FACTORS AFFECTING NUTRIENT RETENTION RECOVERY AND GREENHOUSE GAS EMISSIONS IN RESTORED AGRICULTURAL FLOODPLAIN WETLANDS

A Dissertation

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AN ABSTRACT OF A DISSERTATON

FACTORS AFFECTING NUTRIENT RETENTION RECOVERY AND GREENHOUSE GAS EMISSIONS IN RESTORED AGRICULTURAL FLOODPLAIN WETLANDS

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Doctor of Philosophy in Environmental Science

Restoration initiatives in the Lower Mississippi River Basin (LMRB) have been implemented through programs such as the Wetlands Reserve Program (WRP) and Wetland Reserve Enhancement Partnership (WREP). These efforts aim to improve essential wetland functions, including water quality improvement and reducing nutrient-rich agricultural runoff into the Gulf of Mexico. Understanding the factors that affect wetland function post-restoration is crucial for improving future restoration practices. Using field and simulation experiment data, this dissertation analyzed maximum nutrient retention potentials and greenhouse gas (GHG) emissions in various restored agricultural floodplain wetlands across Kentucky and Tennessee. In this study, natural regeneration habitat showed the highest potential N_2 production, and this production generally increased with longer water residence time. Although dissolved nitrogen (N) and phosphorus (P) retention was also related to water residence time, varying patterns with inundation duration were observed. Overall, results suggest that immediately after flooding, soil properties, hydrology, and vegetation can individually or interactively influence nutrient retention. However, as flooding duration increases, these effects can weaken, signifying water residence time as the primary regulator for nutrient retention. Nevertheless, an extended water residence time might contribute to elevated methane (CH_4) production. Furthermore, this study establishes close correlations between CH_4, carbon dioxide (CO_2), and nitrous oxide (N_2O) production with specific soil properties, and specifically soil moisture. Habitat types showed diverse responses to temperature increase in terms of GHG production. In general, natural wetland appeared to be least affected by temperature increase compared to other restored habitats, emphasizing their greater resilience to withstand temperature fluctuations.

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FACTORS AFFECTING NUTRIENT RETENTION RECOVERY AND GREENHOUSE GAS EMISSIONS IN RESTORED AGRICULTURAL FLOODPLAIN WETLANDS

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DEDICATION

I dedicate this dissertation to my beloved grandfather, Ganesh Dutta Duwadi (गणेश दत्त दुवाडी), whose wisdom, love, and unwavering support have been a constant source of inspiration throughout my academic journey. Though he is no longer with us, his memory and influence continue to guide me in my pursuit of knowledge and accomplishment.

बा, म आउँछु भन्दैथिएँ
तर तपाईं जानुभयो

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CHAPTER 1: BACKGROUND

Human activities involving alteration of landscape, habitat, and vegetation destruction have led to changes in processes controlling water quality and water balance (Peters & Meybeck, 2009). The most problematic water quality problem worldwide is eutrophication which is characterized by excessive plant and algal growth due to the increased availability of one or more limiting growth factors needed for photosynthesis (Schindler, 2006), such as sunlight, carbon dioxide (CO_2), and nutrients (Chislock et al., 2013). Eutrophication occurs mainly due to high nitrogen (N) and phosphorus (P) loads from agricultural runoff, fossil fuel combustion, and domestic sewage and industrial effluents disposal into the aquatic systems (Diaz & Rosenberg, 2008; United Nations, 2014). Eutrophication contaminates drinking water (Fan & Steinberg, 1996), causes hypoxia (Rabalais et al., 2002), decreases aquatic and riparian biodiversity (Carpenter et al., 1998), promotes loss of ecosystem stability, degrades recreational opportunities, causes water systems disruption, shellfish contamination, fish kills, and reduces aquaculture production (Carpenter et al., 1998; Kay et al., 2009; Schindler, 2006; Withers & Haygarth, 2007). The annual estimated cost of damage mediated by eutrophication in the United States (US) alone is approximately $2.2 billion (Dodds et al., 2009).

Eutrophication is prevalent, particularly in marine coasts connected to large, nutrient-rich rivers (e.g., Mississippi River and the Gulf of Mexico; Susquehanna River and the Chesapeake Bay) and has affected over 400 near-shore systems (Diaz & Rosenberg, 2008). The Mississippi River has the largest watershed of all rivers discharging into the Gulf of Mexico and drains 40% of the continental US (Kemp et al., 2011). The Mississippi River contributes substantially to freshwater inflow in the Gulf of Mexico and dominates the ecosystem processes (Kemp et al.,

2011). Historically, the Mississippi Alluvial Valley (MAV) was comprised of vast expanses of bottomland hardwood (BLH) forest and was the largest BLH ecosystem in North America, providing extensive wetland functions such as nutrient and sediment retention (King et al., 2006). Today, less than 25% of original BLH remains (King et al., 2006; Rudis, 1995; Twedt & Loesch, 1999) due to the large-scale conversion of forests to cropland (MacDonald et al., 1979). Commercial agricultural activities and concentrated animal operations (Jackson et al., 2000) have degraded the Mississippi River and the Gulf of Mexico due to increased sediment transportation, N and P loads, and diminished biodiversity (Downing et al., 1999). Most of the nutrients reaching the Gulf of Mexico originate in the corn belt of the upper Mississippi River Basin (MRB) (Alexander et al., 2008; Turner & Rabalais, 2003). Fertilizers are added during cropping, and the soil is artificially drained to optimize aeration. Although these modifications enhance crop productivity, they also promote the transport of excess N to the Gulf of Mexico (Johnson et al., 1997; Randall et al., 1997).

Excess N, along with other nutrients in an aquatic system can lower dissolved oxygen due to the increase in algal biomass and associated higher respiration rates (Rabalais et al., 1996). The growth and metabolism of other oxygen-requiring species are often impacted by hypoxia, a condition in which dissolved oxygen in water bodies reaches less than 2 mg L^{-1} (Rabalais et al., 2001; Downing et al. 1999). Hypoxia in the Gulf of Mexico during 1985-1992 averaged about 7,000-9,000 km^2 (Goolsby et al., 2000), and hypoxia may severely affect biodiversity in the Gulf (Justic et al., 1997; Turner & Allen, 1982). Today, the hypoxic zone reaches an extent of 20,000 km^2 frequently (Rabalais et al., 2002; Scavia et al., 2003). Nutrient loads from the MRB have been directly linked to the Gulf of Mexico net surface productivity, and the extent of hypoxia (Atwood et al., 1994; Scavia et al., 2003). The loss of wetlands in the

MRB and the separation of Mississippi from its floodplain and deltaic plain have exacerbated the formation of hypoxia in the Gulf of Mexico (Dahl, 1990; Day et al., 2003).

Apart from the formation of hypoxia, an increase in algal biomass can degrade water quality, alter habitat, create physiological dysfunction in other aquatic species, disturb community relationships, and cause negative aesthetic effects such as beach fouling. These events which can be due to both high biomass and/or algal toxin production, are often called harmful algal blooms (HABs) (Fisher et al., 2003; Granéli & Turner, 2006). Exposure to HAB toxins affects both commercial fishery and ecologically valuable species (Fisher et al., 2003). The cost of HABs in the US has been estimated to be $300-700 million annually (Luttenberg et al., 2000) through costs associated with beach cleanup (Paerl, 1988), closing of commercial fisheries (Shumway, 1990), and decreased tourism (Horner et al., 2003). The most common harmful blooms in the Gulf of Mexico are caused by the dinoflagellate *Karenia brevis* (Daugbjerg et al., 2000; Geesey & Tester, 1993). *Karenia brevis* provides a reddish hue to the water in higher concentrations and is described as a 'red tide' (Gilbes et al., 1996; Gunter et al., 1948). The red tide event may persist for a few days to not more than three months (Steidinger & Baden, 1984) but the toxic effect of the algal neurotoxin (brevetoxin) created by *K. brevis*, can remain longer. Brevetoxin paralyzes respiratory function in fish and mammals and can accumulate in edible shellfish. Consumption of shellfish that have accumulated brevetoxin can cause human fatalities. In addition, *K. brevis* blooms adversely affect other valuable resources in the Gulf (Fisher et al., 2003). Therefore, the ultimate fate of the Gulf of Mexico depends in large part on the efficient use of N and P fertilizers in agriculture and effective control measures to reduce nutrient runoff from the Mississippi River Basin to the Gulf.

To minimize the disruption in ecosystem functions caused by nutrient runoff, nutrient reduction strategies have been adopted in the states throughout the MRB. These strategies focus on voluntary nutrient reduction measures and aim to protect MRB watersheds from nitrogen and phosphorus pollution (Mississippi River Collaborative, 2023). While the strategies to minimize nutrient runoff can be expensive, as are the consequences of nutrient pollution (Birch et al., 2011; Van Grinsven et al., 2013), studies involving wetland restoration, basin-wide management, and mandatory reductions in fertilizer application have been conducted to examine N pollution mitigation while minimizing costs (Doering et al., 1999; Ribaudo et al., 2001). These studies suggested that the most economical option to achieve modest N reduction goals in the MRB was a mandatory reduction in fertilizer application, but wetland restoration was needed to achieve more ambitious goals (Doering et al., 1999; Ribaudo et al., 2001). Wetlands minimize nutrient runoff by removing and processing nutrients during downstream transport through several mechanisms (Mitsch et al., 2001; Roley et al., 2012a). Nitrogen removal occurs by assimilatory uptake in the tissue of living beings and via microbially-mediated denitrification (Roley et al., 2012a). Anoxic environments and sediments rich in organic matter promote denitrification in the wetlands (Mitsch et al., 2001). Wetlands are most effective when water flowing into them is sufficiently slow to allow sediments to settle (Jansson et al., 1994). In addition to reducing hypoxia in the Gulf of Mexico, wetland restoration can help in the improvement of MRB ecosystems, enhance wildlife in river corridors, and mitigate the effects of floods (Mitsch et al., 2001).

Restoration of degraded natural wetlands has been carried out by developed countries since the 1970s (Moss, 1983; Zhou et al., 2020). In the MRB, the Wetland Reserve Program (WRP)/ Wetland Restoration Enhancement Partnership (WREP) implemented by the US

Department of Agriculture's Natural Resources Conservation Service (USDA-NRCS) aims to minimize agriculture-related environmental impacts. Under the WRP/WREP guidelines, participating private landowners are financially incentivized to retire marginal farmlands from agricultural production and protect, enhance, or restore wetlands to establish long-term conservation and wildlife practices and protection (Faulkner et al., 2011). It is estimated that wetland restoration in 2-5 million ha in MRB (0.7% to 1.8% of the basin) would be required to see a significant reduction in the nutrient in the Gulf of Mexico (Mitsch et al., 2001).

Dissertation objectives

The information on the overall effectiveness of the WRP/WREP program in retaining nutrients from the watershed is limited (Shrestha et al., 2017) and very few studies have been conducted on WRP/WREP easements within MAV (Faulkner et al., 2011). Limited monitoring practices can hinder broader adoption of the restoration strategies in the future (Galatowitsch & Bohnen, 2021) because decisions regarding if the restoration goals are achieved or future restoration strategies need to be revised depend on effective monitoring (Block et al., 2001). Additionally, the mechanisms responsible for ecosystem functioning in restored wetlands are often unclear (Keddy, 2010).This dissertation aimed to understand the influence of environmental factors on nutrient retention in restored wetlands. Specifically, this dissertation aimed to characterize the structural characteristics of soil. This dissertation also aimed to understand how vegetation species and flooding duration influenced N and P retention in the porewater in wetland mesocosms. While restoration programs aim to predict restoration outcomes (Zedler, 2000; Zedler & Callaway, 1999), there are possibilities that some ecosystem services are promoted at the expense of others which is seldom addressed (Zedler & Kercher,

2005). In my final chapter, I evaluated if certain restored wetland habitats produced more greenhouse gases (GHGs) compared to other remaining habitats.

CHAPTER 2: EFFECTS OF SOIL PROPERTIES AND HABITAT HYDROLOGY ON POTENTIAL DENITRIFICATION IN RESTORED AGRICULTURAL WETLANDS

2.1. Abstract

Riparian wetlands play a crucial role in nutrient cycling and water quality maintenance and are becoming increasingly important in this role as nutrient pollution in runoff and rivers remains high. This study aimed to assess denitrification rates, a major nitrogen removal pathway, across various restored riparian wetland practices (i.e., habitats). Additionally, this study aimed to specifically explore the connections between soil characteristics and denitrification, given the potential for soil properties to exert varying impacts on nitrogen cycling processes in different habitats. Five distinct restoration habitats, natural regeneration, remnant forest, shallow water-dry, shallow water-wet, and tree planting were investigated in 23 restored wetlands in western Kentucky and Tennessee. Study objectives were to understand whether denitrification rates vary among habitats and to identify the key soil properties influencing denitrification rates. Cores for soil denitrification rates and structural measurements were collected adjacent to each other. Maximum potential denitrification rates were assessed at 24 and 48 h, simulating a 2-day flood event. All habitats produced nitrogen gas (N_2) at each timepoint, and the rates were greater at 48 h for all habitats. The mean N_2 gas production was significantly greater in natural regeneration habitat at both 24 h and 48 h. Notably, the shallow water-wet habitat had the least N_2 production rate at both timepoints, yet it exhibited the greatest percentage increase between 24 and 48 h, increasing by 34.3%. Several soil characteristics, including sediment oxygen demand (SOD), soil moisture (SM), soil pH, and soil phosphorus (P) had varying degrees of correlation with N_2 production. These results suggest that all habitats efficiently remove nitrogen (N) over a 48 h

flood period; however, highest N removal rates may depend on how long the particular habitat is flooded and the characteristics of the soil in that habitat.

2.2. Introduction

All parts of the world have been affected by humans, with ecosystem degradation being one of the most impactful (Vitousek et al., 1997). Roughly 50% of natural wetlands have been lost in the contiguous US (Mitsch & Gosselink, 2000). The US lost approximately 330,000 ha of wetlands between 2001 and 2016, but 306,000 ha of wetland construction and restoration have helped to advance the federal objective of "no net loss" of wetlands established in 1977 (Taylor & Druckenmiller, 2022). Much of this restoration has occurred by converting agricultural cropland that was originally wetlands, back to wetlands. The USDA's Wetland Reserve Program (WRP)/ Wetland Restoration Enhancement Partnership (WREP) has spent more than $ 4.2 billion to restore and protect wetlands since it was established in the 1990 US Farm Bill (Hansen et al., 2015). Wetland restoration often requires hydrological intervention, but it is not as simple as returning water to the system or planting vegetation (Hunter & Faulkner, 2001; Zedler, 2003). The co-restoration of both wetland structure and function is common goals, but often one or the other is a priority.

The idea of the best ways to restore both wetland structure and function often meets with contradicting opinions, as most studies seem to be structurally driven and use the assumption of restore the ecosystem first, and the function will follow (Sutton-Grier et al., 2010). According to Palmer et al. (1997), this method of restoration is called the "Field of Dreams" approach (meaning "If you build it, they will come"), or the principle of self-design (Mitsch et al., 1998; Mitsch & Wilson, 1996). This approach follows an assumption that restoring physical structures

such as habitat conditions and hydrology will help in the reestablishment of soil biological factors, including soil microorganisms and wetland flora and fauna, as well as processes that cycle carbon (C) and N and transfer energy (photosynthesis and decomposition). These processes will then ultimately restore wetland functions to pre-disturbance levels (Palmer et al., 1997; Sutton-Grier et al., 2010). But there is a need to rigorously test these assumptions across different wetland habitats to advance restoration efforts where the restoration of wetland functions as the ultimate restoration goal.

Many wetland components interact synergistically to create a functioning system (Sutton-Grier et al., 2010). Therefore, successful restoration of wetland function (e.g., denitrification) may depend on soil physicochemical properties, hydrology, habitat, etc., and their interactions. Denitrification is the bioconversion process that transforms nitrate (NO_3^-) into N_2, effectively eliminating bioavailable N and releasing it back into the atmosphere. Although the final product of denitrification is N_2, there are also intermediate gaseous forms of N generated during this process (Bernhard, 2010). Other than denitrification, reduced form of iron (Fe) and manganese (Mn) can also reduce NO_3^- to N_2 during anaerobic ammonium oxidation (annamox). In the annamox reaction NO_3^- and ammonium (NH_4^+) are converted directly to N_2 and water.

Denitrification is the primary nutrient retention mechanism followed by N sedimentation and biological uptake (Saunders & Kalff, 2001). Denitrification is primarily controlled by the presence of N, anoxia, temperature, and organic C supply (Arango et al., 2007; Beauchamp et al., 1989; Christensen et al., 1990; Mulholland et al., 2009), which are in turn influenced by hydrology, land use characteristics, and underlying g geology (Osborne & Wiley, 1988; Stanley & Boulton, 1995). It has been reported that spatial variation of soil properties such as moisture, NO_3^-, and the amount of soluble organic carbon affects denitrification directly (Ball et al., 1997;

Grundmann et al., 1988; Robertson et al., 1988) and could improve our understanding of the spatial variation in denitrification (van den Pol-van Dasselaar et al., 1998).

Studies suggest that soil characteristics play an important role in regulating denitrification potential in wetlands (Kaden et al., 2021; Yu et al., 2012). Nutrient-rich soil contains a significant amount of organic C, which is used by denitrifying microbes as an energy source (Seitzinger et al., 2002; Sutton et al., 2010; Tiedje et al., 1983). Although organic C is essential for N removal via denitrification, wetland restoration projects hardly consider soil conditions (Bruland et al., 2003; Willems et al., 1997). However, organic C is critical to wetland ecosystem functioning, and therefore, restoring soil organic C in degraded wetlands may be critical to effective restoration (Sutton-Grier et al., 2009). Organic amendments increase SM and P sorption, stimulate nutrient cycling and microbial community development, and decrease bulk density (BD) in both coastal and inland restored wetlands (Bruland et al., 2009; Bruland & Richardson, 2004; Burgin & Hamilton, 2007). Xiong et al. (2015) reported that both potential and unamended denitrification rates in riparian wetlands were positively related to soil moisture, which may be the function of anoxia. Denitrification was also related positively to the percentage of the fine substrate, organic matter, and N contents but was related negatively to soil pH and BD (Xiong et al., 2015).

Few studies have suggested that the amount of soil available P is directly related to the denitrification rate (Song et al., 2019a), but there is some evidence that it can be important in specific situations. In a free water surface constructed wetland, denitrification was positively correlated to the P concentrations of water incoming from the treated river (Li et al., 2018). Kim et al. (2017) found that unimpacted sediments in Everglades wetlands had lower denitrification rates than P enriched sites. Similar conclusions were made by (White & Reddy, 2003) based on

the presence of a positive correlation between the total P of the enriched wetland soil and potential denitrification rates. Phosphorus can further influence denitrification by influencing plant growth (Berthold et al., 2018; Kao et al., 2003). High P availability causes an increase in plant growth, availability of organic C from plants, and the suitable surface for attachment for denitrifiers (Song et al., 2019a).

Although denitrification in wetlands is likely ultimately regulated by physical and microbial aspects, vegetation type and cover (e.g., riparian grasses versus wetland plants) and duration of inundation can indirectly influence denitrification (Brix, 1997; Roley et al., 2012b). One of the primary reasons natural wetlands are important for N removal is because forested habitats in natural wetlands support high denitrification rates and, in certain cases, transform the majority of NO_3^- inputs to N_2 (Jacobs & Gilliam, 1985; Lowrance, 1992). Furthermore, forested habitat has shown high spatial variability in denitrification rates (Casey et al., 2001; Dhondt et al., 2004; Pinay et al., 1993; Schipper et al., 1993) due to the presence of patches of organic matter and anaerobic microsites in the soil profile (Casey et al., 2004; Gold et al., 1998; Parkin, 1987), thus creating denitrification hotspots (Christensen et al., 1990; Parkin, 1987). The studies showing the positive influence of highly productive emergent vegetation on N removal in wetlands further emphasize the importance of the effects of vegetation on N removal (Bastviken et al., 2009; Weisner & Thiere, 2010). Although plants help in N removal by assimilating nutrients from the water and litter/sediment into their tissues (Gottschall et al., 2007; Jampeetong et al., 2012), plants contribute to the organic C pool, promoting an anoxic environment by litter accumulation and decomposition, and provide attachment surfaces to denitrifiers, which are favorable for denitrification (Stottmeister et al., 2003; Weisner et al., 1994).

Flood waters can also influence denitrification rates. Denitrification can increase when the N-rich stream water enters the floodplains during flooding (Roley et al., 2012b) because N concentration plays an important part in denitrification rates and efficiency (Gift et al., 2010; Mulholland et al., 2008; Pinay et al., 1993). In addition, inundation creates lower soil redox conditions favorable for denitrification (Ensign et al., 2008), and potentially enhance microbial synthesis of new denitrifying enzymes in response to changing environment (Brock, 1961).

Habitats can interact with hydrology to influence nutrient t cycling rates (Hunter & Faulkner, 2001; Mitsch et al., 2015), and denitrification can significantly vary among habitats based on the interactions (Faulkner et al., 2011). The plant cover and structure and the growth of microbes on them are largely dependent on water depth in wetlands (Maine et al., 2007; Tournebize et al., 2017). Shallow water, in particular, promotes the development of highly productive emergent vegetation (Lou et al., 2016; Vretare et al., 2001). Moreover, wetland vegetation type may contribute to a markedly significant difference in water residence times (Wörman & Kronnäs, 2005) and promote N removal.

Research needs on the effects of habitat, hydrology, and soil properties on N removal from restored wetlands

The WRP/WREP program emphasizes habitat and hydrology restoration when restoring agricultural wetlands. Therefore, it is imperative to understand the interaction among habitat, hydrology, and environmental factors and how these interactions influence nutrient retention in restored wetlands and help in reducing downstream nutrient export. Since denitrification physically removes N from the wetland and puts it into the atmosphere, this process is critical to the improvement of water quality by permanently removing N pollution in runoff. Determining

the underlying mechanisms related to habitat type and soil characteristics with denitrification potential will help managers to develop future restoration strategies and their effective implementation

2.3. Objectives

i) evaluate the variability in soil properties among habitats in restored riparian wetlands.

ii) evaluate if N_2 production varies among habitats in restored riparian wetlands.

iii) determine the relationship between soil characteristics and habitats, and N_2 production.

2.4. Hypotheses

i) Soil properties will differ among habitats. Following the shallow water habitat, remnant forest habitat will exhibit the highest SM due to factors such as the shading and canopy cover in forests, which reduce evaporation. Additionally, the presence of a litter layer in forests helps retain moisture within the soil. Soil moisture will be lowest in tree planting habitat. Bulk density (BD) will be lowest in the shallow water-wet habitat because of high SM and clay content. Soil TC and TN will be highest in shallow water-wet habitat because prolonged saturation of the soil and anaerobic conditions slows organic matter decomposition and leads to organic matter accumulation. Alternatively, slow organic matter decomposition results in the addition of a lower number of acidic cations to the soil, which will increase the soil pH of shallow water-wet habitat.

ii) Nitrogen gas production will differ among habitats and correlate to habitat specific edaphic conditions. Nitrogen gas production will be highest for the submerged (i.e., shallow water- wet) habitat because of higher initial chemical and microbial demand for

oxygen (O_2) compared to other habitats. When the demand for O_2 for cellular respiration exceeds its solubility and diffusion, anoxic conditions predominate, and denitrifying microbes use the O_2 present in the N compounds (NO_3^- and NO_2^-) as a terminal electron acceptor. Upon incubation with nutrient-rich water, dissolved inorganic N will be used immediately by denitrifying microbes in cores from submerged habitat, which leads to the production of more N_2 gas compared to other habitats. Nitrogen gas production will be the lowest for the habitat with the lowest SM. Following the shallow water-wet habitat, N_2 production will be highest from remnant forest habitat. This is attributed to the extensive root system in the remnant forest that releases various organic compounds (root exudates) into the soil in greater quantities, providing a rich C source for denitrifiers. Furthermore, remnant forest habitat benefits from greater organic matter input from leaf litter and other plant debris, making additional substrate available for denitrifiers and resulting in increased N_2 production.

iii) Nitrogen gas production will be affected by soil properties. Soil moisture, soil T N, and TC will be the most influential soil properties. Soil moisture will correlate positively with N_2 production. Soil moisture content has been identified as one of the major drivers of denitrification (Ballantine et al., 2014) because high SM helps in the creation of an anaerobic environment and redox conditions favorable for complete denitrification to N_2 (Ensign et al., 2008; Wilcock & Sorrell, 2008). Nitrogen gas production rates will correlate positively with soil TN because N-rich sediment favors higher N_2 production rates (Groffman & Hanson, 1997). Nitrogen gas production rates will relate positively to soil TC because sediment rich in C can hold more moisture, provide labile C to denitrifying microbes, improve soil texture, and promote plant growth, which are all

conducive for denitrification. High BD will affect N_2 production negatively due to low SM, which restricts denitrifying microbial activity. Soil pH will relate positively to N_2 production rates. Studies have shown that lower amounts of organic C and mineral N are available to the denitrifying population under acidic conditions, which leads to a decrease in N_2 production (ŠImek & Cooper, 2002; Xiong et al., 2015).

2.5. Methods

2.5.1. Easement selection criteria

Twenty-three easements were selected from NRCS's WRP/WREP easements in conjunction with Natural Resources Conservation Service (NRCS) and The Nature Conservancy (TNC). Easements were located along river that were direct tributaries of the Mississippi River, including the Mayfield Creek, Obion Creek, Bayou de Chien, Obion River, Forked Deer River, and Hatchie River (Figure 2.1).

