Impacts of Inundation and Season on Greenhouse Gas Fluxes from a Low-Order Floodplain

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Abstract

The global climate is changing and much of this is attributed to the greenhouse effect, which has been exacerbated by increased anthropogenic releases of greenhouse gases (GHGs). However, important GHGs, carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4), are produced naturally in the soil during the metabolism of many soil microbial and plant communities. The generation rate of GHGs depends on many factors, including soil community composition, nutrient availability, temperature, and soil moisture. Predicted climate variability is expected to alter temperature and rainfall patterns, which can impact the factors regulating natural generation of GHGs. With changing fluxes of GHGs, the natural feedback loops between GHG generation and climate may change. Increased emissions from natural sources would exacerbate climate change, whereas decreased emissions may mitigate its impacts. Floodplains may be particularly susceptible to climate change, as their biogeochemical processing is driven by hydrology. For this study, ten mesocosms were installed on the floodplain of Stroubles Creek in southwest Virginia. A flood event was simulated in half of these mesocosms in both early spring and mid-summer, which represent extremes in soil moisture and primary productivity on the floodplain. Headspace gases were monitored for CO_2, N_2O, and CH_4. Efflux of CO_2 and N_2O was higher in summer than spring, and also increased following wetting events. Methane production was greater in the spring, with no detectable change with wetting. Increases in summer rainfall events could increase the release of important GHGs to the atmosphere, potentially at levels significant to climate change.

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1 Introduction

1.1 Background and Motivation

The global climate is changing. Temperatures are rising, glaciers are melting, and weather events are predicted to become more extreme [*IPCC*, 2007]. Much of this is attributed to the greenhouse effect, which has been exacerbated by increased releases of greenhouse gases (GHGs) from anthropogenic sources. However, many GHGs, including carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4), have significant natural sources. Many soil microbial communities produce these gases as intermediaries or end products of their metabolism. The rate of generation of each of these gases can be dependent on a large number of factors, including soil microbial community composition, vegetation, soil carbon content, nutrient availability, temperature, and soil moisture content. Many of these factors are readily influenced or directly dependent on the local climate. Thus, it is hypothesized that changes in climate may impact the release of these GHGs from soils. With changing flux rates of GHGs, there is a potential for feedback loops to form. Increased emissions from natural sources will exacerbate climate change, whereas decreased emissions may help mitigate its impacts.

Floodplains in particular may be susceptible to this influence. Lying on the interface of aquatic and terrestrial habitats, floodplains tend to be particularly rich and diverse habitats. The periodic inundation of these habitats is a key driving force of many biogeochemical processes occurring in floodplains, including those producing GHGs. As the hydrology that drives these landscapes is altered with changing rainfall patterns due to climate change, there is potential for net fluxes of GHGs to be altered as well. Depending on the direction of change – increased or decreased production – the implications to the feedback loops between natural GHG emissions and climate can be great.

These potential changes in soil microbial production of GHGs are incompletely understood. Some studies have indicated increases in CO_2 flux due to wetting events [*Birch*, 1964; *Sánchez-Andrés et al.*, 2010; *Valett et al.*, 2005]. Release of N_2O has been linked to nitrification, denitrification, and nitrifier denitrification, which are dependent on the redoximorphic characteristics of the soil pore space [*Galloway et al.*, 2003; *Morse et al.*, 2012; *Wrage et al.*, 2001]. Methanogenesis is also dependent on highly reduced conditions [*Pulliam*, 1993; *Whalen*, 2005]. The specific responses of these fluxes to wetting events, such as inundation from flooding and large rainfall events, are incompletely understood. Additionally,

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most studies of soil microbial response to flooding have been done on the floodplains of large rivers. Floodplains of low-order streams tend to have shorter hydroperiods than high-order rivers. Few studies have focused on small streams, and fewer still have observed the impact of the timing of wetting events on GHG generation. A more complete understanding of the processes causing natural GHG generation is needed to better inform our current models of climate change. Improved predictions could better inform management practices to minimize effects of climate change.

1.2 Research Objectives

This research aimed to determine if fluxes of CO_2, N_2O, and/or CH_4 from low-order floodplains are altered by wetting events in different seasons. The timing and magnitude of precipitation events are expected to change with changing climate, and we explored the potential reactions of soil microbial communities to these changes in hydrology. To begin elucidating the effects of climate change on GHG production, we took a two-pronged approach in looking at the impacts of wetting events on GHG fluxes during different seasons. Artificial wetting events were conducted in both the early spring and mid-summer and GHG generation rates were compared to determine any potential impacts or interactions. To summarize, the specific objectives of this study are:

(1) To determine if fluxes of CO_2, N_2O, and/or CH_4 are altered by wetting events;

(2) To determine if season impacts the fluxes of CO_2, N_2O, and/or CH_4; and

(3) To determine if there is an interaction between the impacts of wetting events and season on the fluxes of CO_2, N_2O, and/or CH_4 from floodplain soils.

1.3 Organization of Thesis

This document is organized around a journal article that will be submitted for publication in the *Journal of Geophysical Research – Biogeosciences*. A comprehensive literature review precedes the article, providing an overview of the biogeochemical and hydrological cycles that influence the fluxes of greenhouse gases into and out of floodplains. Though the article in Chapter 3 forms the core of the thesis, it can stand alone with a full abstract, introduction, methods, results, discussion, conclusions and references. The article is followed by a summary of the research, conclusions drawn, and areas recommended for future work. Supporting information for the thesis, including additional tables and figures, as well as detailed methods are included in the appendices.

Chapter 3

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2   Literature Review

2.1   Introduction

An increasingly human-modified landscape, in conjunction with global climate change, continues to change the hydrology of river-floodplain systems. Floodplains and wetlands, being at the intersection of aquatic and terrestrial systems, are sites of intense biogeochemical processing. These systems provide important ecological functions, such as sediment and nutrient control and providing habitat for various growth stages of both aquatic and terrestrial fauna, which may be impacted by altered hydrology. Changes to the frequency, timing, duration, and magnitude of peak flows and their subsequent flood pulses can impact the timing and rate of these processes. There is great potential for the natural feedback loops between climate and the various biogeochemical processes in floodplains generating different quantities of greenhouse gases (GHGs) to change as climate change continues its course. Increases in the amounts of GHGs generated in floodplains could exacerbate climate change, whereas drops in generation could potentially slow its course. There is much debate, however, as to the extent and likelihood that these feedback loops will be altered.

This review begins with a brief examination of floodplains and overview the pertinent points of the carbon and nitrogen cycles, which are roughly outlined in **Figure 2-1**. It will then continue into a review of studies focusing on the potential for climate change and human impacts to change hydrologic regimes and thus alter generation rates of greenhouse gases.

2.2   Floodplains, Flood Pulses, and Climate Change

Riparian zones encompass the interface between aquatic and terrestrial ecosystems. A diverse array of species and environmental processes occur in riparian zones. Floodplains, periodically flooded areas in riparian zones, often harbor particularly diverse and rich ecosystems. Periodic inundation of floodplains in riparian zones is a key driver of this heterogeneity [*Naiman and Décamps*, 1997]. The flood pulse concept of *Junk et al.* [1989] emphasizes the importance of floodplain inundation as a source of connectivity between the aquatic and terrestrial ecosystems, facilitating the exchange of nutrients and substrate between the two. The spatial and temporal dynamics of a given flood pulse can be highly complex and encompass both surface water and groundwater fluctuations [*Krause et al.*, 2007; *Mertes*, 1997]. Large systems, such as the Amazon River, often have very predictable flood pulses lasting months and altering the groundwater dynamics throughout the system. Smaller systems, such as

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low-order headwater streams, have much shorter flood pulses which may each correspond to individual storm events.

The variability in flow necessary for maintaining diversity and productivity of riparian zones has been dramatically impacted throughout the world via flood management controls (e.g. levees and impoundments), changes in land use, and increasing water withdrawals in dry regions [*Benke*, 1990; *Dynesius and Nilsson*, 1994; *Hupp et al.*, 2009; *Nilsson et al.*, 2005]. These disturbances limit the natural interaction between river and floodplain, disrupting many of the biogeochemical processes that would naturally occur. This necessitates the preservation and study of the few remaining rivers left relatively undisturbed [*Benke et al.*, 2000]. These studies of undisturbed systems need to be combined with research on managed systems to understand and manage human impacts on ecosystem services.

Beyond the direct impacts that humans have had on the natural flows of rivers, the indirect effects of climate change on river-floodplain systems are becoming more widely acknowledged. Global temperature is expected to rise by 2-4 °C by the end of the century [*IPCC*, 2007]. Increased drought in many already dry areas is expected, along with increased severity of storms [*IPCC*, 2007]. Changes in flood timing and return period could alter the potential of floodplains to be either sinks or sources for key greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O).

Global atmospheric concentrations of CO_2, CH_4, and N_2O are now far greater than pre-industrial levels detected in Antarctic ice cores [*Leuenberger and Siegenthaler*, 1992; *Petit et al.*, 1999]. These three gases alone constitute more than 87% of the increase in atmospheric radiative forces due to greenhouse gases [*IPCC*, 2007]. Future changes in the emissions of these gases from floodplains may have huge impacts on the future of the global climate. Increases in fluxes of these gases due to changes in hydrology could provide increased positive feedback into the global system, amplifying the effects of climate change. However, there is also potential for floodplains to be sinks for these gases, which could dampen the Earth's response to these forcings.

2.3   Impacts on Carbon Cycling

Changing climate may alter the carbon cycle in many different manners, from complex changes in rainfall patterns to basic alterations in overall temperature. The response of soil carbon stocks to increasing temperature due to global climate change is unclear [*Conant et al.*,

2011]. Temperature rise may increase decomposition rates, which would increase the flux of CO_2 into the atmosphere. However, the decomposition of soil organic matter is a complex process involving many different biogeochemical reactions and different pools of soil carbon (e.g. labile and recalcitrant). The net effect of temperature on these processes is difficult to determine or predict, especially when considering the importance of soil microbial communities as regulators of soil organic matter decomposition and CO_2 generation [*Cleveland et al.*, 2007], and their sensitivity to many other environmental factors. The seasonality of soil respiration, with greater CO_2 flux rates in the summer, indicates that temperature increases will increase soil respiration, at least to a certain threshold [*Pulliam*, 1993; *Rustad et al.*, 2001; *Wu et al.*, 2011]

Moisture also plays a key role in the cycling of soil carbon stocks. Extremes of soil moisture can limit soil microbial activity, overwhelming any correlation with temperature. In addition to evidence of both upper and lower thresholds of moisture where respiration was seen to drop, *Suseela et al.* [2012] saw hysteretic responses to changing soil moisture: as volumetric soil moisture dropped below ~15% or rose above ~26%, soil respiration dropped dramatically, but then rose gradually as moisture returned to a more ideal level. *Almagro et al.* [2009] also observed soil moisture thresholds acting on soil respiration, though the thresholds differed by land use. Below a threshold value of soil moisture, soil respiration appears to be limited by the availability of water for microbial processes, whereas above a certain moisture level, temperature appears to be the controlling factor. Beyond a certain level of wetness, the respiration of soil microbes is also limited by the ability of oxygen to diffuse through the wet soil [*Suseela et al.*, 2012].

Along with rising temperatures and changing hydrology, human impacts and climate change are causing an increase in atmospheric carbon dioxide [*IPCC*, 2007]. The growth of most plants is enhanced by elevated CO_2, but to different extents and with varying impacts on tissue chemistry [*Vitousek et al.*, 1997]. How these impacts carry through and impact the soil microbial communities may be of even greater importance. Soil microbial communities are highly sensitive and adaptive to changes in temperature and moisture, as well as the quality and quantity of root exudates into the rhizosphere [*Haichar et al.*, 2008]. The main impacts of elevated atmospheric CO_2 on soil microbial communities are expected to occur indirectly through increased plant metabolism and root secretions, directly influencing only those communities in close proximity to the root system [*Drigo et al.*, 2008; *Perry et al.*, 2012]. Direct additions of dissolved organic

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matter have been found to stimulate soil CO_2 flux much more than addition of water alone, and also caused profound shifts in the makeup of the soil microbial community [*Cleveland et al.*, 2007], supporting the idea that changes in plant root secretions will impact the soil microbial community response to climate change.

It is likely that the increase in atmospheric CO_2, perhaps the most important human enhancement to the greenhouse effect, will be the driver of substantial climate change in the coming century [*IPCC*, 2007]. A reduction in surface carbon uptake is anticipated as the global climate continues to change, which would enhance the climate forcing by further increasing the atmospheric CO_2 concentration [*Denman et al.*, 2007]. *Cox et al.* [2000] predicted that terrestrial ecosystems will continue to act as a sink for carbon until approximately 2050, at which point they are expected to switch from a sink to a source of carbon to the atmosphere.

Wetlands in particular are of interest when discussing the global carbon budget, as their soils hold a much higher carbon content than those of other ecosystems [*Post et al.*, 1982]. Wetlands commonly act as both carbon sinks and sources. Carbon dioxide is incorporated into living organic matter in these highly productive landscapes and sequestered during the formation of stable organic soil compounds. However, the anoxic conditions in many wetland soils are ideal for the generation of methane by microbial methanogens, releasing carbon back into the atmosphere [*Pulliam*, 1993; *Whalen*, 2005]. Due to the heterogeneity of natural systems, in wetlands and other areas where anoxia may occur such as saturated floodplains, the reducing conditions spurring methane production are often incomplete. As much as half of the methane produced in anaerobic subsurface zones of wetlands may be consumed as it diffuses to the surface, passing through aerated areas where it is oxidized [*Pulliam*, 1993; *Whalen*, 2005]. However, many wetland plants have developed aerenchyma, specialized tissue with air channels to supply their roots with oxygen. Aerenchyma tissue also serves as a pathway for methane produced in the anaerobic areas below the surface to escape without passing through aerobic areas where it would be oxidized. It is estimated that 90% or more of the methane that makes it out of the soil is transported through wetland plants [*Schimel and Holland*, 2005].

Temperature has also been shown to impact methane production in wetlands. *Pulliam* [1993] found that at temperatures below 15 °C, no significant CH_4 emissions occurred. Though CH_4 emissions were generally positive above this threshold, they showed no further correlation with temperature. This threshold effect was not found to be a limitation of the microbial

community directly, but rather an indication of the threshold of oxygen supply and demand [*Pulliam*, 1993]. The temperature, position of the water table, and plant and microbial community composition are all important controls on wetland CH_4 emission [*Whalen*, 2005], thus it is difficult to predict the future of methane emissions with changing climate and hydrology.

2.4 Impacts on Nitrogen Cycling

The carbon and nitrogen cycles are inextricably linked. Both elements are essential for life to flourish, being key components in the proteins, lipids, and carbohydrates that form living beings. It is logical that changes in the nitrogen cycle, such as overabundance of one compound or another, could alter the balance of the carbon cycle, and vice versa.

Although vast pools of nitrogen exist in the atmosphere, the interaction of terrestrial and aquatic ecosystems with this pool is limited, as is the quantity of reactive forms of nitrogen, such as ammonia and nitrate which are readily available for use by flora and fauna, available for biological cycling. In recent decades, humans have been adding greater and greater amounts of reactive nitrogen to the environment [*Cowling et al.*, 1998]. The development of techniques such as the Haber-Bosch process caused additions of manufactured nitrogen fertilizers to increase exponentially in the mid-20th century, with over 6 million metric tons now being added annually to the Mississippi River basin alone [*Goolsby et al.*, 1999]. These additions of reactive nitrogen cascade through the landscape, sometimes accumulating in reservoirs such as growing stands of vegetation [*Galloway et al.*, 2003]. Accumulation of nitrogen in soils, living organisms, etc. constitutes a lag in the movement of nitrogen through the system, but not a true removal. The nitrogen will eventually continue moving through the cascade as described by *Galloway et al.* [2003], potentially exacerbating the effects of reactive nitrogen on the environment. For example, in exceeding the ability of the landscape to utilize the reactive nitrogen humans apply to agricultural fields, this nitrogen has leached into aquatic habitats, causing widespread algal blooms and hypoxia [*Goolsby et al.*, 1999].

The only way to remove excess reactive nitrogen derived from human activities is by converting its various forms back into inert nitrogen gas (N_2) via denitrification. Denitrification is mediated by certain microbial organisms under suboxic conditions, generally in landscapes such as wetlands or along subsurface hydrologic flow paths where dissolved oxygen is depleted. Much of the denitrification in the environment tends to happen in hot spots and hot moments, for

example when nitrogen rich flows from uplands encounter anoxic zones on floodplains [*Groffman et al.*, 2009; *Harms and Grimm*, 2008; *Harms et al.*, 2009; *McClain et al.*, 2003; *Vidon et al.*, 2010].

An active soil microbial community is integral to nitrogen retention and removal, as indicated by seasonal patterns found in nutrient chemistry [*Harms and Grimm*, 2008]. Nitrogen-rich subsurface flows can move through anoxic landscapes, but without an active microbial community no removal will occur. A supply of readily oxidizable carbon is also vital for optimal denitrification [*Hedin et al.*, 1998; *Holmes et al.*, 1996]. The consequences of climate change on hot spots and moments of nitrogen cycling are difficult to predict and will likely vary greatly by location.

Increasing river-floodplain connectivity is looked at as one potential strategy in an effort to reduce nitrate loadings in streams and rivers [*Kaushal et al.*, 2008]. Human development has drastically changed hydrology in urban areas, replacing natural surfaces with pavement which increases high flows and causes hydrology to become 'flashier'. The construction of levees and straightening of channels limits the connectivity between rivers and their floodplains. Increasing the interaction between rivers and floodplains has the potential to remove excess nitrate via denitrification. Encouraging flow of nitrate-rich stream water through subsurface flow paths can increase the number of molecules encountering anoxic hotspots where the reactive nitrogen can be reduced to inert nitrogen gas [*Vidon et al.*, 2010].

One of the more potent greenhouse gases, nitrous oxide (N_2O), is generated naturally during the process of denitrification, as it is an intermediary in the sequence of transformations from nitrate to nitrogen gas [*Galloway et al.*, 2003; *Morse et al.*, 2012]. When anoxia is incomplete or nitrate is highly available, the denitrification process becomes 'leaky', releasing intermediaries such as N_2O into the environment [*Morse et al.*, 2012; *Schimel and Holland*, 2005]. Nitrous oxide produced elsewhere can also be consumed by the denitrification process when nitrate availability is low and the area highly anoxic [*Chapuis-Lardy et al.*, 2007].

