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1. Title Page

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Title:

Separate effects of flooding and anaerobiosis on soil greenhouse gas emissions and redox sensitive biogeochemistry

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2. Key Points:

- Soils were incubated in crossed anaerobic headspace and flooding treatments
- Flooding stimulated or suppressed anaerobic greenhouse gas fluxes
- Flooding had additional biogeochemical effects independent of oxygen depletion

3. Abstract:

Soils are large sources of atmospheric greenhouse gases, and both the magnitude and composition of soil gas emissions are strongly controlled by redox conditions. Though the effect of redox dynamics on greenhouse gas emissions has been well studied in flooded soils, less research has focused on redox dynamics without total soil inundation. For the latter, all that is required are soil conditions where the rate of oxygen (O₂) consumption exceeds the rate of atmospheric replenishment. We investigated the effects of soil anaerobiosis, generated with and without flooding, on greenhouse gas emissions and redox-sensitive biogeochemistry. We collected a Histosol from a regularly flooded peatland pasture and an Ultisol from a humid tropical forest where soil experiences frequent low redox events. We used a factorial design of flooding and anaerobic dinitrogen (N₂) headspace treatments applied to replicate soil microcosms. An N₂ headspace suppressed carbon dioxide (CO₂) emissions by 50% in both soils. Flooding, however, led to greater anaerobic CO₂ emissions from the Ultisol. Methane emissions under N₂ were also significantly greater with flooding in the Ultisol. Flooding led to very low N₂O emissions after an initial pulse in the Histosol, while higher emission rates were maintained in control and N₂ treatments. We conclude that soil greenhouse gas emissions are sensitive to the redox effects of O₂ depletion as a driver of anaerobiosis, and that flooding can have additional effects independent of O₂ depletion. We emphasize that changes to the soil diffusive environment under flooding impacts transport of all gases, not only O₂, and changes in dissolved solute availability under flooding may lead to increased mineralization of C.

4. Keywords
Soils are globally significant sources of the atmospheric greenhouse gases carbon dioxide (CO₂), nitrous oxide (N₂O), and methane (CH₄). Soils are responsible for annual CO₂ emissions that are an order of magnitude greater than industrial sources [Raich and Potter, 1995] and produce 70% of total N₂O emissions, and 60% of natural CH₄ emissions [Conrad, 1996]. Redox potential strongly controls the magnitude and composition of soil greenhouse gas emissions. Under oxic conditions, soil respiration is dominated by the reduction of molecular O₂ due to its abundance and thermodynamic favorability as an electron acceptor, while anaerobic respiration pathways using alternative terminal electron acceptors (TEAs) are inhibited [Ponnamperuma, 1972]. Following O₂ depletion, a cascade of alternative TEAs is utilized by a diverse set of facultative or obligate anaerobic microorganisms [Megenigal et al., 2004]. Reduction of alternative TEAs typically follows the sequence: nitrate (NO₃⁻), manganic manganese (Mn⁢³⁺/Mn⁴⁺), ferric iron (Fe³⁺), sulfate (SO⁴²⁻), and CO₂ [Takai and Kamura, 1966; Peters and Conrad, 1996]. The reduction of O₂ and alternative TEAs can lead to CO₂ production via coupled oxidation of labile organic carbon (C) compounds [Lovley et al., 1991; Roden and Wetzel, 1996; Dubinsky et al., 2010]. Reduction of NO₃⁻ and CO₂ leads to the production of N₂O and CH₄. Though these two gases are generally produced in much smaller quantities, their per-molecule solar-radiative forcing effects are 298 and 25 times greater than CO₂, respectively, over 100 years [Forster et al., 2007]. Thus, the global warming potential of soil gas emissions is closely related to redox conditions.

Investigations of the effects of redox on greenhouse gas emissions have been conducted predominantly with flooded soils due to the close in situ coupling between flooding and anaerobic conditions [Freeman et al., 1993; Regina et al., 1999; De-Campos et al., 2011]. Flooding is one of the...
dominant mechanisms leading to O$_2$ depletion and low redox conditions. By greatly retarding the
diffusion rate of O$_2$ in the soil matrix, flooding can cause O$_2$ demand to exceed rates of diffusive
resupply leading to anaerobic conditions over timescales of hours to days [Takai and Kamura, 1966].