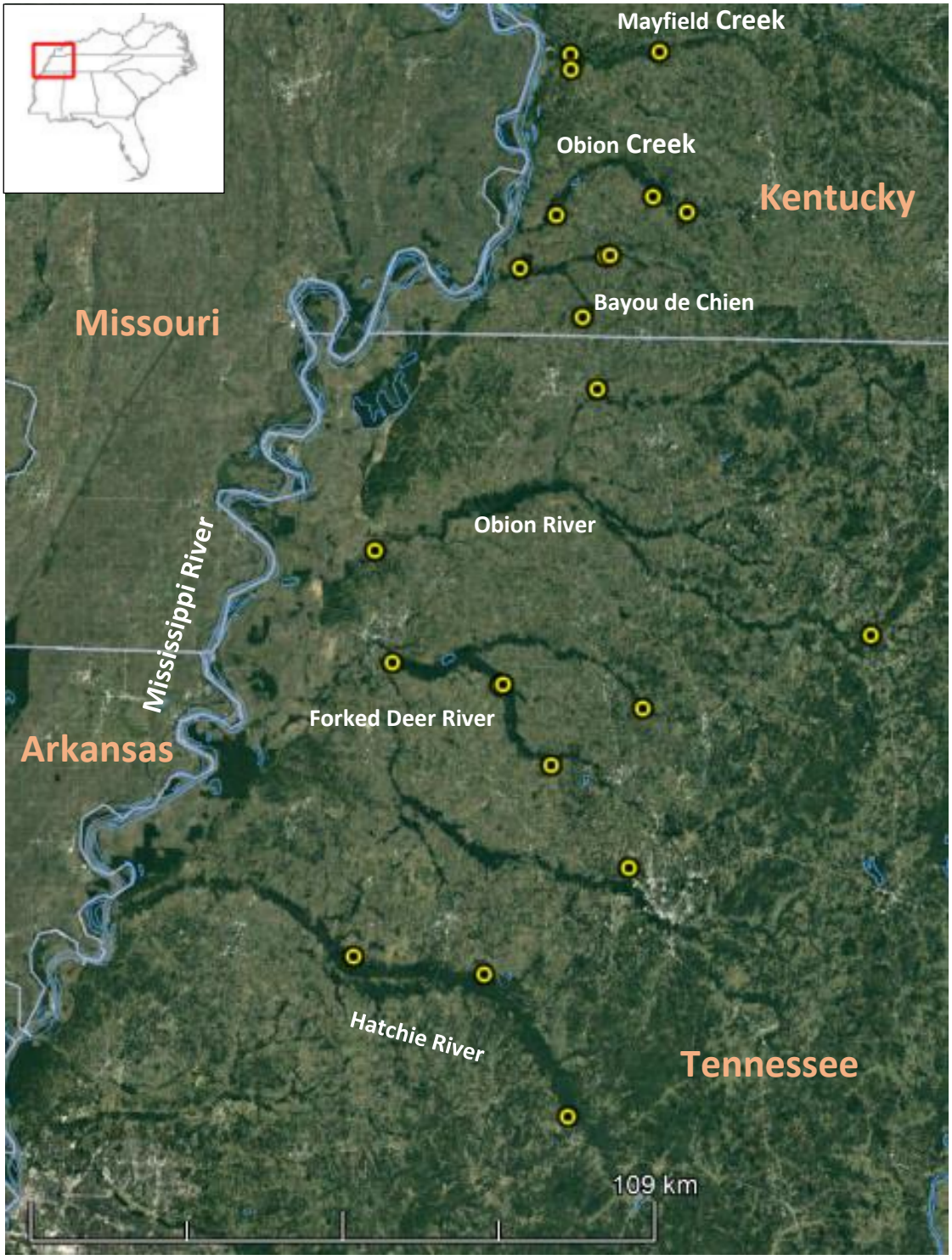

Figure 2.1.

Map of study sites in Kentucky and Tennessee. WREP easements are represented by yellow pins.

Wetland easement selection criteria used to select study sites:

- Easement age (year of entry into the WRP/WREP program and year of restoration). The easements were categorized as new (1-5 years old), middle-aged (6-10 years old), upper middle-aged (11-15 years), and old (>15 years old).
- Hydrologic connection (emphasis on sites where hydrological restoration occurred).
- Easement history (known condition of the easement when it entered the program and past land use).
- Proximity to other easements.
- Management practices used.
- A similar size of easements as possible.
- Current and pre-easement soil and vegetation characteristics.

2.5.2. Habitat characterization

The habitats in the easements were representative of the restoration practices. Major habitats in the easement were identified based on satellite images and field visits on the day of the core collection. Most common habitats covering a significant area of the easement included natural regeneration, shallow water area, tree planting, and remnant forest (Figures 2.2 & 2.3). The hydrology in shallow water areas is often actively managed using water control structures (USDA NRCS, 2012). Thus, shallow water habitat was determined as dry or wet based on the absence or presence of water at the time of sampling. Sediment cores collected from the dry edge of shallow water-wet habitat were also classified as shallow water-dry cores. Natural regeneration habitat includes areas that revegetate on their own through the natural process of plant succession. Plant sources colonizing the site are derived from propagules present in the soil

seed bank and/or dispersed by wind, animals, water, or other natural means of delivering plant materials (USDA NRCS, 2003). Tree planting habitat includes areas where trees/shrubs are planted to supplement forest stand regeneration in locations where natural regeneration of desired species is not possible (USDA NRCS, 2016). Remnant forest habitat includes areas containing native tree species that has not been in recent agricultural production as determined by areal images dating back to the 1980s and 1990s. However, all habitats were not present in every easement.

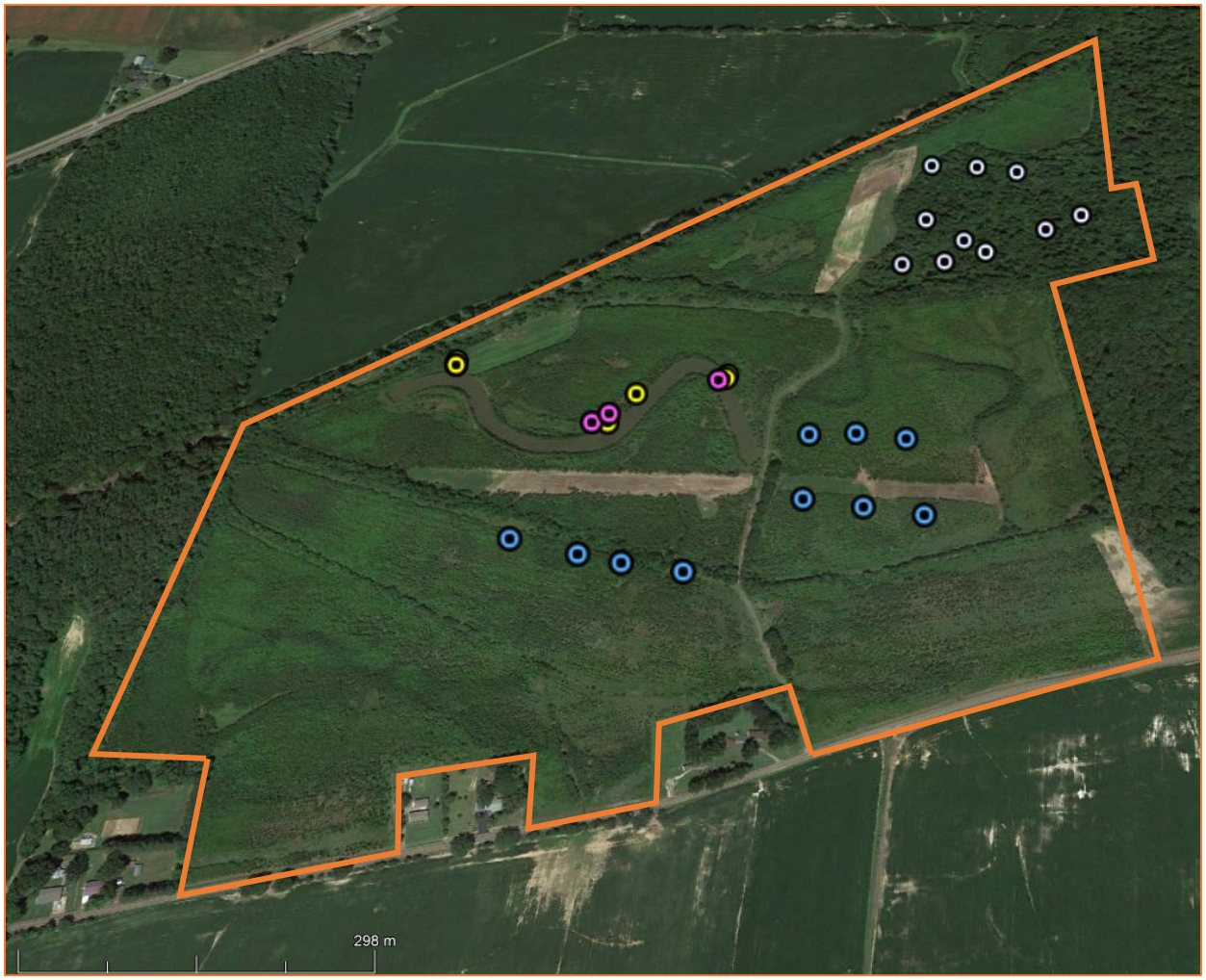

Figure 2.2.

Map of an easement in western Tennessee with locations of sediment cores collected from selected habitats. Habitats were classified as shallow water-dry (yellow pins), shallow water-wet (pink pins), tree planting (blue pins), and remnant forest (white pins). Orange outline represents the easement boundary.

Figure 2.3.

Examples of each habitat type sampled in the study site.

2.5.3. Core collection

Soil/sediment cores were collected from the easements from May through August from 2020 through 2022. Thirty paired soil function and soil structure cores (60 total) were collected from representative habitats from each of 23 easements. Soil function cores were used to determine potential denitrification rates. Soil structure cores were collected next to function cores and were used to determine soil physicochemical characteristics. An equal number of cores were collected from each habitat whenever feasible. Within each habitat, cores were distributed as evenly as possible to help account for spatial variation across a habitat. However, in the shallow water habitat, sampling was limited to the edge due to sample limitations and safety concerns of deeper water, making uniform sampling unfeasible (Figure 2.2). Approximately 15

cm deep soil/sediment cores were taken by inserting acrylic tubes (6.76 cm diameter ×30 cm height) into the soil.

Cores from compacted soil were collected in acrylic tubes housed inside a welded steel coring device (Figure 2.4A) and hammered with a sledgehammer. Cores in soft sediments were collected by pushing acrylic tubes into the sediments by hand or by using a PVC coring device (Figure 2.4B). Cores from the shallow water-wet habitat were filled with water on-site to minimize the disturbance of sediment surface during transportation and to maintain in-situ soil conditions. All cores were sealed with rubber bottoms secured with pipe straps and plastic tops. Cores were labeled, placed in a backpack cooler, and transferred to the cooler with ice to restrict microbial activities during transportation. GPS coordinates of collection points were recorded. Sampling time were recorded to account for the effect of holding time between sampling and incubation. Photographs of the collection site and surrounding habitat were taken to record the visual vegetation and hydrological characteristics while sampling. Soil structure cores were collected within 15 cm from incubation cores following a similar protocol as soil function core collection (Figure 2.4B). Upon returning to the lab, soil function cores were placed in the environmental chamber at incubation temperature (24^{o}C) to acclimate overnight. Incubations were started the following morning. Soil structure cores were transferred to a walk-in-cooler maintained at 4^{o}C and processed the following day.

Figure 2.4.

A) Welded steel corer assembly and B) paired soil function and soil structure cores.

2.5.4. Incubation water preparation and core incubation

Laboratory made incubation water was prepared according to the historical average water quality data of Bayou de Chien, Kentucky and the Obion River, Tennessee (Table 2.1). However, the concentration of NO_3^- -N and PO_4^{3-} -P were increased to 10 mg L^{-1} and 1 mg L^{-1}, respectively, to saturate nutrient uptake rates and provide consistent nutrient availability across easements. Therefore, the rates derived from incubation are maximum potential denitrification rates as opposed to ambient rates.

Table 2.1.

Average water quality data of Bayou de Chien (United States Geological Survey Station 07024000, Hickman County, KY) and Obion rivers (United States Geological Survey Station 07026040, Obion County, TN).

Compounds added to the water	Concentration (mg L^{-1})
Major minerals	
Potassium chloride (KCl)	3
Potassium phosphate (KH_2PO_4)	4.4
Magnesium sulphate ($MgSO_4*7H_2O$)	27
Calcium chloride ($CaCl_2$)	20
Sodium nitrate ($NaNO_3$)	60.5
Sodium bicarbonate ($NaHCO_3$)	70
Trace metals	
Manganese chloride ($MnCl_2$)	0.5
Iron (II) ammonium sulphate ($Fe(NH_4)_2(SO_4)_2*6H_2O$)	3
Cobalt chloride ($CoCl_2*6H_2O$)	0.1
Zinc sulphate ($ZnSO_4*7H_2O$)	0.05
Copper chloride ($CuCl_2*2H_2O$)	0.02
Sodium molybdate ($Na_2MoO_4*2H_2O$)	0.03
Dissolved organic C	
Glucose ($C_6H_{12}O_6$)	1

Immediately after returning to the lab from core collection, water from shallow water cores was siphoned out carefully, and the outside of acrylic tubes were wiped clean. Plastic tops were replaced with acrylic lids equipped with inflow and outflow ports (i.d. 1 mm and 1.25 mm, respectively) and secured with pipe straps (Figure 2.5). The incubation took place in a walk-in environmental chamber. A temperature of 24^{o}C was maintained throughout the incubation process to simulate average summer regional air temperature. The cores were set in the environmental chamber overnight in the dark. Incubation using a continuous flow through system started at 8 am the day following collection. Cores were housed in wooden boxes equipped with PVC frames and gutters for draining outflow water (Figure 2.6). Three water-only control cores were incubated to account for changes in nutrients and gases in the water due to the incubation process. Lab water was delivered to individual cores at a rate of 2 mL min^{-1} through an inflow tubing connected to a Masterflex L/S peristaltic pump. Water flowed out of the cores through a second tube into sample containers, or into PVC gutters that drained into a bucket between sampling times. Green lights were used while working in the chamber throughout the incubation process to reduce photosynthesis and oxygen production in the cores.

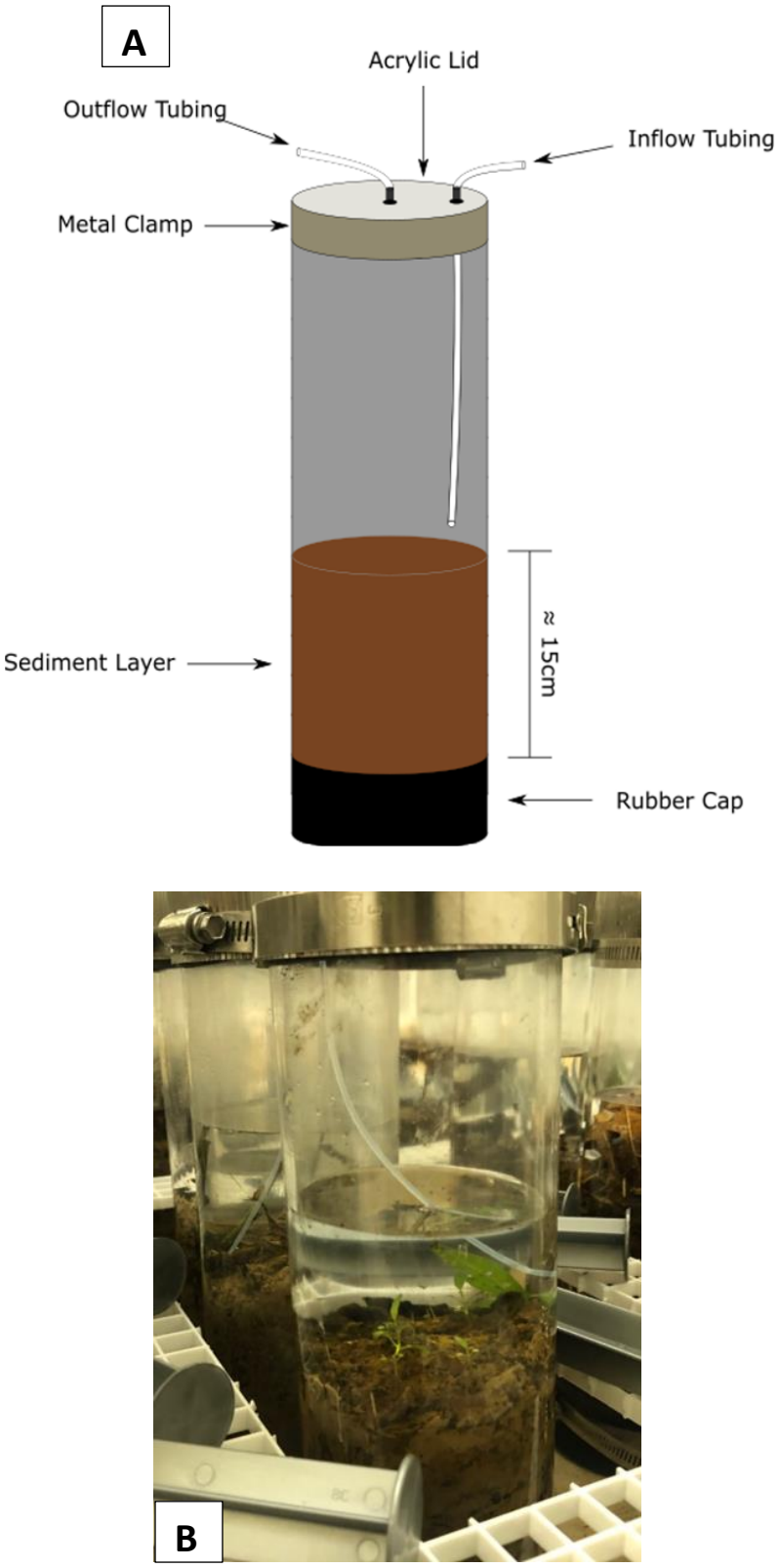

Figure 2.5.

A) Diagram of an incubation core and B) image of incubation cores.

Figure 2.6.

Flow-through incubation setup in an environment chamber with peristaltic pumps and the recirculating system.

2.5.5. Dissolved gas sampling and analysis

Water samples were collected from outflow tubes in triplicate 12 mL exetainers at 24 and 48 h after the start of the incubation. Vials were allowed to overflow three times before collecting the samples. Afterward, the outflow tubes were slowly and carefully removed from the vials to prevent headspace formation and reduce contamination of dissolved gas with atmospheric air. After removing the tube, samples were dosed immediately with 180 µL zinc chloride ($ZnCl_2$) to restrict microbial activities. Vials were capped quickly and turned upside down a few times to check if air bubbles were trapped in the water sample and to ensure the

uniform distribution of $ZnCl_2$ in the sample. The samples were stored underwater at 4^{o}C and analyzed within a month of collection.

Dissolved gas concentrations in water samples were determined using a Membrane Inlet Mass Spectrometer (MIMS) (Kana et al., 1994). The instrument measured dissolved N_2, O_2, and Argon (Ar) concentrations in the water using the MIMS Faraday detector in 2020 and 2021 and MIMS Secondary Electron Multiplier (SEM) in 2022. No difference in N_2 O_2, or Ar concentration measurements was found between the two methods. Each gas was analyzed according to its atomic mass-to-charge-ratio as follows: N_2 at *m/*z 28, O_2 at *m/z* 32, and Ar at *m/z* 40. Standards for dissolved gas were prepared by continuously stirring deionized water to make sure that the gas concentrations were in equilibrium with the atmosphere. The deionized water was kept in a 1 L round-bottomed flask and placed in a water bath set at 24°C.

Triplicate standards were measured after every six samples to correct for the drift in MIMS signal overtime and to calculate a calibration factor. To determine the concentration of each gas, the thermodynamically expected concentration at 24°C, which was adjusted for atmospheric pressure (Weiss, 1970) was divided by the average *m/z* signal obtained from triplicate standard measurements. The slope between each set of standards was used as a calibration factor to adjust m/z signal of N_2, O_2, and Ar in each sample before calculating concentrations of each gas. The Ar ratio method in R (*mimsy* package) was used to correct for physical effects on N_2 and O_2 concentrations (Kelly, 2020). Because Ar only changes due to physical processes, while O_2 and N_2 respond to both physical and biological processes, the effects of physical changes on the measured gas concentrations can be corrected using Ar ratio as follows:

$$[BG]_{sample} = \left(\frac{uncorrected[BG]_{sample}}{[Ar]_{sample}} \times [Ar]_{expected}\right)\left(\frac{[BG]_{expected}/[Ar]_{expected}}{[BG]_{standard}/[Ar]_{standard}}\right)$$

where,

$[BG]_{sample}$ = corrected concentration of biologically active gas (N_2 and O_2),

$uncorrected[BG]_{sample}$= concentration of N_2 and O_2 before Ar ratio correction,

$[Ar]_{sample}$ = Ar concentration in the same sample,

$[Ar]_{expected}$ = thermodynamic Ar concentration at 24°C,

$[BG]_{expected}/[Ar]_{expected}$ = thermodynamically expected N_2: Ar and O_2: Ar measurements at 24°C, and

$[BG]_{standard}/[Ar]_{standard}$ = N_2: Ar and O_2: Ar measurements averaged over three triplicate standards.

The output gas concentrations from MIMS were expressed as µg L^{-1} of N_2 and O_2 (Kana et al., 2006). The areal N_2 flux for each sediment core was calculated according to (Speir et al., 2017) as follows:

$$Aeral\ Flux\ (mg\ m^{-2}\ h^{-1}) = \left(\frac{[(Core)_{out} - (Core)_{in}] * Q_{\ core}}{A\ (m^2)}\right)$$

where,

$(Core)_{out}$ = outflow concentration (mg L^{-1}) of N_2 and O_2 in incubation core,

$(Core)_{in}$ = inflow concentration (mg L^{-1}) of N_2 and O_2 incubation core,

$Q_{\ core}$ = flow rate of incubation core (L h^{-1}), and

A = surface area of soil (m^2).

Positive flux indicates a net gain (production or release) of N_2 or O_2 in the water column and negative flux indicates a net loss (consumption or removal) of N_2 or O_2 from the water column. More negative O_2 flux rates correspond to higher sediment oxygen demand (SOD).

2.5.6. Soil structure core processing

Supplemental cores were processed for determining soil physicochemical properties, including soil moisture (SM), bulk density (BD), pH, total carbon (TC), total N (TN), and extractable phosphorus (P). The cores were transferred to a clean aluminum sheet and weighed. Water from the shallow-water cores were carefully siphoned out before transferring the soil to the aluminum sheet. Using the forceps, detritus (dead plant materials and dark brown to black in color) was removed from the soil surface. Similarly, vegetation (alive and green in color) was clipped on the soil surface using scissors and removed. Each core was divided at 10 cm depth from the soil surface (0-10 cm) using a spackle knife and soil below 10 cm depth was discarded. After removing the coarse materials such as woody root pieces and gravel, each soil section was homogenized manually to get a uniform mixture through repeated mixing. The homogenization process is as follow:

1. The soil core was first broken down by hand as much as possible and spread on aluminum foil.
2. A spackle knife was used to break up large clumps while working in sections.
3. After breaking large clumps, the bulk soil sample was mixed thoroughly using a spackle knife and hands alternately.
4. Process 1-3 were repeated (spreading, breaking, mixing) until the soil texture was consistent throughout the sample.

5. Once the soil was homogenized, subsamples were taken for analysis.

Subsamples were taken to measure SM, BD, pH, TC, TN, and P. Soil properties data for each supplemental core was presumed to be representative of the corresponding incubation core**.**

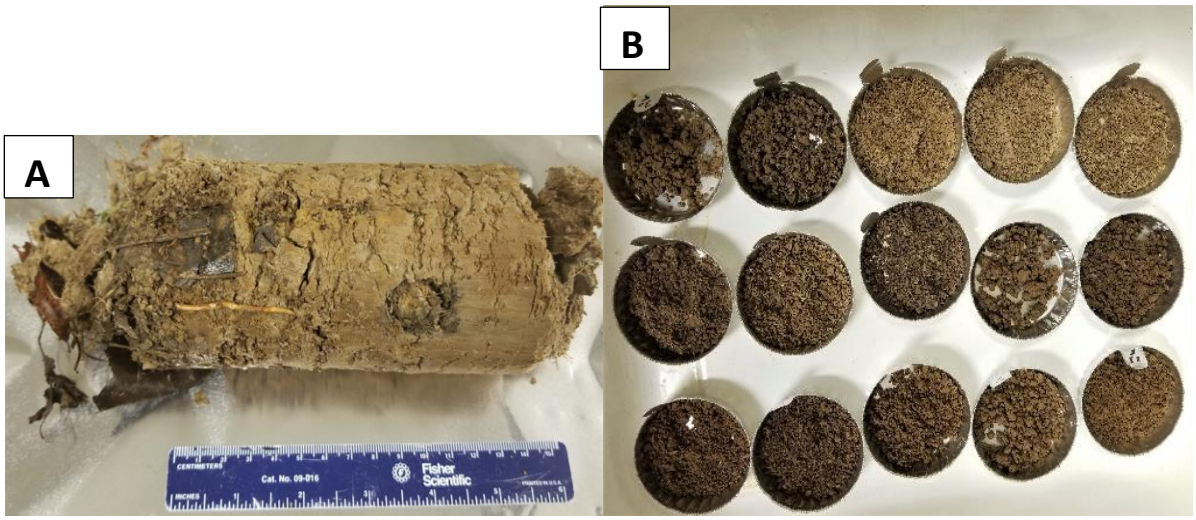

Figure 2.7.

A) A 15 cm sediment core, B) samples from different cores.