Nitrous oxide is also an intermediary in nitrification, the transformation of ammonium (NH_4^+) into usable NO_3^-. This process can also 'leak' N_2O into the system when reactions are incomplete [*Galloway et al.*, 2003; *Morse et al.*, 2012]. Often, nitrification and denitrification are coupled closely in the environment, though the processes are carried out by different classes of microbes. In addition to these joint paths, there is also a process known as nitrifier

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denitrification, in which the entire transformation from NH_4^+ to N_2 is carried out by a single group of organisms [*Wrage et al.*, 2001]. It is estimated that nitrifier denitrification, though less commonly recognized than coupled nitrification-denitrification, accounts for up to 30% of the total N_2O production [*Wrage et al.*, 2001]. Again, these processes can be sinks as well as sources of nitrous oxide, depending on the availability of different forms of nitrogen, the presence or absence of certain microbial groups, and environmental factors such as water table levels, temperature, and level of anoxia.

2.5 Importance of the Hydroperiod

The integrity of river-floodplain systems and their biogeochemical processes, such as those comprising the carbon and nitrogen cycles, depends heavily on natural flood dynamics [*Poff et al.*, 1997]. The dynamic hydroperiod of these systems influences ecological function and structure, providing subsidies between the terrestrial and aquatic ecosystems [*Junk et al.*, 1989], as well as providing the disturbance often necessary for the establishment and growth of native riparian vegetation [*Molles et al.*, 1998]. The magnitude, frequency, duration, and timing of peak flows and the flood pulses they create are integral to maintaining the health and productivity of river-floodplain systems. Changes to the natural flood pulse dynamics of river systems can have profound repercussions which may not be fully understood [*Benke et al.*, 2000].

The timing and volume of river discharges respond strongly to changes in precipitation, and are affected to a lesser degree by changes in evaporation rates and storage in soils and/or reservoirs [*Hamilton*, 2010]. The combination of global climate change and human interference will continue to alter river hydrological regimes in the future. Although there is much variability in the predictions made for future precipitation regimes as the global climate continues to change, one consistent trend is that precipitation events will become more extreme [*IPCC*, 2007]. Droughts will become more prolonged and rainfall events will become more intense, even if total precipitation levels do not change. Changes in precipitation regime will no doubt alter the pattern and frequency of flood inundation [*Meehl et al.*, 2007]. Altered inundation patterns will directly influence the extent and level of anoxia in floodplain systems, which would have profound impacts on the biogeochemical cycling of river-floodplain systems.

Impacts of changing precipitation regimes take on a special role in cold climates. Depth of soil freezing typically exhibits an inverse relationship with depth of snow cover [*Fitzhugh et al.*, 2001]. Decreased snow accumulation or a late accumulating snow pack result in deeper soil

freezing for a longer duration than when a thick snow pack accumulates early in the season. Thus, reduced snow cover resulting from a warmer climate will likely lead to increases in the frequency, severity, and extent of soil freezing events. This is important because freezing releases large pulses of nutrients from soils and may contribute to microbial mortality, which can inhibit the uptake of these freshly released nutrients before they make their way into the stream network [*Fitzhugh et al.*, 2001; *Noe and Hupp*, 2007].

Changes in precipitation regime can also have direct impacts on soil processes. *Harper et al.* [2005] found that soil fluxes of CO_2 were reduced more by altered rainfall timing (increasing length of dry intervals between rainfall events) than by reduced rainfall amount, though combining the two alterations caused the greatest reduction. The changes they saw were consistent with changes in plant productivity, which directly influences soil respiration, as up to half of soil respiration is attributed to plant root respiration [*Ryan and Law*, 2005]. *Chen et al.* [2008] also found that the size of a rainfall pulse impacts the subsequent soil respiration.

Pulses of increased soil respiration rates have been observed immediately after rainfall events in semiarid environments [*Huxman et al.*, 2004]. These pulses in respiration following rewetting are often referred to as the 'Birch effect', after the first scientist to describe and study the phenomenon [*Birch*, 1958, 1964]. Birch found that decomposition of plant residues was increased by intermittent drying of the soil as compared to soil maintained at a constant level of moisture [*Birch*, 1964]. Carbon isotopes were more recently used to isolate the mechanism behind the 'Birch' pulse. The pulse of CO_2 efflux immediately following rewetting was caused by the rapid mineralization of recently lysed microbial biomass and/or osmoregulatory substances released by soil microbes to avoid lysis due to osmotic stress [*Unger et al.*, 2010].

When the wetting event is of a greater magnitude, such as from inundating flood waters rather than a single rainfall event, the soil community responds differently. During extended flood events (on the order of months), after a period of acclimation soil respiration is seen to rise to levels much higher than prior to the flood event [*Valett et al.*, 2005]. The drying out of the soil after such extended inundation produces a dramatic drop in soil respiration followed by another pulse of increased respiration [*Sánchez-Andrés et al.*, 2010]. The drop in respiration is likely due to extensive microbial lysing caused by the rapid change in soil moisture content. The pulse of respiration that follows can be attributed to the microbial community 'catching up' after the extensive cell death, combined with the improved availability of nutrients normally bound tightly

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to soil aggregates during dry spells. Flooding has been shown to significantly alter the makeup of the soil microbial community [*Wilson et al.*, 2011]. Changes in the microbial community could be part of the forcing behind the pulse of increased respiration after a sustained change in soil moisture.

This touches on another important concept: the inter-flood interval, or dry period between subsequent flooding events [*Molles et al.*, 1998]. In the absence of regular inundation, organic matter accumulates on the floodplain, isolated from the stream system. When inter-flood intervals are long, this supply of accumulated organic matter can cause an increase in respiration during the eventual flood event. However, this abundance of available organic matter also drives the flood waters to become anoxic, whereas an area with a shorter inter-flood interval and thus less accumulated organic matter would not become depleted of oxygen [*Molles et al.*, 1998; *Valett et al.*, 2005]. Thus differences in inter-flood interval, whether caused by changes in climate or human activity, can translate into differences in redoximorphic conditions and biogeochemical cycling. In a long-term study of wetlands in China, *Song et al.* [2009] found that wetlands which were only seasonally inundated had greater ecosystem respiration than permanently inundated wetlands, but the production of CH_4 and N_2O was greater in the permanently inundated wetlands. *Altor and Mitsch* [2008] also found that intermittent inundation produced lower methane fluxes from hydric soils than did continuous inundation.

The accumulation of organic matter during the inter-flood interval highlights the asynchrony of resource availability often observed in floodplain systems. Along with large debris accumulating on the surface of the floodplain, mineral nitrogen and other nutrients can accumulate in the soil during dry periods, especially in arid ecosystems [*Austin et al.*, 2004]. The microbial processes generating nitrate can continue even after the soil moisture drops below levels necessary for plant uptake of the nutrient, causing an accumulation of nitrate in the soil [*Voroney*, 2007]. This accumulation leads to increased loss of labile N via leaching and other processes when the soil is rewetted [*Austin et al.*, 2004]. This asynchrony of resource availability due to pulsed hydrology may have significant long-term effects on the soil nutrient pools, especially with the changes in hydrology expected to result from climate change.

These pulses of carbon and nitrogen efflux can often outweigh the uptake of these compounds by the system, causing net loss over time. It is often difficult to get a fully accurate view of these fluxes, however, as these pulsed releases of carbon and nitrogen are episodic in

nature and spatially variable. Many high-magnitude events are of short duration and can be easy to miss with standard sampling regimes [*Song et al.*, 2009]. Thus it is possible that many studies already completed present conservative estimates of gas fluxes. The distribution and variability of the microbial community also play a key role in biogeochemical cycling within floodplains [*Wilson et al.*, 2011]. Environmental disturbances impact different microbial community structures differently, making an understanding of microbial dynamics necessary to understand the impacts of climate change and disturbances on biogeochemical cycling [*Blodau and Moore*, 2003]. The driving forces of these pulses, notably rainfall patterns, are also highly spatially variable, making modeling of global trends of gaseous fluxes challenging.

The spatial variability of the floodplain habitat itself must also be taken into consideration. Many studies have been performed on long hydroperiod floodplains, commonly associated with large rivers, where inundation lasts weeks to months [*Molles et al.*, 1998; *Sánchez-Andrés et al.*, 2010; *Valett et al.*, 2005], but little is known about the reactions of short hydroperiod floodplains often associated with low-order streams to inundation. *Noe and Hupp* [2007] studied nutrient processing in a short hydroperiod stream, and while there was a great deal of variability in their results, they found that the floodplain generally exported inorganic nutrients. This is the opposite of the exportation of organic nutrients and retention of inorganic nutrients generally found on longer hydroperiod floodplains. The floodplain *Noe and Hupp* [2007] studied was characterized by brief inundation periods, low residence times, and low nutrient loadings, very different from the long hydroperiod floodplains generally studied. These differences may be central to the balance of uptake and release of inorganic nutrients by floodplains, and a shift from release to uptake by floodplains may be apparent when moving from headwater streams down gradient to larger rivers. These differences in functionality between low order and high order streams may also be expressed in the generation of greenhouse gases by the soil microbial community.

Wet-dry cycles, both due to flood inundation and rainfall events, are important to soil community metabolism, which can be an important natural source of greenhouse gases. Changes in flood dynamics will inevitably change the biogeochemical processing within floodplains and thus the fluxes of greenhouse gases from them. Climate change can alter the magnitude, frequency, duration, and timing of storm events and the flood pulses they create, having repercussions on greenhouse gas fluxes from floodplains. These changes could potentially alter

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the feedback loops between climate and natural GHG emissions. Increases in the amounts of greenhouse gases generated in floodplains could exacerbate climate change, whereas drops in generation could potentially slow the process.

2.6 Conclusion

The production rates of greenhouse gases, such as CO_2, CH_4, and N_2O, by soil microbial processes in floodplains, are dependent on highly variable processes governed by a wide range of different and often interacting factors. Changes in global climate and human activities both stand to impact gas fluxes from floodplains in a number of ways. Direct effects of changes in driving forces such as temperature may be overshadowed by effects from altered precipitation regimes, and synergistic effects of changes in these and other driving force are highly likely. Inundation of the floodplain by high flows can have different effects on gaseous fluxes depending on the timing, duration, and nutrient loadings of the flood pulse, as well as the properties of the floodplain itself. These interactions may play out differently depending on the scale of the floodplain, with smaller floodplains potentially reacting differently than better understood larger floodplains.

All of these processes and interactions are incompletely understood. On top of these complexities, human interferences and climate change continue to alter the hydroperiod of river-floodplain systems as they are studied. These interactions have the potential to cause either an increase or decrease in net fluxes of greenhouse gases, which can in turn trigger positive or negative feedback to global climate change. Further research is necessary to identify which way the balance may swing.

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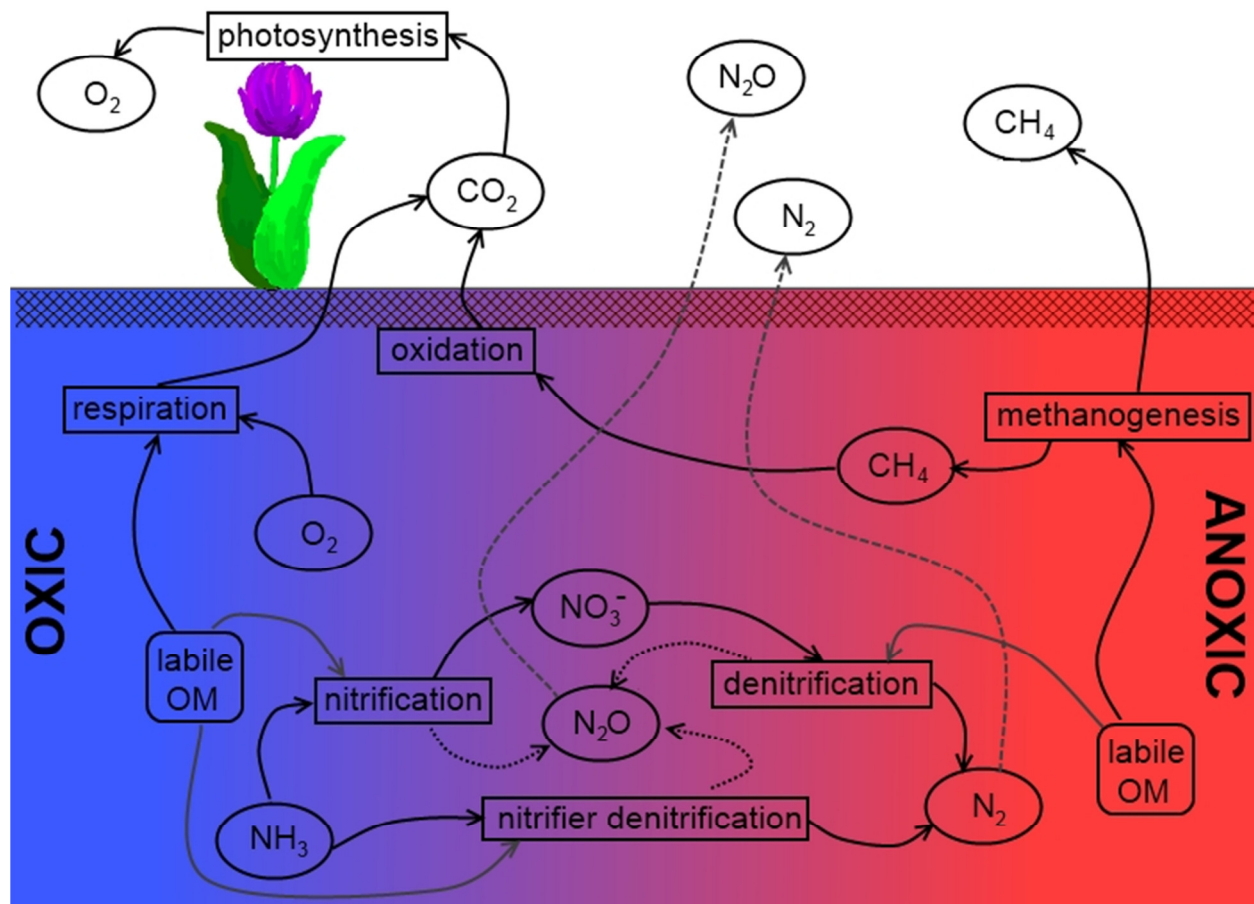
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Figure 2-1: A cross-section of pertinent processes in the carbon and nitrogen cycles and their relative relationships to redoximorphic state. Processes placed more to the left require more oxic conditions to occur, while processes placed closer to the right of the figure require more reduced conditions.

3   Seasonal Impacts of Inundation on Greenhouse Gas Fluxes from a Low-Order Floodplain

3.1   Key Points

Potential for climate change feedback from floodplain greenhouse gas generation

Season and water availability impact nitrous oxide and carbon dioxide fluxes

Short wetting events are insufficient to prompt methane production

3.2   Abstract

While anthropogenic processes have exacerbated the greenhouse effect by adding greenhouse gases (GHGs) to the atmosphere, key GHGs CO_2, N_2O, and CH_4 are also produced naturally by the soil microbial community. Production rates of GHGs in the soil may be sensitive to climate change attributed to the greenhouse effect. Floodplain systems may be especially sensitive as the hydrologic processes driving productivity are dependent on climate. Altered timing, frequency, duration, and magnitude of inundation events can significantly impact biogeochemical processes within floodplains, including production of GHGs. This study explores the potential for changing hydrologic regimes to significantly alter the net flux of GHGs from low-order floodplains by simulating inundation events in different seasons. Ten mesocosms were installed in situ on the floodplain of Stroubles Creek, a second order stream in the Ridge and Valley physiographic province of southwest Virginia. In early spring (March) and mid-summer (July) 2012, flood events were simulated in half of the mesocosms by maintaining a head of water for 2 hours. Headspace gas concentrations were monitored for three weeks. Both CO_2 and N_2O had greater flux rates from the floodplain in summer than in spring, and both showed pulses of increased production following summer wetting events. Methane production was greater in spring when the entire floodplain was saturated, and showed no response to simulated flooding. There is potential that hydrologic variability due to changing climate could significantly alter the net flux of GHGs out of low-order floodplains and exacerbate or mitigate feedback loops on climate change.

3.3   Introduction

The global climate is changing: temperatures are rising, glaciers are melting, and weather events are predicted to become more extreme [*IPCC*, 2007]. Much of this change is attributed to the greenhouse effect, which many believe has been thrown out of equilibrium by anthropogenic additions of greenhouse gases (GHGs) to the atmosphere, especially through the burning of fossil

fuels and changing agricultural practices. Global atmospheric concentrations of CO_2, N_2O, and CH_4 are far greater now than pre-industrial levels detected in Antarctic ice cores [*Leuenberger and Siegenthaler*, 1992; *Petit et al.*, 1999]. These three gases alone constitute more than 87% of the increase in atmospheric radiative forces due to GHGs [*IPCC*, 2007]. In addition to anthropogenic sources, however, these three main GHGs are also generated naturally by the soil microbial communities, providing a natural feedback to climate change. The production of CO_2, N_2O, and CH_4 has been shown to be influenced by a number of factors, including soil microbial community structure, temperature, soil moisture, and redoximorphic potential. Studies have indicated increases in CO_2 flux during flooding, as well as during in the drying process following inundation events [*Birch*, 1964; *Sánchez-Andrés et al.*, 2010; *Valett et al.*, 2005]. Release of N_2O has been linked to nitrification, denitrification, and nitrifier denitrification [*Galloway et al.*, 2003; *Morse et al.*, 2012; *Wrage et al.*, 2001], the rates of which are dependent on the redoximorphic characteristics of the soil pore space. Methanogenesis is also dependent on highly reduced conditions, which are often associated with extended wet periods in which the soil pores become oxygen depleted [*Pulliam*, 1993; *Whalen*, 2005].

Floodplain systems are highly heterogeneous landscapes at the interface between upland landscapes and streams. These systems tend to be hot spots of biogeochemical processing, where nutrients being transported from upland areas are intercepted by different microbial communities and heterogeneous redoximorphic conditions [*McClain et al.*, 2003; *Vidon et al.*, 2010]. As hotspots of biogeochemical processing, floodplains can be both sources and sinks of GHGs. The heterogeneity and productivity of floodplains are driven by the flood pulses of their associated rivers and streams [*Junk et al.*, 1989; *Naiman and Décamps*, 1997]. These wetting events are drivers of biogeochemical processing in floodplains; therefore, alterations in the hydrology of the systems could logically alter the generation of GHGs by these processes. Large river systems tend to be well-studied and often have a hydrology dependent on seasonal precipitation changes more so than local precipitation events (e.g. the annual flooding of the Amazon River during the rainy season[*Junk et al.*, 1989]). Smaller, low-order streams tend to respond readily to local precipitation events, with floodplain inundation events being associated with stream stage rise due to individual storms and/or the floodplain being wetted directly by rainfall.