Observations of microbial activity in agricultural soils support a simple model where activity
decreases due to O$_2$ limitation as soil moves from field capacity to saturation [Linn and Doran, 1982],
however flooding may also impact soil biogeochemistry and greenhouse gas emissions independent of
the direct redox changes. For example, flooding radically alters the soil physicochemical environment;
the pore-space phase-change from gas to liquid slows diffusion of dissolved gases in general, while it
may also expedite solute transport and availability by making diffusion paths less tortuous. Moreover
soil structure and micro- and macro-porosity can be affected by changes in moisture primarily via
swelling and shrinking of clay minerals (Mitchell and Soga, 1976). The effects of flooding on soil
matrix aggregation have also been studied, but have not been distinguished from the effects of O$_2$
depletion alone [Kirk et al., 2003; De-Campos et al., 2011], and we know of no studies that have
experimentally separated the effects of flooding and anaerobic conditions on greenhouse gas emissions.

Notably, anaerobic conditions can arise in the absence of flooding, even in upland soils. Humid and
finely textured or organic soils displaying sufficiently high biological activity or low gas diffusivity can
deplete soil O$_2$ and drive low redox reactions [Grable and Siemer, 1968; Magnusson, 1992; Silver et
al., 1999; Schuur, 2001; Liptzin et al., 2010; Hall et al., 2013; Silver et al., 2013]. Anaerobic microsites
are likely to exist even in well-drained soils and explain the observation of net CH$_4$ production in
upland soils [Teh et al., 2005].

Soil disaggregation and reductive dissolution of organo-mineral complexes under flooded
conditions may enhance the availability of carbon (C) substrates for degradation [Ponnampерuma,
1972; Suarez et al., 1984; Kirk et al., 2003; Thompson et al., 2006; De-Campos et al., 2009]. If soil
aggregation and organo-mineral associations previously acted as a barrier between microorganisms and
C substrates, then these changes could theoretically impact both CO$_2$ and CH$_4$ emissions [Teh and Silver, 2006]. Similarly, increased soil matrix connectivity under flooded conditions could connect microbes to dissolved solutes; nitrate (NO$_3^-$) bioavailability, for example, could be enhanced by flooding due to lower soil tortuosity [Nye, 1979; Kirk et al., 2003] and this could stimulate NO$_3^-$ reduction and associated N$_2$O production relative to a non-flooded anaerobic soil. Alternatively, flooding may dilute nutrients and C substrates in soil water, reducing bioavailability for microbes and leading to lower rates of soil respiration [Cleveland et al., 2010]. Flooding could also decrease N$_2$O emissions due to slower dissolved gas-phase diffusivity which increases the probability of microbial reduction of N$_2$O in the soil matrix and shifts the proportion of gaseous nitrogen (N) emissions from N$_2$O toward N$_2$ [Patrick and Reddy, 1976; Firestone and Davidson, 1989].

In this study, we hypothesized that anaerobiosis under flooded and unflooded conditions may have experimentally distinguishable effects on soil greenhouse gas emissions. We used soils from two ecosystems that experience fundamentally different soil redox regimes: a periodically flooded temperate peatland Histosol and an Ultisol from an upland, clay rich humid tropical forest. Our experiment was designed to explore the separate and combined effect of flooding and anaerobiosis on greenhouse gas emissions and related soil biogeochemical characteristics.

2. Method

We collected soil samples at the water table interface (80-100 cm deep) in a drained peatland pasture on Sherman Island, in the Sacramento-San Joaquin Delta, USA (38.04 N, 121.75 W) and from an Ultisol in a lower montane wet tropical forest in Luquillo Experimental Forest, Puerto Rico (18.18 N, 65.50 W). The drained peatland pasture soil is classified as a fine, mixed, superactive, thermic Cumulic Endoaquoll, consisting of a 25 to 92 cm oxidized layer exhibiting ~20-30 % soil carbon overlying a 151 to 292 cm thick organic peat horizon [Drexler et al., 2011; Teh et al., 2011]. We
collected soil from the intact peat layers only and refer to the soil as a Histosol hereafter. Soils from the tropical forest were clay-rich Ultisols exhibiting 12 % soil organic C and a mineral fraction dominated by Al and Fe oxides [Beinroth, 1982; Silver et al., 1999].