Soil moisture and bulk density

Soil moisture was measured by the thermogravimetric method, which is based on the weight measurement of a wet sample before and after oven drying (Evett et al., 2008). A 30 g fresh subsample after homogenization was weighed in a pre-weighed aluminum tin and placed in the oven at 105°C. Soil moisture was reported as g g^{-1} of dry soil and calculated as:

$$SM = \frac{weight\ of\ water\ lost\ after\ oven\ drying\ fresh\ soil\ (g)}{weight\ of\ dried\ soil\ (g)}$$

Assuming that every pore size within the soil sample of each core is uniform, moisture lost by 30 g of fresh soil due to drying was used to calculate the moisture lost by total soil mass of predetermined volume (volume of the acrylic tube). Bulk density of each soil core was reported as g cm^{-3} calculated as:

$$BD = \frac{mass\ of\ dried\ soil\ (g)}{soil\ volume\ (cm3)}$$

Gravimetric SM was compared with field SM readings to confirm the accuracy of field measurements

Soil pH

Soil pH was measured using a 1:2 fresh soil-to-water ratio after equilibrium for 30 minutes (Reddy et al., 2013). The pH meter was calibrated using buffer solutions of pH 4.0, 7.0, and 10.0. After removing the protective cap, the electrode was washed using deionized water. Calibration started with a pH 7.0 buffer solution, followed by pH 4.0 and 10 buffer solutions. The electrodes were washed in between calibrations. Before measuring the pH of the samples, the pH of the deionized water that was used to prepare soil suspension was measured to ensure that the deionized water was neutral. Then, 10 g homogenized soil subsample was weighed in a 50 ml beaker, and 20 ml deionized water was added. The suspension was stirred for approximately 10 seconds every 5 minutes using a stainless-steel mini whisk during the next 30 minutes. The suspension was then allowed to settle for 30 minutes. The pH of the samples was measured by immersing the electrode in a supernatant solution. The pH values were recorded when the reading stabilized (usually after 1 minute).

Soil nutrients

Approximately half of the homogenized soil sample from each 0-10 cm soil core were used for soil nutrients analysis to ensure uniform soil horizon representation for all habitats. The soil samples were dried in an oven at 60^{o}C. Because soil is defined as having a particle size of less than 2 mm, dried soil samples were ground to pass a 2 mm mesh screen. A subset of ground soil was transferred to fill a labeled 20 ml plastic scintillation vials and shipped to the Soil Testing Laboratory at Kansas State University where TC, TN, and extractable P were analyzed. Total C and TN were analyzed by dry combustion (Wright & Bailey, 2001). In this method, the samples are combusted at high temperature in the presence of O_2, and the resulting carbon dioxide (CO_2) and N_2 are measured using non-dispersive infrared (NDIR) analyzer and a thermal conductivity detector (TCD), respectively. The amount of CO_2 and N_2 detected are used to calculate the amount of TC and TN in the sample. The Mehlich-3 extraction procedure was used to determine "plant-available" P (orthophosphate) using colorimetry (Mehlich, 1984). The Mehlich-3 extractant is a mixture of acetic acid (CH_3COOH), ammonium nitrate (NH_4NO_3), ammonium fluoride (NH_4F), nitric acid (HNO_3) and ethylenediaminetetraacetic acid (EDTA) at pH 2.5. The Mechlich-3 extractant mixture works by breaking down the chemical bond between the soil particles and adsorbed P, releasing the P into the solution. The solution is then filtered and extracted P is quantified using colorimetry. Soil nutrient concentrations were expressed in dry weight equivalent.

2.5.7. Data processing

At each sampling timepoint, boxplots and dot charts were used to visualize the presence of outliers in both N_2 and O_2 flux measurements. Additionally, N_2 and O_2 flux rates, along with soil properties for the cores, were assessed for normal distribution at each respective sampling timepoint. Given the non-normal distribution of the dataset, flux rates specific to each habitat and sampling timepoint were averaged, resulting in a singular representative value for each habitat within the easement. This approach effectively mitigated variations in the dataset and simultaneously averted the issue of pseudo-replication. Thus, the habitats represented experimental factors, while the cores functioned as individual measurement units, in line with the methodology mentioned by (Zuur et al., 2009). Before averaging flux rates, any individual core-level data points that deviated beyond the 2.5% and 97.5% quantiles at each sampling timepoint were excluded from the analysis. Excluding data points beyond the 2.5% and 97.5% quantiles was expected to reduce the influence of extreme values during data analysis. This helps ensure that the analysis is more representative of the typical or expected behavior of the system, as extreme outliers can distort the results and lead to misleading conclusions. Furthermore, cores with missing data were excluded from the averaging process. Initially, there were 1426 data points, but following the removal of outliers and subsequent averaging, the dataset was reduced to 149 data points (Supplemental Table 2.1).

2.5.8. Response variable, treatment, and covariates

In this study, the functional response variable of interest was N_2 flux rate. The focal point of investigation was to analyze the effect of habitats on N_2 production rate. Therefore, habitat was treated as the key factor. Additionally, various soil properties were used as covariates

including, SOD, SM, BD, pH, soil TC, soil TN, and soil P. These covariates may contribute to a comprehensive understanding of N_2 flux patterns by accounting for potential confounding variables that might influence the observed associations. Integrating these covariates would ensure an investigation of interplay between N_2 flux rates, habitat types, and soil properties and how they impact each other.

2.5.9. Statistical Analysis

Statistical analysis was performed using R statistical software from the R Core Team (2022). Soil properties data were tested for normal distribution and variance homogeneity. For multiple comparisons of soil properties, Analysis of Variance (ANOVA) was used when the variance was homogeneous and Kruskal Wallis test was used as when variances were either inhomogeneous or data was not normally distributed. The effects of soil properties and habitats on N_2 flux rates was analyzed using linear mixed model (LME). The steps involved were as follows:

- Running linear mixed effects model (LME): The effects of soil properties and habitats on N_2 flux rates was analyzed using linear mixed effects models (R package *lme*), conducted separately at both the 24 h and 48 h timepoints. Easement was incorporated as a random factor in the analysis. Interaction components were chosen utilizing ecological insights and thorough examination of the data. Backward selection approach was applied, iteratively refining the model until all soil properties exhibited p-values < 0.05. Final models were fit using restricted maximum likelihood (REML) estimations.
- Model validation and refinement: Standardized residual plots and normality plots (QQplot) were generated to assess the appropriateness of the model assumptions visually,

followed by Variance inflation factor (VIF) assessment to check multicollinearity between predictor variables. The complete model, containing main terms and interactions, was further evaluated.

- Additional model assumptions check: ANOVA, AIC, and BIC tests were done to ensure final model simplicity and goodness of fit compared to the initial model. Normality in residuals were verified visually (QQplot, histograms) and by using normal distribution test (Shapiro and skewness tests). Homogeneity assumptions were confirmed after plotting standardized residuals vs. fitted values and standardized residuals vs. each predictor variable in the final model.
- Final ANOVA: ANOVA with type III sum of squares was used to analyze the effect of predictor variables included in the final model on mean N_2 flux rates. Type III sum of squares was employed due to the presence of interaction in the model and unbalanced sample size in different habitats (natural regeneration n = 6, remnant forest n = 17, shallow water-dry n = 15, shallow water-wet n = 18, and tree planting n = 18).
- Predicted mean calculation: Predicted mean N_2 flux rates were computed using the *emmeans* function in R. Subsequently, post hoc tests were performed using Tukey's HSD to discern differences between habitat categories.

2.6. Results

2.6.1. Soil properties

Habitat types exhibited significant differences across multiple soil properties. These differences were observed in SM (Kruskal-Wallis $\chi^2_{(4)} = 24.777$, $P<0.0001$), pH (ANOVA $F_{(4)} = 2.622$, $P = 0.0420$), soil TC ($\chi^2_{(4)} = 15.314$, $P = 0.0041$), and soil TN ($\chi^2_{(4)} = 10.356$, $P = 0.0348$). No difference in BD ($F_{(4)} = 1.203$, $P = 0.317$) and soil P ($F_{(4)} = 2.388$, $P = 0.0593$) was found among habitat types at 0.05 significance level. The mean SM and pH showed the highest values in the shallow water-wet habitat. On the other hand, tree planting habitat exhibited the highest mean BD. In the remnant forest habitat, the mean values of soil TC and TN were the highest. Natural regeneration and remnant forest habitats displayed the highest mean levels of soil P (Table 2.2).

Soil properties exhibited considerable variability both within and among different habitats (Table 2.2). Notably, soil P displayed the least variation within and among the habitats. Within the habitat, soil TC showed the highest level of variability in remnant forest habitat (10.60-53.60 mg g^{-1}). Among the drier habitats, natural regeneration habitat had the highest mean SM. In comparison, shallow water-wet habitat had 0.25 g g^{-1} (54%) more SM compared to the natural regeneration habitat. The variability in SM (0.11-1.01 g g^{-1}), BD (0.83-1.21 g cm^{-3}), and pH (4.20-6.07) were most pronounced within the natural regeneration habitat. As for soil TN, the variability was most pronounced within the remnant forest habitat (1.38-2.42 mg g^{-1}). While the mean SM was notably high in the shallow water-wet habitat, the mean BD remained relatively consistent across all habitats (Table 2.2). Although the natural regeneration habitat displayed a high variability in soil pH, the mean soil pH was nearly similar for all habitats. Soil P variability was highest in both remnant forest and shallow water-dry habitat (0.01-0.09 mg g^{-1}).

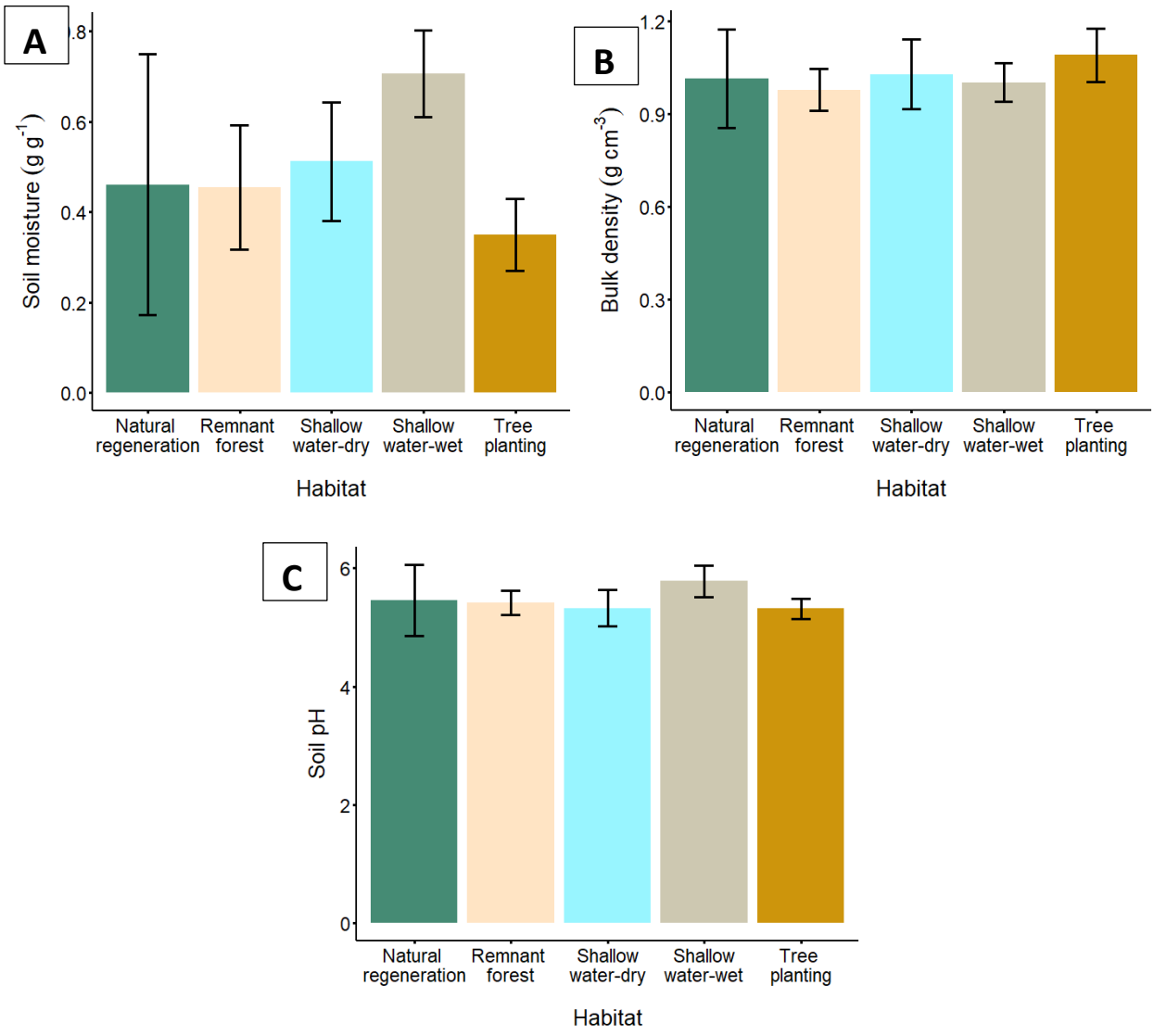

Figure 2.8.

Mean A) soil moisture, B) bulk density, and C) soil pH. Habitat types were natural regeneration, remnant forest, shallow water-dry, shallow water-wet, and tree planting. Error bars represent 95% confidence interval.

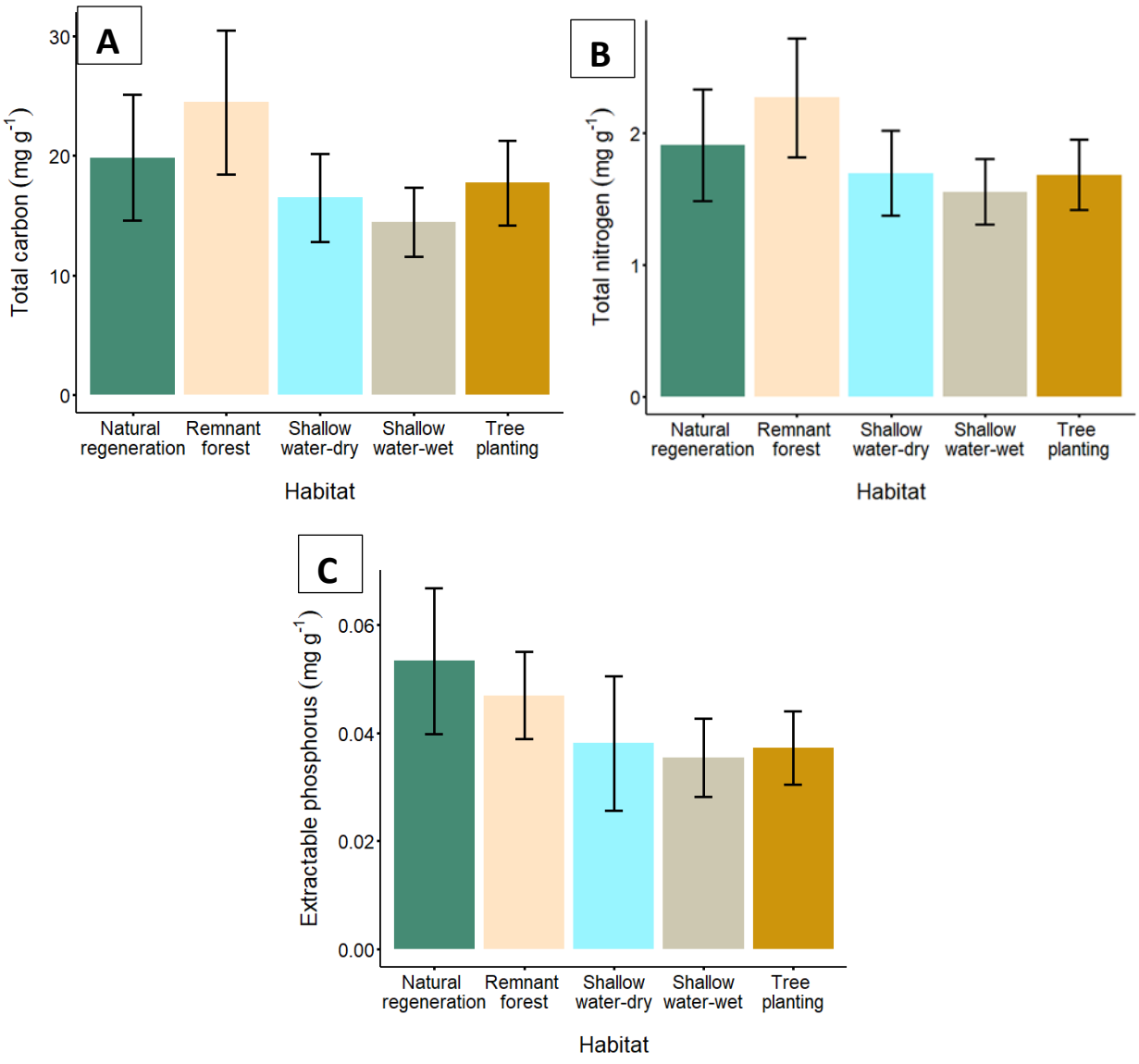

Figure 2.9.

Mean A) total carbon, B) total nitrogen, and C) extractable phosphorus. Habitat types were natural regeneration, remnant forest, shallow water-dry, shallow water-wet, and tree planting. Error bars represent 95% confidence interval.

Table 2.2.

Soil properties means ± SE for 23 easements and 5 habitat types. The soil properties include soil moisture, bulk density, pH, total carbon, total nitrogen, and extractable phosphorus.

Habitat	n	Soil moisture ($g\ g^{-1}$)	Bulk density ($g\ cm^{-3}$)	pH	Total carbon ($mg\ g^{-1}$)	Total nitrogen ($mg\ g^{-1}$)	Extractable phosphorus ($mg\ g^{-1}$)
Natural regeneration	7	0.46 ± 0.12	1.01 ± 0.07	5.46 ± 0.25	19.82 ± 2.15	1.90 ± 0.17	0.05 ± 0.005
Remnant forest	17	0.45 ± 0.06	0.98 ± 0.03	5.42 ± 0.09	24.46 ± 2.83	2.27 ± 0.21	0.05 ± 0.003
Shallow water-dry	15	0.51 ± 0.06	1.03 ± 0.05	5.33 ± 0.14	16.48 ± 1.71	1.69 ± 0.15	0.04 ± 0.006
Shallow water-wet	18	0.71 ± 0.05	1.00 ± 0.03	5.78 ± 0.13	14.43 ± 1.38	1.55 ± 0.12	0.04 ± 0.003
Tree planting	18	0.34 ± 0.04	1.09 ± 0.04	5.32 ± 0.08	17.72 ± 1.68	1.68 ± 0.13	0.04 ± 0.003

2.6.2. Nitrogen gas (N_2) flux between 24 h and 48 h

Nitrogen gas was produced from all habitats at both 24 h and 48 h sampling timepoint. Nitrogen gas production exhibited an upward trend throughout the incubation period, except for the remnant forest habitat. Nitrogen gas production rates for natural regeneration, remnant forest, shallow water-dry, shallow water-wet, and tree planting habitats were 6.86, 4.43, 5.23, 3.44, and 5.31 mg m^{-2} h^{-1} at 24 h, and 8.10, 4.83, 5.95, 4.62, and 5.66 mg m^{-2} h^{-1} at 48 h sampling timepoint, respectively. Notably, the natural regeneration habitat displayed the highest production rate at both sampling timepoints. Shallow water-wet habitat showed the lowest production at both sampling timepoints (Figure 2.10). Nitrogen gas production rates did not significantly change between the 24 h and 48 h sampling timepoints. Shallow water-wet habitat exhibited the highest percentage increase in N_2 production by 34.3%. In natural regeneration, remnant forest, shallow water-dry, and tree planting habitats, the release increased by 18.1%, 9.0%, 13.8%, and 6.6%, respectively.

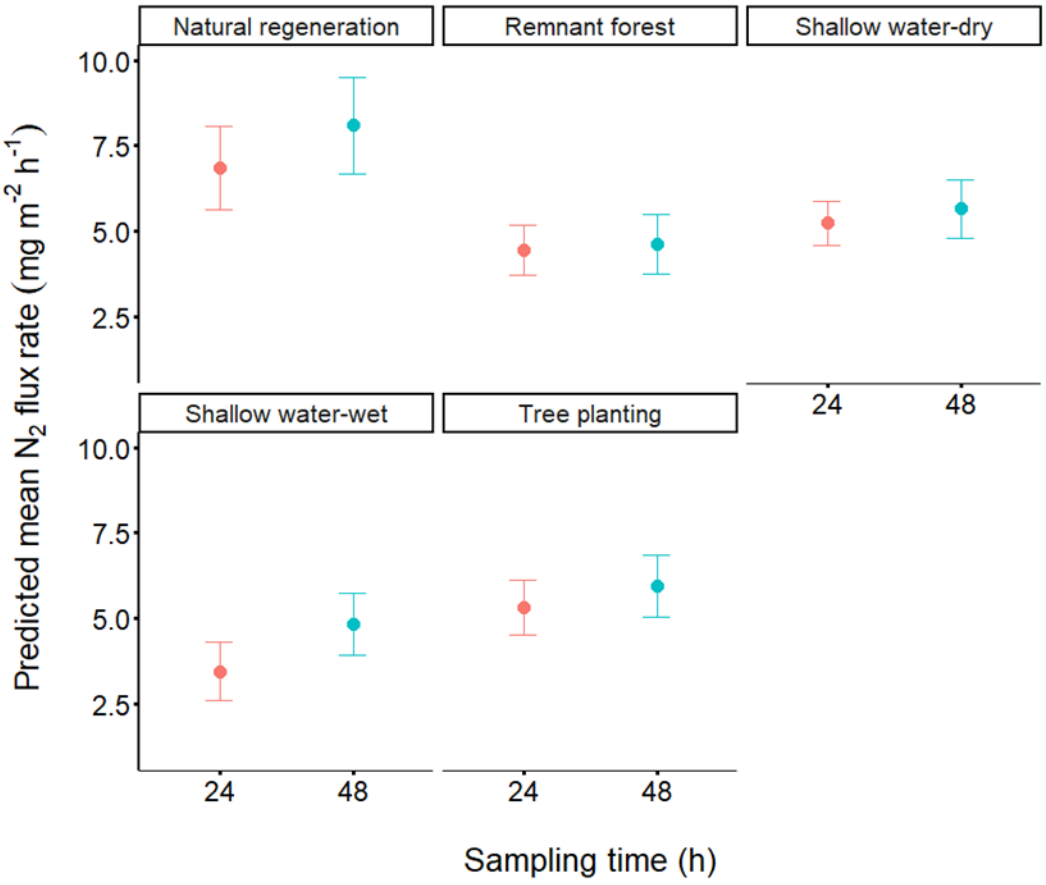

Figure 2.10.

Predicted mean N_2 flux rate at 24 and 48 h sampling timepoint by habitat. Habitats were natural regeneration, remnant forest, shallow water-dry, shallow water-wet, and tree planting. Error bars represent the 95% confidence interval.

2.6.3. Nitrogen gas (N_2) flux at 24 h

At the 24 h sampling timepoint, N_2 was produced from all habitats. The estimated mean N_2 production rate exhibited significant differences among the habitat types (Chi-squared $_{(4,53)}$ = 16.79, P = 0.0021) (Table 2.3). Upon conducting pairwise comparisons, it was found that the mean N_2 production rate was notably lower in the shallow water-wet habitat (3.44 mg m^{-2} h^{-1}), followed by remnant forest habitat (4.43 mg m^{-2} h^{-1}). Conversely, the natural regeneration habitat had the highest mean N_2 production at 6.86 mg m^{-2} h^{-1}. The mean N_2 production rate was nearly identical for shallow water-dry (5.23 mg m^{-2} h^{-1}) and tree planting (5.31 mg m^{-2} h^{-1}) habitats (Figure 2.11)

The estimated mean N_2 production was significantly influenced by SOD (Chi-squared $_{(4,53)}$ = 22.70, P<0.0001), SM (Chi-squared $_{(4,53)}$ = 3.99, P = 0.0459), and soil P (Chi-squared $_{(4,53)}$ = 6.10, P = 0.0135). However, there was no significant effect of soil pH alone on N_2 production. Nonetheless, a significant interaction between habitat and soil pH on N_2 production was observed (Chi-squared $_{(4,53)}$ = 22.20, P = 0.0001). Additionally, a significant interaction of habitat with SM (Chi-squared $_{(4,53)}$ = 11.26, P = 0.0238) and soil P was observed (Chi-squared $_{(4,53)}$ = 25.47, P<0.0001) (Table 2.3). This interaction effect implies that the relationship between N_2 production and SM, pH, and soil P is dependent on the specific habitat type, and the impact of these soil properties on N_2 production may vary depending on the habitat conditions.

Table 2.3.

Chi-squared statistics from ANCOVA for habitat, sediment oxygen demand (SOD), soil moisture, soil pH, soil phosphorus, and the interaction between habitat and soil moisture, habitat and soil pH, and habitat and soil phosphorus at 24 h sampling timepoint.