The timing, frequency, duration, and magnitude of these precipitation events are expected to change with changing climate; droughts have been predicted to become more prolonged and

precipitation events are expected to become more extreme, even though the overall amount of precipitation delivered to an area may not change [*IPCC*, 2007]. Generation of GHGs on the floodplains of low-order streams is less well studied than on floodplains of large rivers, but this responsiveness to local climate may make them more sensitive to changing climate.

In this study we explore the potential that changes in climate, notably those expressed in altered precipitation regimes, could impact the natural fluxes of GHGs from floodplains of low-order streams strongly enough to alter the feedback loop between natural GHG generation and global climate – either mitigating or enhancing global climate change. Specifically, the study focuses on the impacts of wetting events and season on the generation of CO_2, N_2O, and CH_4 from the floodplain of a second order stream in southwest Virginia.

3.4   Methods

Stroubles Creek is a second-order stream in southwest Virginia whose headwaters encompass the Virginia Tech campus and much of the Town of Blacksburg before flowing through landscapes dominated by agricultural and forestry land uses. Blacksburg and Stroubles Creek lie in the Ridge and Valley physiographic province of Virginia. Climate data collected at the Corporate Research Center on the Virginia Tech campus provides an annual daily average temperature of 10.8°C (51.5°F) and an average annual precipitation of 103 cm, with 52.6% of the precipitation occurring from May to October [*Committee et al.*, 2006]. The study site, shown in **Figure 3-1** lies on the floodplain of a restored reach of the creek at the Stream Research, Education, and Management (StREAM) Lab downstream of the Virginia Tech campus [*Thompson et al.*, 2012].

Ten mesocosms were constructed from 25.4 cm inner diameter Sch. 40 PVC pipe and installed in situ on the floodplain of Stroubles Creek in two rows of five within a 6 m by 15 m grid (**Figure 3-1**). All mesocosms were placed at least 3 m apart to minimize influence of flooding treatment on any adjacent non-flooded mesocosms. The particular section of the floodplain used was chosen for its relative homogeneity of microtopography and vegetative cover. The collars were pushed into the soil to a depth of approximately 5 cm, providing a good seal with the soil to minimize gas loss during sampling without severing too many of the root connections to adjacent plants (**Figure 3-2**).

Flood events were simulated on 21st of March and 6th of July 2012. These months represented early spring, just before the vegetation on the floodplain began actively growing, and

mid-summer, near the height of vegetative growth. Time and resource constraints restricted the study to two runs of the experiment, but the two time periods chosen provide a wide range of environmental conditions, as shown in **Figure 3-3**. In the spring it is cooler, the water table is at or above the surface of the soil and rainfall events are generally smaller and more frequent. In the summer it is warmer, the water table has dropped to more than one meter below the surface and rainfall events are generally larger and less frequent. In addition, evapotranspiration is generally lower in the spring as the growing season is just beginning and significantly higher in the summer when the vegetation on the floodplain is actively growing. The difference in vegetative growth can be seen in **Figure D-10** and **Figure D-11**.

Half of the mesocosms were chosen randomly for the artificial floods, with the other five remaining as controls. Water for each flood was taken directly from adjacent Stroubles Creek and used to maintain a water depth of 10.2 cm inside the collars of the chosen mesocosms for 2 hr. Mesocosms chosen for the flooding treatment were each surrounded by a 50 cm diameter outer ring of stiff plastic inserted 5 cm into the soil (**Figure 3-2**). This outer ring performed similarly to the outer ring of a double-ring infiltrometer setup, as a 10.2 cm head was also maintained between the rings as a buffer between the flooded treatment area and the surrounding, non-flooded soil. The flood simulation was modeled after a large flood event that occurred in June of 2006 and visual evidence of flooding in the recent past. For both the spring and summer experiments, flood water was sampled and analyzed for total nitrogen and dissolved organic carbon content (Shimadzu TOC-V) as well as concentration of nitrate (Dionex IC).

Ambient climate conditions and rainfall data were available from the StREAM Lab meteorological tower, located on the study site, through the StREAM Lab website (http://streamlab.bse.vt.edu/). Hourly rainfall, air temperature, soil temperature, soil moisture, solar radiation, barometric pressure, and wind direction and speed data were used from the meteorological tower to quantify key differences between conditions on the floodplain during spring and summer experiments (rainfall: Texas Electronics Rain Gage TE525WS, air temperature: CS215 Temperature and Relative Humidity Sensor, soil temperature: 107-LC Temperature Probe, soil moisture: CS616 Water Content Reflectometer, solar radiation: CS300 Pyranometer, barometric pressure: CS106 Barometric Pressure Sensor, wind direction and speed: 034B Met One Wind Set). Headspace gases were monitored in flooded and non-flooded mesocosms for three weeks following the artificial flood event to ensure any response to the

flooding treatment would be captured (full data sets shown in **Appendix D**). However, all significant responses occurred within the first seven days of the experiments, thus only the first seven days of data collection are discussed herein. Additionally, a diurnal sampling was performed 3-4 days after each flood to capture diurnal responses in GHG flux and potential differences in response between flooded and non-flooded treatments. This timing was chosen before performing the experiments and learning that responses do not last beyond the third day, but the data still provide important insight into the processes occurring.

Before each sampling, actively growing vegetation inside the mesocosm was trimmed to within 1 cm of the surface to minimize the effects of photosynthesis on the makeup of the headspace gas mixture. Samples were taken 3-4 times during an hour-long incubation period at the same time each day (11:00 to 12:00), during which the mesocosms were capped and samples were drawn via syringe through a sampling port. The caps also incorporated vent tubes designed following guidelines presented by *Hutchinson and Livingston* [2001] to equilibrate the mesocosms with atmospheric pressure while minimizing leakage of gases.

Concentrations of CO_2, N_2O, and CH_4 over the hour-long incubation periods were determined using a Shimadzu GC-2010 gas chromatograph and associated GC Solution software. The instrument was outfitted with an FID detector to analyze the CO_2 and CH_4 fractions of the samples and an ECD detector to analyze the N_2O fraction. Changes in concentration of each gas over the incubation period were used to calculate the flux rate of the gas in μmol of gas m^{-2} day^{-1} by fitting a linear regression to the data points. These flux rates were compared between flooded and non-flooded treatments on individual days using Wilcoxon rank sum tests. Cumulative generation of each gas over the first three days post-flood was also calculated for each mesocosm using the trapezoidal method. This three-day integration encompassed the significant responses seen in GHG flux to flooding treatment and after three days all flux rates in flooded mesocosms were back to background (control) levels. These cumulative generation values were compared using a 2-way ANOVA assessing the significance of season (spring vs. summer), treatment (flooded vs. non-flooded), and interactions between the two factors. All statistical tests were performed (R version 2.14.1) at a significance level of $\alpha = 0.05$.

3.5 Results

3.5.1 Flood Events

The floodplain of Stroubles Creek varied dramatically from spring to summer (**Table 3-1**). The first experiment represented conditions of early spring, before the growing season. The average soil temperature over the spring sampling period (18-28 March 2012) was 13.3°C with an average volumetric water (VW) content of 0.66. Very little vegetation was growing on the floodplain, and there was standing water throughout the area during the entire sampling period. In the summer sampling, 3-13 July 2012, the floodplain was flush with growth, making evapotranspiration a key driving force. No standing water was present during the summer sampling, with average soil temperatures of 20.7°C and average VW content of 0.31. Images of the floodplain during the spring and summer experiments are shown in **Appendix D**. On the third day post-flooding of the spring sampling (24 March 2012) there was light rain throughout the day, not exceeding 5 mm/hr and generally at a rate of 0.9 mm/hr, totaling 22 mm over the 24 hour period. Another, smaller event occurred on the following day from 8:00 – 19:00 totaling 5.5 mm. During the summer sampling, a small rainfall event occurred on the fourth day post-flood (10 July 2012) from 7:00-10:00 totaling 6.4 mm of precipitation.

The March flood simulation took approximately 75 L of water, while the July flood used over 8000 L of water, as the floodplain was saturated in spring and much drier in summer. The difference in water table elevation is illustrated in **Figure 3-3**. The water for both floods came directly from Stroubles Creek, and basic analyses are presented in **Table 3-1**. It should be noted that a natural flood would not use as much water as the simulated floods, as the water table would be elevated and the head of water would be uniformly distributed across the entire floodplain.

Generation of GHGs was tested in all ten mesocosms 2-3 times within the week prior to each flood simulation to assess the variability between mesocosms in both seasons. Using a Wilcoxon rank sum test at a significance of 0.5, no significant difference between mesocosms in flooded and non-flooded treatments was seen pre-flood, indicating that the mesocosms were similar enough to act as replicates within treatments.

3.5.2 Carbon Dioxide

The CO_2 flux rate was significantly greater in both the flooded and non-flooded treatments in summer than in spring (**Table 3-2** and **Figure 3-4**). Additionally, the flooded

mesocosms showed significantly greater effluxes of CO_2 than the non-flooded mesocosms for the first two days after the summer flood simulation. Cumulative generation over the first three samplings after the flood simulation was calculated for each mesocosm and analyzed using a 2-way ANOVA. Results from ANOVA analysis showed significant interaction effects between treatment and season (**Table 3-3**). A clear diurnal pulse in CO_2 can be seen in the summer diurnal sampling, but not in the spring (**Figure 3-5**).

3.5.3   Nitrous Oxide

The N_2O flux rates were significantly greater overall for the summer sampling than in the spring (**Table 3-2**). A distinct peak in N_2O efflux from the flooded mesocosms is visible for two days following the flood simulation, as can be seen in **Figure 3-6**. A smaller peak, especially prominent in the non-flooded mesocosms, occurred on the fourth day after the summer flood, which closely follows a 5 mm rainfall event. A diurnal sampling was performed on that day (**Figure 3-7**), which shows the increased efflux of N_2O following wetting due to rainfall, but only in the non-flooded mesocosms. The increased flux rate is also shown to last throughout the afternoon and evening, though this pulse does not seem likely to last as long as the pulse attributed to the flood simulation as the increased generation is already falling off by the end of the day. As with CO_2, ANOVA results comparing the cumulative generation over the first three days post-flood showed that both season and treatment were significant, as well as there being a significant interaction effect between the two (**Table 3-3**).

3.5.4   Methane

There was net efflux of CH_4 during the spring, while uptake of CH_4 by the floodplains soils is apparent in summer (**Figure 3-8**). The cumulative generation over the 3 days following the flood simulation was significantly greater in the spring than summer (**Table 3-2**). The simulated flooding did not show any significant impacts on CH_4 flux in either experiment.

3.6   Discussion

Differences in flux rates of CO_2, N_2O, and CH_4 between spring and summer speak to the annual variation in GHG emissions from floodplains. Temperatures were higher and soil moisture contents lower in summer than in spring. These may be driving forces behind the increased efflux of CO_2 and N_2O, as increasing temperature tends to increase soil microbial metabolism, along with optimal productivity being subject to upper and lower limits of soil moisture. *Rustad et al.* [2001] found that CO_2 emissions were positively correlated with

temperature in 17 different locations throughout North America, South America, and Europe. Upper and lower moisture limits on soil respiration have also been observed by *Suseela et al.* [2012]. Generation of N_2O is linked to nitrification, denitrification and nitrifier denitrification [*Galloway et al.*, 2003; *Morse et al.*, 2012; *Wrage et al.*, 2001]. These processes occur at varying redoximorphic potentials, and often occur jointly within soil micropores with different potentials, thus the less uniform saturation provided in the summer may provide conditions that increase total release of N_2O by combining these processes.

However, focusing on simple environmental factors such as moisture and temperature overlooks the key role that vegetation can play. Plants are actively growing and evapotranspiration is much higher in summer than in spring on the Stroubles Creek floodplain. Root exudates are an important source of labile organic matter for soil microbial communities and are released at greater rates during the growing season than when the plants are dormant [*Haichar et al.*, 2008]. Soil microbial metabolism is dependent on the availability of labile organic matter in addition to appropriate temperature and moisture levels, so the increases in CO_2 and N_2O flux may also be linked to vegetative growth.

The greater efflux of CH_4 during the spring run than the summer run can be attributed to the saturated conditions on the floodplain. Extended periods of saturation found on the Stroubles Creek floodplain enable the soil microbial community to use up the more energy-efficient electron acceptors, such as O_2, Fe^{3+}, and NO_3^-, depleting portions of the soil pore space of these compounds and inducing highly reducing conditions which would encourage methanogenesis [*Sylvia et al.*, 2004]. In the summer, however, the soil was dry enough that oxygen can diffuse into most of the soil matrix. Any CH_4 that would be produced in the summer would likely be oxidized as it moved through the soil to the surface, and there also appeared to be net consumption of CH_4 from the atmosphere (**Figure 3-8**). The artificial flood treatments in this experiment were not long-lived enough for the soil matrix to become anoxic, which could have resulted in a net efflux of CH_4.

While the wetting events of this study were not sufficient to induce the level of anoxia necessary for production of CH_4, responses to the flooding treatment were observed in both CO_2 and N_2O. A prolonged increase in CO_2 flux was observed in the flooded mesocosms as compared to the non-flooded mesocosms in the summer, the effects lasting a full two days following the flood simulation. Interestingly, though the soil moisture was significantly higher in the flooded

mesocosms than the non-flooded mesocosms for a full seven days following the flood simulation (**Figure D-9**), the response of the soil community only lasted a few days. Various processes may be responsible for this response. Short wet-dry pulses have been shown to release pulses of CO_2 in a phenomenon known as the 'Birch effect' [*Birch*, 1958]. These short pulses of CO_2 efflux immediately following rewetting have been shown to be derived from the rapid mineralization of lysed microbial biomass and/or osmoregulatory substances released by soil microbes to avoid lysis due to osmotic stress [*Unger et al.*, 2010]. The scale of the wetting front used by *Unger et al.* [2010] was several orders of magnitude smaller than the simulated flood from this work. In other studies, longer flood events have shown potentially different processes occurring. *Sánchez-Andrés et al.* [2010] saw dramatic drops in soil respiration after floods lasting 15-21 days, which were followed by increases in respiration rates, often exceeding those in control plots. This drop before the pulse may be due to inhibition of soil microbial metabolism due to high soil water content, perhaps preventing sufficient oxygen from reaching the soil community. The following pulse may be similar to the Birch effect, caused by the rapid mineralization of lysed microbes, but may also be due to the improved availability of nutrients previously bound tightly to soil aggregates or may even due to adjustments in the soil microbial community structure promoting the growth of certain groups of microbes over others [*Sánchez-Andrés et al.*, 2010]. It is possible that a depression of CO_2 efflux occurred in the study presented here, but was not captured by the sampling protocol. The scale of the present study lies somewhere between the two past studies discussed, so the cause of the pulse of respiration may be due to a combination of these effects. The importance of autotrophic respiration to soil CO_2 fluxes should not be ignored, however, as about half of soil respiration is associated with autotrophic metabolism [*Ryan and Law*, 2005]. Addition of flood water in the summer may have increased the respiration associated with plant root systems, as plant metabolism may have been moisture limited in the dry summer season. It is also possible that the addition of water and some nutrients associated with it encouraged the release of greater amounts of soil root exudates, which would feed the metabolism of the soil microbes in the surrounding areas. No visible changes in plant growth were observed in the field, however, though no measurements of plant biomass or other plant metrics were taken.

Extrapolating the results of this study, the flooded mesocosms produced an additional 2.41×10^5 μmol CO_2 m^{-2} beyond the non-flooded mesocosms, on average, over the three days following the flood simulation (**Table 3-2**). Along the 330m stretch of Stroubles Creek that the

study site was located on, there are over 36,000 m^2 of floodplain similar in elevation, vegetation, and soil makeup to the study site. This means that there is a potential for at least 380 kg of CO_2 to be released beyond the background flux of this section of floodplain over three days following an event similar to the one simulated here. While it may appear small compared to the 1040 kg CO_2 that would be released normally, this additional 380 kg CO_2, when added up over large areas this addition could cause a significant positive forcing on climate change.

The pulse of increased N_2O efflux in the flooded mesocosms following the summer flood event was also detectable for a full two days after the simulated flood, but was much more prominent the first day than the second (**Figure 3-6**). Nitrification, denitrification, and nitrifier denitrification all have N_2O as an intermediary of the processes, and thus all three processes can 'leak' N_2O into the system [*Galloway et al.*, 2003; *Morse et al.*, 2012; *Wrage et al.*, 2001]. The amount of N_2O released from these processes depends on a variety of factors, including level of anoxia, concentration of reactants, and availability of labile organic matter as substrate. It is likely that the wetting event of the simulated flood produced a wider range of redoximorphic conditions than were previously in the well-aerated soils, increasing the likelihood of a combination of these processes to be occurring at once, thus increasing the output of N_2O from the system.

There was a rainfall event four days after the summer flood simulation, lasting from 7:00 – 10:00, totaling about 6 mm of precipitation. This event had some interesting implications for N_2O fluxes. The flux rate of the flooded mesocosms remained fairly steady, while the non-flooded mesocosms showed an increase in N_2O efflux lasting all afternoon and evening (**Figure 3-7**). We postulate that the readily bioavailable nutrients and organic matter for nitrification and denitrification in the soils of the flooded mesocosms were depleted following the flood simulation and had not recovered fully by the subsequent rainfall event, thus limiting the reaction of the soil microbial community to the wetting event. The soils of the non-flooded mesocosms, meanwhile, contained a ready supply of nutrients and labile organic matter for microbes to consume, allowing for a detectable response in N_2O generation.

3.7   Conclusions

This study found season and inundation to have strong interaction effects on generation of CO_2 and N_2O, and found season to correlate significantly with the generation of CH_4. The flux

of CO_2 was greater in mid-summer (July) than in the early spring (March), and was increased even further in mesocosms subject to the summer flooding treatment. The flux of N_2O followed a similar response, with very distinct peaks following wetting events in the summer. Wetting events in the spring did not show any impact on fluxes of any of the GHGs studied, as the floodplain was already fully saturated during the spring run of the experiment.

The generation of CH_4 was significantly greater in the spring run than in the summer run, as the saturated condition of the floodplain in spring was more conducive to the anoxia and highly reducing conditions necessary to initiate methanogenesis on a detectable scale. The artificial flood events did not last long enough to induce any appreciable methanogenesis from the system.

The results highlight the responsiveness of GHG generation from floodplain soils to both season and wetting events. The changing climate and the potential changes in distribution of precipitation and associated floodplain inundation could appreciably alter the net flux rates of GHGs from floodplain soils, though the complexity of the processes involved makes it difficult to state one way or the other. Release of GHGs from floodplains following an inundation event may be counteracted by increased uptake later on. Also, floodplains are highly heterogeneous landscapes that can contain hot spots for production of GHGs in close proximity to an area of increased GHG uptake. These hot spots may be overlaid by hot moments, where production of GHGs may be influenced by the time of year or time since an inundation event. The results of this study indicate that the timing of flood events can be very important.