We intentionally selected two highly contrasting soil types that both experience periodic anaerobiosis due to different drivers. Oxygen depletion in the peat soil occurs primarily as a result of water table fluctuations and soil saturation, whereas in the tropical forest Ultisol gas-phase O₂ can be depleted without soil inundation [Silver et al., 1999]. The Histosol samples were transported in Ziploc™ bags from the Sacramento Delta and the Ultisol samples were shipped overnight from Puerto Rico. Both soils were prepared for incubation in the laboratory within 24 hours of arrival. Soils were homogenized with gentle mixing, and roots, rocks, and plant litter were removed. Subsamples of 250 g fresh soil were transferred to 1-quart Mason jars and placed in light-tight boxes to prevent phototrophic metabolism.

The experimental design employed a full factorial of two manipulations to produce four treatment groups (n = 6): ambient (21 % O₂) headspace and field moisture (control), ambient headspace and flooded (flooded), anaerobic headspace and field moisture (N₂), and anaerobic headspace and flooded (flooded N₂). We flooded the soils by inserting a funnel through the soil and gradually adding DI H₂O at ambient temperature until the entire soil was inundated while minimizing the depth of overlying water. Soil was flooded from the bottom up which has a tendency to maximize displacement of gas using DI H₂O equilibrated with either ambient air or pure N₂ for flooded and flooded N₂ treatments respectively. To produce the N₂-headspace we placed jars in a glovebox and purged the headspace for 30 min with ultra-pure N₂ gas (flow rates and timing determined a priori) then maintained N₂ flow at a lower flow rate for the duration of the incubation. Soil in field moisture (control and N₂) treatments was initially at field capacity at the time of collection and was maintained
gravimetrically by DI H$_2$O additions from bottles equilibrated either with ambient air or the pure N$_2$

glovebox headspace.

Gas samples were collected 11 times over 20 days for the Histosol and 8 times over 15 days for the Ultisol. Gas samples were collected by isolating the headspaces of the jars with lids fitted with rubber septa, mixing the headspace by gently pumping a 30 ml syringe three times, then sampling 30 ml of headspace. Samples were taken immediately after sealing, and after one hour. The gas samples were placed in 20 ml, pre-evacuated, helium-flushed glass vials crimped with rubber septa. Approximately 5 ml of gas was analyzed for CO$_2$, CH$_4$, and N$_2$O concentration using a Shimadzu GC-14A gas chromatograph (Shimadzu Scientific Inc., Columbia, Maryland, USA) within 48 hours of sampling. Concentrations were converted to molar quantities using the ideal gas law and headspace volume, and fluxes modeled assuming a linear change in concentration over the course of the 1 hr incubation.

Soil pH, mineral nitrogen, and HCl-extractable ferrous Fe ($\text{Fe}^{2+}$) and Fe$^{3+}$ were measured at the end of the incubations for all treatments. We chose to examine patterns in N and Fe as previous research had shown both sites to be rich in these redox-active species [Silver et al., 1999; Pett-Ridge et al., 2006; DeAngelis et al., 2010; Yang et al., 2011]. Soil pH was measured in 2:1 water/soil slurry. A 10 g subsample of fresh soil was oven-dried to a constant weight at 105 °C to determine moisture content. Concentrations of ammonium ($\text{NH}_4^+$) and nitrate ($\text{NO}_3^-$) were measured after extracting soil in 2 M KCl, shaking for an hour at 180 rpm, and running filtered extracts on a Lachat QC8000 flow injection analyzer using a colorimetric analysis (Lachat Instruments, Milwaukee, Wisconsin). A concentrated phosphate solution was added to KCl extracts prior to analysis to eliminate Fe interference [Yang et al., 2012]. The most labile Fe fraction was extracted in 0.5 M HCl and Fe$^{2+}$ concentrations were determined colorimetrically by diluting 100 µL of extracted sample in 100 µL DI H$_2$O and adding 1.8 mL of ferrozine solution (1 g/L ferrozine in 50 mM HEPES buffer, pH 8) then...
measuring absorbance at 562 nm. Ferric Fe concentrations were determined with the same colorimetric method by substituting 100 µL of 10% hydroxylamine for DI H₂O [Stookey, 1970; Viollier et al., 2000].