Source	**df**	**Chi-squared**	***P***
Intercept	1	3.30	0.0694
Habitat	4	16.79	0.0021
SOD	1	22.70	<0.0001
Soil moisture	1	3.99	0.0459
Soil pH	1	0.02	0.8747
Soil P	1	6.10	0.0135
Habitat: soil moisture	4	11.26	0.0238
Habitat: soil pH	4	22.20	0.0001
Habitat: soil P	4	25.47	<0.0001

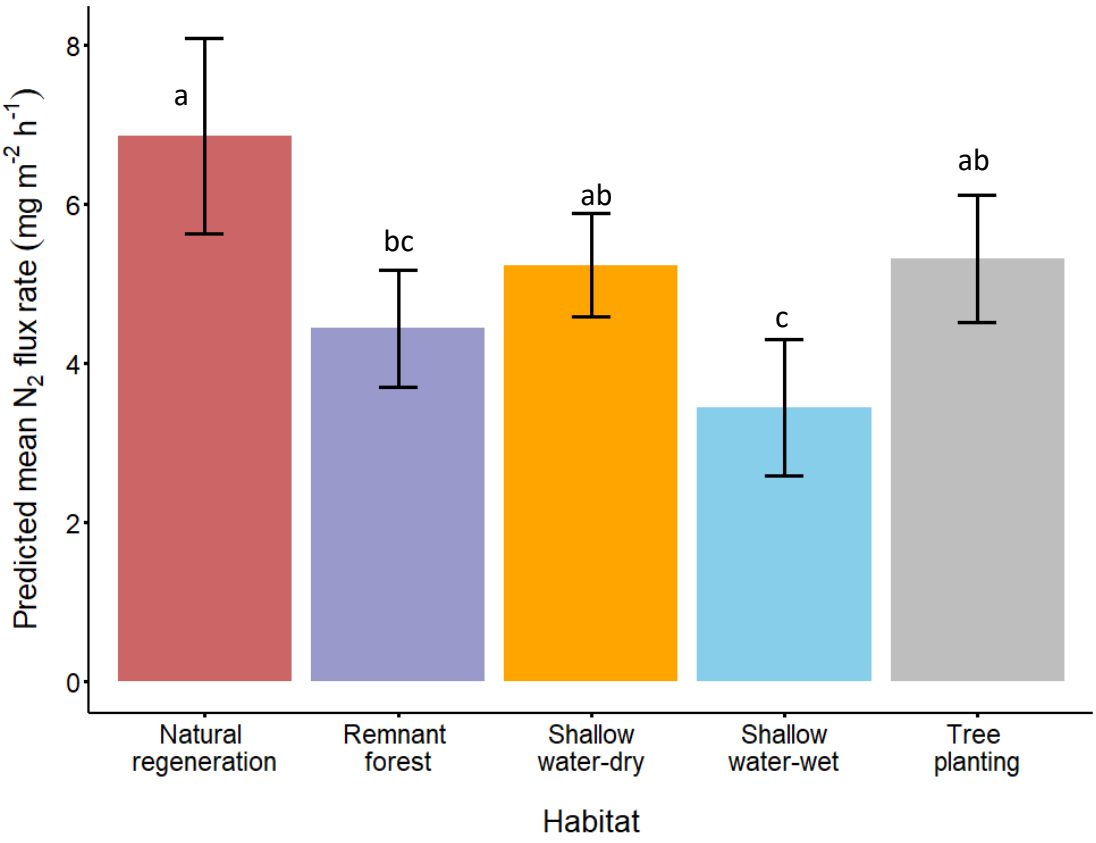

Figure 2.11.

Predicted mean N_2 flux rate among the habitats at 24 h sampling timepoint. Habitats were natural regeneration, remnant forest, shallow water-dry, shallow water-wet, and tree planting. Error bars represent 95% confidence interval. Means associated with different lowercase letter are significantly different by post hoc analysis using Tukey's Honestly Significant Difference.

Interaction between habitat and soil moisture (SM) at 24 h

In the natural regeneration and shallow water-wet habitat, increase in SM negatively correlated with N_2 production, while in remnant forest habitat increase in SM correlated positively N_2 production. The negative correlation between increasing N_2 production and SM observed in the shallow water-dry and tree planting habitats was likely the result of an outlying SM value (Figure 2.12).

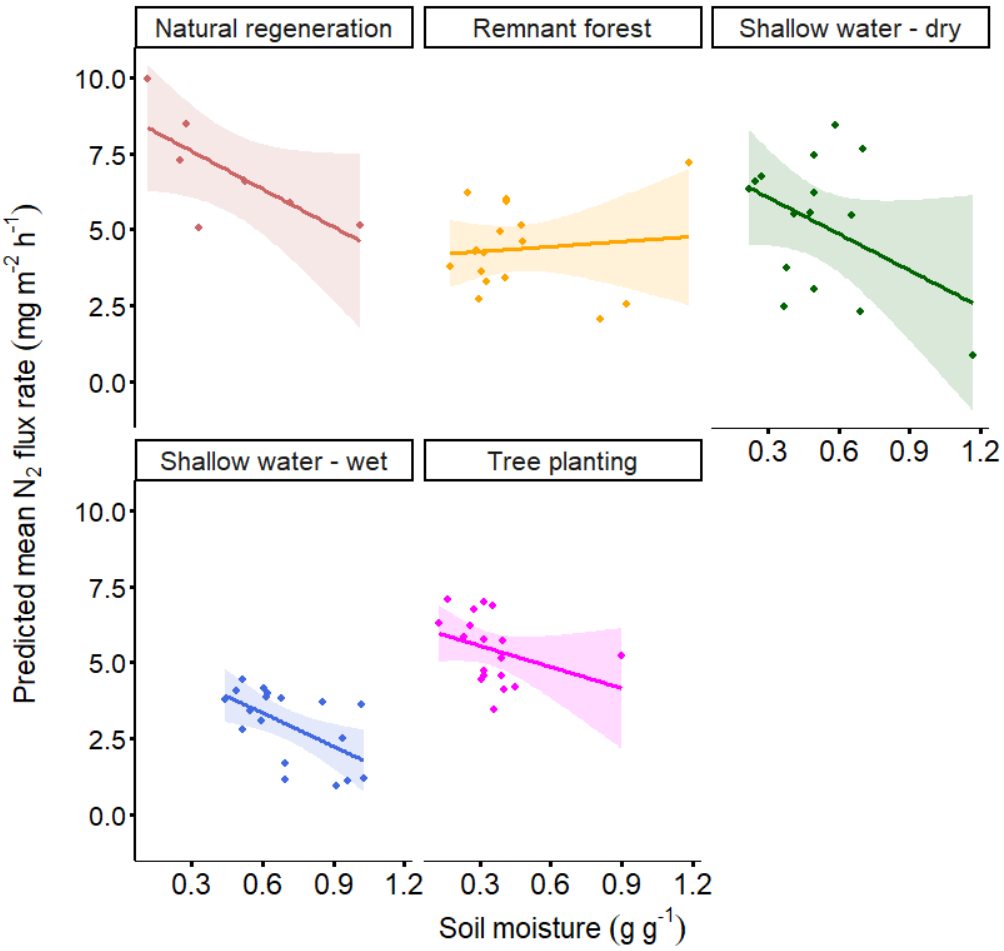

Figure 2.12.

Predicted mean N_2 flux rate among the habitats as affected by soil moisture at 24 h sampling timepoint. Habitats were natural regeneration, remnant forest, shallow water-dry, shallow water-wet, and tree planting. Bands represent 95% confidence intervals.

Interaction between habitat and soil pH at 24 h

In most habitats, the impact of soil pH on N_2 production was minimal, except in the shallow water-dry habitat, where an increase in soil pH led to a decrease in the N_2 production rate (Figure 2.13). Additionally, it is worth noting that the strong correlation between soil pH and N_2 production in the shallow water-dry habitat could have potentially exaggerated the significance of this interaction term in the overall model.

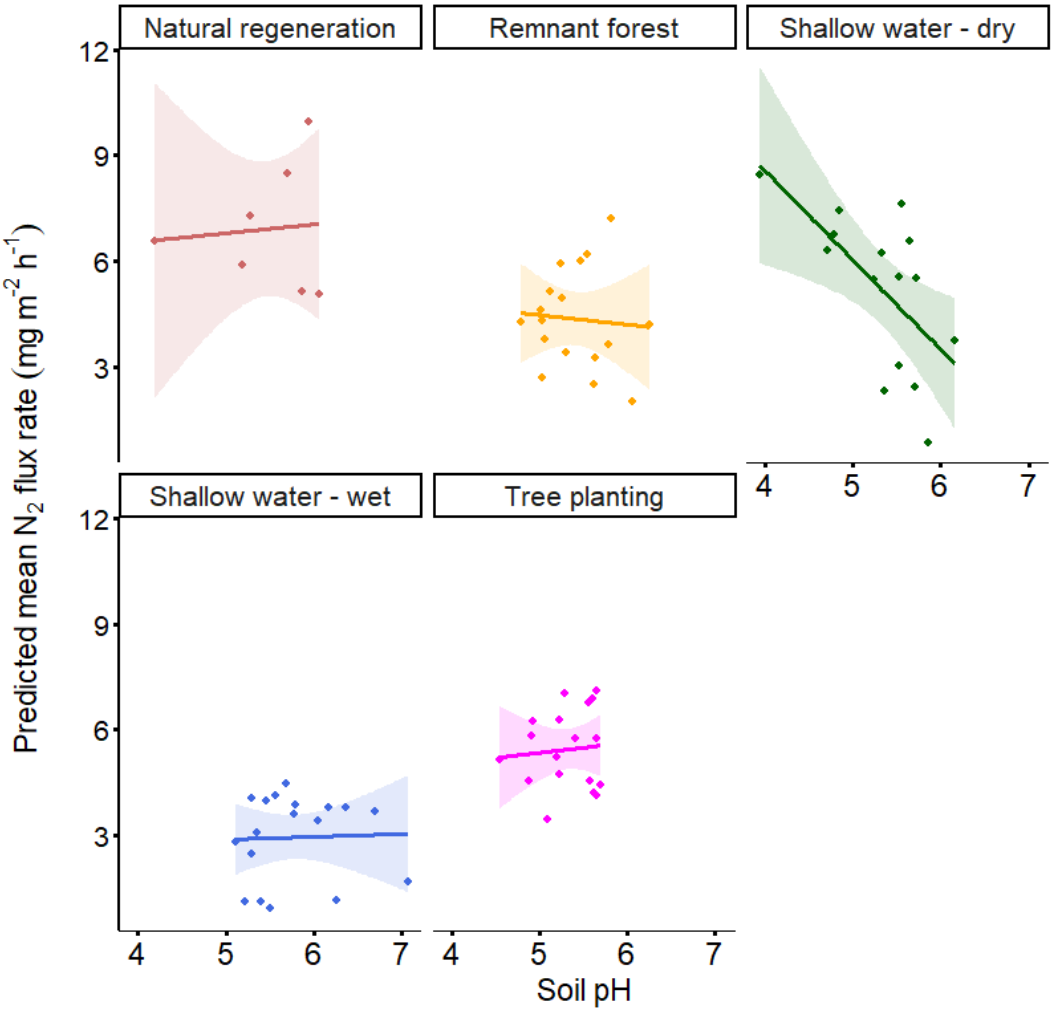

Figure 2.13.

Predicted mean N_2 flux rate among the habitats as affected by soil pH at 24 h sampling timepoint. Habitats were natural regeneration, remnant forest, shallow water-dry, shallow water-wet, and tree planting. Bands represent 95% confidence intervals.

Interaction between habitat and soil phosphorus (P) at 24 h

An increase in soil P showed a negative correlation with N_2 production in natural regeneration habitat and positive correlation with N_2 production in remnant forest, shallow water-dry, and tree planting habitats. In shallow water-wet habitat, the effect of soil P on N_2 production was minimal with little change in flux rates observed across the soil P gradient (Figure 2.14).

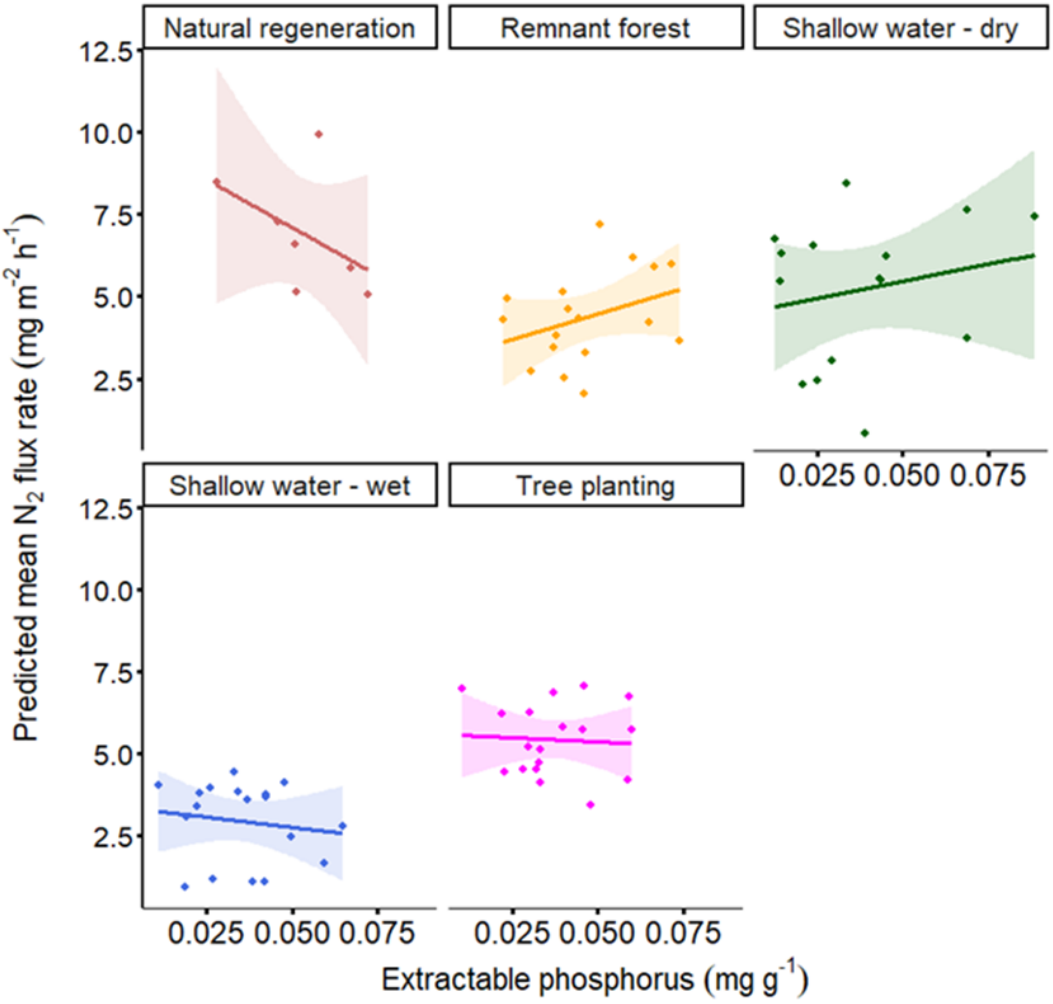

Figure 2.14.

Predicted mean N_2 flux rate among the habitats as affected by soil phosphorus at 24 h sampling timepoint. Habitats were natural regeneration, remnant forest, shallow water-dry, shallow water-wet, and tree planting. Bands represent 95% confidence intervals.

2.6.4. Nitrogen gas (N_2) flux at 48 h

The estimated mean N_2 production at 48 h sampling timepoint was significantly affected by habitat type (Chi-squared $_{(4,62)}$ = 12.60, P = 0.0134) (Table 2.4). The mean N_2 production rates were significantly low in the remnant forest (4.83 mg m^{-2} h^{-1}), shallow water-dry (5.95 mg m^{-2} h^{-1}), shallow water wet (4.62 mg m^{-2} h^{-1}), and tree planting (5.66 g m^{-2} h^{-1}) habitats when compared with natural regeneration habitat (8.10 mg m^{-2} h^{-1}). The mean N_2 production rates were nearly similar for remnant forest and shallow water-wet habitats, as well as for shallow water-dry and tree planting habitats (Figure 2.15).

Additionally, the estimated mean N_2 production rate was significantly affected by SOD and the interaction between habitat and soil P. Increases in SOD enhanced N_2 production across all habitat types (Chi-squared $_{(4,62)}$ = 15.34, P < 0.0001). The relationship between N_2 production and soil P was dependent on the habitat type (Chi-squared $_{(4,62)}$ = 9.85, P = 0.0431). An increase in soil P showed a negative correlation with N_2 production in natural regeneration and positive correlation with N_2 production in remnant forest, shallow water-dry, and tree planting habitats. In natural regeneration and shallow water-wet habitats, the effect of soil P on N_2 production was minimal with little change in flux rates observed across the soil P gradient (Figure 2.16).

Table 2.4.

Chi-squared statistics from ANCOVA for habitat, sediment oxygen demand (SOD), soil phosphorus (P), and the interaction between habitat and soil phosphorus (P) at 48 h sampling time point.

Source	**df**	**Chi-squared**	***P***
Intercept	1	1.20	0.2738
Habitat	4	12.60	0.0134
SOD	1	15.34	<0.0001
Soil P	1	3.31	0.0689
Habitat: soil P	4	9.85	0.0431

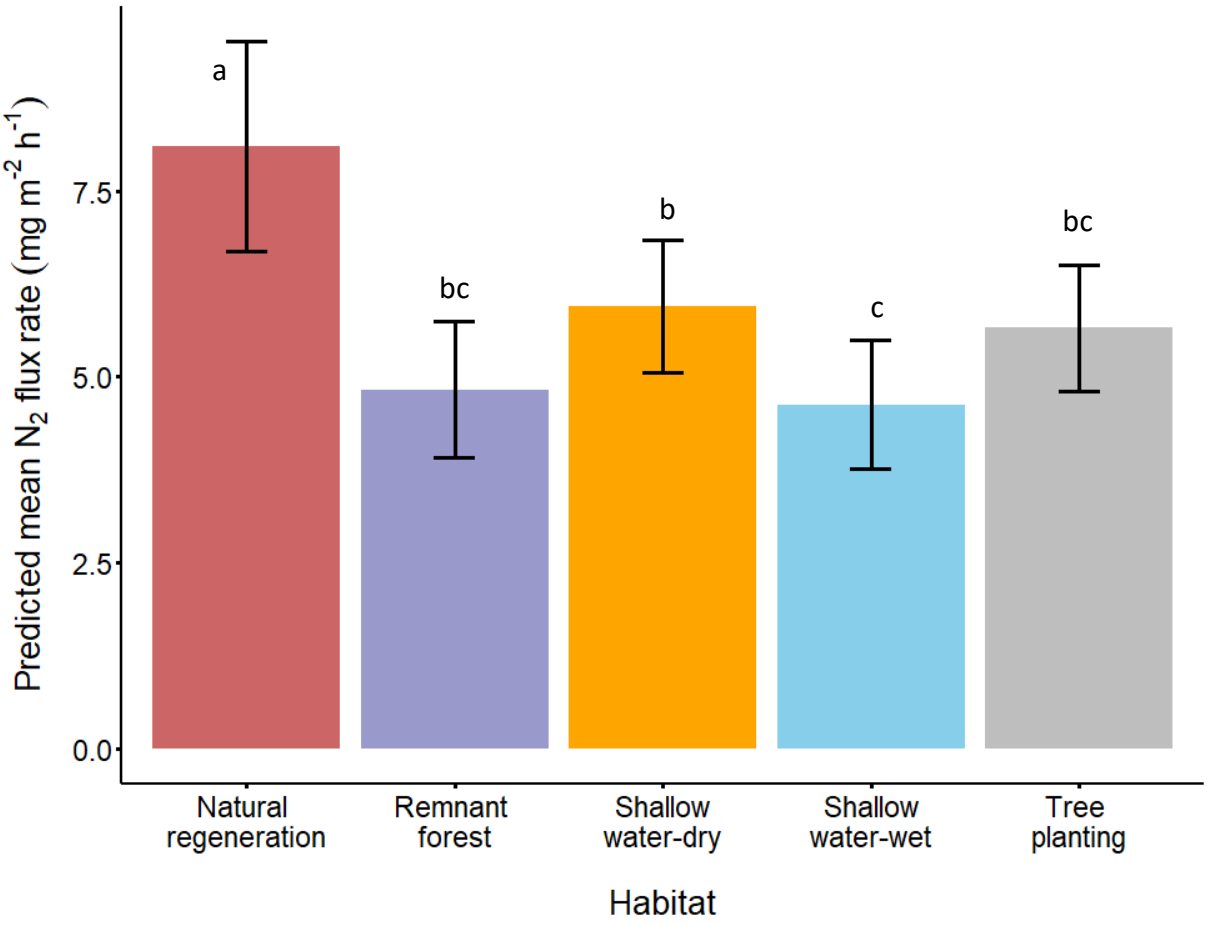

Figure 2.15.

Predicted mean N_2 flux rate among the habitats at 48 h sampling timepoint. Habitats were natural regeneration, remnant forest, shallow water-dry, shallow water-wet, and tree planting. Error bars represent 95% confidence interval. Means associated with different lowercase letter are significantly different by post hoc analysis using Tukey's Honestly Significant Difference.

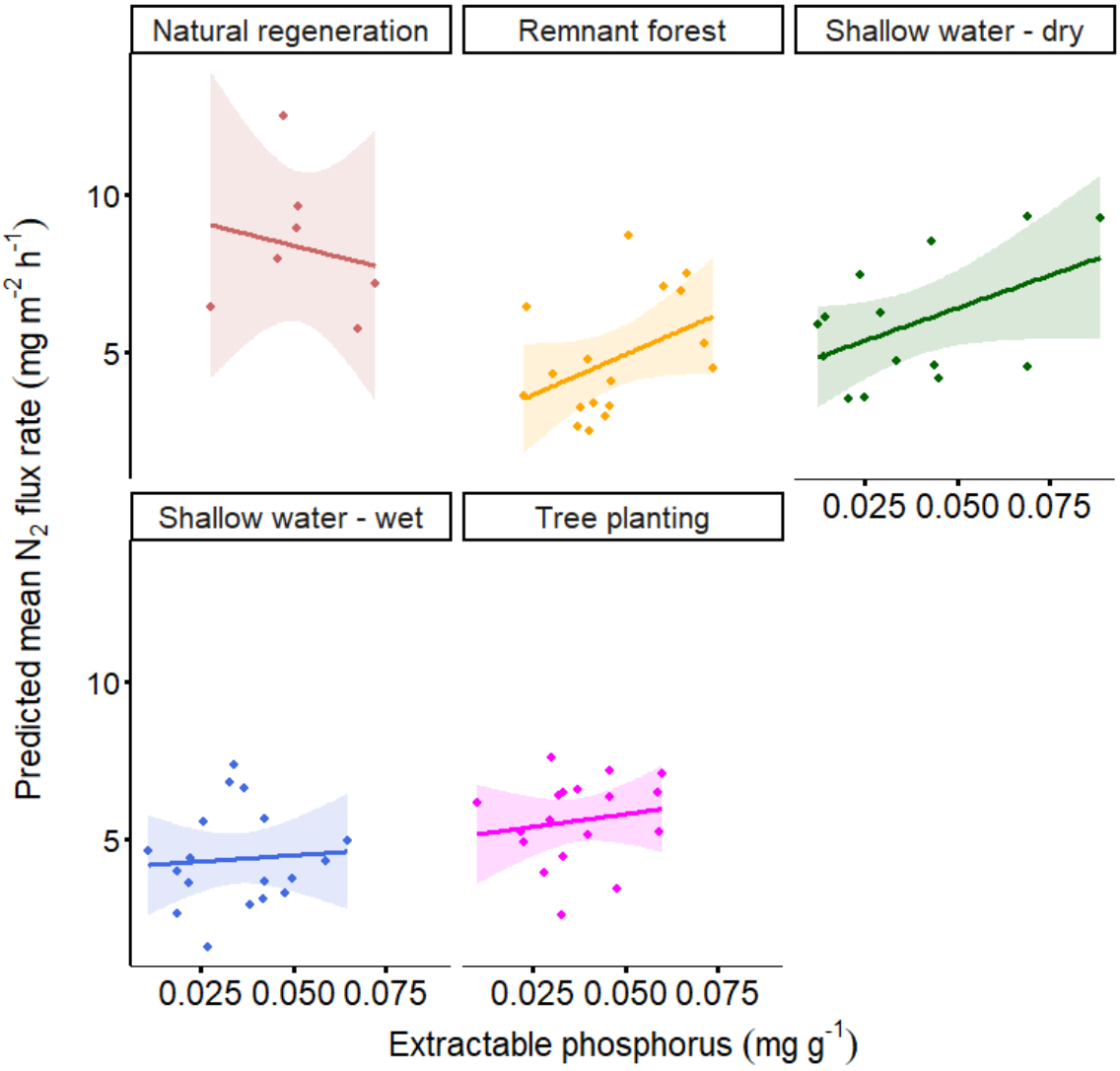

Figure 2.16.

Predicted mean N_2 flux rate among the habitats as affected by soil phosphorus at 48 h sampling timepoint. Habitats were natural regeneration, remnant forest, shallow water-dry, shallow water-wet, and tree planting. Bands represent 95% confidence intervals.

2.7. Discussion

2.7.1. Hypotheses evaluation

Hypothesis i: Soil properties will differ among habitats.

This study aimed to assess whether soil properties and N_2 production rates differ among various habitats in restored riparian wetlands and to establish the connection between soil characteristics and N_2 production rates. My hypothesis that among drier habitats remnant forest habitat will exhibit the highest SM and tree planting habitat will have lowest SM were supported. Despite significant variations in SM across habitat types, the average BD remained relatively consistent at around 1.02 g cm^{-3}. My hypothesis that soil pH would be highest in shallow water-wet habitat was supported. Contrary to my hypothesis that soil TC and TN would be highest in the shallow water-wet habitat, it was actually the lowest. Notably, mean soil P levels were quite similar across the habitats.

Hypothesis ii: Nitrogen gas production will differ among habitats.

My hypothesis that N_2 gas production will vary among habitats was supported at both 24 h and 48 h. However, the results did not align with my initial hypothesis, which anticipated the highest N_2 production in the habitat with the highest SM (i.e., shallow water-wet habitat). Instead, N_2 production from the shallow water-wet habitat was the lowest at both 24 h and 48 h. Conversely, N_2 production was highest in the natural regeneration habitat at both sampling time points, which possessed an intermediate SM content.

Hypothesis iii: Nitrogen gas production will be affected by soil properties.