The IPCC predict an increase in annual precipitation in eastern North America, which includes the study site [*Christensen et al.*, 2007]. A future increase in precipitation could mean an increased likelihood of events similar to the flood simulated in the summer experiment. A flood event in the summer of a similar magnitude to the one modeled here could cause a release of an additional 380 kg CO_2 over three days from a 36,000 m^2 section of floodplain associated with a 330 m stretch of stream. Releases of this magnitude may seem small compared to the background generation rates, but added up over the distributed lengths of similar streams they could become very significant indeed. Although the floodplains of low-order streams are often smaller than those of higher-order streams, low-order streams cover significantly more mileage, contributing more floodplain area overall [*Freeman et al.*, 2007; *Leopold*, 1962].

Significant net changes in GHG fluxes from floodplains of low-order streams could cause changes in the feedback loop between natural GHG generation and climate. If changes in precipitation regime were to cause net increases in GHG generation, there is potential for net positive feedback forcing climate to change even further. However, if net fluxes were lowered due to climate change, there would be a drop in the forcings, mitigating the severity of climate change. Fully understanding the balance between uptake and release of GHGs from floodplains, however, is a complex problem. The frequency and duration of wetting events are shown to be important to GHG emissions from floodplains, but the exact relationships need to be studied more closely. More intensive studies are necessary to better understand the processes being affected, but a more complete understanding will inform our climate change models and allow us to make more realistic predictions and management decisions

3.8 References

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Table 3-1: Environmental conditions and flood water analysis for spring (19-28 March 2012) and summer (3-13 July 2012) experiments.

		Spring	Summer
Environmental Conditions			
Avg Air Temp	(deg C)	13.7	22.6
Avg Soil Temp	(deg C)	13.3	20.7
Avg VW		0.68	0.30
Avg Max Daily Solar Radiation	(kW/m^2)	0.77	0.80
Avg Daily ET	(mm/day)	5.70	6.12
Flood Water Analysis			
NO$_3$-N	(ppm)	2.09	0.81
DOC	(ppm)	1.80	4.71
TN	(ppm)	2.34	1.64

[a]ET, evapotranspiration calculated using Bowen ratio.
[b]DOC, dissolved organic carbon.
[c]TN, total nitrogen.

Table 3-2: Cumulative generation of greenhouse gases from study site over three days following artificial flood event.

	Spring			Summer		
	mean ± standard deviation			mean ± standard deviation		
	$\mu mol\ CO_2\ m^{-2}$					
flooded	9.08E+04	±	2.73E+04	9.00E+05	±	6.88E+04
non-flooded	9.41E+04	±	4.98E+04	6.59E+05	±	1.01E+05
	$\mu mol\ N_2O\ m^{-2}$					
flooded	-1.6	±	6.4	180.8	±	102.1
non-flooded	-7.4	±	3.6	34.4	±	28.9
	$\mu mol\ CH_4\ m^{-2}$					
flooded	83.9	±	167.7	-62.1	±	29.0
non-flooded	118.0	±	132.8	-83.1	±	19.2

Table 3-3: Results of 2-way analysis of variance (ANOVA) tests on effects of season (spring vs summer) and flooding treatment (flooded vs. control non-flooded) on cumulative generation of greenhouse gases for three days post-flood simulation. Asterisk (*) indicates significance at an α of 0.05.