A mixed-effects ANOVA statistical model was developed using the lme package in R to test the significance of the effects of treatments, time, and their interaction, on CO₂, CH₄, and N₂O fluxes. The model consisted of a fixed treatment effect (Treatment) and a random time effect (Day), including a treatment-temporal interaction (Treatment*Day). A Tukey range multiple-comparison test was used to assess which treatments differed significantly on each day whenever all three effects (Treatment, Day, and Treatment*Day) were all found to be significant in the mixed effects model. We treated the two study sites separately as our goal was not to directly compare the Histosol and Ultisol, but to explore how each responded to the range of treatments applied. Significant treatment effects on redox sensitive soil characteristics measured at the end of the incubation were tested using fixed-effects ANOVA and a Tukey range multiple-comparison test in R. Statistical significance was determined at P < 0.05 unless otherwise noted. Values reported in the text are means ± one standard error.

3. Results

3.1 Treatment Effects on the Histosol

For the Histosol, rates of soil CO₂ emissions were approximately 50% lower than the control throughout the incubation under flooded, N₂, and flooded N₂ treatments (P < 0.0001 for all treatments, Figure 1a; Table 1). Flooding initially decreased CO₂ emissions relative to the unflooded N₂ treatment, but the effect did not persist past the fourth day of the experiment. Methane emissions were close to the experimental detection limit (< 1 ng C g⁻¹ hr⁻¹) throughout most of the study in the Histosol, ranging from -0.77 ng C g⁻¹ hr⁻¹ to 1.82 ng C g⁻¹ hr⁻¹ (Figure 1c). Nitrous oxide emissions differed significantly across treatments and through time (Figure 1e; Table 1). Emissions of N₂O dropped to zero by Day 1 in
the flooded N\textsubscript{2} treatment and did not increase throughout the remainder of the incubation. In contrast, net N\textsubscript{2}O emissions occurred throughout the experiment in the control and N\textsubscript{2} treatments. In the flooded treatment, N\textsubscript{2}O spiked between Day 2 and Day 11, and peaked on Day 5 with an N\textsubscript{2}O emission rate of 19.0 ± 2.2 ng N g\textsuperscript{-1} hr\textsuperscript{-1}.

Soil pH was significantly greater in flooded N\textsubscript{2} (6.5 ± 0.02) and N\textsubscript{2} (6.7 ± 0.04) treatments than the control (6.0 ± 0.02) in the Histosol (P < 0.05, Table 2). Nitrate concentrations were high (130 ± 4.5 µg N g\textsuperscript{-1}) in the control and below detection in all other treatments. The pattern was reversed for NH\textsubscript{4}\textsuperscript{+}, with concentrations below detection (< 0.5 µg N g\textsuperscript{-1}) in the control treatment and significantly higher in all other treatments. Soils NH\textsubscript{4}\textsuperscript{+} concentrations were highest in the flooded N\textsubscript{2} treatment, followed by the N\textsubscript{2} treatment, and lowest in the flooded treatment. Iron reduction was stimulated in the flooded and flooded N\textsubscript{2} treatments with 60 to 70 % of HCl-extractable Fe in the reduced phase and lower reduced fractions observed in the N\textsubscript{2} and control treatments (Table 2).