Nitrogen gas production was influenced by soil properties in general. I predicted that SM would correlate positively with N_2 production. Interestingly, SM correlated positively with N_2 production only in remnant forest habitat at 24 h. In natural regeneration and shallow water-wet habitat, SM correlated negatively with N_2 production at 24 h. There was no significant effect of SM on N_2 production at 48 h. No effect of BD was observed on N_2 production. My assumption that soil pH would correlate positively with N_2 production rates was not supported. The impact of soil pH was only evident in the shallow water-dry habitat at 24 h, where the correlation was negative. Moreover, no effect of soil TC and TN was observed on N_2 production rates. Soil P was the only soil nutrient that affected N_2 production rate estimates for the habitats, and the strength of this effect was variable across sampling time points.

2.7.2. Evaluation of soil properties

Sediment oxygen demand

Sediment oxygen demand would significantly affect denitrification rates across all time points was supported as SOD influenced mean flux rates among the habitats at both 24 h and 48 h. The significant effect of SOD on N_2 production suggests that redox potential which is a measure of the oxidation/reduction status of sediments influenced N_2 flux rates. Redox potential is one of the most important factors governing denitrification in wetlands (Seo & DeLaune, 2010). When SOD is high, it leads to rapid O_2 consumption in the sediment, creating an anoxic low redox environment suitable for optimal denitrification (Cornwell et al., 1999). Therefore, higher SOD levels increase denitrification rates in aquatic sediments (McCarthy et al., 2007; Seitzinger, 1988).

In many freshwater sediments, the major source of NO_3^- for denitrification is NO_3^- produced within the sediments themselves, rather than NO_3^- diffusing from the overlying water (Seitzinger, 1988). Therefore, positive correlation between N_2 production rates and SOD may also be because higher SOD promotes the production of NO_3^- within the sediments during the mineralization of organic matters, providing a greater substrate for denitrification (Seitzinger, 1988). In conclusion, the positive correlation between N_2 production and SOD can be attributed to the promotion of anoxic conditions and the production of NO_3^- within sediments. Sediment oxygen demand may be a reliable predictor of the N_2 production potential given its significant effect on predicting mean N_2 production rates among the habitats during 48 h incubation.

Soil moisture (SM)

Initial SM content strongly affected mean N_2 production estimates during the first 24 h of flooding, but this effect was no longer preset at 48 h. At 24, SM was correlated positively with N_2 production in remnant forest habitat. This impact is likely due to the lower redox potential in moist soils (Keddy, 2010), which supports more denitrifying microbes and their metabolic activity (Klemedtsson et al., 1988; Ma et al., 2020). Interestingly, SM was correlated negatively to N_2 production in natural regeneration and shallow water-wet habitats. Higher SM may have led to the leaching of substrates (i.e., organic matter), reducing their availability for denitrifiers. This limitation in substrate availability can result in decreased denitrification rates (Wang et al., 2023; Xiong et al., 2017) and ultimately N_2 production.

Soil pH

The effect of soil pH on N_2 production was found only at 24 h sampling timepoint. There was no significant effect of soil pH alone on N_2 production rates but significant interaction between habitat and soil pH on N_2 production was observed. Surprisingly, the effect was only seen in shallow water-dry habitat. These relations were absent in natural regeneration, remnant forest, shallow water-wet, and tree planting habitats.

The pH of soil from shallow water-dry habitat ranged between 4.3 and 6.5 (Table 2.2), showing a typical, acidic characteristic of the soil. The highest N_2 production via denitrification in the shallow water-dry habitat was observed when the soil pH was the lowest. The relationship between soil pH and denitrification has been extensively researched, with most studies finding higher denitrification rates at circumneutral pH (Thomsen et al., 1994; Wijler & Delwiche, 1954), so it is notable that the opposite was true in this study for shallow water-dry habitat. However, in few studies similar results have been observed in low pH (≤ 4.5) soils of tropical rainforests (Tiedje et al., 1983), created nontidal freshwater wetlands (Peralta et al., 2013), and mixed hardwood and heath wetlands (Seitzinger, 1994) revealing that denitrifying bacteria can be more active in strongly acidic soils (Schlesinger, 1997). Studies have shown that edaphic conditions, including pH, can directly and indirectly regulate denitrification rates by altering denitrifier abundance and activity (Xiong et al., 2017). Therefore, it is possible that in the shallow water-dry habitat, the microbial communities may be adapted to more acidic conditions and the increase in soil pH may not favor the growth of acidophilic microbial taxa, which could result in decreased denitrification activity. Additionally, denitrification enzyme activity may be inversely related to soil pH in the acidic environment (Ahn & Peralta, 2012), which may decrease the denitrification activity.

In summary, it is important to note that the relationship between pH and N_2 production during denitrification is complex and can be influenced by various factors, including specific environmental conditions, organic matter content, nutrient availability, and microbial community composition (ŠImek & Cooper, 2002). The specific differences observed between shallow water-dry habitat and other remaining habitats may be influenced by variations in environmental conditions and microbial communities between the habitats, but it needs a further investigation.

Soil nutrients

Nitrogen gas production was not affected by soil TC and TN. Except in the natural regeneration and shallow water-dry habitats, soil P showed a positive correlation with N_2 production. The positive correlations between soil P and N_2 production observed in this study was consistent with those of O'Neill et al. (2022) and Zhang et al. (2012), who found that elevated soil P levels enhance denitrification and increased NO_3^- retention. An increase in soil P can stimulate microbial growth and metabolic activity since P serves as an essential nutrient for microbial growth and activity and can exert direct and indirect influences on denitrification rates (Henderson et al., 2010; Houlton & Bai, 2009). In the natural regeneration habitat N_2 production estimates were negatively correlated with soil P. This negative correlation could be influenced by interactions with other soil properties that were not accounted for in this study.

2.8. Conclusion

Between the 24 h and 48 h sampling timepoints, an increase in N_2 production was observed, with percentages ranging from 6.6% in tree planting habitat to 34.3% in the shallow water-wet habitat. This suggests a pivotal role of flooding duration in determining N_2 production from restored wetlands. As flood duration extended, the influence of soil properties on N_2 production became less prominent. Furthermore, the effect of soil properties on N_2 production was found to vary among different habitat types, highlighting the complexity of the restoration process and the diverse ecological interactions within these habitats. The interactions of flood duration, habitat type, redox state, and soil properties suggest complexities in predicting N_2 production in restored agricultural floodplain wetlands. Moving forward, a comprehensive analysis encompassing variations in soil properties, microbial communities, and the presence of localized hotspots and microsites becomes essential for effectively predicting outcomes of restoration efforts.

CHAPTER 3: EFFECTS OF VEGETATION AND FLOOD DURATION ON POREWATER NITROGEN AND PHOSPHORUS RETENTION IN WETLAND MESOCOSMS

3.1. Abstract

Wetland restoration initiatives commonly emphasize the restoration of the natural hydrologic regime and the reestablishment of native vegetation. This research undertook a wetland mesocosm experiment to assess the impacts of wetland habitat types and hydrological conditions on the retention of nitrate (NO_3^-) and phosphate (PO_4^{3-}), as well as the rates of nitrogen gas (N_2), methane (CH_4), and nitrous oxide (N_2O) production in soil porewater during a simulated flood. Vegetation types were bare soil as a control, native grass represented by rice cutgrass (*Leersia oryzoides* L.), and tree plantings represented by bald cypress (*Taxodium distichum* (L.) Rich) and river birch (*Betula nigra* L.). The hydrology treatments included 3-day and 3-week inundation flood regimes. Following an eight week of flood cycles, mesocosms were flooded with nutrient-rich water, and the concentration of porewater NO_3^- and PO_4^{3-} was measured over five days. Following porewater measurements, sediment cores were incubated in a flow-through system and potential dissolved gas flux rates were assessed over 48 h. Initial porewater NO_3^- concentrations were significantly different between hydrology levels, while initial PO_4^{3-} concentrations varied significantly among vegetation types. Nitrate flux was significantly affected by habitat types and initial NO_3^- concentration in the porewater. Specifically, the native grass habitat retained a significantly higher amount of NO_3^- when compared to the bare soil and tree planting habitats. Habitat exerted independent effect on PO_4^{3-} flux during the initial 24 h, after which it interacted with hydrology. During the initial 24 h post-dosing, there was a mean

PO_4^{3-} release in bare soil (both hydrology levels) and tree planting subjected to 3-day flooding. Notably, native grass habitats exhibited the highest efficiency in reducing NO_3^- and PO_4^{3-}, achieving >95% and >85% reduction, respectively, on average by day 3 for both hydrology levels. Nitrogen gas production increased consistently across all habitats and hydrology treatments during the 48 h incubation period, signifying the importance of water residence time in maximizing nitrogen (N) removal. At 12 h, N_2 production was affected by sediment oxygen demand (SOD) and soil total nitrogen (TN), while at 24 h and 48 h, only SOD was significant. Minimal production of N_2O and CH_4 was observed in all treatments throughout the 48 h incubation period. These findings suggest that wetland vegetation and hydrology can impact nutrient retention in the porewater and gas production, either independently or through their combined effects. Moreover, as the duration of flooding extended, the differences among treatments tended to decrease, indicating that the water residence time becomes more important than vegetation as flood duration increases.

3.2. Introduction

The USA has lost 54% of its original 87 million ha of wetlands (Tiner, 1984), primarily to drainage for agricultural production. Wetland drainage changes the water flow path (Holden et al., 2006). The creation of a drainage channel contradicts with natural flow pattern and causes less water to reach various parts of wetlands leading to lower water tables, especially in sloping wetlands (Holden et al., 2004). Studies have shown that drained peatlands demonstrate relatively less overland flow and more subsurface flow (Holden & Burt, 2003). Water has always been the key to the management of the Lower Mississippi River Basin (LMRB). Despite frequent flooding, LMRB was recognized as an agricultural hotspot due to the availability of nutrient-rich

soil (USDA, 2012). Subsequently, in the mid-19th century, flooding was controlled, and 75% of riparian forests were clear-cut to develop LMRB for agriculture (Faulkner et al., 2011). While agriculture is still the dominant land use in LMRB, clearing the forest has destabilized uplands (USDA, 2012) and caused downstream eutrophication in the Gulf of Mexico due to rapid drainage of floodwater containing agricultural nutrients (Venterink et al., 2002). Therefore, restoring former agricultural lands into wetlands is challenging due to the lasting impacts of extensive cultivation, which can result in enriched and subsided soils, subsequently influencing hydrology and plant communities (Wong et al., 2011). This emphasizes the critical importance of considering both vegetation and hydrology in wetland restoration, particularly while restoring agricultural wetlands. Johnson et al. (2013) further emphasizes that wetland restoration can be successful when land managers pair vegetation and hydrology correctly.

Wetland restoration in the LMRB has focused on both restoring the natural hydrologic regime and restoring native vegetation. To restore hydrological conditions, wetland managers have blocked drainage in many wetlands (Armstrong et al., 2010; Howie et al., 2009; Wallage et al., 2006). Alternatively, introducing vegetation adjacent to farmland, floodplains, and wetlands might help to reduce nutrient-rich agricultural loads. However, limited studies have been done on how vegetation affects nutrient removal from floodwater. Nevertheless, the relation between floodwater residence time and degree of nutrient removal is well documented (Karim et al., 2013). A comprehensive understanding of interactions between vegetation and hydrology may help to guide restoration initiatives and promote nutrient retention in restored wetlands.

Vegetation can reduce the velocity of water flowing across wetlands (Holden et al., 2007) and increase floodwater residence time. Surface vegetation cover could be of greater importance, especially for upland wetlands (Ballard et al., 2011; Lane & Milledge, 2013). Type of vegetation

influences floodwater resistance and consequently the reduction in flow rate (Acreman & Holden, 2013). Harvey et al. (2009) reported that floodwater velocities in densely vegetated ridges were 29% lower than in sloughs in the Everglade wetlands. Wetlands with trees, in particular, store more floodwater during wet periods and therefore reduce flood peaks and increase water travel time (Thomas & Nisbet, 2007). An increase in water residence time can allow longer contact time between nutrient-rich floodwater and sediments both on the surface and in the soil pores. This condition will allow more opportunities to remove nutrients from the water due to microbial assimilation and plant uptake. Alternately, hydroperiod (wetting and drying cycles) can affect microbial degradation and leaching of litter and soil organic matters (Battle & Golladay, 2001, 2007; Chow et al., 2006; Watt & Golladay, 1999). Therefore, understanding the role of water residence time, drying/wetting cycle, vegetation, and their interaction with natural biogeochemical cycles and nutrient processing in restored wetlands can provide an important benchmark to guide the USDA-Natural Resources Conservation Service (NRCS) wetland restoration goals.

Few studies suggest that plants modify the sediment environment, which may indirectly play a crucial role in nutrient removal via denitrification (Caffrey et al., 2007). The sediment environment is altered by plants either by competing for water and NO_3^- (Kirk & Kronzucker, 2005), introducing oxygen (O_2) from root diffusion (Cufrey & Kemp, 1992), consuming O_2, regulating litter input and quality, or by producing labile organic carbon (C) compounds that shape microbial community in the rhizosphere (Malique et al., 2019). The type of vegetation used for restoration is likely to affect denitrification in restored wetlands since vegetation type favors the growth of specific bacterial communities in the rhizosphere compared to bulk soil (Costa et al., 2006; Drigo et al., 2009; Herrmann et al., 2009; Trias et al., 2012). Plants like bald

cypress and rice cutgrass inhabit wetlands with nutrient-rich soil and slow-moving water or waterlogged conditions. Therefore, they are excellent vegetations for wetland restoration and remediation (Darris & Bartow, 2004; Parresol, 2002). Bald cypress, in particular, helps in maintaining high regional water, provides flood control and groundwater recharge, and helps in wastewater treatment (Ewel, 1990). It also accumulates inorganic sediments and plays an important role in nutrient retention and maintenance of water quality (Parresol, 2002).

Research needs on effects of vegetation and hydrology in porewater nutrient retention in restored wetland

Increasing the water residence time in restored wetland is important not only for reducing nutrient flow downstream but also for promoting the infiltration of nutrient-rich water into the soil pores. This dual action serves to prolong the duration of water residence while simultaneously improving nutrient sequestration. Furthermore, it facilitates nutrient uptake by wetland vegetation and microbial communities, thereby contributing to overall health and ecosystem function of restored wetlands. Since wetlands act as natural filters, the retention of nitrogen (N) and phosphorus (P) in the soil pores will ultimately help to trap and remove excess nutrients from the surface water. This retention mechanism, in turn, helps to improve water quality by preventing nutrient overload downstream.

Although assessments of nutrient retention in restored riparian wetlands have received global attention, the focus has primarily centered on nutrient retention at the sediment-water interface, as evident in numerous studies (Jordan et al., 2003; Newcomer Johnson et al., 2016; Reddy et al., 1999). Some investigations have explored porewater chemistry in restored tidal wetlands (Hoffmann et al. 2011), restored bogs (Howson et al., 2021), and boreal peatlands

(Haapalehto et al., 2014). Others have primarily concentrated on porewater P (Surridge et al., 2007), salinity (Lee et al., 2021), and herbicide concentration (Lawrence et al., 2015). Therefore, there is growing need to research on porewater nutrient retention in restored riparian wetlands, with a specific emphasis on understanding how vegetation and hydrology interact to influence nutrient retention.

Nonetheless, field studies face challenges when attempting to isolate the effects of specific factors of interest due to the presence of potential confounding factors that can influence the response variable, potentially leading to biased results if not adequately addressed (Howards, 2018). Moreover, ecological systems are inherently dynamic and subject to natural variability, making it difficult to control for all potential sources of variation (Ziegel et al., 1995). Therefore, it is essential to establish a robust experimental design that minimizes confounding and ensures that the factor of interest is the primary driver of the observed responses (Rothery & Hairston, 1991). This could be achieved through a controlled experimental setting that enables the independent manipulation of vegetation type and hydrology. Gaining a deeper understanding of how vegetation and hydrology interact to impact nutrient retention will provide valuable insights to help land managers make informed decisions regarding the choice of vegetation types and flood duration management in future restoration projects.

3.3. Objectives

i) determine if inorganic N and P retention rates in the porewater and N_2, N_2O, and CH_4 production rates differ among bare soil, native grass, and tree planting habitats

ii) determine the effects of flooding duration in porewater N and P retention rates and N_2 production rates

iii) determine the effects of soil properties in porewater N and P retention rates and N_2 production rates

3.4. Hypotheses

i) Nitrogen and P retention in the porewater and N_2, N_2O, and CH_4 production will be affected by habitat types. Bare soil treatment will retain the least N and P because of an absence of vegetation for nutrient uptake and assimilation from the soil pores (Taylor et al., 2015). Nitrogen and P retention will be highest for the native grass habitat due to the presence of extensive root system which enables more availability of labile C from roots and enhances N_2 production during denitrification (Gift et al., 2010; Pinay et al., 1993). Consequently, increased organic matter inputs and quick decomposition of labile C by microbial activity will favor higher CH_4 production in the native grass habitat (del Aguila Pasquel, 2017; King et al., 1998; Liu et al., 2020). Native grass habitat will also promote higher N_2O production rates due to their potential to enhance denitrification processes (Liu et al., 2020). The production of N_2O will be lowest in tree planting habitat compared to bare soil and native grass habitats because bald cypress and river birch have mycorrhizal association which helps to increase nutrient uptake and assimilation by

ii) Nitrogen and P retention in the porewater and N_2 production will be affected by flooding durations. Nitrate and PO_4^{3-} retention will be higher for 3-week flooding because flooding duration can affect the nutrient limitation of wetland plants. Longer flooding durations can lead to decreased nutrient limitation, as the continuous presence of water can enhance nutrient availability and uptake by plants (Lan et al., 2021). Furthermore, longer flooding durations can result in more reducing soil porewaters, which can enhance nutrient retention by reducing the mobility and leaching of nutrients. Additionally, reducing conditions can promote denitrification (Lan et al., 2021), leading to increased N_2 production.

iii) Nitrogen and P retention in the porewater and N_2 production will be affected by soil properties. Soil total carbon (TC) and TN will be the most influential soil properties for NO_3^- retention and N_2 production, and soil P for PO_4^{3-} retention. Nitrate retention in the porewater will correlate positively with soil TN because nutrient-rich sediment favors higher N_2 production (Groffman & Hanson, 1997; Warneke et al., 2011). Phosphate retention in the porewater will correlate negatively with soil P. Soils with high P content have been associated with increased P release upon rewetting (Bostic & White, 2007).

3.5. Methods

3.5.1. Mesocosms setup

The experiment was conducted in an outdoor set-up in Shipley Farm, Tennessee Technological University. Thirty-six 379 L tubs (61 cm x 61 cm x 122 cm) were established according to the protocols of Tyler et al. (2012) and Taylor et al. (2015). The tubs were arranged in four rows of nine and filled with sand up to 30 cm from the bottom (Figure 3.1 & 3.2).

Another 15 cm was filled with soil obtained from the stream restoration project. Mesocosms were seeded equally by spreading a small amount of wetland soil on top of the 15 cm sediment. Two holes were drilled to fit discharge hoses (0.95 x 0.64 cm). One hole was drilled corresponding to the soil surface and another near the bottom to maintain the water level. Discharge hoses were plugged during hydrological treatments to maintain approximately 10 cm water column on top of the sediment. Cookeville municipal water was supplied to the mesocosms by a gravity-fed system from one storage tank (568 L) per row placed on the top of the cinderblock tower. Water was dechlorinated with sodium thiosulfate in each storage tank. Storage tanks were connected to PVC pipes (i.d. 3.81 cm) with outflows controlled by ball valves at each mesocosm.

3.5.2. Plant and soil preparation

Saplings of river birch (*Betula nigra* L.) and bald cypress (*Taxodium distichum* (L.) Rich), and rice cutgrass (*Leersia oryzoides* L.) were used for this study. These plants were selected because the trees are among the species planted by NRCS during wetland restoration, all of these species are abundant in the WRP easements in Kentucky (KY) and Tennessee (TN), and all are fairly tolerant to prolonged root saturation. Bald cypress and river birch were purchased from a local nursery. Rice cutgrass was collected from a riparian area of Little Creek in Cookeville Tennessee, USA (Latitude: 36.196383, Longitude: -85.529292). The soil was obtained from a wetland restoration project in west Tennessee and was provided by the West Tennessee River Basin Authority. 60 cm deep Falaya topsoil (Coarse-silty, mixed, active, acid, thermic Aeric Fluvaquents) was collected from a restoration site in west Tennessee adjacent to the Middle Fork Forked Deer River, Tennessee, and delivered to Shipley Farm, Tennessee

Tech University. Falaya soil is somewhat poorly drained and available widely in the Southern Mississippi Valley Silty Uplands floodplain (National Cooperative Soil Survey, 2013). Saturated wetland soil from a Natural Resources Conservation Service (NRCS) conservation easement in western Kentucky was collected to seed mesocosms with relevant microbial communities. The microbial communities in the wetland soil are representative of those found in restored flood plain wetlands in Tennessee (TN) Kentucky (KY) and were expected to help in the establishment of microbial communities in the mesocosm.

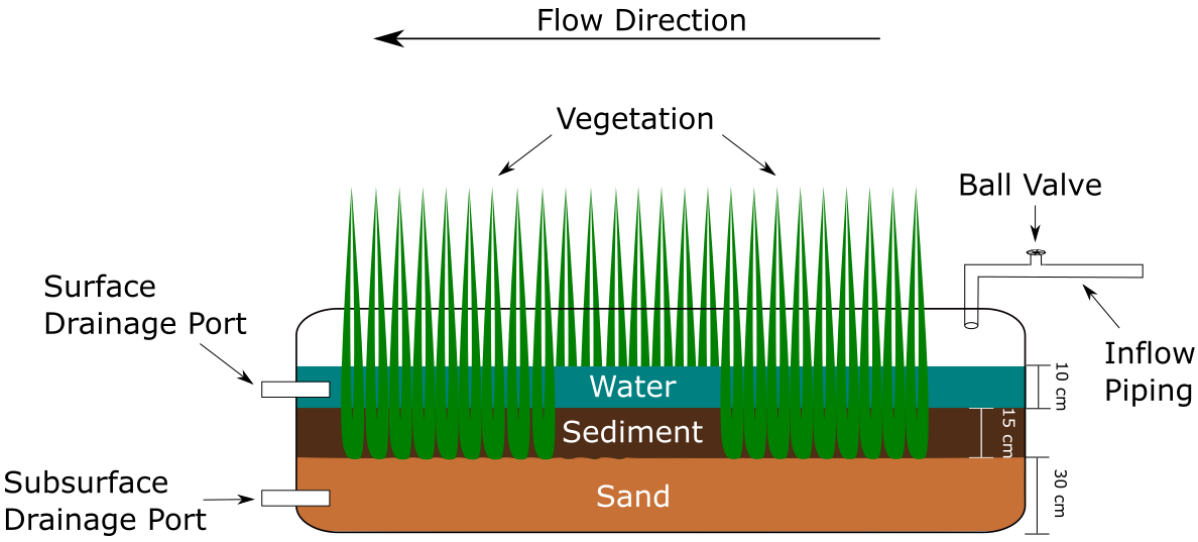

Figure 3.1.

Diagram of a mesocosm.

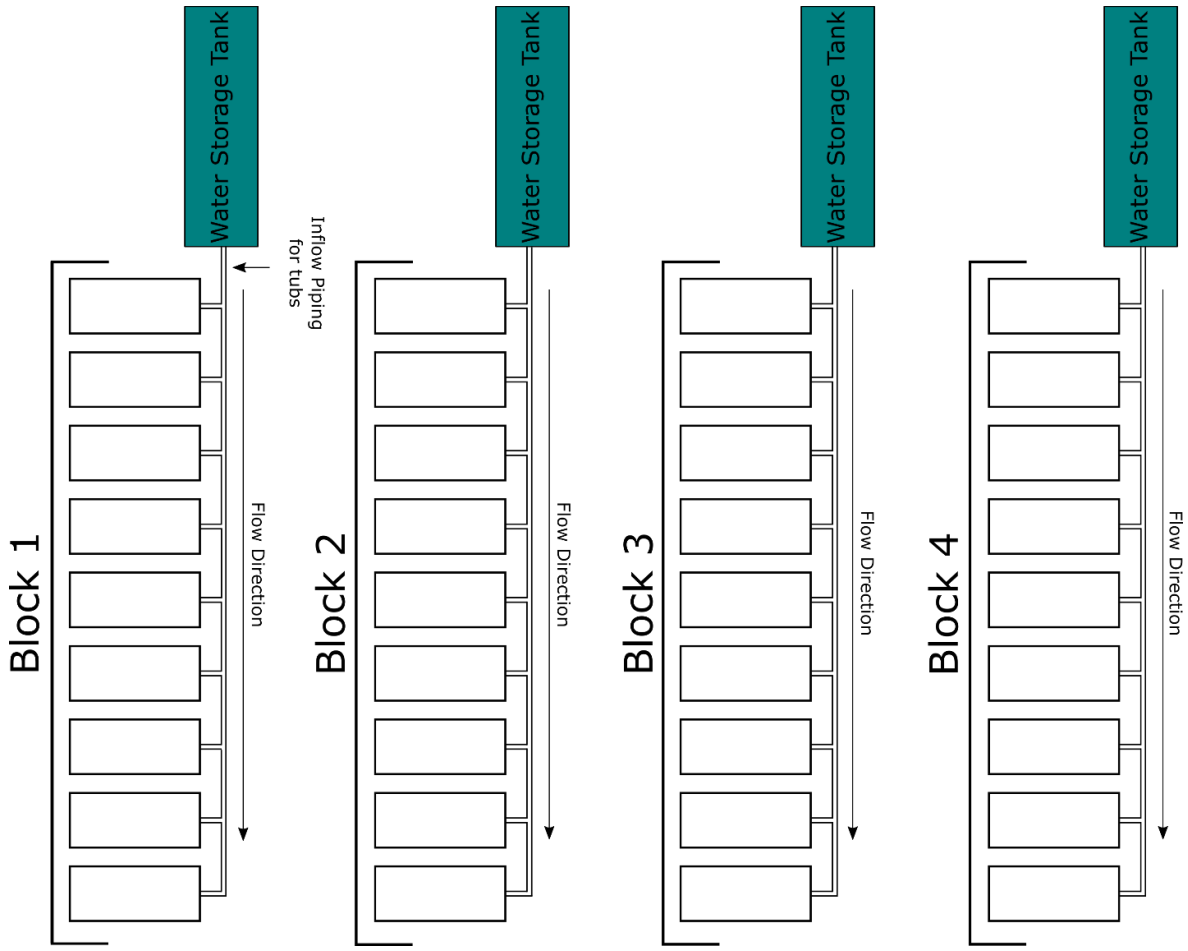

Figure 3.2.