	2-way ANOVA p-values		
	season	flooding	interaction
CO_2	< 0.0001*	0.0012*	0.0009*
N_2O	0.0002*	0.0056*	0.0093*
CH_4	0.0025*	0.8941	0.5768
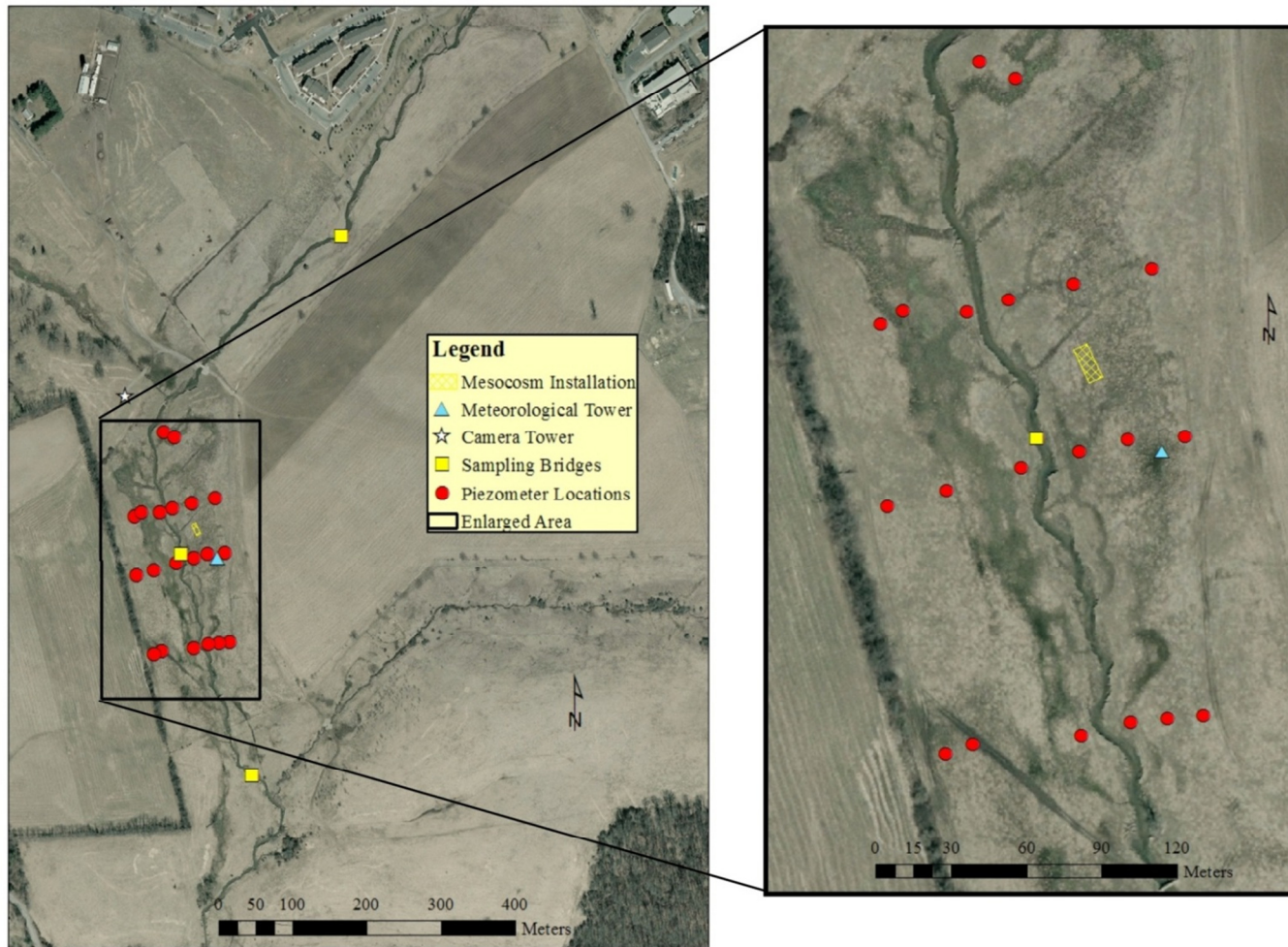
Figure 3-1: Map of StREAM Lab and study site ('Mesocosm Installation'). Blacksburg, VA lies directly to the northeast of the study site.
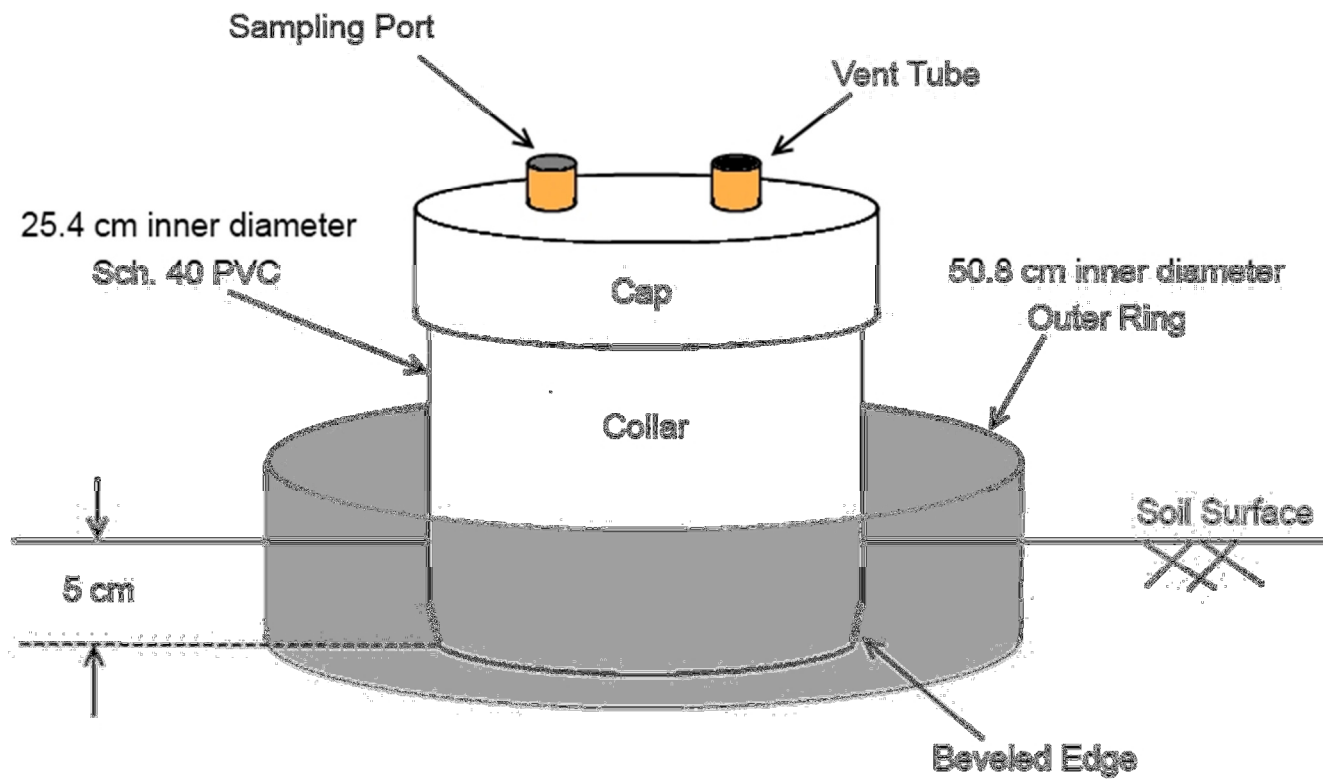
Figure 3-2: Mesocosm setup, not to scale. Outer ring and collar both installed 5 cm into floodplain soil. Vent tube modeled after *Hutchinson and Livingston* [2001]
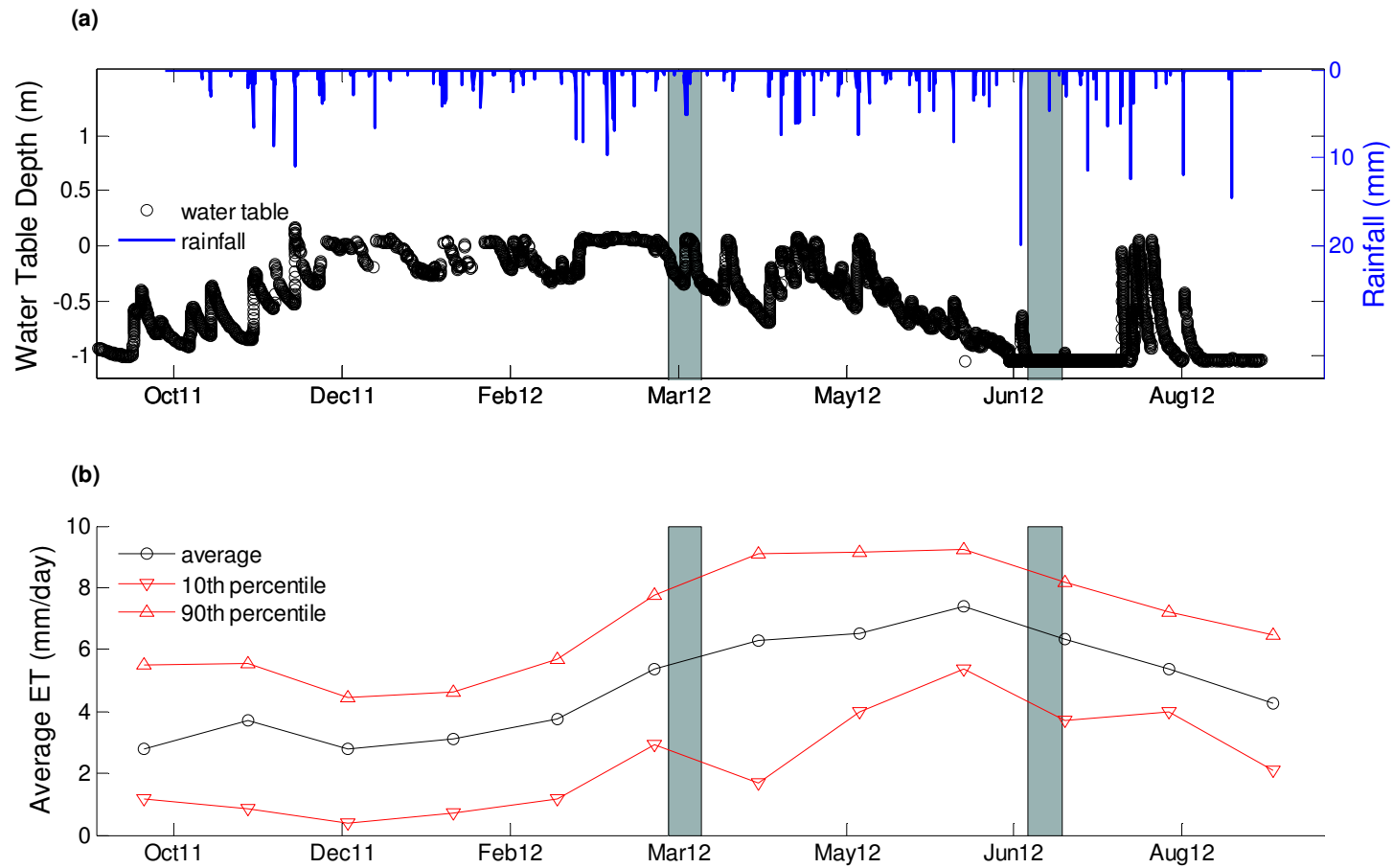
Figure 3-3: Water table depth in reference to soil surface and hourly rainfall data (a) above average evapotranspiration (ET) (b).for StREAM Lab in Blacksburg, VA from October 2011 to September 2012. Highlighted areas represents spring (19-28 March 2012) and summer (3-13 July 2012) experiments. Water table data from piezometer installation on floodplain with a maximum depth of 1.05 m, thus no water table values below this depth are available. ET calculated using Bowen ratio. The 10th and 90th percentile data are shown as well as the monthly average ET.
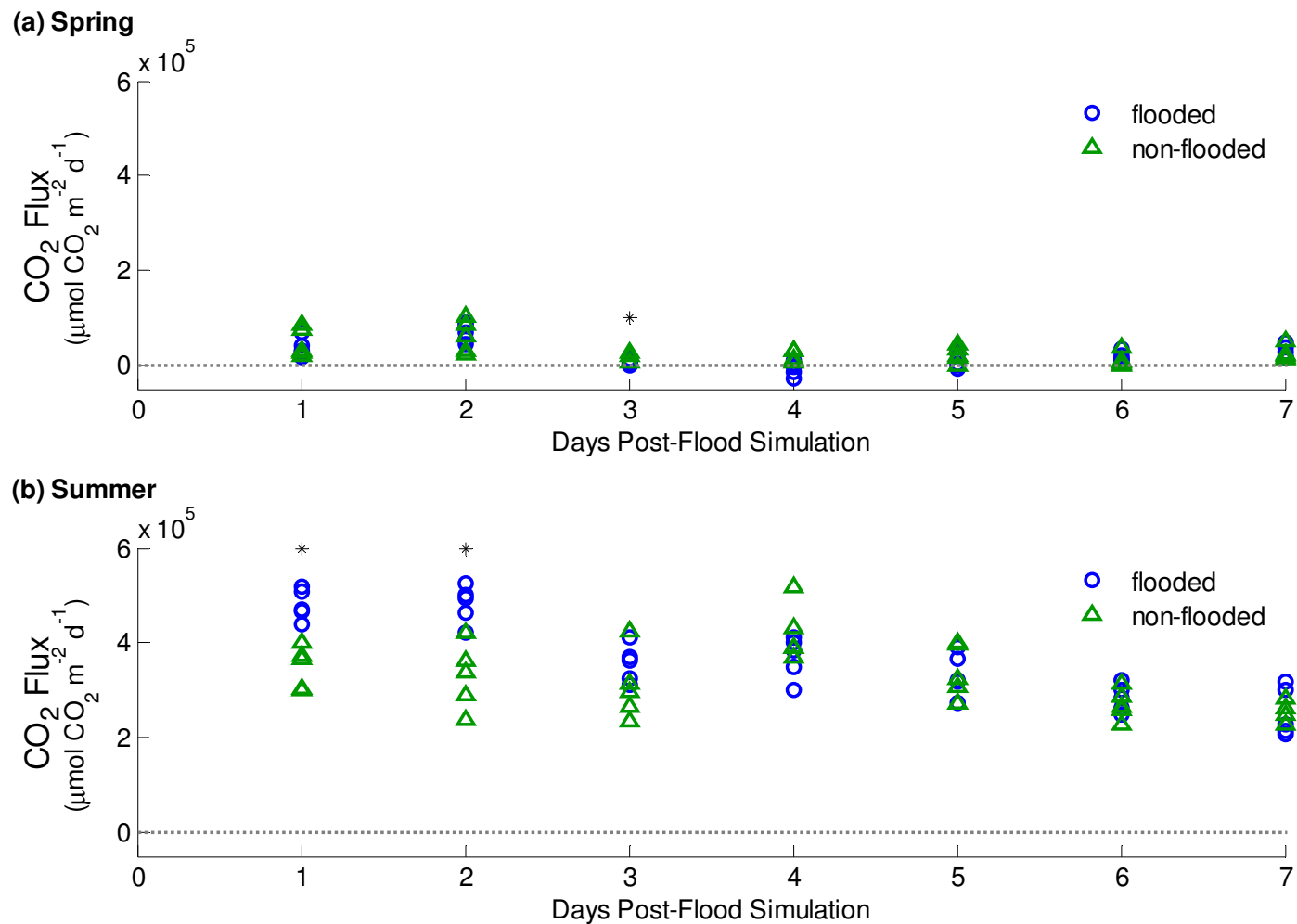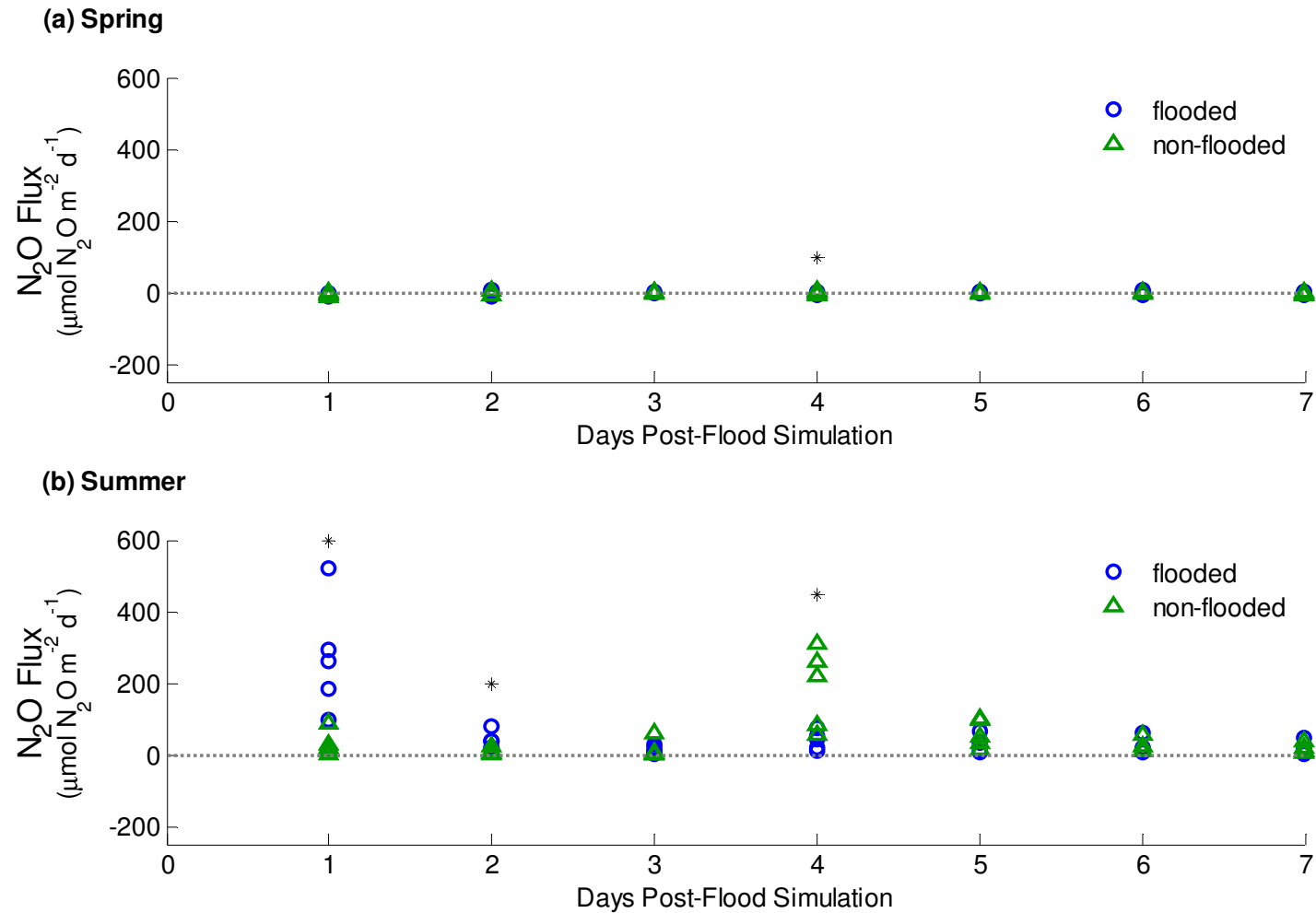
Figure 3-4: Flux of CO_2 in spring sampling (a) and summer sampling (b). Spring flood event occurred on 21 March 2012, summer flood event on 6 July 2012. Blue circles represent mesocosms subjected to the flood treatment, green triangles represent non-flooded controls. Asterisks (*) indicate days on which there was significant difference between flooded and non-flooded mesocosms using Wilcoxon rank sum test ($\alpha=0.05$). Horizontal dotted line on both plots represents no net flux.
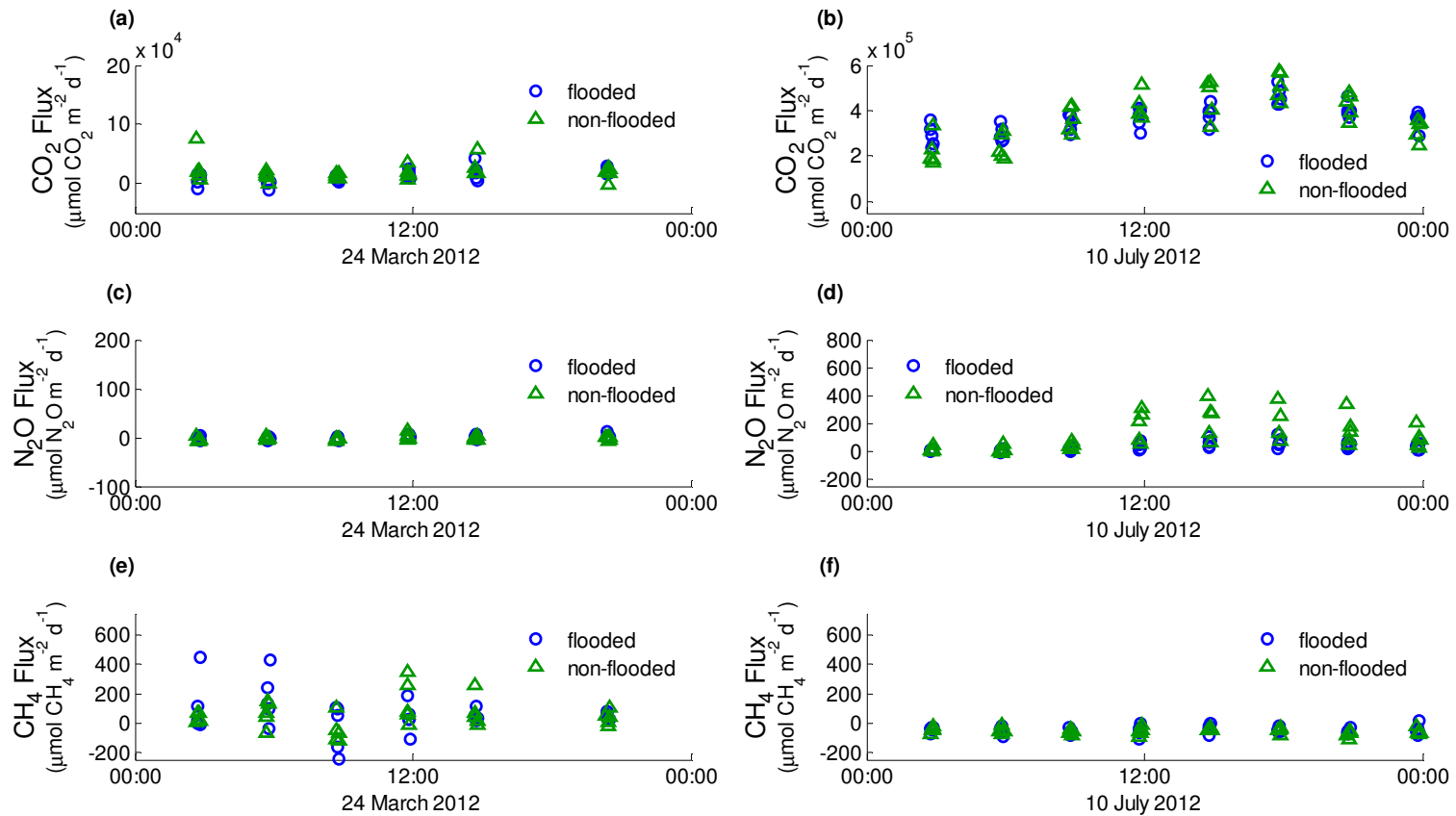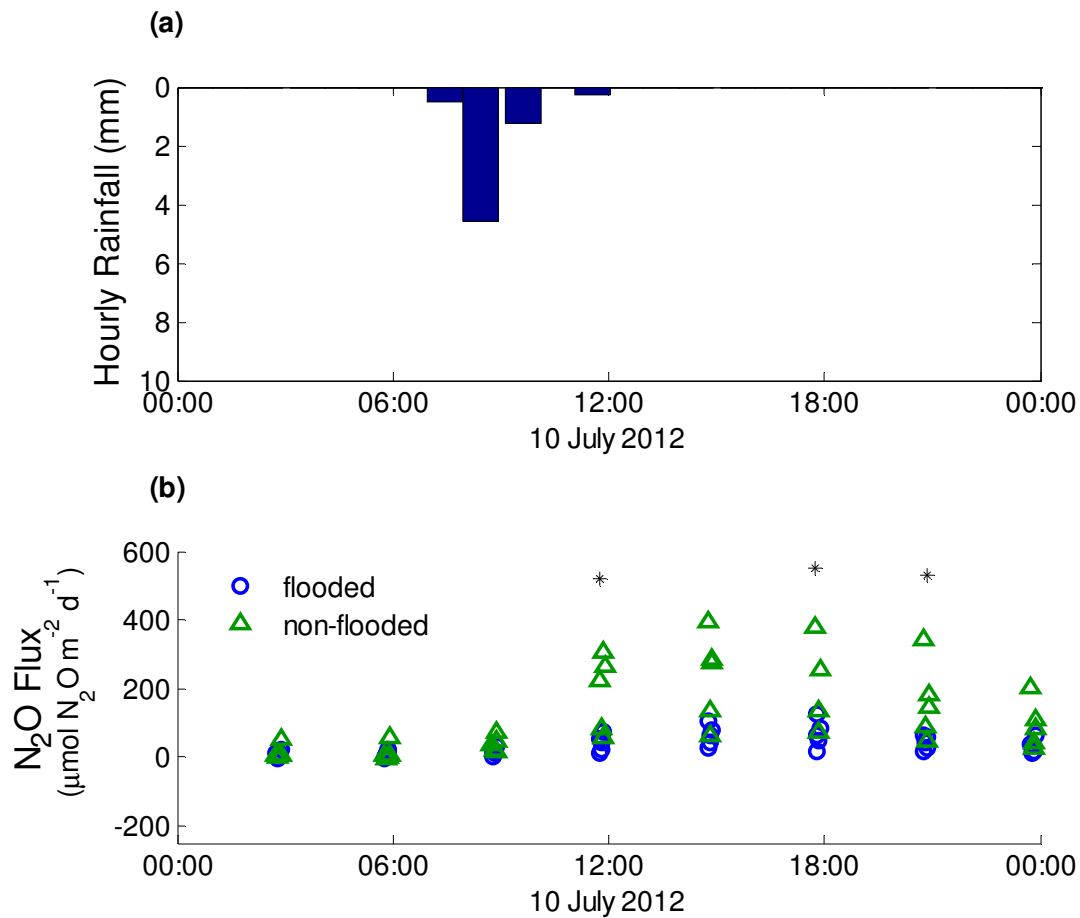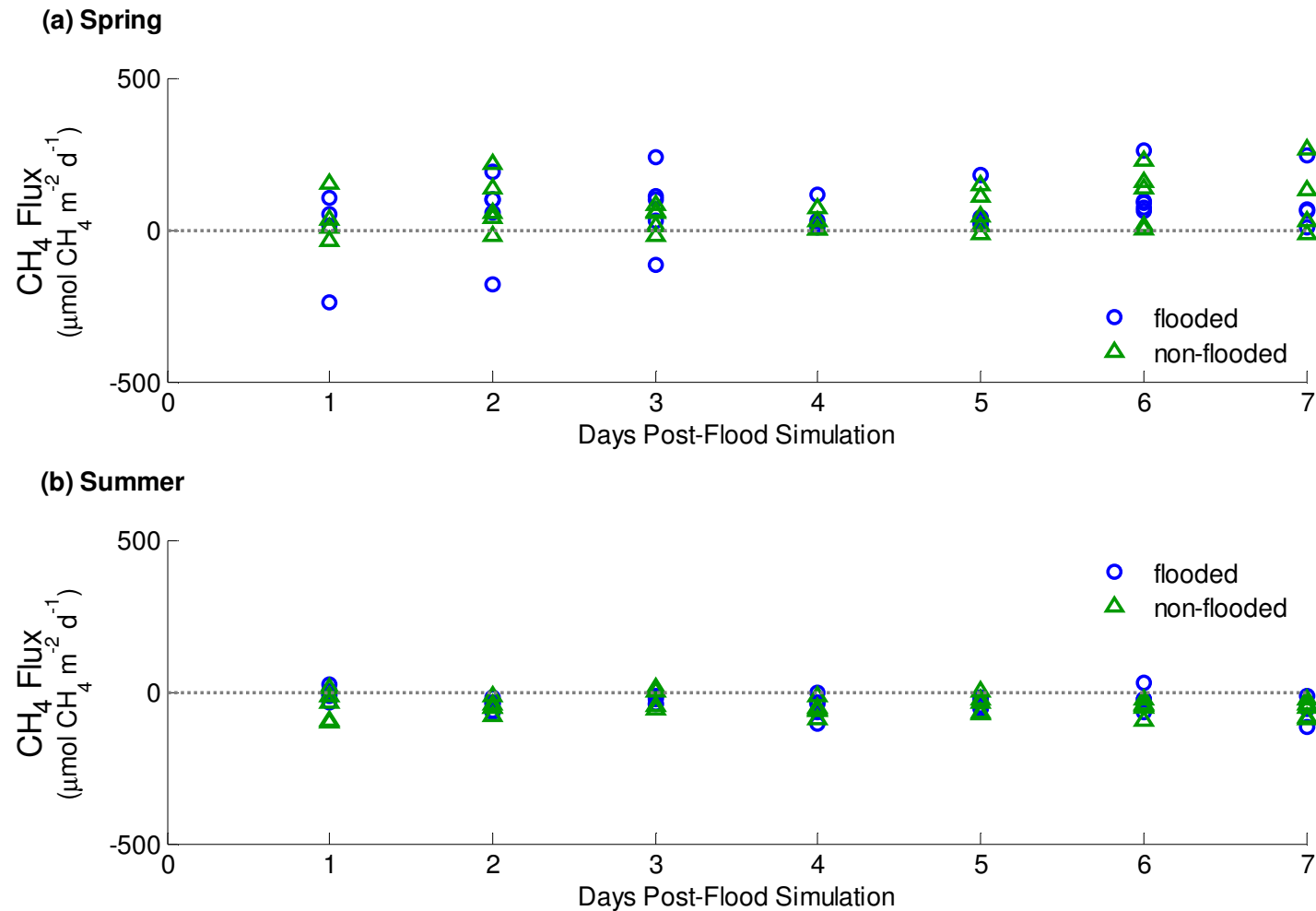
Figure 3-5: Diurnal sampling flux rates for CO_2 (a and b), N_2O (c and d), and CH_4 (e and f). Blue circles represent mesocosms subjected to the flood treatment, green triangles represent non-flooded controls. Plots a, c, and e represent the spring diurnal sampling, which took place three days after the flood simulation (21 March 2012). Plots b, d, and f represent the summer diurnal sampling, which took place four days after the flood simulation (6 July 2012). Note that not all y-axes are to the same scale.

Figure 3-6: Flux of N$_2$O in spring sampling (a) and summer sampling (b). Spring flood event occurred on 21 March 2012, summer flood event on 6 July 2012. Blue circles represent mesocosms subjected to the flood treatment, green triangles represent non-flooded controls. Asterisks (*) indicate days on which there was significant difference between flooded and non-flooded mesocosms using Wilcoxon rank sum test (α=0.05). Horizontal dotted line on both plots represents no net flux.

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Figure 3-7: Hourly rainfall (a) and flux of N_2O (b) on the 10th of July, four days after the summer flood event on 6 July 2012. Blue circles represent mesocosms subjected to the flood treatment, green triangles represent non-flooded controls. Asterisks (*) indicate samplings for which there was significant difference between flooded and non-flooded mesocosms using Wilcoxon rank sum test ($\alpha=0.05$).

Figure 3-8: Flux of CH$_4$ in spring sampling (a) and summer sampling (b). Spring flood event occurred on 21 March 2012, summer flood event on 6 July 2012. Blue circles represent mesocosms subjected to the flood treatment, green triangles represent non-flooded controls. Asterisks (*) indicate days on which there was significant difference between flooded and non-flooded mesocosms using Wilcoxon rank sum test (α=0.05). Horizontal dotted line on both plots represents no net flux.

4   Conclusions and Future Research

4.1   Carbon Dioxide

Carbon dioxide was found to be influenced by both season and inundation (**Table 3-3**). Baseline efflux rates were greater in the summer experiment (July) than in the spring experiment (March) (**Table 3-2**). This was likely due to a combination of increased temperature, moderate soil moisture content, and greatly increased plant productivity in July as compared to March. The artificial flooding event in the summer contributed to an increase in CO_2 efflux in the flooded mesocosms lasting several days, while the spring event did not result in any significant change (**Figure 3-4**). The wetting event may have mobilized nutrients previously bound tightly in soil aggregates and increased the productivity of nearby plants which would in turn increase both plant root respiration and the amount of root exudates available for microbial consumption. Flooding and subsequent drying may have also caused the lysing of many soil microbes and a shift in the microbial community structure, which could have caused the significant jump in soil microbial respiration.

4.2   Nitrous Oxide

Both season and inundation also significantly influenced the generation of N_2O (**Table 3-3**). Baseline net production of N_2O in summer was greater than in spring, similar to the relationship of CO_2, but to a lesser degree (**Table 3-2**). N_2O is a by-product of nitrification, denitrification, and nitrifier denitrification, and can be released to the atmosphere during these processes. Thus, the heterogeneous nature of the floodplain in summer, as opposed to the uniformly saturated conditions in spring, in combination with increased plant productivity fueling microbial metabolism likely caused the increased net production rate in summer as compared to spring. Sharp peaks in N_2O production were observed after the summer flood simulation (**Figure 3-6**). These may be attributed to a combination of increased availability of reactants and labile organic matter as well as an increased range of redoximorphic conditions, supporting nitrification, denitrification, and nitrifier denitrification which combine to increase the release of N_2O.