3.2 Treatment Effects on the Ultisol

Different trends in fluxes were observed in the Ultisol. Rather than converging over time, soil CO\textsubscript{2} emissions from the flooded treatments diverged from the N\textsubscript{2} treatment and, on Day 15, were not significantly different from the control (Figure 1b). Soil CO\textsubscript{2} emissions in the N\textsubscript{2} treatment dropped gradually over time to a level approximately 50 % of the control. Significant CH\textsubscript{4} emissions were observed in all but the control treatment, ranging from zero initially in all treatments to a maximum of 14.1 ± 2.4 ng C g\textsuperscript{-1} hr\textsuperscript{-1} by Day 15 in the flooded N\textsubscript{2} treatment (Figure 1d). Emissions of CH\textsubscript{4} from the N\textsubscript{2} and flooded N\textsubscript{2} treatments differed significantly by the end of the incubation, with rates 3 times greater in the latter by Day 15. For most of the incubation period N\textsubscript{2}O emissions were very low from the Ultisol (Figure 1f), and were close to the experimental precision (< 2 ng N g\textsuperscript{-1} hr\textsuperscript{-1}).
Soil pH was significantly greater under flooding and N₂-headspace treatments (6.3 ± 0.07 to 6.6 ± 0.09) relative to the control (5.8 ± 0.01) in the Ultisol (Table 2). Soil NO₃⁻ concentrations were below the detection limit of the analytical instrumentation in all treatments (< 0.2 µg N g⁻¹). Ultisol NH₄⁺ concentrations were highest in the flooded N₂ treatment, followed by the N₂ treatment, and then the flooded control. Iron reduction was observed with 75 to 95 % of HCl-extractable Fe in the reduced phase in the flooded and flooded N₂ treatments and significantly lower reduced fractions observed in the N₂ and control treatments.

4. Discussion

4.1 Separate Effects of Flooding and Anoxia

In the Histosol, which experiences regular flooding events, the impact of the N₂-headspace on soil respiration was equal to the effect of flooding for most of the incubation. This suggests that the principle cause of lower CO₂ emissions following flooding in the Histosol was lower O₂ availability and inhibition of aerobic respiration. An additional suppression of CO₂ emission rate was observed between flooded and N₂ treatments initially (prior to Day 4) which may be due to the dissolution of CO₂ into added water, rather than an effect on CO₂ production. In the tropical forest Ultisol, that rarely experiences flooding under natural conditions, the unflooded anaerobic treatment (i.e. N₂ treatment) decreased CO₂ emissions by ~50 % over the incubation, whereas flooding resulted in only a short-term decline followed by an increase in CO₂ emissions that equaled the control treatment by the end of the incubation. Soil respiration increased in both flooded treatments between Day 8 and Day 15, while under an N₂-headspace alone soil respiration continued to decline. These results are evidence that flooding and anoxia can have distinct effects on soil respiration.

There are several potential mechanisms that could have contributed to the patterns observed in the Ultisol. Flooding may have enhanced the availability of non-O₂ TEAs leading to more anaerobic
respiration and CO$_2$ production. Both greater methanogensis and greater Fe reduction observed in the flooded N$_2$ treatment could be the source of additional CO$_2$. Increases in soil pH during reduction can lead to solubilization of C and has been shown to be an important mechanism in highly weathered soils [Thompson et al., 2006; Wagai and Mayer, 2007], however pH changes from an initial analysis in the present study were modest (0.4 – 0.8; data not shown). Flooding may have facilitated the destabilization of organo-mineral complexes and increased labile C availability relative to the unflooded, but anaerobic soil. Past studies have found that flooding can lead to soil disaggregation, dissolution of soluble constituents, and concurrent increases in soil solution dissolved organic C (DOC) availability [Ponnampерума, 1972; Suarez et al., 1984; Kirk et al., 2003; De-Campos et al., 2009]. The Ultisol is characterized by high Fe-oxide content and organo-mineral associations in these soil types can contribute substantially to C storage [Silver et al., 1999; Dubinsky et al., 2010]. Density fractionation performed on surface (0-10 cm) samples of the same Ultisol found that 78-88 % of total soil C was in the mineral-associated (dense) fraction (Hall et al., unpublished data). In contrast, free-light and occluded-light C fractions dominate Histosols, which did not exhibit a similar stimulation of CO$_2$ or CH$_4$ emission. We therefore propose that the physical disaggregation or reductive dissolution of organo-mineral complexes could have led to a release of formerly protected C that was then exposed to mineralization processes under flooding. In this way, flooding may act to influence soil redox conditions, not only by changing the dominant TEA processes, in this case O$_2$ availability, but also by influencing the availability of C as electron donors. Our results show that flooding maintained elevated CO$_2$ emissions relative to an N$_2$ headspace treatment alone and thus we demonstrate a separate effect of flooding on anaerobic soil respiration rates in the Ultisol.