Diagram of experimental design and plumbing for the mesocosms.

3.5.3. Treatment application

The experimental factors were hydrological regime and vegetation type. The hydrological factor had two levels: 3-day flooding and 3-week flooding. The mesocosms receiving 3-day flooding were flooded for 3 days, drained and dried for 4 days, and flooded again. Those receiving 3-week flooding were flooded for 3 weeks, drained and dried for 1 week, and flooded again. The flooding and drying process took place for 8 weeks prior to the experiment. Vegetation types had three levels: bare soil as control, native grass habitat represented by rice

cutgrass, and tree planting habitat represented by river birch and bald cypress. In a two-factor experimental design, six hydrology/vegetation treatment combinations were distributed among four rows, and each treatment had six replicates. A random number generator was used to assign treatments. Two river birch and two bald cypresses were planted in each mesocosms designated for tree planting treatment. The same tree species were planted on either end of the of the mesocosm. Rice cutgrass was planted densely to completely cover the soil surface (Figure 3.4). The trees were allowed to acclimatize to the new environment for one year before starting the hydrological treatment.

Table 3.1.

Treatment factor combinations (BS = bare soil, NG = native grass, TP = tree planting; 3-days = 3-day flooding, 3-weeks = 3-week flooding).

Hydrology	**Habitat**		
	Bare soil	**Native grass**	**Tree planting**
3-day flooding	BS×3-days	NG×3-days	TP×3-days
3-week flooding	BS×3-weeks	NG×3-weeks	TP×3-weeks

Figure 3.3.

Assembled mesocosms with assigned habitat treatments. Note the bottom right mesocosm is not part of the study and was used to house extra trees to replace trees that died during the experiment setup. However, all trees survived, and none needed to be replaced.

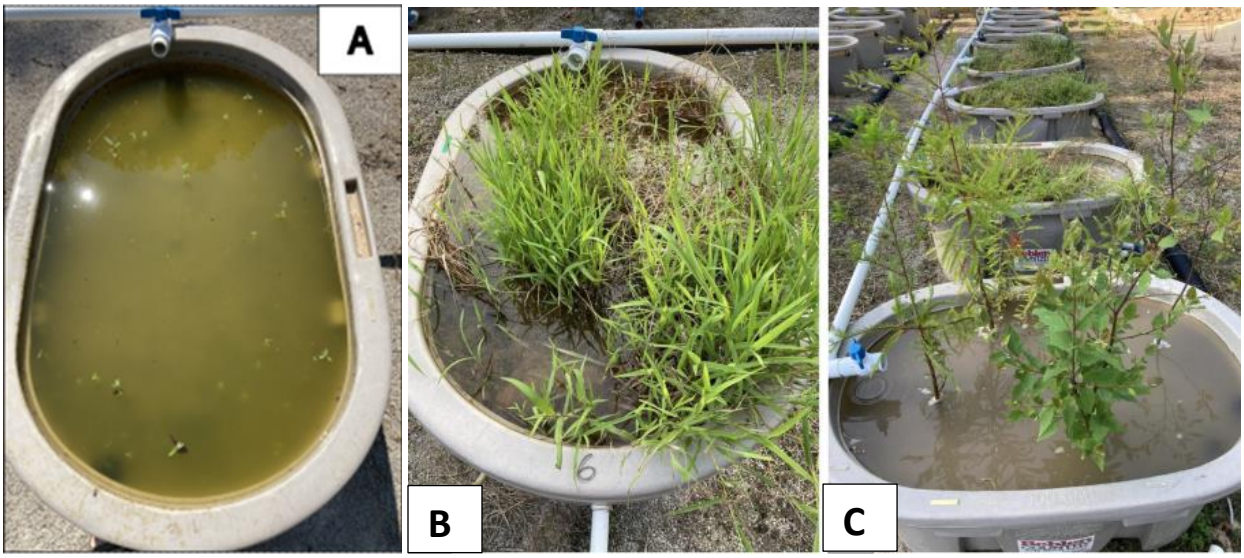

Figure 3.4.

A) Bare soil, B) native grass, and C) tree planting habitats.

3.5.4. Nutrient enrichment and porewater sampling and analysis

Following an 8-week flooding cycle, mesocosms were inundated with high nutrient water to simulate flooding by nutrient-rich river waters as occurs in the WRP easements in TN and KY. Sodium nitrate ($NaNO_3$) and potassium phosphate (KH_2PO_4) were added to dechlorinated water in the storage tanks creating a final dosing concentration of approximately 10 mg L^{-1} and 1 mg L^{-1} of N and P, respectively. The high concentrations were expected to ensure nutrient uptake saturation, giving maximum N and P uptake potential of mesocosms, and reducing nutrient competition between plants and soil microbes.

One porewater sampler per mesocosm was installed to a depth of 10 cm in the middle of each tub one day before porewater collection (Figure 3.5). Porewater samplers were made using a 25 cm metal tube (i.d.5 mm) with the tapered tip. The tube was drilled with ten-1 mm diameter holes spaced 1 cm apart. The lowest hole was drilled 1 cm above the tip. The tapered tip helped in the easy installation of samplers in mesocosms. An adjustable silicone collars was fitted to samplers to reduce surface water movement down the tube during sample collection. The end protruding from the sediment surface was connected to tubing and the free end of the tubing was attached to a Luer lock connector.

One porewater sample per mesocosm was taken immediately after high-nutrient water flooding and at approximately 24 hour intervals for the next 5 days. 45 mL porewater samples were drawn by connecting a 50 mL syringe to the Luer lock connector and slowing drawing in water from the sampler. Water was transferred to centrifuge tubes. Samples were transferred to the lab on ice and vacuum filtered using a 0.7 µm glass fiber filter. The filtrate was immediately placed in a freezer at -20^{o}C and stored until analyzed. The filtrate was analyzed for dissolved organic carbon (DOC) using a Shimadzu TOC-L/TN analyzer (Shimadzu Corporation, Kyoto,

Japan). Nitrate and PO_4^{3-} concentrations were measured via colorimetric analysis using a SEAL AQ400 Discrete Analyzer. Nitrate was measured via cadmium coil reduction to NO_2^- followed by a sulfanilamide reaction (EPA Method 353.2). Phosphate (reported as soluble reactive phosphorus (SRP) was measured using the ascorbic acid method (EPA Method 365.1).

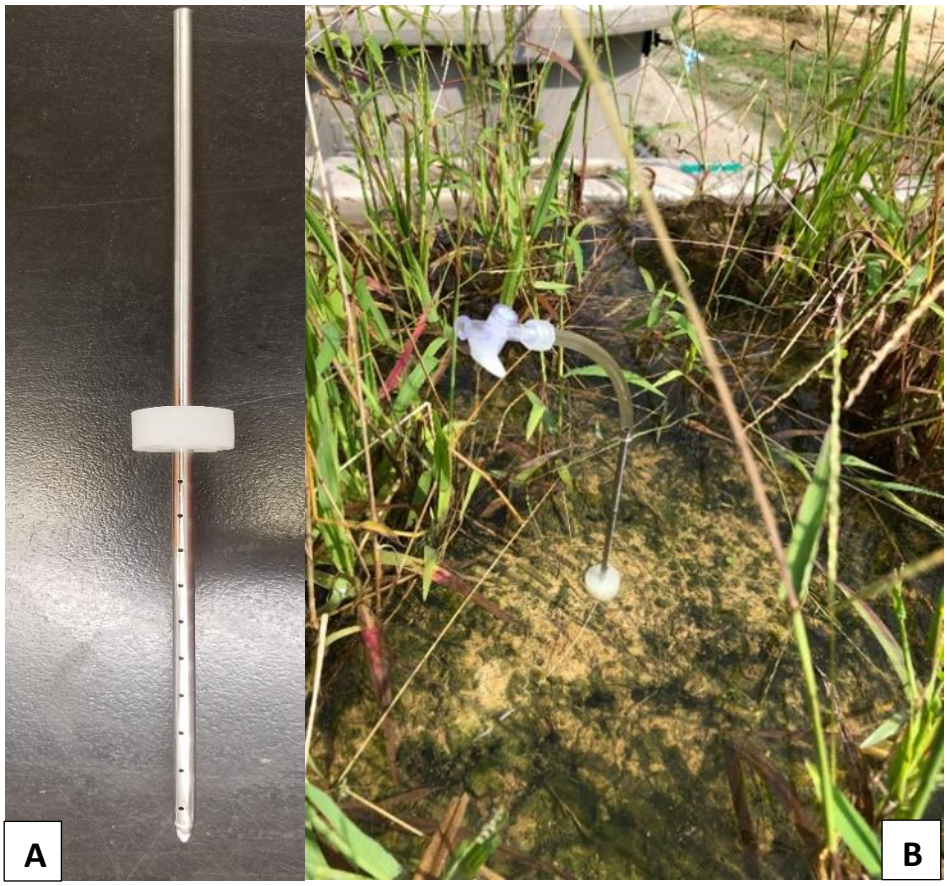

Figure 3.5.

A) Porewater sampler with holes and silicone collar and B) Installed pore water sampler.

3.5.5. Soil sampling and analysis

A 10 cm deep soil core for assessing background soil nutrient conditions was collected using a hand auger (i.d. 2.54 cm) before nutrient dosing. The cores were collected again on day 5 after completing porewater sampling and draining mesocosms. Three soil cores, one each from the head (towards pipe supplying water to mesocosms), middle, and outflow (towards water drainage hose) were collected and then pooled in a Ziploc bag. The bags were placed in a cooler and transported to the lab for processing. The soil samples were dried in an oven at 60^{o}C and then ground to pass a 2 mm mesh screen. A subset of ground soil from each sample was transferred to labeled 20 mL plastic scintillation vials and shipped to the Soil Testing Lab at Kansas State University where soil TC, soil TN, and extractable P (soil P) were analyzed as described in Chapter 2.

3.5.6. Algae biomass and ash-free dry mass analysis

After completing porewater collection, tubs were left to drain overnight. On the subsequent morning, samples were collected for soil surface chlorophyll-*a* (chl-*a*) and ash-free dry mass (AFDM) content. To collect these samples, the surface area of each tub was divided into 25 individual grids, organized as 5 x 5 transects (Figure 3.6). A random number generator was used to choose one of the 5 available grids along a row for collecting both chl-*a* and AFDM samples. A 50-mL centrifuge tube (2.7 cm diameter) was used to collect samples by pushing it into the sediment surface to a depth of 1 cm (equivalent to 1.35 cm² of soil per transect). Two samples were collected at each location, one for chl-*a* and one for AFDM. This process was repeated once along each of the 5 transects, yielding approximately 6.75 cm² of sample area per tub.

Chlorophyll-*a* samples were frozen for a minimum of 24 h. Chlorophyll-*a* extraction involved submerging each sample in a 95% ethanol solution and then heating the samples to 78°C using a water bath. The samples were then stored in dark for 24 h to complete the extraction process. The extracted samples were analyzed using the Welschmeyer method (non-acidification) on a Turner Designs Trilogy fluorometer (Welschmeyer, 1994) and scaled to chl-*a* mg cm^{-2} using the calculated surface area of the collection tube, which was aggregated across the 5 subsamples. Ash-free dry mass samples were first dried at 60° C for 24 h upon returning to the lab and subsequently combusted in the muffle furnace at 500ºC to determine AFDM.

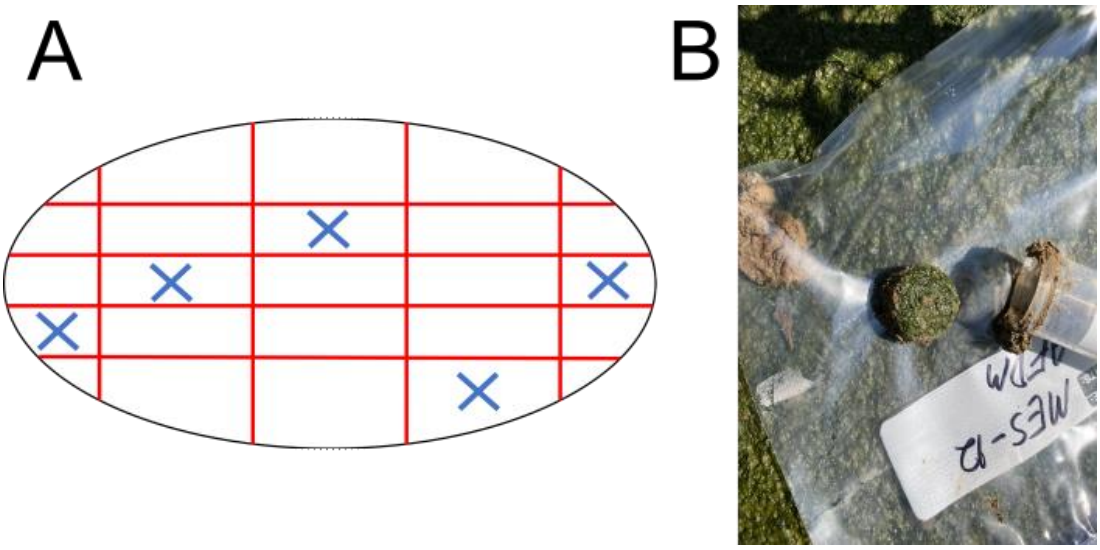

Figure 3.6.

Diagram of the sampling grid used for algal biomass and organic matter sampling. A) blue Xs represent randomly selected sampling locations, B) An example of a soil sample collected from one grid.

3.5.7. Core incubation and gas sampling

A 15 cm deep sediment was collected using an acrylic core (6.76 cm×30 cm) after completing chl-*a* samples collection. One sediment core each was collected near one river birch and one bald cypress from tree planting mesocosms (total 2 cores from each tree mesocosm). This was done to capture any within-mesocosm variability due to differences in tree species. One sediment core/tub was collected from the middle of the tub in bare soil and native grass mesocosms. The acrylic cores were sealed with lids fitted with sampling ports and secured with pipe straps. Cores were incubated using a flow-through incubation method at 24^{o}C (described in Chapter 2). Dechlorinated municipal water containing 10 mg L^{-1} NO_3^--N and 1 mg L^{-1}, PO_4^{3-}-P, was used during the incubation. The first round of dissolved gas samples was collected 12 h after incubation, and the other two rounds were collected at 24 h and 48 h. Samples were dosed immediately with 180 µL sodium hydroxide (NaOH) followed by 157 µL zinc chloride ($ZnCl_2$) to precipitate dissolved carbon dioxide (CO_2) that can interfere with CH_4 measurements and to restrict microbial activities, respectively. Vials were capped quickly and turned upside down a few times to check if air bubbles were trapped in the water sample and to ensure the uniform distribution of added chemicals. The samples were stored underwater at 4^{o}C and analyzed for N_2, O_2, N_2O, and CH_4 within one month of collection as described in Chapter 2.

3.5.8. Data analysis

Nutrient flux

Mesocosm porewater dissolved nutrient (DOC, NO_3^-, and PO_4^{3-}) flux rates were calculated as mg L^{-1} day^{-1}. To determine these rates, porewater nutrient concentrations analyzed immediately after the initial dosing (on day 0) was subtracted from the concentrations on the final day. This difference was then divided by the number of days it took for the flux rates to became non-linear. In order to analyze the daily changes in porewater concentration, mean percent changes in nutrient flux rates among the treatments across sampling days were calculated.

$$Nutrient\ flux\ rate\ (mg\ L^{-1}\ day^{-1}) = \frac{Concentrtion_{final\ day} - Concentration_{after\ inital\ dosing}}{Number\ of\ days\ until\ depletion}$$

$$\%\ change\ in\ mean\ daily\ flux\ rate = \left(\frac{Concentration_j - Concentration_i}{Concentration_i}\right) * 100$$

where $Concentration_j$ represents nutrient concentration on a day j, and $Concentration_i$ represents nutrient concentration of the preceding day.

Phosphate flux

The phosphate concentration data were segregated into two distinct groups and analyzed independently. The first dataset contained PO_4^{3-} flux rates calculated from data up to day 1. This division was made because, within the initial 24 hours after dosing, there was a notable mean release of PO_4^{3-} in treatments involving bare soil, both under 3-day and 3-week flooding conditions, as well as in the tree planting habitat subjected to 3-day flooding. The second dataset

comprised PO_4^{3-} flux rates calculated from data collected during days 2-3. Data beyond day 3 was not included in the statistical model, as almost all of the added P was removed by day 3.

Missing data

For all analyses, specific data exclusions were made for various reasons. First, data from mesocosm #4 (bare soil habitat– 3-day flooding) were omitted due to missing soil nutrient data and an erroneous chl-*a* value caused by incomplete extraction. Furthermore, during the analysis of PO_4^{3-} from day 2-3, data from mesocosms # 1 and 15 were removed (tree planting habitat– 3-day flooding, tree planting habitat– 3-week flooding) because they were large outliers. In the context of soil core incubations, no data was available from mesocosm #33's soil core (native grass habitat – 3-week flooding) during the 12 h sampling timepoint, due to a pump issue. The issue was resolved before the 24 h sampling event, and consequently, dissolved gas data for mesocosm #33 was included in 24 h and 48 h sampling timepoints. The resulting sampling size were: n = 35 for DOC and NO_3^-, n = 35 for 24 h PO_4^{3-}, n = 33 for 2-3 days PO_4^{3-} , n = 34 for 12 h N_2, N_2O, and CH_4, and n = 35 for 24 h and 48 h N_2, N_2O, and CH_4 analyses.

3.5.9. Statistical analysis

Response variable, treatment factors, and covariates

In this study, the response variables were DOC, NO_3^-, PO_4^{3-}, and N_2 flux rates. The objective was to analyze the effects of vegetation and hydrology on DOC, NO_3^-, PO_4^{3-}, and N_2 flux rates. Therefore, habitat, hydrology, and the interaction between habitat and hydrology were used as treatment factors. Additionally, soil properties were used as covariates. These covariates included soil TC, soil TN, soil P, chl-*a*, and AFDM. Soil nutrient values (soil TC, TN, and P)

derived from samples collected prior to porewater collection were used to assess their effects on DOC, NO_3^- and PO_4^{3-} flux rates, while values from samples collected post-porewater collection and sediment oxygen demand (SOD) were used for analyzing their effects on N_2 flux rates. Furthermore, the initial porewater NO_3^-, concentrations assessed immediately after nutrient dosing were used for analyzing their effects on NO_3^- flux rates, respectively. Integrating these covariates contribute to a comprehensive understanding of nutrient flux patterns by controlling potential confounders, ultimately improving the validity and interpretability of the results.

Starting concentrations

The difference in the concentrations of DOC, NO_3^-, and PO_4^{3-} among mixing tanks, as well as initial porewater concentration among habitat types and between hydrology levels were analyzed using ANOVA.

Flux rate estimates

Statistical analysis was performed using R statistical software from the R Core Team (2022). The steps involved were as follows:

- Running generalized least squares model (GLS): The impacts of habitat, hydrology, and soil properties on the flux rates of DOC, NO_3^-, PO_4^{3-}, and N_2 were analyzed using GLS models (R package *nmle*) (Pinheiro et al., 2022). Variance structures used in the models were either habitat or hydrology. Backward selection approach was applied, iteratively refining the model until all soil properties exhibited p-values <0.05. Final models were fit using restricted maximum likelihood (REML) estimations.

- Model validation and refinement: Standardized residual plots and normality plots (QQplot) were generated to assess the appropriateness of the model assumptions visually, followed by Variance inflation factor (VIF) assessment to check multicollinearity between predictor variables. The Breusch-Pagan Test was applied to ascertain the absence of heteroscedasticity in the model. The complete model, containing main terms and interactions, was further evaluated.
- Additional model assumptions check: ANOVA, AIC, and BIC tests were done to ensure final model simplicity and goodness of fit compared to the initial model. Normality in residuals were verified visually (QQplot, histograms) and by using normal distribution test (Shapiro and skewness tests). Homogeneity assumptions were confirmed after plotting standardized residuals vs. fitted values and standardized residuals vs. each predictor variable in the final model.
- Final ANOVA: ANOVA with type III sum of squares was used to analyze the effect of predictor variables included in the final model on mean DOC, NO_3^-, PO_4^{3-}, and N_2 flux rates. Type III sum of squares was employed due to the presence of interaction in the model and unbalanced sample size in different habitats (bare soil n = 11, native grass n = 12, tree planting n = 12, 3-day flooding n = 17, and 3-week flooding n = 18).
- Predicted mean calculation: Predicted mean DOC, NO_3^-, PO_4^{3-}, and N_2 flux rates were computed using the *emmeans* function in R. Subsequently, post hoc tests were performed using Tukey's HSD to discern differences between habitat and hydrology categories.

Soil properties and greenhouse gas production

Wilcoxon Ranked-Sign paired t-test was conducted to assess the differences between soil nutrients before and after dosing within a specific habitat or hydrology. The production rate of N_2O and CH_4 were too low for analysis using parametric methods, and therefore, they were solely analyzed based on observed trends during the 48 h incubation period. Consequently, no statistical comparisons were conducted to assess differences in the means of N_2O and CH_4 production rates among the various treatments.

3.6. Results

3.6.1. Dissolved nutrients starting concentrations

The average concentrations of DOC, NO_3^-, and PO_4^{3-} in the mixing tanks were 0.9, 10.5 and 1.2 mg L^{-1}, respectively. While there was a marginal significance observed in the initial NO_3^- concentration among the mixing tanks ($P = 0.05$), subsequent post-hoc pairwise comparisons showed no differences. There were no differences in DOC and PO_4^{3-} concentrations among the tanks.

Table 3.2.

Mean nutrient concentrations in the mixing tanks. The tanks were filled twice, denoted as A and B for the first and second fillings, respectively.

Mixing Tank ID	DOC (mg L^{-1})	NO_3^- (mg L^{-1})	PO_4^{3-} (mg L^{-1})
1A	0.8852	10.90	1.35
1B	0.7940	11.80	0.95
2A	0.8545	9.88	1.15
2B	1.0330	9.54	1.06
3A	0.9541	10.30	1.26
3B	0.7953	9.33	1.26
4A	0.9548	11.40	1.40
4B	0.9547	11.20	0.98

After flooding the mesocosms, nutrient-rich water infiltrate into the soil pores and mix with the water already present in the soil pores. The average concentrations of DOC, NO_3^-, and PO_4^{3-} in the porewater across all mesocosms analyzed immediately after dosing were 2.1, 7.9 and 0.16 mg L^{-1}, respectively. There were no differences in the initial porewater DOC among habitat (P= 0.2990) and hydrology levels (P=0.9530). The initial porewater NO_3^- concentration was significantly higher (P=0.0263) in 3-week flooded (8.2 mg L^{-1}) compared to 3-day flooded mesocosms (7.5 mg L^{-1}) (Figure 3.7). The initial porewater PO_4^{3-}concentration was significantly different among habitat types (P<0.0001). Post-hoc pairwise comparisons showed that initial

PO_4^{3-}concentration in native grass habitat (0.28 mg L^{-1}) was significantly higher compared to bare soil (0.06 mg L^{-1}) and tree planting habitats (0.14 mg L^{-1}) (Figure 3.7).

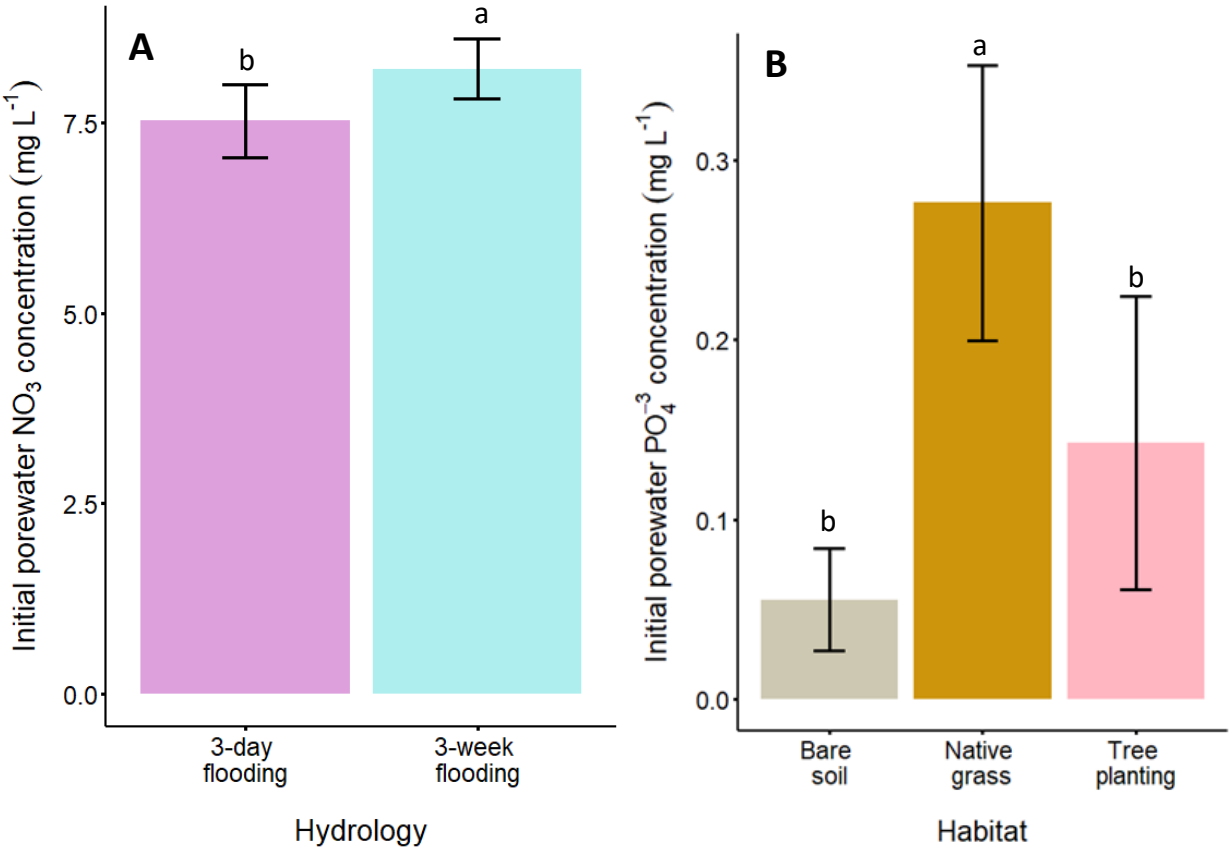

Figure 3.7.