The pulse of N_2O on the fourth day following the flood simulation in only the non-flooded mesocosms (**Figure 3-6**, **Figure 3-7**) can be attributed to a 5 mm rainfall event earlier that day. We theorize that the readily bioavailable nutrients and organic matter for nitrification, denitrification, and nitrifier denitrification in the flooded mesocosms were depleted following the

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flood simulation and had not recovered fully by the subsequent rainfall event, thus the generation of additional N_2O was limited in the flooded mesocosms. The soils of the non-flooded mesocosms, meanwhile, contained a ready supply of nutrients and labile organic matter for microbes to consume, allowing for a detectable response in N_2O generation.

4.3 Methane

Methanogenesis takes place only under anoxic conditions. While anoxic microsites may be present throughout the soil strata, CH_4 produced in anoxic sites is often oxidized as it passes through better aerated soil pores as it move through the soil column. Thus, a predominance of anoxia is necessary to see net generation of CH_4 in soils. In the spring experiment, the floodplain had been saturated for an extended period of time, leading to a predominance of anoxic zones and a net efflux of methane. In summer, however, conditions were predominantly oxygenated and there appeared to be a net consumption of CH_4 by the system (**Figure 3-8**). The artificial flood event was not sufficient in either season to increase the production of methane.

4.4 Potential for Feedback Loops

The generation rates of CO_2, N_2O, and CH_4 may all be responsive to changes in climate. All three are impacted by season overall, which can be thought of as a summation of factors including temperature, soil moisture, solar radiation, and primary productivity. In addition, CO_2 and N_2O generation rates can be influenced by wetting events, either from direct inundation by flood waters or wetting from rainfall events. Thus changes in the timing, duration, magnitude, and frequency of precipitation events and any associated flood events can potentially alter the generation rates of GHGs from floodplains. With changing flux rates of GHGs, there is a potential for feedback loops between natural GHG production and climate to change. Increased emissions from natural sources may exacerbate climate change, whereas decreased emissions may mitigate its impacts. Although smaller individually, the cumulative area of floodplains of low-order streams is much larger than the land area of floodplains associated with larger rivers. This larger area could allow potential feedbacks on small scales to become large-scale impacts.

4.5 Directions for Future Research

This study only scratches at the potential of GHG fluxes from floodplains to alter the course of climate change. Expanding our knowledge of these processes and interactions can better inform our climate change models, allowing us to make better informed predictions and management decisions.

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More in-depth studies should be performed to tease out the relationships between the different factors, e.g. vegetation, soil moisture, temperature, and the timing and frequency of wetting events. Large numbers of replicates are necessary for results of studies to be acceptable for use in the framework of large, high impact models such as those now being used in climate change prediction. The generation of GHGs in other landscapes needs to be more fully explored, beyond floodplains and wetlands. Complex interactions, such as those between generation processes and the lag time between wetting events, need to be studied more closely. There are a great deal of future research paths that could begin with this work, if only given enough time and resources.

Appendix A: Mesocosm Construction

A complete list of materials for constructing the mesocosms is included in **Appendix C: Parts List**. The mesocosm collars and caps were constructed based on a design developed by Dr. Brain Strahm of Virginia Tech's Department of Forest Resources and Environmental Conservation.

The mesocosm collars were constructed out of 10" Sch. 40 PVC pipe cut to 7 ½ inch lengths. One end of the collars was beveled using a grinder to make installation in the field less labor-intensive (see **Figure A-1**). For field installation, the collars were pushed into the soil surface 2 inches, providing a good seal with the soil to minimize gas loss without severing too many of the root connections which provide a major source of food for soil microorganisms. For flood studies such as this one, mesocosms chosen for the flooding treatment were each surrounded by a 20 in diameter outer ring of stiff plastic inserted 2 in into the soil. This outer ring performed similarly to the outer ring of a double-ring infiltrometer setup, as a 10.2 cm head was also maintained between the rings as a buffer between the flooded treatment area and the surrounding, non-flooded soil.

Additionally, 2 inch wide rings were cut from the PVC to serve as spacers inside of the caps. When installed inside the caps, these spacers left a ½ inch gap for joining the cap to the collar, while preventing the caps from slipping so far onto the collars that they were difficult to remove. In each cap, two ports were drilled out and fittings installed, as can be seen in **Figure A-2**. One port served as a sampling port, with a rubber septum installed between the shaft fitting and nut. Installed in the other port was a fitting with a male connector on the inside of the cap to mount the vent tube (**Figure A-3**). Each vent tube was constructed out of two 2 inch lengths of flexible PVC tubing connected by an elbow joint and mounted to the male connector in the cap via zip ties.

Design of vent tubes and depth of collar installation follows guidelines presented in Hutchinson and Livingston (2001):

Hutchinson, G. L., and G. P. Livingston (2001), Vents and seals in non-steady-state chambers used for measuring gas exchange between soil and the atmosphere, *European Journal of Soil Science*, 52(675-682).
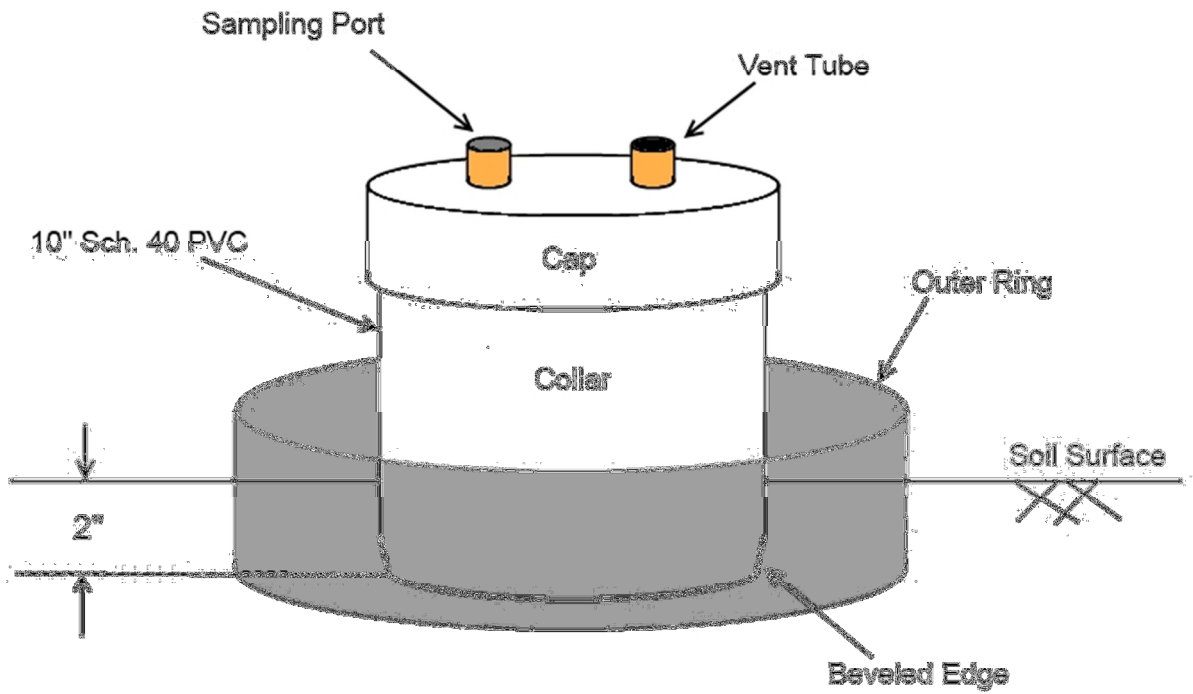
Figure A-1: Mesocosm design, not to scale. Outer ring and collar both installed 2" into floodplain soil. Vent tube modeled after *Hutchinson and Livingston* [2001].

Figure A-2: Overhead view of mesocosm cap. Sampling port to left with visible grey septum, vent tube port to right. Sharpie® in image for scale.
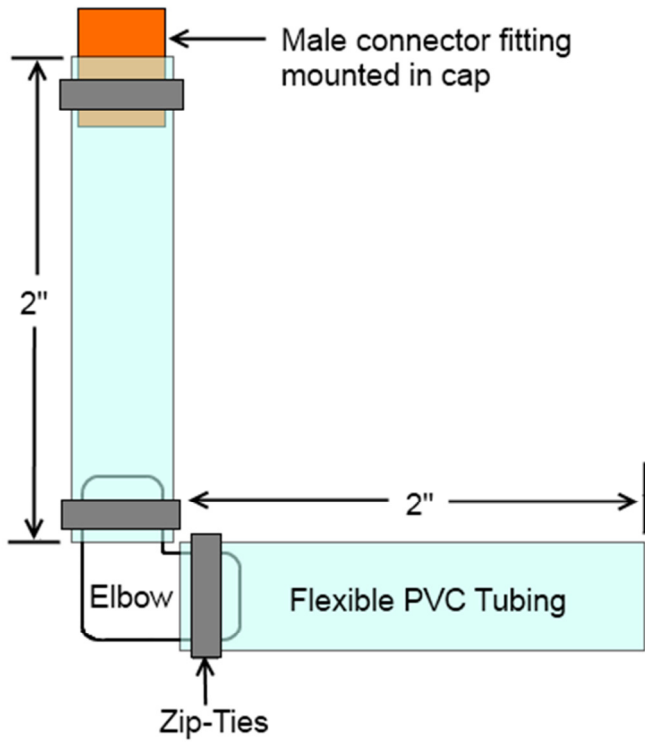
Figure A-3: Vent tube design, not to scale. Modeled after guidelines laid out by *Hutchinson and Livingston* [2001]

Appendix B: Sampling Procedure

A full list of supplies for sampling is included in **Appendix C: Parts List**.

Preparation work:

Before any sampling can begin, the vials must be assembled and prepped. Septa are inserted into vials and aluminum seals are fitted using a hand crimper. Once the sampling vials are assembled, they must be evacuated using a setup similar to the one shown in **Figure B-1**, which was designed and constructed by Dr. Brian Strahm of Virginia Tech's Department of Forest Resources and Environmental Conservation. The vials are iteratively filled with dinitrogen gas and evacuated using an air pump three times, being left evacuated. Vials are not evacuated more than four days prior to use.

When vials are prepped, three vials for each run of sampling are filled with a known mixed gas standard. This standard should be near the median concentration expected in samples. These vials serve as check samples to be stored and taken out into the field with the sample vials to monitor changes in sample concentration that may indicate leakage due to changes in environmental variables.

Collars should be installed 2 inches into the soil surface at least two weeks prior to sampling. See **Appendix A: Mesocosm Construction** for details of mesocosm collar and cap construction and installation. Before each sampling, the actively growing vegetation should be trimmed to the soil surface. This will minimize the uptake of carbon dioxide via photosynthesis, which would skew the fluxes estimate for this gas due to soil microbial respiration.

Samples are taken four times over an hour incubation period, from which concentrations can be determined and flux rates calculated. Take an appropriate number of prepped and evacuated vials into the field in addition to the check samples. For this experiment, all mesocosms were sampled during the same hour period, so the vials and sampling equipment were carried along in a bag for time efficiency.

Field sampling procedures:

If there is standing water in the mesocosm, the depth should be recorded. The presence of standing water will change the volume of the headspace, altering the calculations of flux.

Place cap on collar. Secure rubber band, sealing cap to collar. This works best if the band is wrapped around the cap and then flipped down so that half of the band is in contact with the

cap and the other half in contact with the collar (**Figure B-2**). Take care not to disturb the collar's seating in the soil.

Take first sample. Using needle with air tight syringe, pierce the rubber septum of the sampling port, pushing the needle as far through the sampling port as possible, and pull approximately 15 mL of headspace gas. Pull needle out of sampling port. Express gas sample into atmosphere until 12 mL of sample remains. Puncture septum of evacuated sample vial and fill with the 12 mL of sample gas. Labeling the vials after filling is recommended to prevent mix-ups. Over-filling the sample vials prevents contamination of samples later. If there were to be leakage through the septum, sample would leak out rather than external air moving in, preserving the sample from contamination.

If sampling from an array of mesocosms placed nearby, head to next mesocosm, cap it and take the first sample. This experiment was performed with an array of 10 mesocosms within a 6 m x 15 m grid, so a circuit could be performed sampling from all 10 mesocosms before the next round of sampling needed to start.

To take four samples over an hour-long incubation, a 15 minute period between samples is recommended. Incubation length and number of samples can be adjusted according to system characteristics, e.g. a shorter or longer incubation period may be warranted if the flux rates are significantly faster or slower, respectively, than those presented here.

After final sample is pulled, remove cap and band from collar, again taking care not to disturb the collar. If performing a diurnal sampling, a two hour 'breathing' period is recommended between hour-long incubations to allow the system to re-equilibrate to ambient gas concentrations.

Sample analysis:

Samples should be analyzed as soon as possible. A Shimadzu GC-2010 gas chromatograph and associated GC Solution software outfitted to analyze greenhouse gases housed in the Department of Forest Resources and Environmental Conservation was used to analyze the samples for this experiment. The instrument was outfitted with an FID detector to analyze the CO_2 and CH_4 fractions of the samples and an ECD detector to analyze the N_2O fraction.

Mixed gas standards were obtained from Scotty Analyzed Gases at three different levels. The 'low' standard contained 371 ppm CO_2, 1.01 ppm CH_4, and 0.1 ppm N_2O. The 'medium'

standard contained 2500 ppm CO_2, 5.0 ppm CH_4, and 1.0 ppm N_2O. The medium level standard was the one used in this experiment as the field standard. The 'high' standard contained 5000 ppm CO_2, 10 ppm CH_4, and 5.0 ppm N_2O. Appropriate QA/QC methods should be followed, as with any analysis.

<u>Data analysis:</u>

Field standard values for each gas were averaged. If this value was not within 10% of the known concentration, the difference used to adjust the field standard values was applied to all samples associated with the field samples. The raw ppm values were converted to μmol values, taking into consideration the volume of headspace actually present during each sampling, less any volume comprised of standing water. A linear fit was applied to each set of four samples associated with an individual incubation sampling. These curves and associated data points were individually assessed to ensure that the concentration measurements did not level off towards the end of the incubation, which would indicate that the concentration of the gas(es) had reached a steady-state, rendering the last sample(s) invalid. Using these generation rates, the flux of each gas in μmol (gas)$\cdot$m^{-2}$\cdot$day^{-1} was calculated.
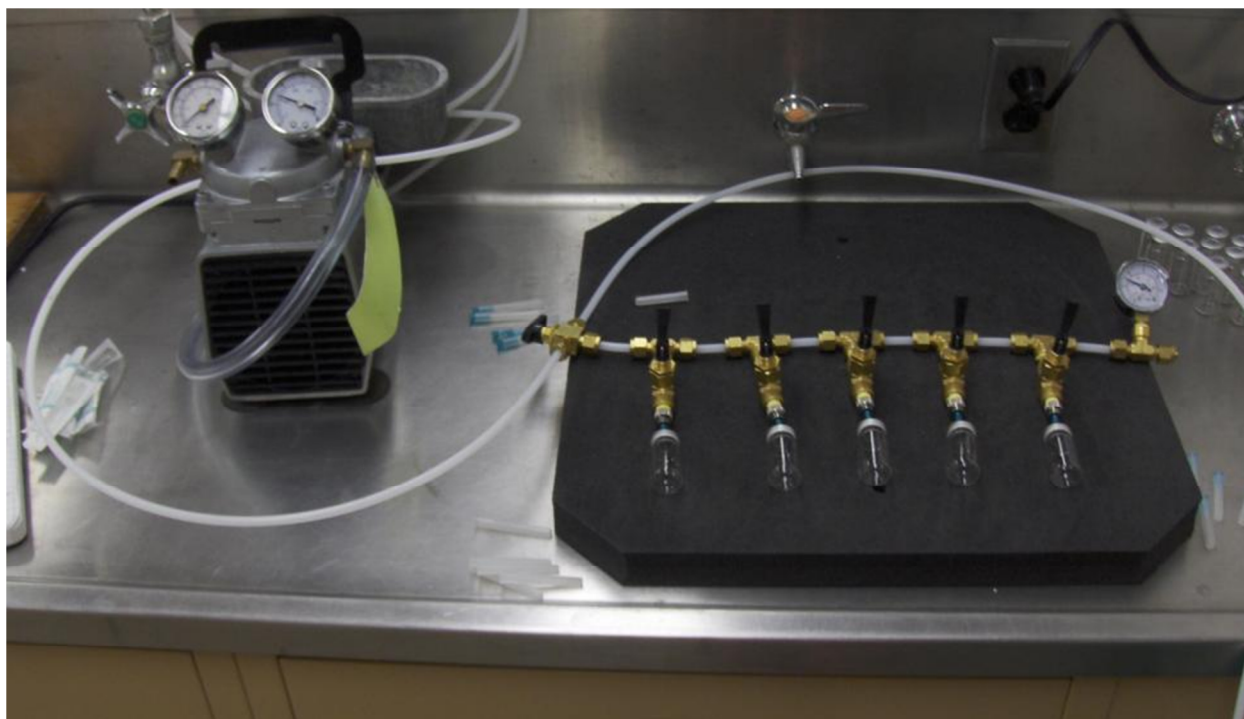
Figure B-1: Vial prep/evacuation setup. Air pump shown in figure, tank of dinitrogen gas connected via tubing that runs out of the image.

Figure B-2: Mesocosm capped and ready for incubation sampling.

Appendix C: Parts List

**Table C-1:** List of supplies and vendors used for experiment.

Vendor	Part Number	Description	Number	Unit Cost	Total Cost
collar and cap construction					
Fisher	50-121-5190	Septum Stopper 13MM HUNGATE (pack of 100)	2	$39.25	$78.50
Swagelok (Dibert Valve & Fitting Co., Inc.)	B-600-11-6	Brass Swagelok Tube Fitting, Bulkhead Male Connector, 3/8 in. Tube OD x 3/8 in. Male NPT	10	$5.60	$56.00
Swagelok (Dibert Valve & Fitting Co., Inc.)	B-600-R1-6	Brass Swagelok Tube Fitting, Bulkhead Reducer, 3/8 in. Tube OD	10	$7.10	$71.00
Cole-Parmer	S-96605-08	PVC Tubing, 3/8 X 1/2", 50-ft/pk	1	$26.00	$26.00
Cole-Parmer	DK-30622-78	Elbow, Polyethylene, 3/8", 10 PACK	1	$9.60	$9.60
Cole-Parmer	S-06830-66	Ties 4" 100/pk	1	$2.55	$2.55
Best Priced Products, Inc.	2418-10	Cando band loop, 15" silver, 10 pack	1	$44.99	$44.99
Harco Fittings/Consolidated Pipe	--	10" Schedule 40 PVC 'Solvent Weld Cap'; a special order of a component of the typical cap/plug	10	$30.00	$300.00
Ferguson	--	20' sections of 10" Schedule 40 Pipe @$7.23/foot	1	$144.60	$144.60
sampling vials and septa					
MicroLiter Analytical Supplies, Inc.	20-1100	Vial, Clear 22x46mm 10mL, Flat Crimp, 100/PK	11	$38.87	$427.57
MicroLiter Analytical Supplies, Inc.	20-0025-B	Septa, 20mm gray Butyl stopper, 1000/PK	2	$96.50	$193.00
MicroLiter Analytical Supplies, Inc.	20-0000AS-B	Seal, 20 mm Standard Aluminum, Bulk, 1000/PK	2	$74.29	$148.58
Kebby Industries, Inc	20001-00-C01A	Kebby Hand Crimper, 20mm hand crimper for one piece seals	1	$152.00	$152.00
Kebby Industries, Inc	D-20	Kebby Plier Decapper with grips, 20mm plier decapper	1	$42.50	$42.50
sampling equipment					
Fisher Scientific	14-821-13F	BD* PrecisionGlide* Disposable Needles Turquoise 23 Gauge 1-1/4 in 100pk	2	$10.26	$20.52
Fisher Scientific	14-817-33	AirTite* Norm-Ject* Syringes without Needles; 20cc, Luer-Lock tip 100pk	1	$42.70	$42.70

Appendix D: Extended Data Plots and Additional Figures.