We detected no CH$_4$ emissions from the Histosol. These soils have shown methanogenesis under flooded conditions in the field [Teh et al., 2011] and the lack of net CH$_4$ production during the 30 day laboratory incubation was surprising. However, other peatland soil incubation studies have
observed delays of > 30 days for the onset of methanogenesis after re-flooding of experimentally dried soil [Estop-Aragonés and Blodau, 2012] or partly drained peatland soil [Jerman et al., 2009]. Iron reduction may have contributed to a competitive inhibition of CH₄ production [Teh et al., 2006], as at least 30 % of the acid-extractable Fe was still present as Fe³⁺ by the end of the experiment in these Fe-rich peatland soils. Flooding stimulated net CH₄ emissions under anaerobic conditions in the Ultisol. If CH₄ production was predominantly via acetate-cleavage rather than hydrogenotrophic CO₂ reduction [Conrad, 1999; Chasar et al., 2000; Ye et al., 2012], then increased labile C availability from flooding could have been responsible for the patterns observed.

Separate effects of flooding and O₂ depletion alone were observed for Histosol N₂O fluxes with sustained net N₂O emissions in the N₂ treatment and zero N₂O emission under flooding. Disappearance of N₂O emissions under flooding may have been caused by more rapid NO₃⁻ depletion; inhibiting further denitrification to N₂O. The continued net N₂O emission in the absence of flooding may be attributable to faster diffusion in the gas-filled pore spaces of the field moisture treatment. This interpretation follows from the ‘hole-in-the-pipe’ conceptual model proposed to explain patterns in NO, N₂O, and N₂ soil gas emissions [Firestone and Davidson, 1989]. The model proposes that soils with gas-phase pore spaces are more ‘leaky’ to gaseous intermediates during denitrification than low porosity or flooded soils [Bollman and Conrad, 1998; Davidson et al., 2000]. Headspace O₂ removal and flooding may both lead to a loss of NO₃⁻ (Table 2) via denitrification, but differences in the rate of NO₃⁻ reduction and differences in soil diffusivity specifically associated with flooding may explain the observed treatment differences in N₂O emission rates.

4.2 Quantitative Importance of Aerobic Respiration
In both soils, headspace O\textsubscript{2} removal (N\textsubscript{2} treatment versus control) resulted in a large (~50 \%) suppression of respiration rates. Suppression was observed immediately (< 1 day) in the Histosol in contrast to a gradual decline in the Ultisol. The large, and sudden, response of the Histosol to reduced O\textsubscript{2} availability supports recent research that has proposed a critical role for O\textsubscript{2} in peatland C degradation. Oxygen is important as a high energy-yield TEA for the final step of C mineralization by soil microbes, but earlier steps are also dependent on available O\textsubscript{2} such as the activity of extracellular oxidative enzymes. The inhibition of oxidative enzymes due to anoxia has been proposed to function as an enzymatic latch on soil C pools, for flooded or low redox soils in particular [Freeman et al., 2001; Sinsabaugh, 2010]. Thus direct inhibition of aerobic respiration likely explains the immediate drop in respiration, but the continued, more gradual, decline could be a result of reduced oxidative enzyme activity.