Mean A) initial porewater NO_3^-, and B) PO_4^{3-} concentrations. Hydrology levels were 3-day and 3-week flooding. Habitat types were bare soil, native grass, and tree planting. Error bars represent 95% confidence interval. Means associated with different lowercase letter are significantly different by post hoc analysis using Tukey's Honestly Significant Difference.

3.6.2. Dissolved organic carbon (DOC) release

The Initial DOC concentrations in the porewater varied among mesocosms, with concentration in individual mesocosms ranging from 1.3 -3.7 mg L^{-1} DOC in bare soil habitat– 3-weeks flooding and tree planting habitat– 3-days flooding, respectively. All treatments resulted in a net release of DOC in the porewater by > 160 % after 5 days of inundation and release rate was generally linear (Figure 3.8). Mesocosms with bare soil subjected to the 3-week flooding treatment exhibited the highest percentage increase in DOC release, with > 545% release after 5 days of inundation. The lowest percentage increase in DOC release was observed in native grass habitat, with 180 % increase in 3-day flooded mesocosms and 161 % increase in 3-week flooded mesocosms (Figure 3.8).

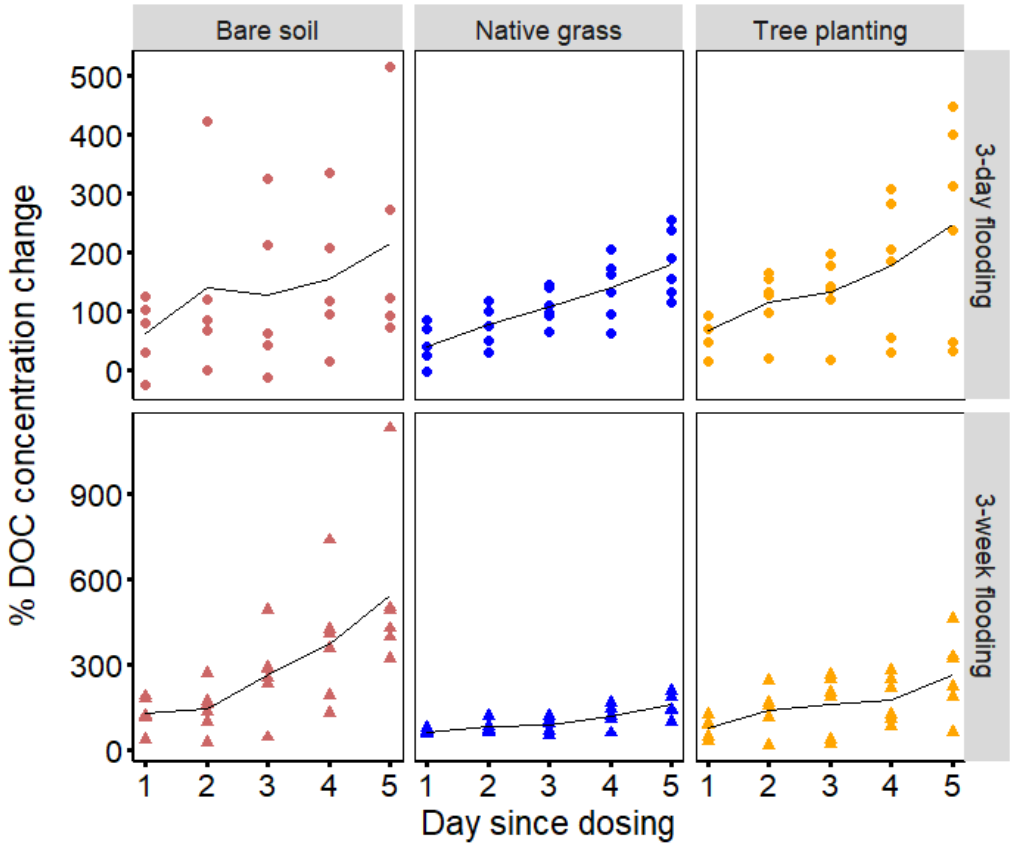

Figure 3.8.

Percent change in DOC concentration over the 5 days inundation period. Lines represent means. Habitat types were bare soil, native grass, and tree planting. Hydrology levels were 3-day and 3-week flooding.

After 5 days of inundation, all treatments experienced a net release of DOC in the porewater, with individual mesocosm flux rates ranging from 0.19 to 2.87 mg L^{-1} day^{-1} (bare soil habitat – 3-day flooding and bare soil habitat– 3-week flooding, respectively). The estimated mean DOC release rate exhibited significant difference between hydrology (Chi-squared $_{(1,26)}$ = 14.12, P = 0.0002) (Table 3.3). The mean DOC release rate was significantly lower in 3-day flooding treatment (0.74 mg L^{-1} day^{-1}) compared to 3-week flooding treatment (1.23 mg L^{-1} day^{-1}) (Figure 3.9).

However, the interaction between habitat and hydrology was also significant (Chi-squared $_{(2,26)}$ = 16.48, P = 0.0003) (Table 3.3). Notably, the significant difference among habitats was only evident in the 3-week flooded mesocosms (Figure 3.10), and the significant difference in DOC between hydrology levels was observed in bare soil habitat only (Figure 3.11). Additionally, the estimated mean DOC release rate was significantly affected by soil P (Chi-squared $_{(1,26)}$ = 26.29, P<0.0001), chl-*a* content (Chi-squared $_{(1,26)}$ = 16.86, P<0.0001), and AFDM (Chi-squared $_{(1,26)}$ = 4.38, P = 0.0364) (Table 3.3). Specifically, the DOC release rate exhibited a negative relation with soil P, while it displayed a positive relation with chl-*a* and AFDM. Although AFDM showed statistical significance in the model, its impact was relatively weak, with an increase in AFDM by 1 mg g^{-1} corresponding to an increase in DOC release by 0.01 mg L^{-1} day^{-1}.

Table 3.3.

Chi-squared statistics from ANCOVA for habitat, hydrology, interaction between habitat and hydrology, soil phosphorus, chlorophyll-a, and ash free dry mass (AFDM) for DOC flux rate.

Source	**df**	**Chi-squared**	***P***
Intercept	1	1.73	0.1884
Habitat	2	0.77	0.6803
Hydrology	1	14.12	0.0002
Habitat: Hydrology	2	16.48	0.0003
Soil P	1	26.29	<0.0001
Log(chl-*a*)	1	16.86	<0.0001
AFDM	1	4.38	0.0364

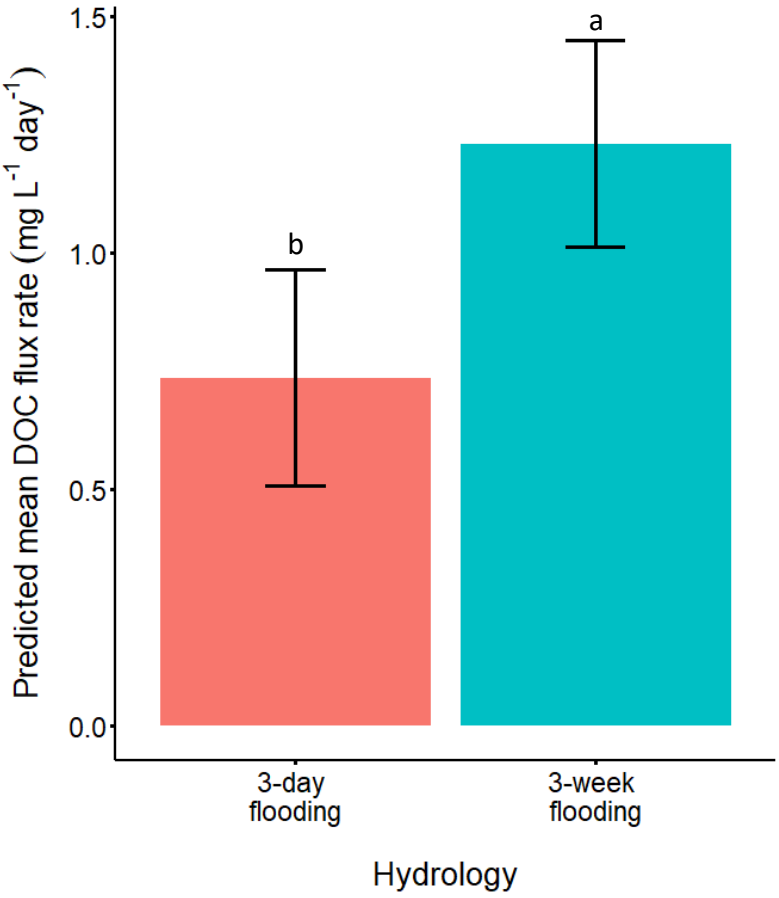

Figure 3.9.

Predicted mean DOC flux rate between the hydrology. Hydrology are 3-day flooding and 3-week flooding. Error bars represent 95% confidence interval. Means associated with different lowercase letter are significantly different by post hoc analysis using Tukey's Honestly Significant Difference.

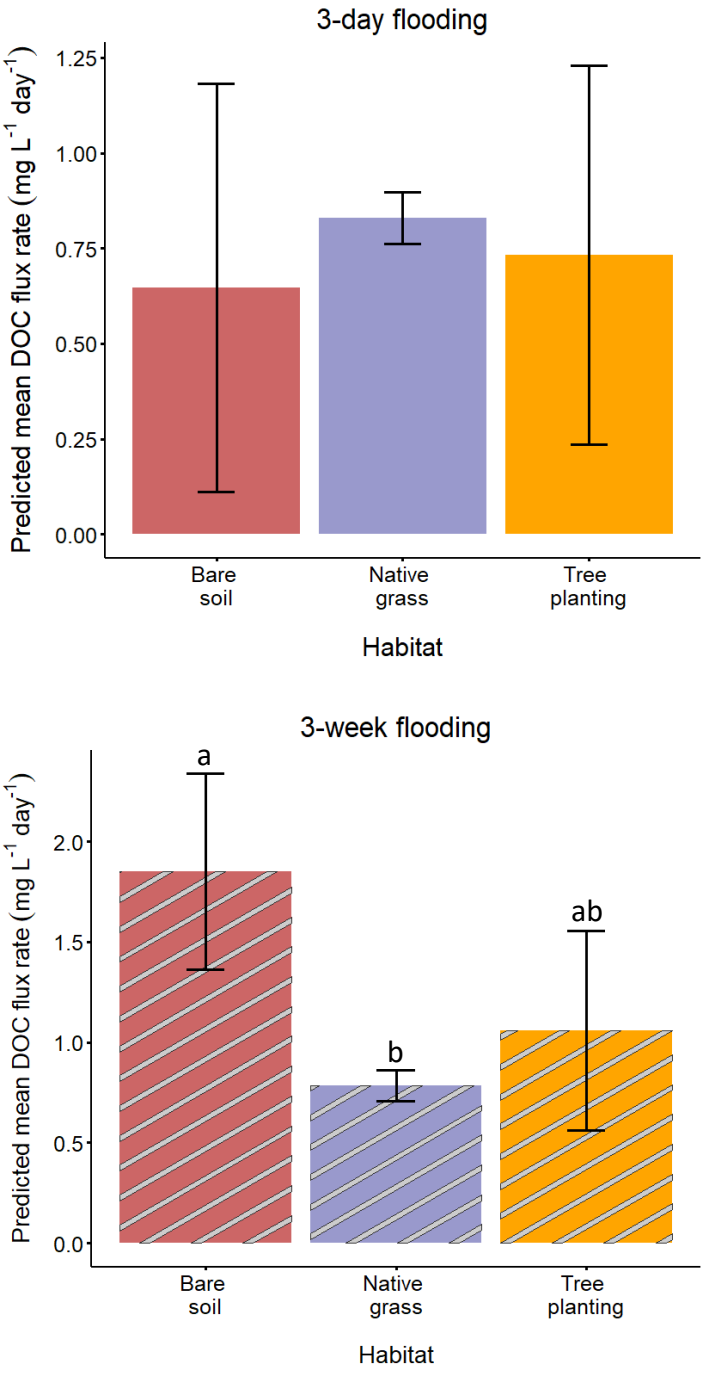

Figure 3.10.

Predicted mean DOC flux rate among habitat types within hydrology levels. Error bars represent 95% confidence interval. Means associated with different lowercase letter are significantly different by post hoc analysis using Tukey's Honestly Significant Difference.

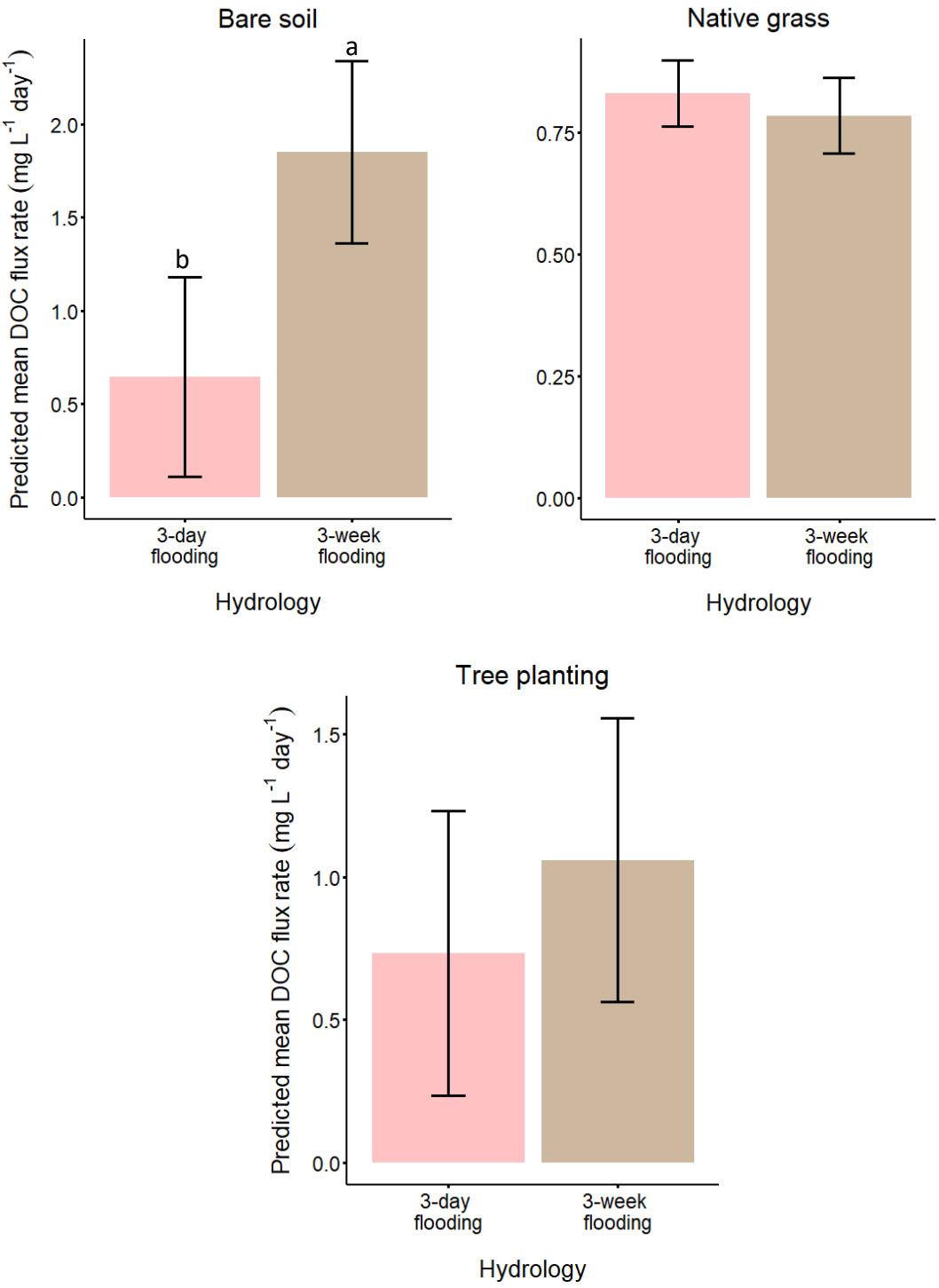

Figure 3.11.

Predicted mean DOC flux rate between hydrology levels within habitat types. Error bars represent 95% confidence interval. Means associated with different lowercase letter are significantly different by post hoc analysis using Tukey's Honestly Significant Difference.

3.6.3. Nitrate (NO_3^-) retention

The first round of porewater sampling was conducted immediately after dosing the mesocosms with nutrient-rich water containing ~10 mg L^{-1} NO_3^-. The initial porewater NO_3^- concentrations varied among mesocosms, with concentrations ranging from 6.1 to 9.9 mg L^{-1} NO_3^- in tree planting– 3-days flooding and bare soil habitat– 3-weeks flooding, respectively. With the exception of mesocosms with bare soil that received a 3-day flooding treatment, all other treatments had a > 95% decrease in NO_3^- concentration in the porewater by 5 days of inundation (Figure 3.12). Mesocosms with bare soil subjected to the 3-day flooding treatment decreased NO_3^- by 83.3 % in the porewater water after 5 days of inundation. Native grass habitats exhibited the highest efficiency in decreasing NO_3^-, achieving > 95% decrease on average by day 3 for both hydrology levels.

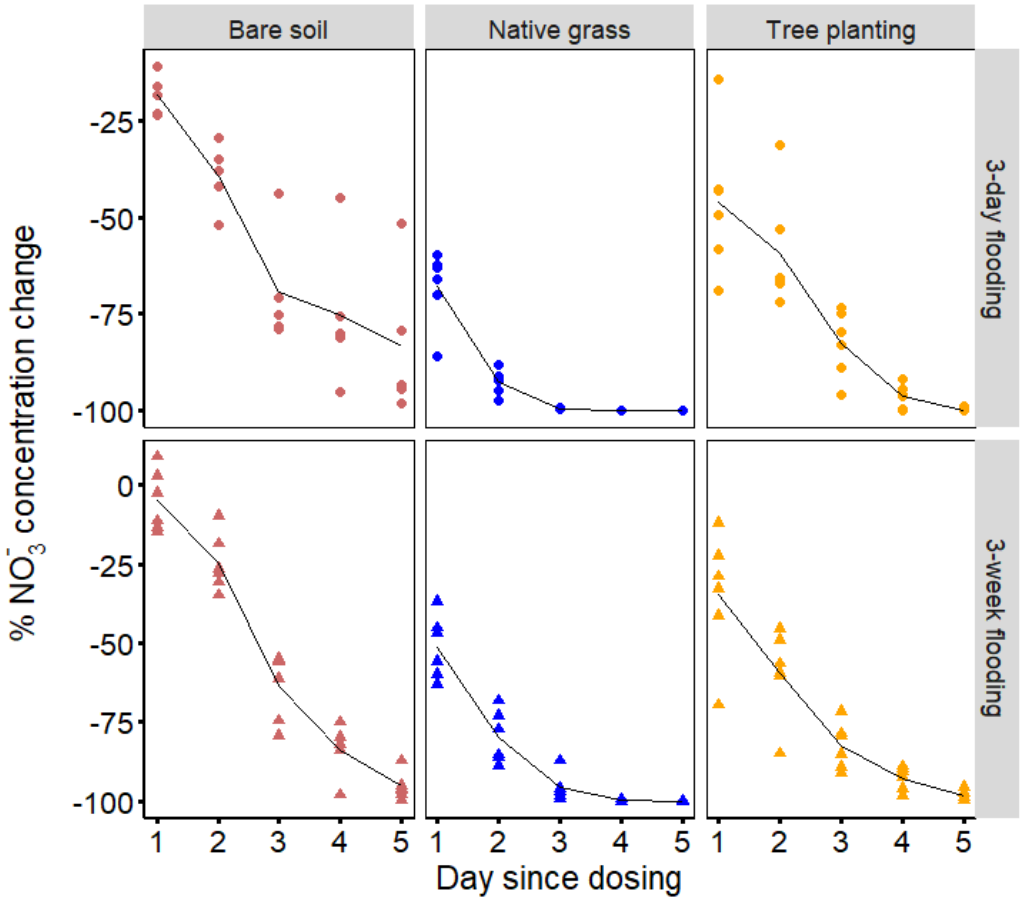

Figure 3.12.

Percent change in NO_3^- concentration over the 5 days inundation period. Lines represent means. Habitat types were bare soil, native grass, and tree planting. Hydrology levels were 3-day and 3-week flooding.

After a 5-day inundation period, all treatments retained NO_3^- in the porewater. Individual mesocosm flux rates ranged from -2.16 to -0.75 mg L^{-1} day^{-1}, in the tree planting habitat subjected to 3-day flooding and the bare soil habitat subjected to 3-day flooding, respectively. There was a significant difference in mean NO_3^- retention rates among habitat types (Chi-squared $_{(2,28)} = 24.88$, $P<0.0001$) (Table 3.4). The mean NO_3^- retention rate in the native grass habitat (-1.99 mg L^{-1} day^{-1}) was significantly higher compared to the bare soil (-1.56 mg L^{-1} day^{-1}) and tree planting (-1.87 mg L^{-1} day^{-1}) habitats (Figure 3.13). No differences in NO_3^- retention rate was observed between hydrology levels, highlighting that hydrology had no measurable effects across habitats. Furthermore, the initial NO_{3-} concentration of porewater significantly influenced NO_3^- retention estimates in the porewater (Chi-squared $_{(1,28)} = 8450.43$, $P<0.0001$) (Table 3.4). These results indicate a positive correlation, wherein higher initial porewater NO_3^- concentrations corresponded to increased dissolved NO_3^- removal rates (Figure 3.14).

Table 3.4.

Chi-squared statistics from ANCOVA for habitat, hydrology, interaction between habitat and hydrology, and nitrate concentration of porewater immediately after dosing for NO_3^-flux rate.

Source	df	Chi-squared	*P*
Intercept	1	15.32	<0.0001
Habitat	2	24.88	<0.0001
Hydrology	1	0.41	0.5213
Habitat: Hydrology	2	4.22	0.1207
Initial porewater NO_3^-	1	8450.43	<0.0001

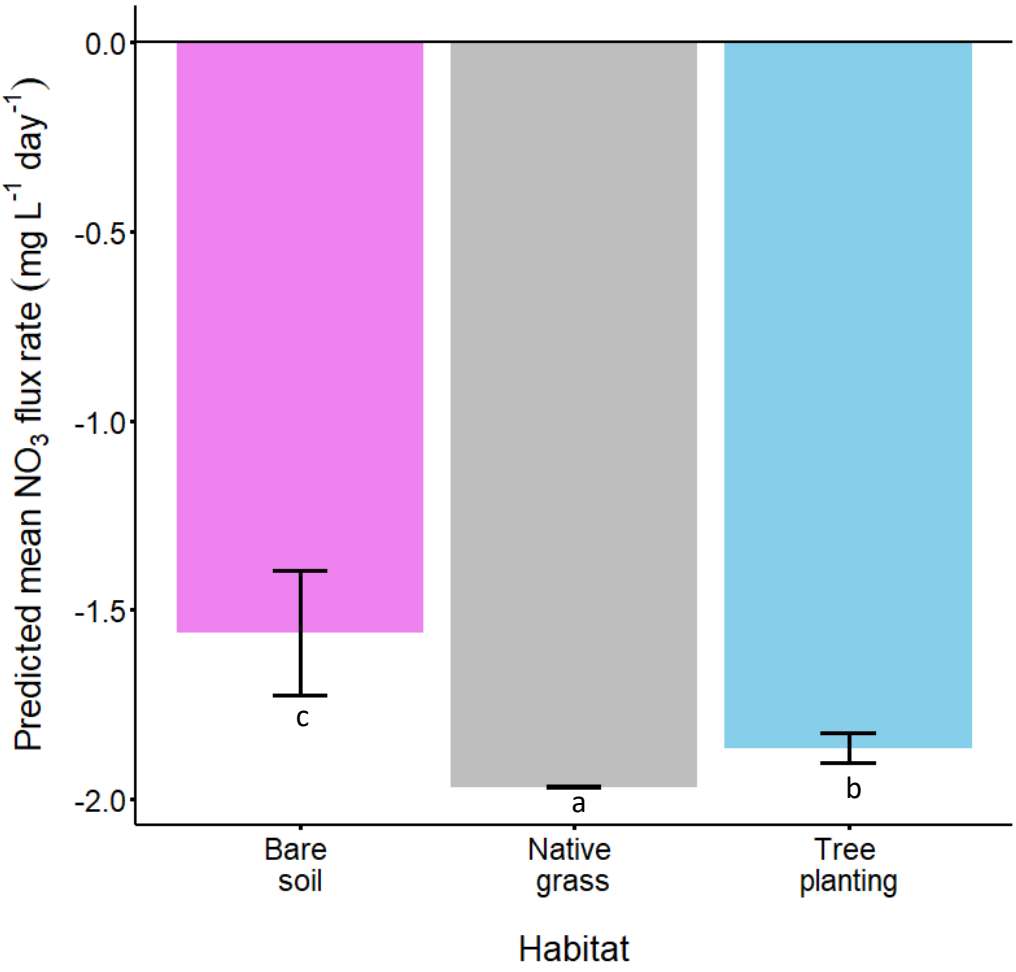

Figure 3.13.