(a) CO$_2$ Flux
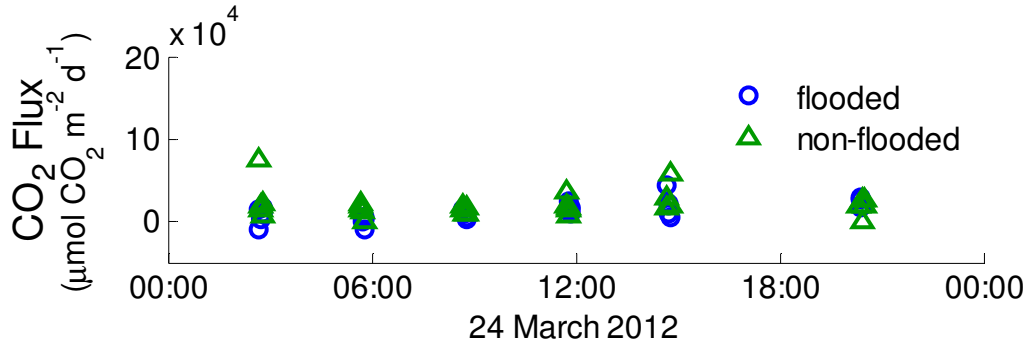
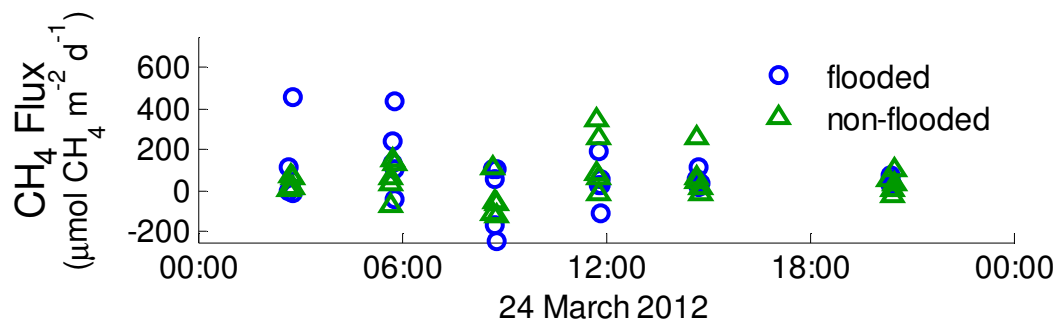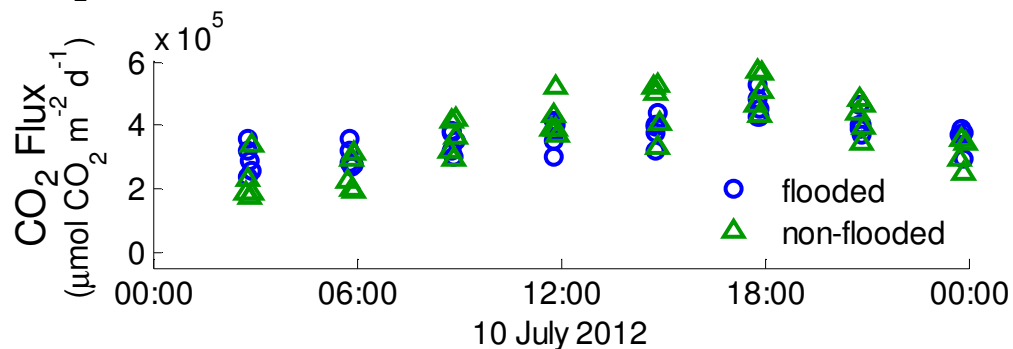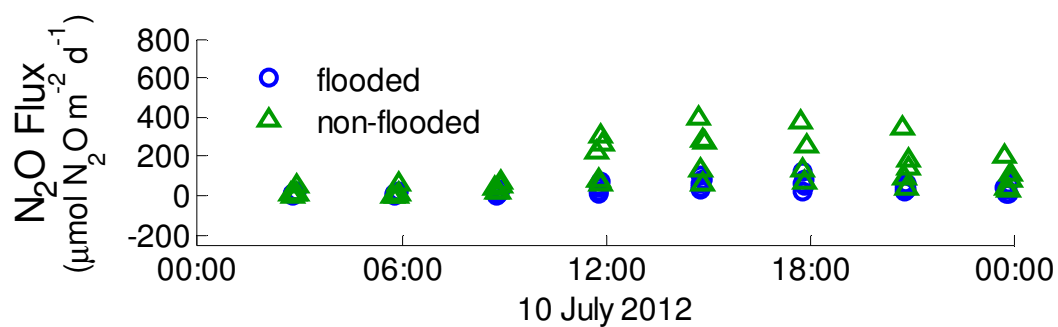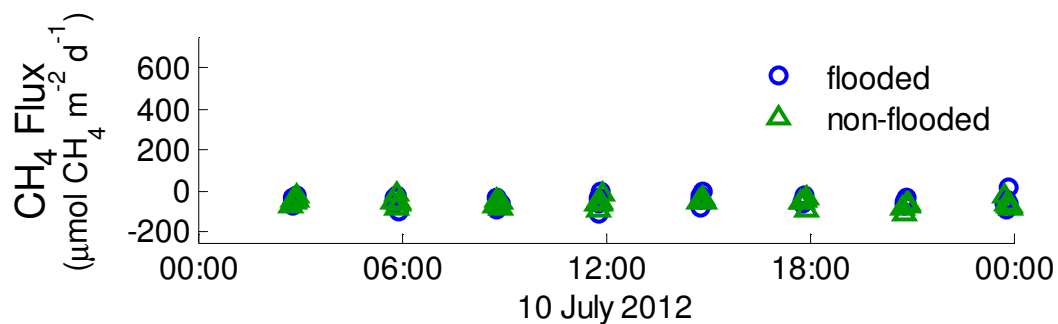**(b) N$_2$O Flux**
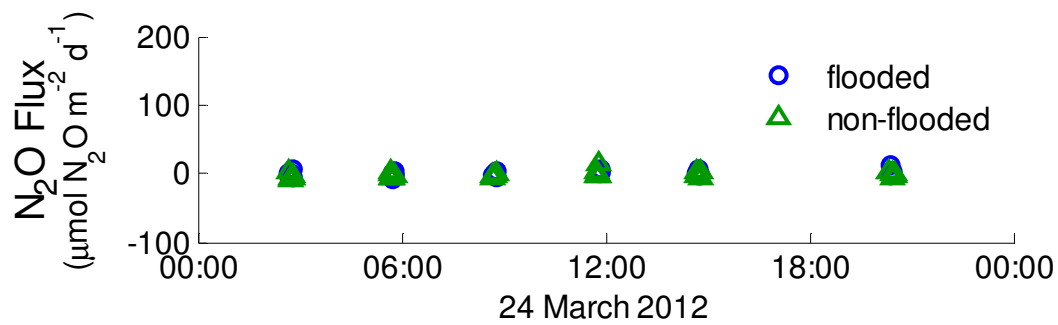
(c) CH$_4$ Flux

Figure D-1: Diurnal sampling data for CO$_2$ (a), N$_2$O (b), and CH$_4$ (c) from the spring diurnal sampling, which took place three days after the flood simulation (21 March 2012). Blue circles represent mesocosms subjected to the flood treatment, green triangles represent non-flooded controls.
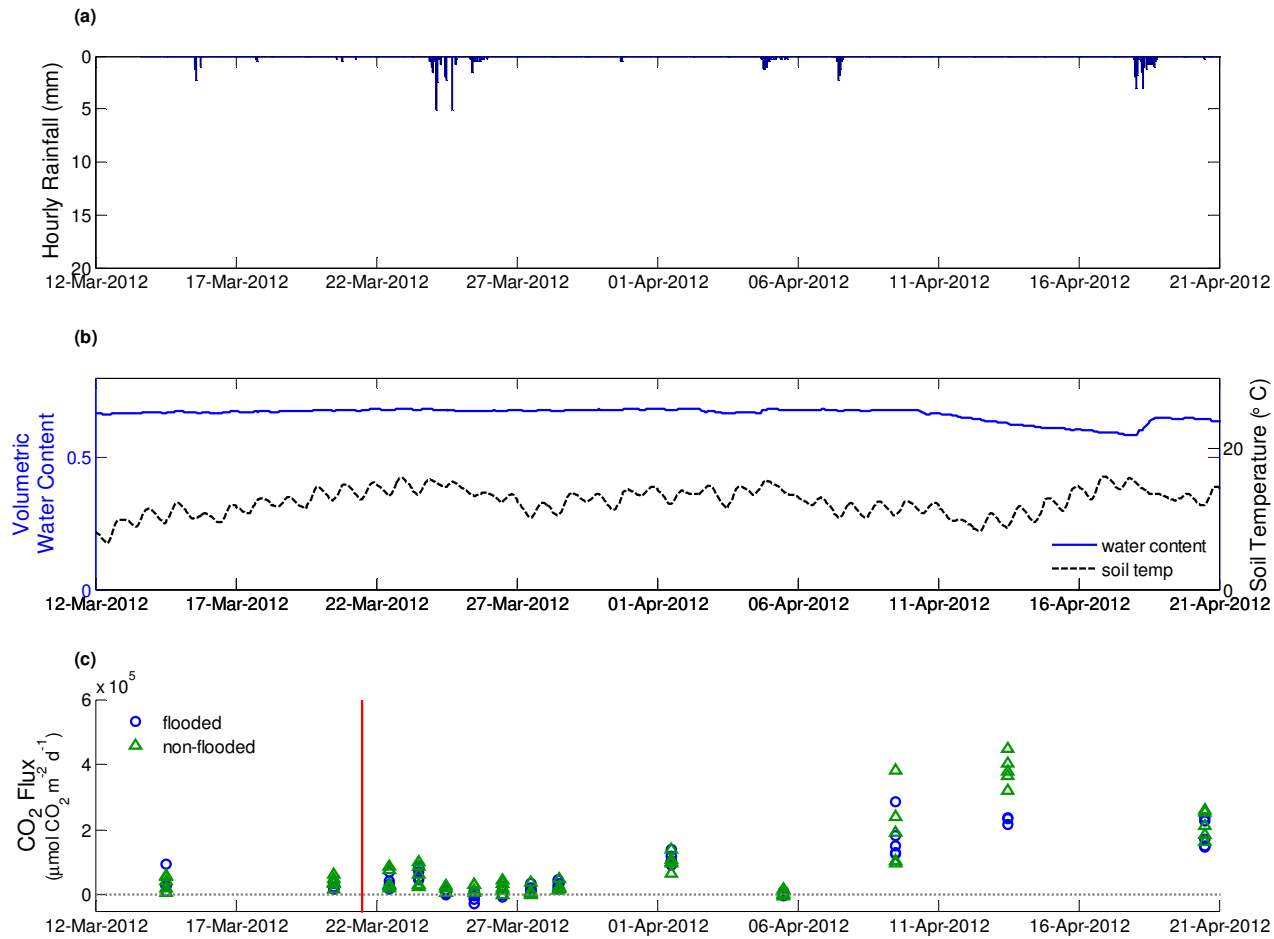
Figure D-2: Diurnal sampling data for CO_2 (a), N_2O (b), and CH_4 (c) from the summer diurnal sampling, which took place four days after the flood simulation (6 July 2012). Blue circles represent mesocosms subjected to the flood treatment, green triangles represent non-flooded controls.
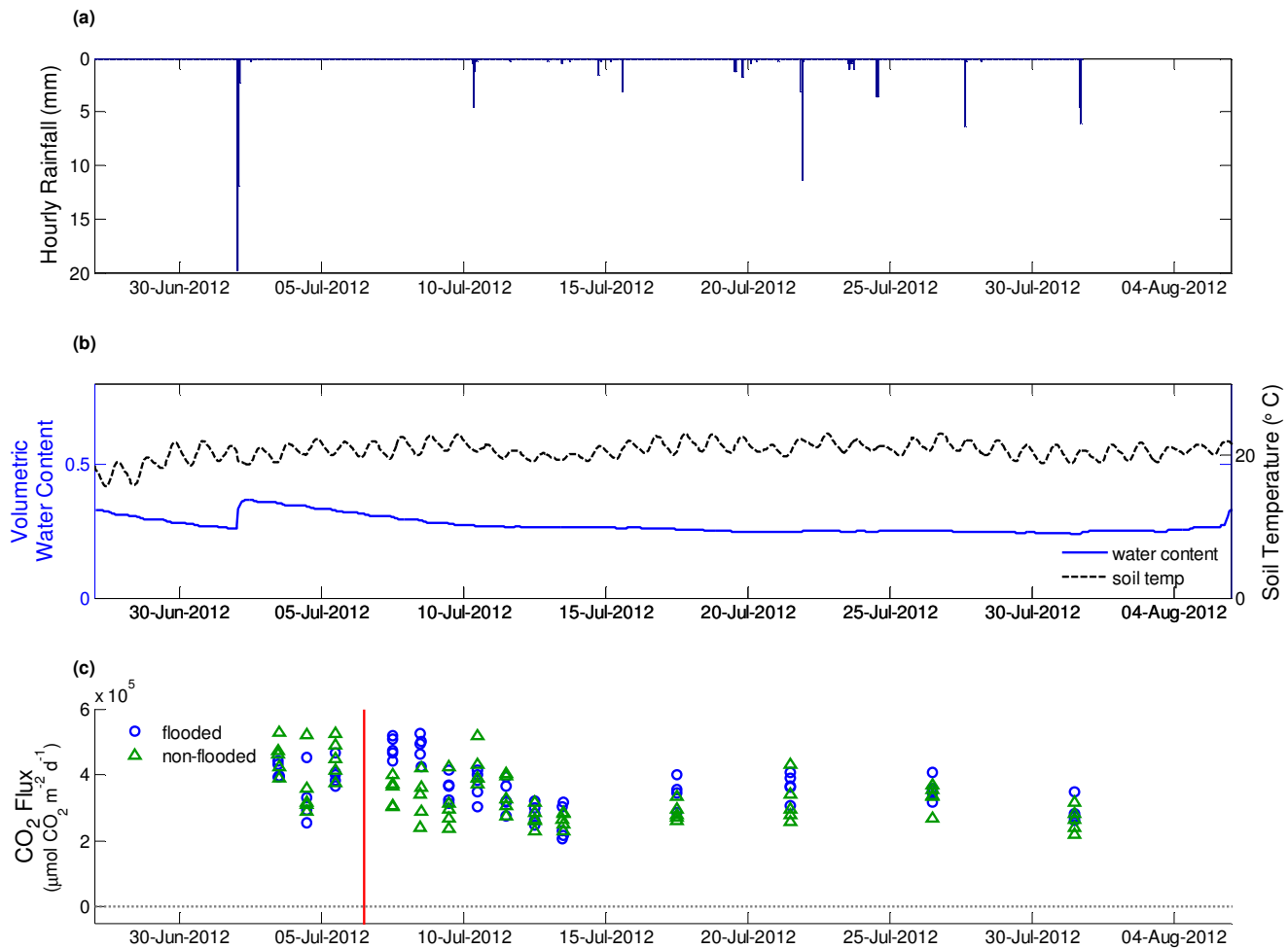
Figure D-3: Full CO_2 data from spring experiment (c) plotted with hourly rainfall (a) and soil temperature and water content (b). Blue circles represent mesocosms subjected to the flood treatment, green triangles represent non-flooded controls. Vertical red line indicates flood date (21 March 2012).
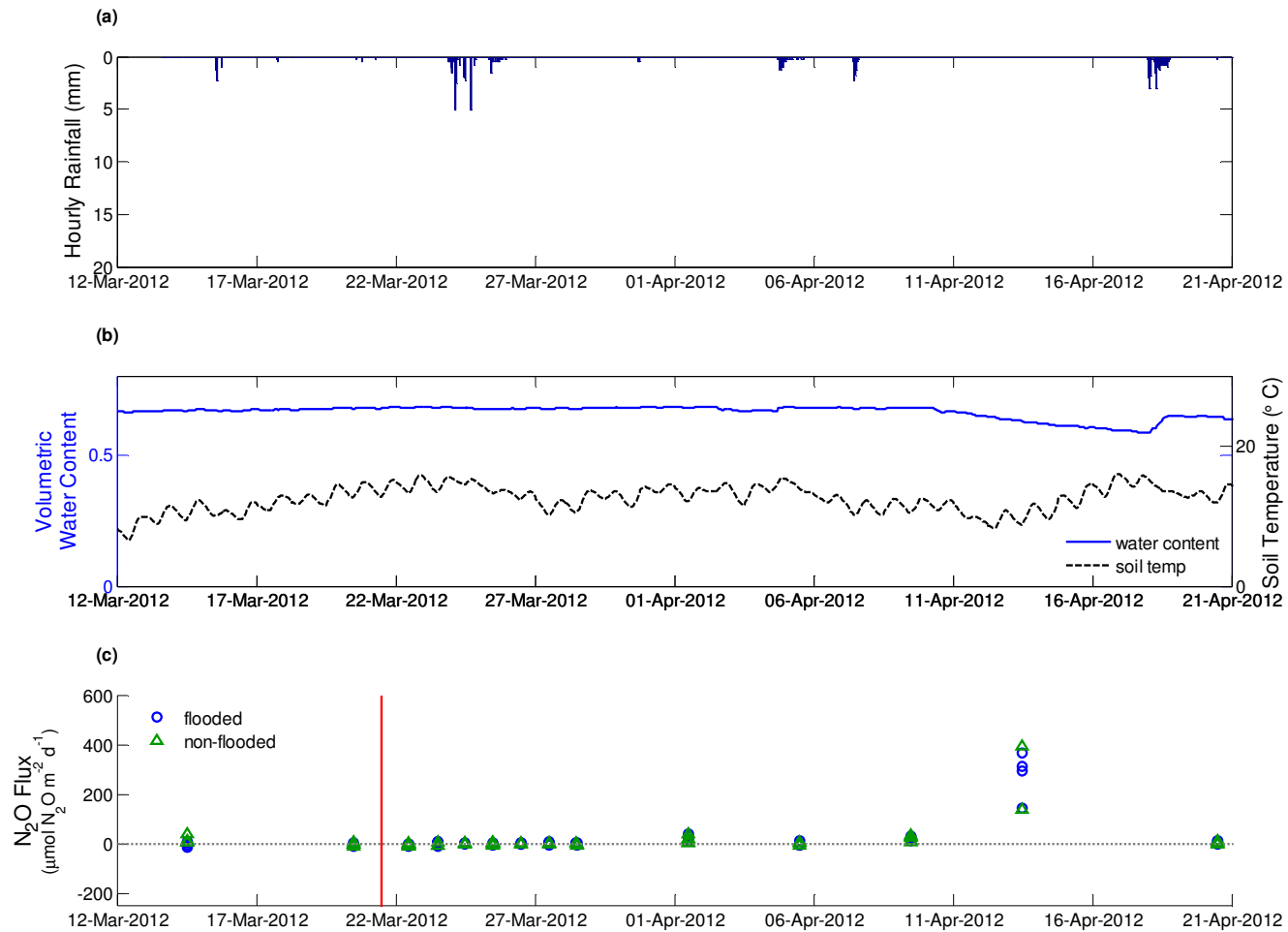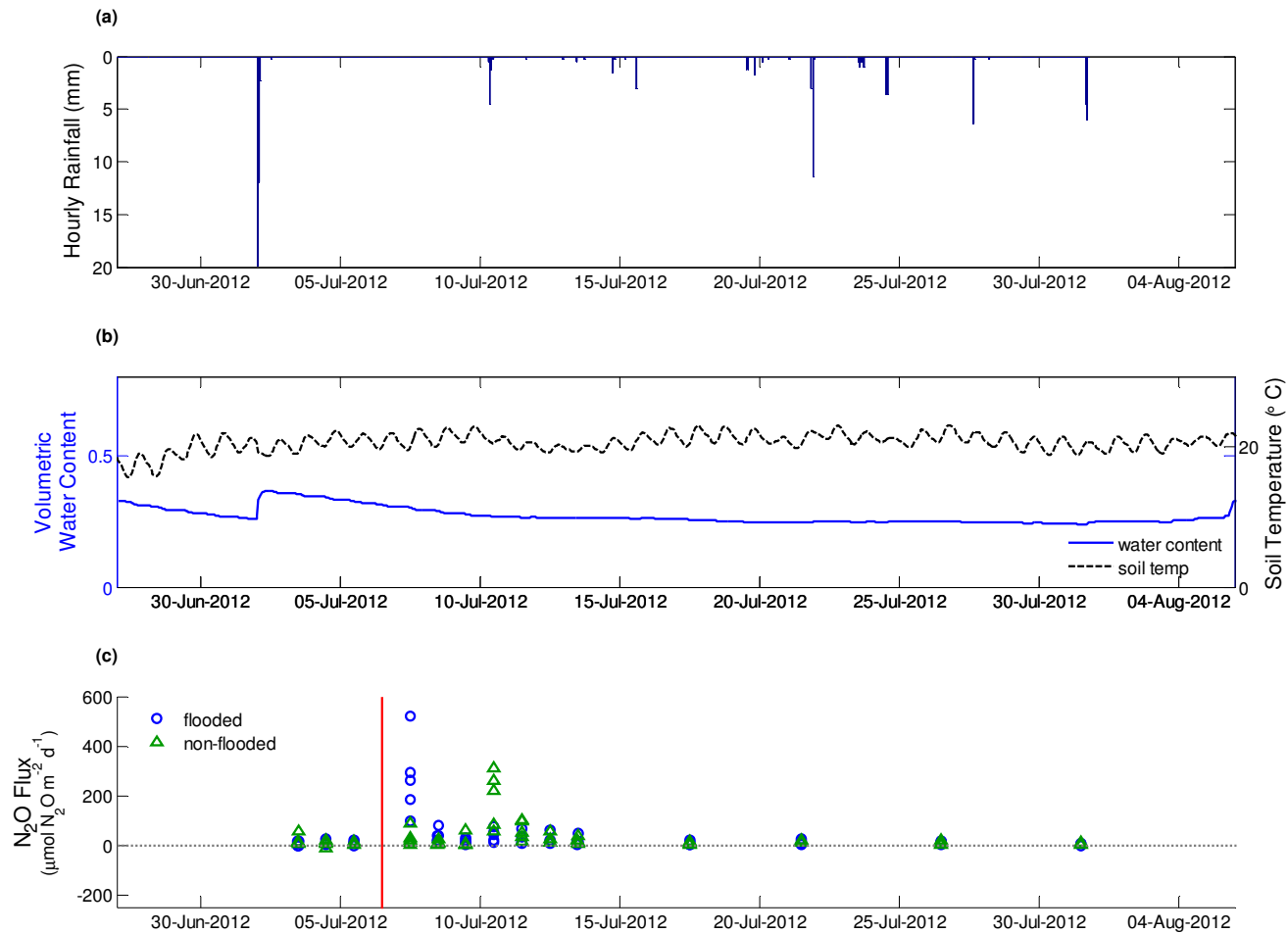
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Figure D-4: Full CO_2 data from summer experiment (c) plotted with hourly rainfall (a) and soil temperature and water content (b). Blue circles represent mesocosms subjected to the flood treatment, green triangles represent non-flooded controls. Vertical red line indicates flood date (6 July 2012).