In the Ultisol a reduction in soil respiration (N\textsubscript{2} treatment versus control) was not observed until Day 3 and increased in magnitude only gradually thereafter. There are several potential explanations for this pattern. First, it is possible that aerobic microsites environments persisted in the high clay soil, and O\textsubscript{2} continued to be consumed over the early period of the incubation. However it is also possible that alternative TEAs, such as the abundant Fe in these soils, dominated respiration even in aerobic conditions (control treatment) where they were regenerated by available O\textsubscript{2}, and that the gradual decline in respiration under N\textsubscript{2} occurred as the alternative TEAs were exhausted. This interpretation is consistent with the emerging view that C cycling in clay-rich Ultisols found in tropical forests is driven by the rotation of the Fe\textsuperscript{3+}-Fe\textsuperscript{2+} redox wheel [Chacón et al., 2006; Dubinsky et al., 2010; Li et al., 2012; Hall and Silver, 2013] and may explain observed decoupling of soil respiration from moisture and O\textsubscript{2} availability \textit{in situ} [Hall et al., 2013].

4.3 Effects of O\textsubscript{2} Availability on Flooded-soil Greenhouse Gas Emissions
The experimental design also allowed us to test the effects of higher versus lower O₂ availability on flooded-soil biogeochemistry (flooded versus flooded N₂ treatment). The flooded Histosol with an oxic headspace had very similar heterotrophic respiration rates to the flooded N₂ treatment, indicating aerobic respiration was not quantitatively important under flooding. Minimal aerobic respiration is consistent with studies of wetland sediments or peatland soils that have measured dissolved O₂ across fine spatial gradients and show depletion within a few millimetres or centimetres of the oxic interface [Takai and Kamura, 1966; Askaer et al., 2010]. In contrast, an oxic headspace was found to significantly suppress flooded-soil CH₄ emissions in the Ultisol. Methanotrophic bacteria can couple the oxidation of CH₄ to the reduction of O₂ [Hanson and Hanson, 1996]. Assuming the treatment difference was entirely due to oxidation, we estimate that up to 80-85 % of CH₄ was consumed during upward diffusion in the microcosm by the end of the incubation. Such strong attenuation of CH₄ emissions has been observed in other systems dominated by diffusive fluxes; oxic-anoxic interfaces at rice-plant rhizospheres can consume up > 90 % of the net CH₄ flux [Holzapfel-Pschorrn et al., 1986] and oxygenated water-columns have also been shown to ameliorate CH₄ emissions by up to 90 % [King, 1990].

The rates of nitrification and denitrification, driven by higher and lower O₂ availability respectively, complement the concept of pore-space diffusivity to explain the distinct N₂O emissions observed between the flooded and flooded N₂ treatments. In the flooded Histosol we observed a large, though temporary, pulse in N₂O emissions. The absence of a similar pulse of N₂O emission in the flooded N₂ treatment suggests the availability of O₂ or an oxic headspace can influence the timing and magnitude of the pulse. We include timing as well as magnitude because we cannot exclude the possibility that we missed a brief pulse in N₂O emission that occurred before Day 1 in the flooded N₂ treatment. Similar pulses have been repeatedly observed during soil wet-up experiments, during periods of high soil water-filled pore space, and during in situ precipitation or flooding events across a range of
soil types [Keller and Reiners, 1994; Hungate et al., 1997; Teh et al., 2011; Jørgensen and Elberling, 2012]. Such events are typically attributed to a stimulation of denitrification during soil reduction [Conrad, 1996] however given the presence of O$_2$ in the water used to flood the soil we cannot exclude a contribution from nitrification in the flooded treatment (Firestone and Davidson, 1989). The greater dissolved O$_2$ present in the flooded treatment initially may have led to greater N$_2$O production by temporarily stimulating nitrification, by favoring incomplete denitrification to N$_2$O, and/or by providing a larger or more persistent NO$_3^-$ supply for denitrification. Though we cannot isolate relative impacts on nitrification versus denitrification, our results indicate that large pulses of N$_2$O emissions associated with soil wet-up or flooding are strongly dependent upon soil O$_2$ availability.

