C:N of plant residues have been shown to relate with lower N<sub>2</sub>O emissions (Huang et al., 2004). Thus, the larger, more recalcitrant root system of *F. arundinacea*, compared to that of *T. repens*, could lower the activity of both denitrifiers and decomposers, thereby reducing N<sub>2</sub>O emissions.

We found that cumulative CH<sub>4</sub> emissions were positively related with aboveground biomass in flooded conditions, a relation which was driven by the presence of *T. repens*. Greater aboveground biomass could lead to relatively more plant tissue death in response to flooding, and more litter entering the soil during and immediately following the flood. An increase in plant biomass (and thus plant litter) serves as a carbon source for methanogens, and has been shown to increase CH<sub>4</sub> emissions (Banger et al., 2012; Bodelier and Steenbergh, 2014). This increase in carbon availability could underlie the increase in CH<sub>4</sub> emissions following flooding, especially after the severe flood. However, since this relation was not significant when considering the communities with only grasses, it should be interpreted with caution, as it may change when a broader range of species is considered.

### 4.4. Plant traits and N<sub>2</sub>O emissions

We found that plant communities with an acquisitive strategy (broad, thin leaves, and nitrogen-rich roots with low tissue density) had higher N<sub>2</sub>O emissions in control conditions, in contrast to our



hypothesis and previous research (Abalos et al., 2018). However, this relation was only significant when *T. repens* was present, and not in communities with only grasses. In flooded conditions, we found that plant communities with a conservative strategy (thinner leaves and denser tissues) had lower N<sub>2</sub>O emissions, in line with our hypothesis. Conservative communities produce slower-decomposing litter (Freschet et al., 2012), and are related to lower nitrogen mineralization (Orwin et al., 2010), and higher nitrogen retention (Grigulis et al., 2013). However, these relations were again driven by the presence of *T. repens* and were not significant in communities with only grasses.

Root C:N ratio was negatively related with cumulative N<sub>2</sub>O emissions in communities with *T. repens* and those containing only grasses. Addition of relatively nitrogen-poor (high C:N ratio) root litter could mitigate N<sub>2</sub>O emissions. Thus, especially in flooded conditions where root turnover is likely higher than in non-flooded conditions, nitrogen-poor root litter could lower the nitrogen source for N<sub>2</sub>O and the rate at which it is produced by the microbial community. Root traits mediating decomposition may play a larger role in N<sub>2</sub>O emitted during flooding and drying cycles than traits related to nitrogen uptake. How traits related to decomposition affect N<sub>2</sub>O emissions during and after flooding could improve our understanding of nitrogen cycling as the frequency and intensity of flooding increases.

Finally, functional traits of plant species and communities, related to nutrient uptake and decomposition, have been shown to influence the soil microbial community, and feedbacks between plants and soil microbes (Baxendale et al., 2014; Cortois et al., 2016; Veen et al., 2019). Plants and microbes strongly influence ecosystem functioning and nitrogen cycling in nitrogen-rich grasslands (de Vries et al., 2015; Legay et al., 2016). Therefore, connecting plant traits to the nitrogen cycling microbial community structure and activity in flooded grasslands is an important next step in understanding flood effects on N<sub>2</sub>O emissions.

## 5. Conclusions

We show that repeated flooding increases the global warming potential of intensively managed grasslands by increasing cumulative N<sub>2</sub>O emissions by 97 fold, and cumulative CH<sub>4</sub> emissions by 1.6 fold on average. This increase in greenhouse gas emissions could perpetuate a positive feedback whereby increased floods related to global warming increase greenhouse gas emissions (Li et al., 2019). We found that plant species identity has consequences for the global warming potential of intensively managed grasslands in flooded conditions. Increasing abundance of *F. arundinacea* was related with significantly lower N<sub>2</sub>O emissions. Nitrous oxide emissions were reduced by 54%, on average, when the *F. arundinacea* relative abundance was 1 (monoculture) compared to 0 (not present). This suggests that in the face of extreme weather events, it is likely an important species to include in grassland mixtures as it can cope with a range of temperature and moisture conditions (Gibson and Newman, 2001), including flooding (Ploschuk et al., 2017) and drought (Wilman et al., 1998), while mitigating the grassland's N<sub>2</sub>O emissions (present study). Our findings suggest that grasses with conservative root traits, such as a high C:N ratio, may become more important in the future to lower the global warming potential of intensively managed grasslands in the face of extreme weather events. Further, consideration of flood tolerant forage legumes, such as *Trifolium fragiferum* or *Lotus corniculatus* (Striker and Colmer, 2017) grown with grasses with conservative traits could contribute to maintaining productivity while reducing the global warming potential of flooded grasslands.

## CRedit authorship contribution statement

**Natalie J. Oram:** Conceptualization, Data curation, Formal analysis, Writing - original draft. **Jan Willem van Groenigen:** Writing - review & editing. **Paul L.E. Bodelier:** Writing - review & editing. **Kristof Brenzinger:** Writing - review & editing. **Johannes H.C. Cornelissen:**

Writing - review & editing. **Gerlinde B. De Deyn:** Writing - review & editing. **Diego Abalos:** Investigation, Writing - review & editing.

## Declaration of competing interest

The authors declare no conflict of interest.

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