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Figure D-5: Full N$_2$O data from summer experiment (c) plotted with hourly rainfall (a) and soil temperature and water content (b). Blue circles represent mesocosms subjected to the flood treatment, green triangles represent non-flooded controls. Vertical red line indicates flood date (21 March 2012).

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Figure D-6: Full N$_2$O data from summer experiment (c) plotted with hourly rainfall (a) and soil temperature and water content (b). Blue circles represent mesocosms subjected to the flood treatment, green triangles represent non-flooded controls. Vertical red line indicates flood date (6 July 2012).
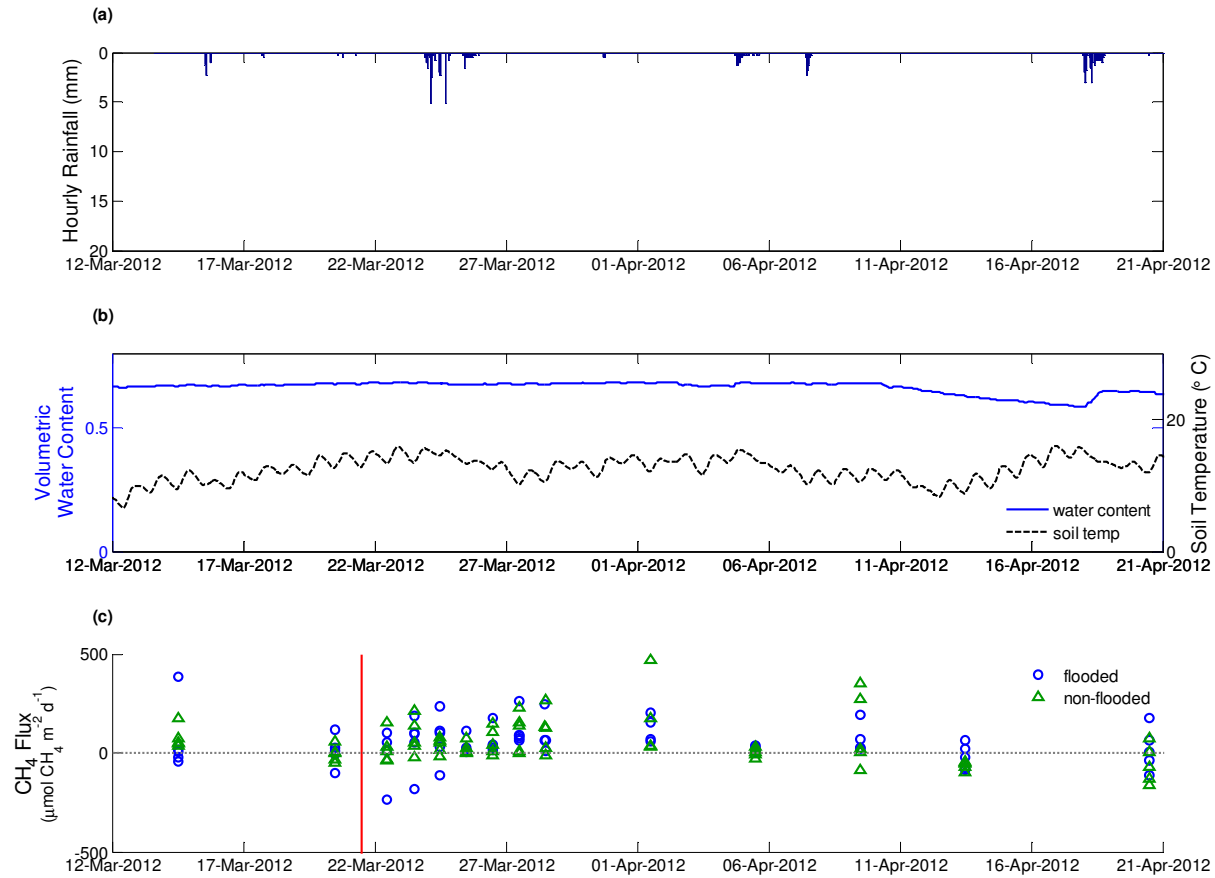
64

Figure D-7: Full CH$_4$ data from summer experiment (c) plotted with hourly rainfall (a) and soil temperature and water content (b). Blue circles represent mesocosms subjected to the flood treatment, green triangles represent non-flooded controls. Vertical red line indicates flood date (21 March 2012).
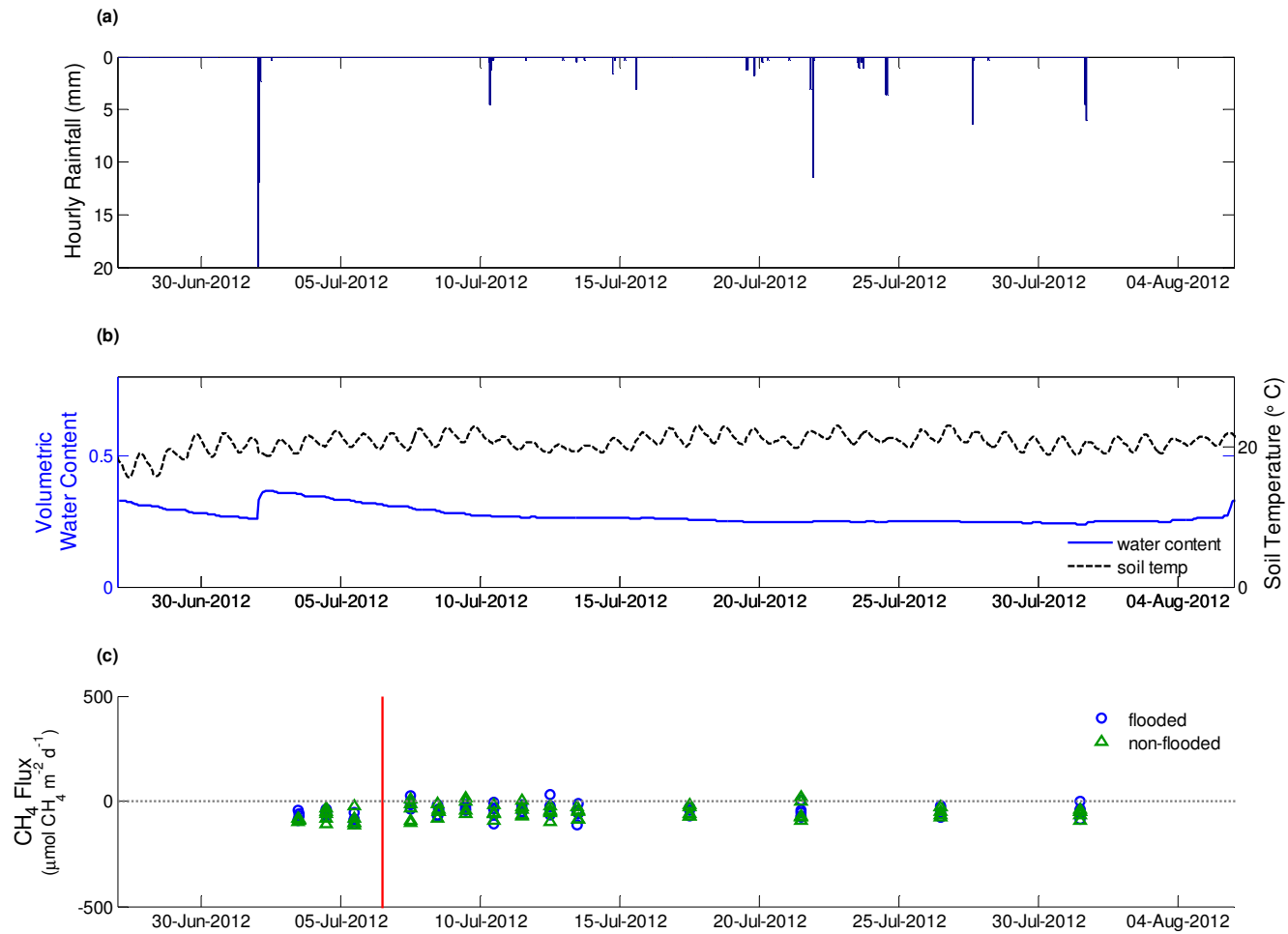
Figure D-8: Full CH$_4$ data from summer experiment (c) plotted with hourly rainfall (a) and soil temperature and water content (b). Blue circles represent mesocosms subjected to the flood treatment, green triangles represent non-flooded controls. Vertical red line indicates flood date (6 July 2012).
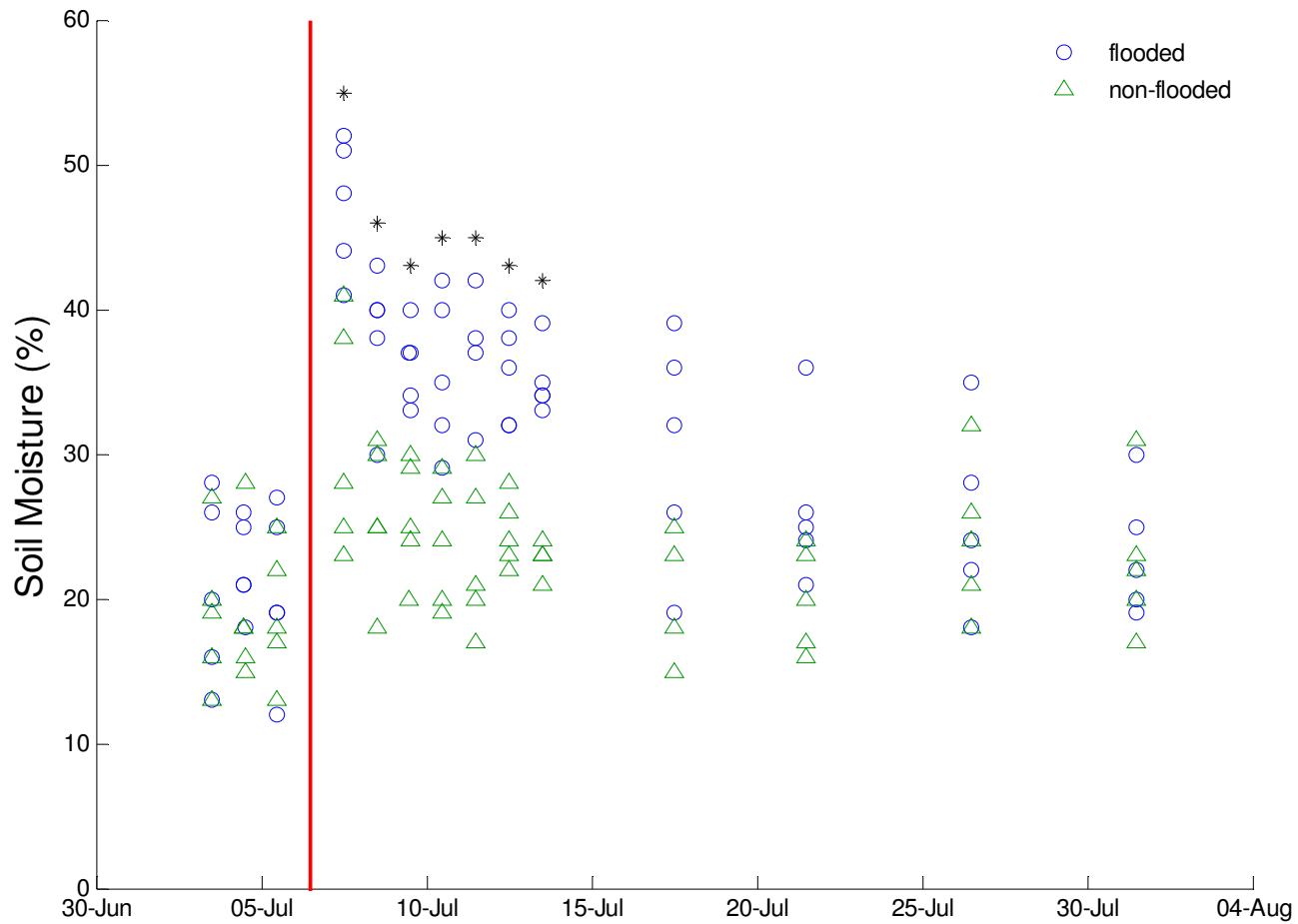
Figure D-9: Soil moisture from each mesocosm at sampling during summer experiment. Vertical red line indicates flood date (6 July 2012). Blue circles represent mesocosms subjected to the flood treatment, green triangles represent non-flooded controls. Asterisks (*) indicate days on which there was significant difference between flooded and non-flooded mesocosms using Wilcoxon rank sum test (α=0.05).

Figure D-10: Image of study site on Stroubles Creek floodplain in spring experiment (late March 2012), note standing water and lack of actively growing vegetation. StREAM Lab meteorological tower visible in background.

Figure D-11: Image of study site on Stroubles Creek floodplain on 3 July 2012, during summer experiment, note dense, actively growing vegetation.