5. Conclusions

Soil greenhouse gas emissions are strongly controlled by soil redox conditions. Flooding is generally assumed to precede redox changes; some soils, however, experience soil gas-phase anoxia without pore-space saturation. Here we asked how gas emissions differ under these distinct scenarios. We found that the size and magnitude of greenhouse gas emissions differ across the headspace and flooding treatments for two biogeochemically distinct soils. We found that in an Ultisol the effects of flooding on soil respiration could be divided into an effect of O$_2$ removal and a separate effect, perhaps due to changes in the transport and/or availability of dissolve solutes following soil inundation. Emissions of N$_2$O in both a Histosol and an Ultisol were likely sensitive to changes in pore-space diffusivity associated with flooding, in addition to the redox manipulations. Interestingly only the Ultisol, and not the Histosol, produced significant CH$_4$ effluxes in the anaerobic incubation and these were significantly greater with flooding. We propose that the observation of elevated anoxic soil respiration and CH$_4$ emission rates under flooding warrants further investigation to better identify the responsible biogeochemical mechanisms.
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Tables.

Table 1. Mixed-effects Model\(^a\)

<table>
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<td>CH(_4)</td>
<td>0.0989</td>
<td>0.1433</td>
<td>0.0002</td>
</tr>
<tr>
<td>Ultisol</td>
<td>CO(_2)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>N(_2)O</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>CH(_4)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^a\) p-values for significance of Treatment, Day, and Treatment*Day effects on each gas, for each soil type

Table 2. Redox-sensitive Soil Characteristics\(^a\)

<table>
<thead>
<tr>
<th>Soil</th>
<th>Treatment</th>
<th>pH</th>
<th>(\text{NH}_4^+) (µg N g(^{-1}))</th>
<th>(\text{NO}_3^-) (µg N g(^{-1}))</th>
<th>Percent Fe(^{2+}) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histosol</td>
<td>Control</td>
<td>6.02 ± 0.02(^a)</td>
<td>0</td>
<td>130 ± 5(^a)</td>
<td>8.3 ± 0.2(^a)</td>
</tr>
<tr>
<td></td>
<td>Flooded</td>
<td>6.08 ± 0.03(^a)</td>
<td>40 ± 2(^a)</td>
<td>0</td>
<td>60.0 ± 8.9(^b)</td>
</tr>
<tr>
<td></td>
<td>N(_2)</td>
<td>6.67 ± 0.04(^b)</td>
<td>65 ± 1(^b)</td>
<td>0</td>
<td>15.6 ± 0.8(^a)</td>
</tr>
<tr>
<td></td>
<td>Flooded N(_2)</td>
<td>6.52 ± 0.02(^c)</td>
<td>100 ± 3(^c)</td>
<td>0</td>
<td>69.3 ± 2.5(^b)</td>
</tr>
<tr>
<td>Ultisol</td>
<td>Control</td>
<td>5.77 ± 0.10(^a)</td>
<td>0</td>
<td>0</td>
<td>3.8 ± 0.4(^a)</td>
</tr>
<tr>
<td></td>
<td>Flooded</td>
<td>6.28 ± 0.07(^b)</td>
<td>5 ± 1(^a)</td>
<td>0</td>
<td>74.9 ± 4.1(^b)</td>
</tr>
<tr>
<td></td>
<td>N(_2)</td>
<td>6.29 ± 0.07(^b)</td>
<td>9 ± 1(^b)</td>
<td>0</td>
<td>54.7 ± 2.3(^c)</td>
</tr>
<tr>
<td></td>
<td>Flooded N(_2)</td>
<td>6.59 ± 0.09(^c)</td>
<td>12 ± 1(^b)</td>
<td>0</td>
<td>95.1 ± 0.8(^d)</td>
</tr>
</tbody>
</table>

\(^a\) Mean ± 1 S.E. Superscript letters denote significant treatment differences within each soil type (P < 0.05)

Figures.
Captions.

Figure 1. Trace gas fluxes for a Histosol (a, c, e) and an Ultisol (b, d, f). Mean CO$_2$ (a and b; $\mu$g C g$^{-1}$ hr$^{-1}$), CH$_4$ (c and d; ng C g$^{-1}$ hr$^{-1}$), and N$_2$O (e and f; ng N g$^{-1}$ hr$^{-1}$) flux over 20 (Histosol) or 15 (Ultisol) days of incubation (Mean ± SE; n = 6). Treatments were: control (open circles); N$_2$ (open triangles); flooded (filled circles); and flooded N$_2$ (filled triangles).