Predicted mean NO_3^- flux rate among habitats. Error bars represent 95% confidence interval. Means associated with different lowercase letter are significantly different by post hoc analysis using Tukey's Honestly Significant Difference.

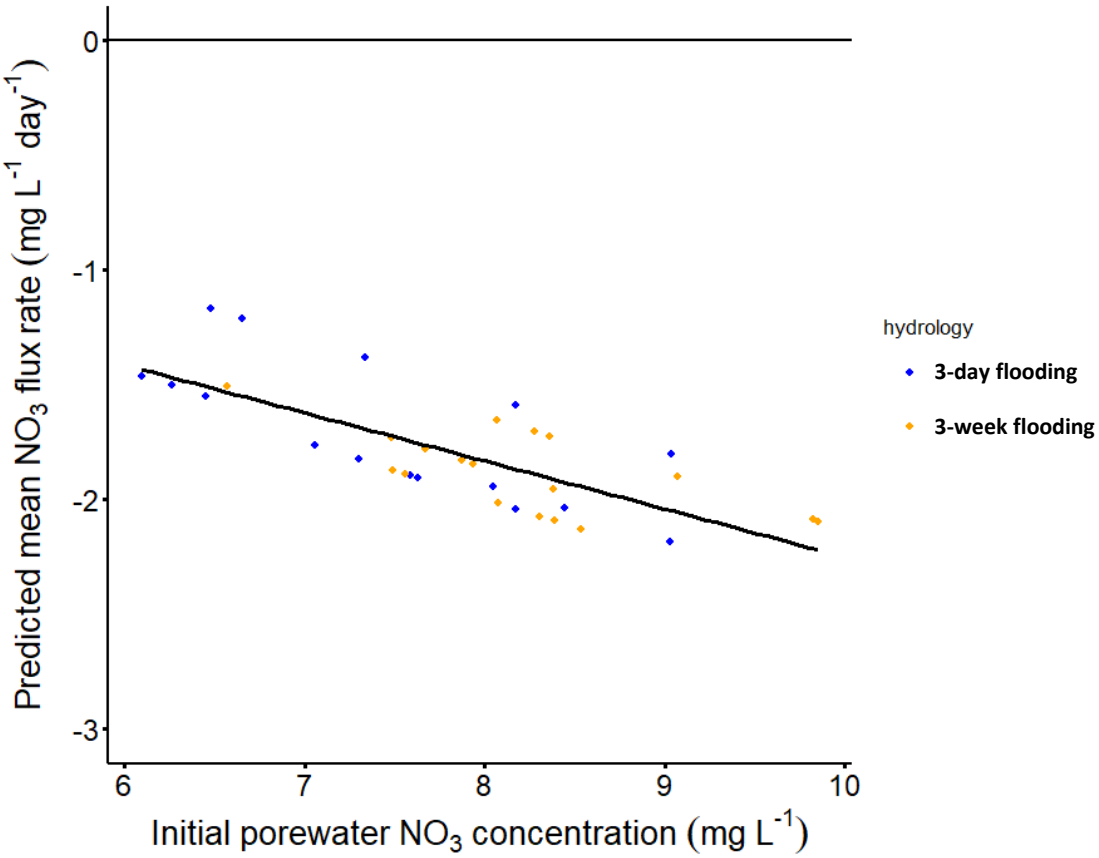

Figure 3.14.

Relation between predicted mean NO_3^- flux rate and initial porewater NO_3^- concentration.

3.6.4. Phosphate (PO_4^{3-}) retention

The first round of porewater sampling was conducted immediately after dosing the mesocosms with nutrient-rich water containing 1 mg L^{-1} PO_4^{3-}. The PO_4^{3-}concentrations in the porewater during initial sampling was substantially lower than surface water concentrations and varied among mesocosms, with individual mesocosms concentrations ranging from 0.014 - 0.485 mg L^{-1} PO_4^{3-} in bare soil habitat– 3-days flooding and native grass habitat– 3-weeks flooding treatments, respectively. In the first 24 h after dosing, there was a mean PO_4^{3-} release in bare soil treatments with both 3-day and 3-week flooding, as well as tree planting with 3-day flooding (Figure 3.15). Bare soil flooded for 3 days exhibited the lowest decrease in PO_4^{3-} concentration, achieving 19% decrease on average by day 3. Native grass habitats demonstrated the highest decrease, achieving > 85% decrease on average by day 3 in both hydrology levels. For all treatments, the uptake rates reached a plateau after 3 days, except for tree planting habitat subjected to 3-week flooding. Beyond day 3, there was no further increase in the uptake rate (Figure 3.15).

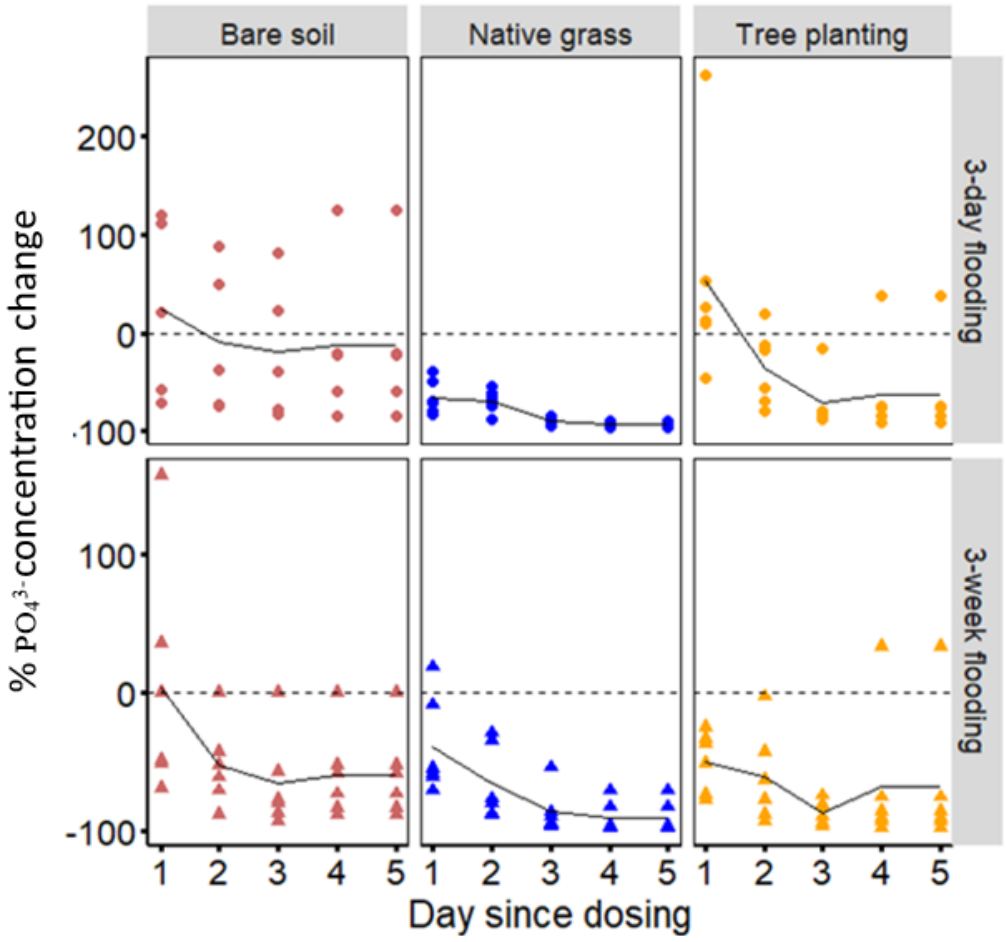

Figure 3.15.

Percent change in PO_4^{3-} concentration over the 5 days inundation period. Lines represent means. Habitat types were bare soil, native grass, and tree planting. Hydrology levels were 3-day and 3-week flooding.

First 24 hours PO_4^{3-} flux

Within 24 h, individual mesocosm PO_4^{3-} flux rates ranged from -0.353 to 0.107 mg L^{-1} day^{-1} in the tree planting habitat subjected to 3-week flooding and the bare soil habitat subjected to 3-week flooding, respectively. There was a significant difference in the estimated mean PO_4^{3-} flux rates among habitat types (Chi-squared $_{(2,29)} = 49.76$, $P<0.0001$) (Table 3.5). The mean PO_4^{3-} retention rate in the native grass habitat (-0.16 mg L^{-1} day^{-1}) was significantly higher compared to the bare soil (-0.02 mg L^{-1} day^{-1}) and tree planting (-0.04 mg L^{-1} day^{-1}) habitats (Figure 3.16). No differences in PO_4^{3-} flux was observed between hydrology levels (Table 3.5).

Table 3.5.

Chi-squared statistics from ANCOVA for habitat, hydrology, and interaction between habitat and hydrology for PO_4^{3-} flux rate at first 24 hours after dosing.

Source	**df**	**Chi-squared**	***P***
Intercept	1	0.17	0.6814
Habitat	2	49.76	<0.0001
Hydrology	1	0.005	0.9465
Habitat: Hydrology	2	4.47	0.1069

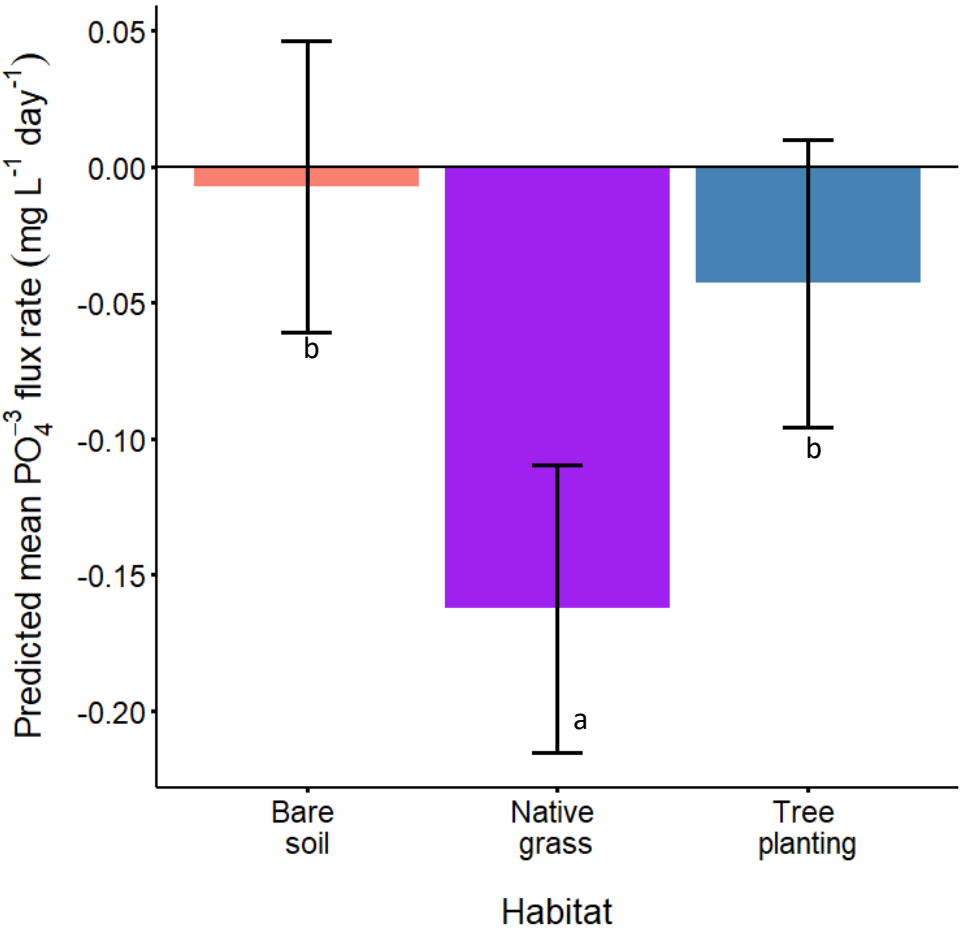

Figure 3.16.

Predicted mean PO_4^{3-} flux rate among habitat types at first 24 hours after dosing. Errors bars represent 95% confidence interval. Means associated with different lowercase letter are significantly different by post hoc analysis using Tukey's Honestly Significant Difference.

Day 2-3 PO_4^{3-} flux

After a 3-day inundation period, all treatments retained PO_4^{3-} in the porewater. The individual mesocosm flux rates varied between -0.089 and -0.004 mg L^{-1} day^{-1}, with the native grass habitat subjected to 3-week flooding and bare soil habitat subjected to 3-day flooding, respectively. A significant difference in the estimated mean PO_4^{3-} retention rates was observed among habitat types (Chi-squared $_{(2,26)} = 13.68$, $P = 0.0012$) (Table 3.6). The average PO_4^{3-} retention rate in the bare soil habitat (-0.014 mg L^{-1} day^{-1}) was significantly lower compared to native grass (-0.045 mg L^{-1} day^{-1}) and tree planting (-0.037 mg L^{-1} day^{-1}) habitats (Figure 3.17). Although PO_4^{3-} flux rate was not affected by hydrology alone, a significant effect due to the interaction between habitat and hydrology was detected (Chi-squared $_{(2,26)} = 6.05$, $P = 0.0487$) (Table 3.6). The significant difference among habitats was evident in both hydrology levels (Figure 3.18). However, due to the extremely low retention rate, it is likely that the interaction of hydrology and habitat is of little ecological significance. Subsequently, comparing hydrology levels within each habitat types show these differences were no longer statistically significant (Figure 3.19). Additionally, the mean flux rate was significantly affected by AFDM (Chi-squared $_{(1,26)} = 5.82$, $P = 0.0158$) (Table 3.6). Despite the statistical significance observed for AFDM in the model, its influence proved to be relatively weak, with an increase in AFDM by 1 mg g^{-1} corresponding to an increase in PO_4^{3-} retention by 0.003 mg L^{-1} day^{-1}.

Table 3.6.

Chi-squared statistics from ANCOVA for habitat, hydrology, interaction between habitat and hydrology, and ash free dry mass (AFDM) for PO_4^{3-} flux rate at day 2-3.

Source	df	Chi-squared	*P*
Intercept	1	4.95	0.0260
Habitat	2	13.68	0.0012
Hydrology	1	2.49	0.1149
Habitat: Hydrology	2	6.05	0.0487
AFDM	1	5.82	0.0158

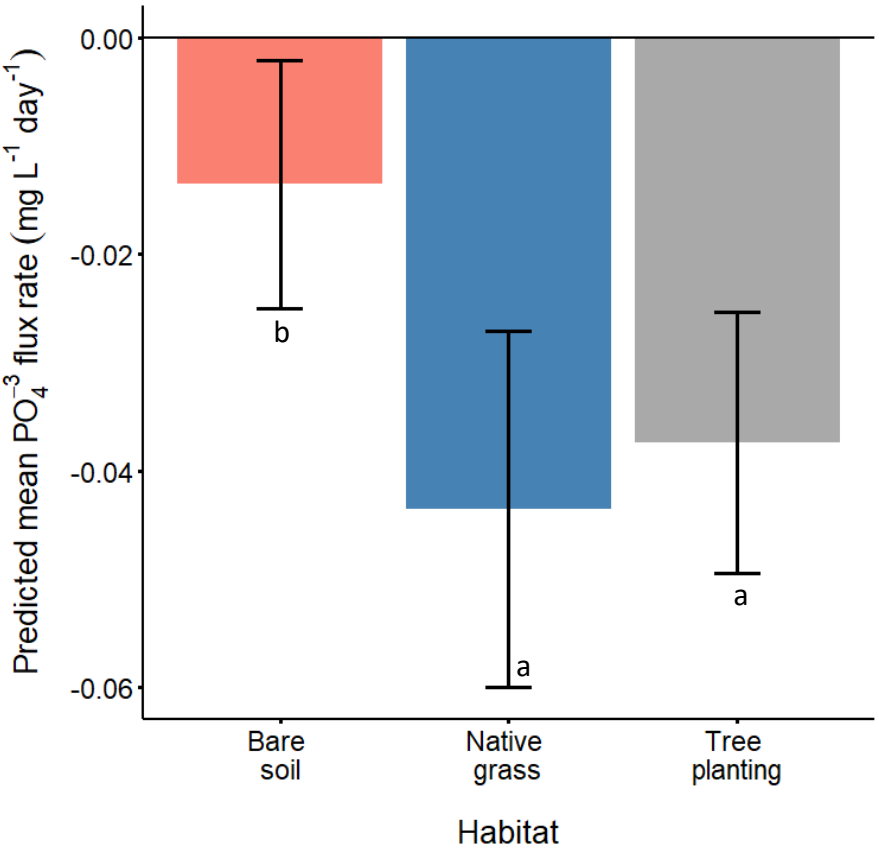

Figure 3.17.

Predicted mean PO_4^{3-} flux rate at day 2-3 among habitat types. Error bars represent 95% confidence interval. Means associated with different lowercase letter are significantly different by post hoc analysis using Tukey's Honestly Significant Difference.

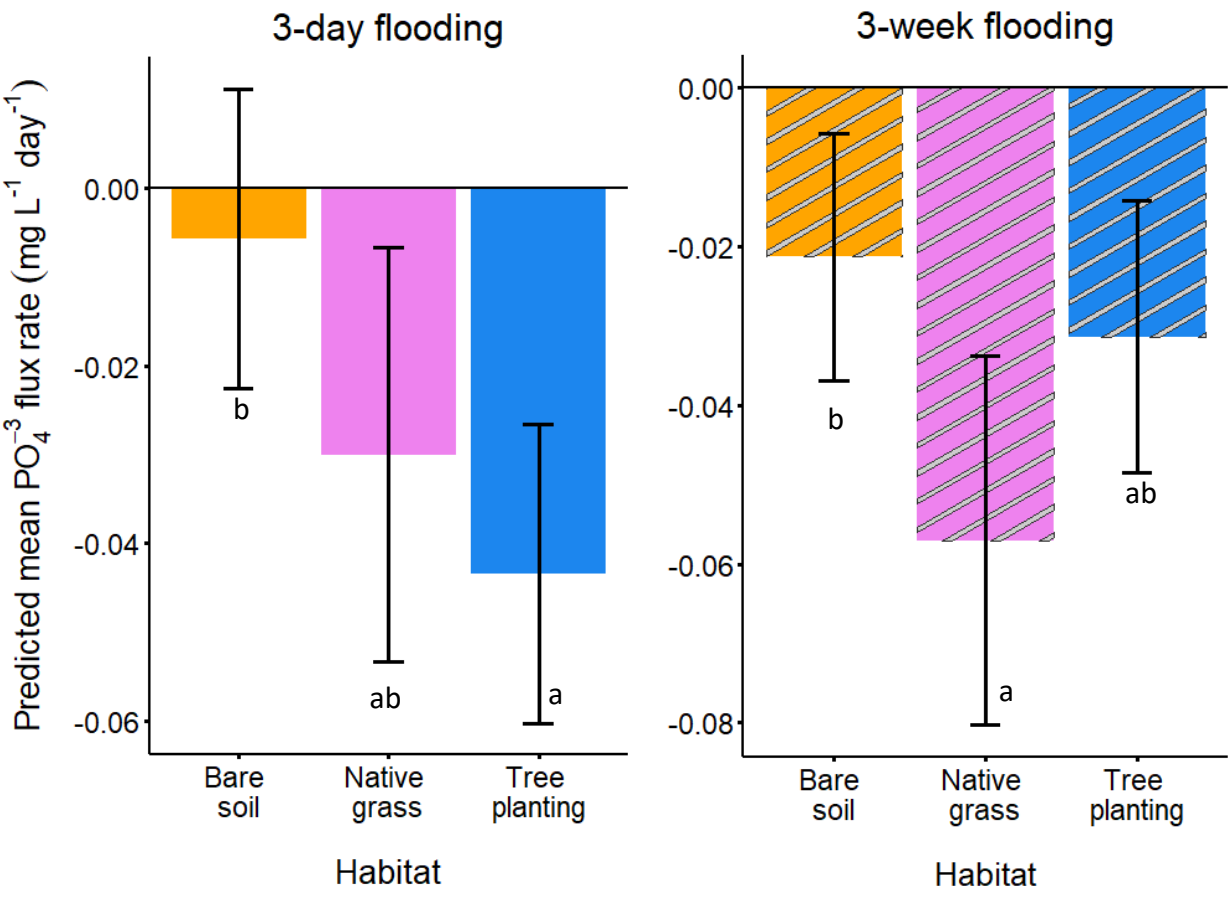

Figure 3.18.

Predicted mean PO_4^{3-} flux rate among habitat types within hydrology levels at day 2-3. Errors bars represent 95% confidence interval. Means associated with different lowercase letter are significantly different by post hoc analysis using Tukey's Honestly Significant Difference.

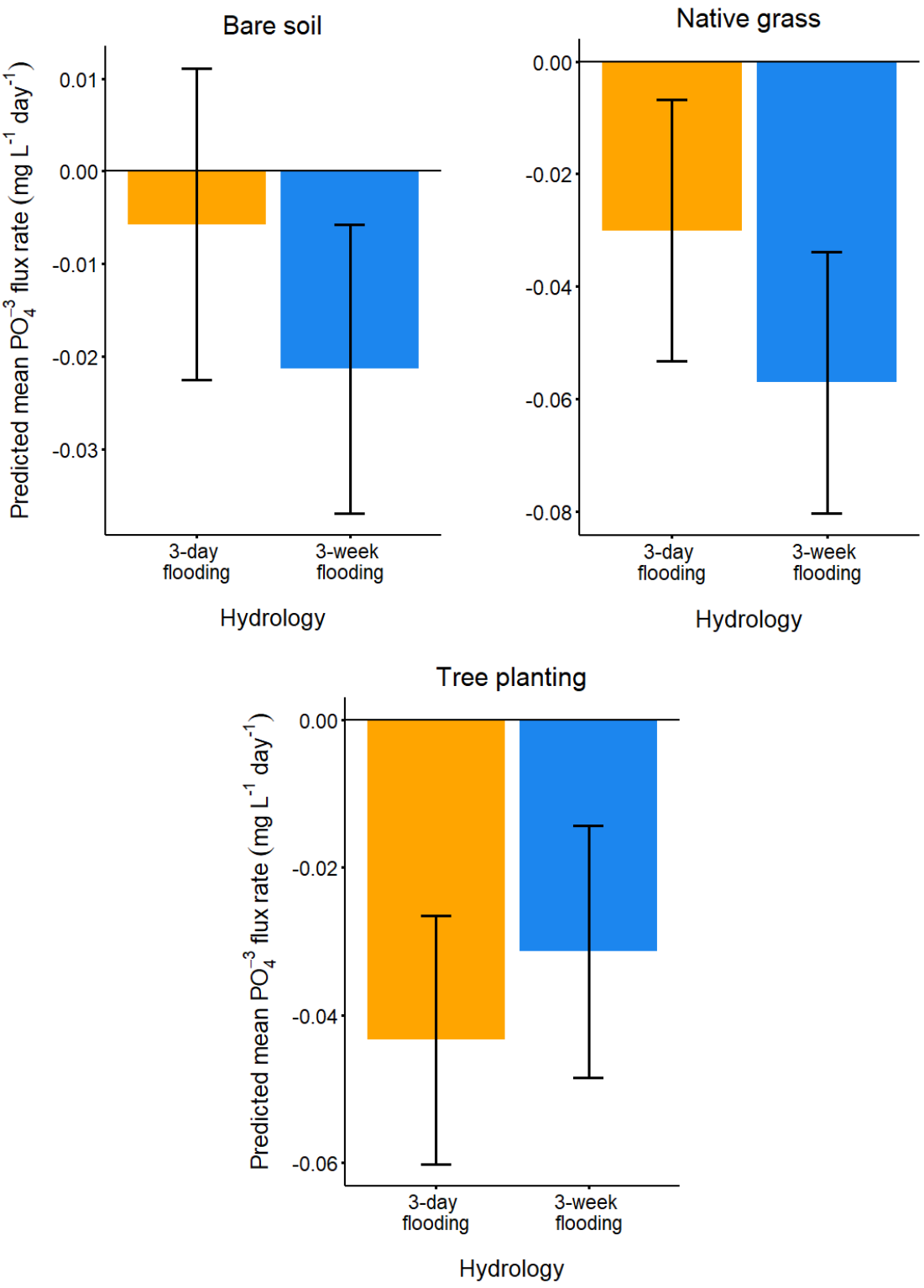

Figure 3.19.

Predicted mean PO_4^{3-} flux rate between hydrology levels within habitat types at day 2-3. Errors bars represent 95% confidence interval.

3.6.5. Nitrogen gas (N_2) flux

N_2 flux at 12 h, 24 h, and 48 h

Nitrogen gas production was observed across all habitats and hydrology treatments at the 12, 24, and 48 h sampling timepoints, displaying a consistent increasing trend during the incubation period (Figure 3.20 & 3.21). Production rates at the 24 h and 48 h sampling timepoints were compared with those at the 12 h sampling timepoint. In the bare soil habitat, production rate increased by 31.1% and 74.8%, while in the native grass habitat, increases of 14.0% and 59.5% were noted. The tree planting habitat exhibited increases of 24.1% and 60.3%. Similarly, under 3-day flooding hydrology, rates increased by 16.4% and 47.3%, whereas in the 3-week flooding hydrology rates increased by 31.9% and 85.6%.

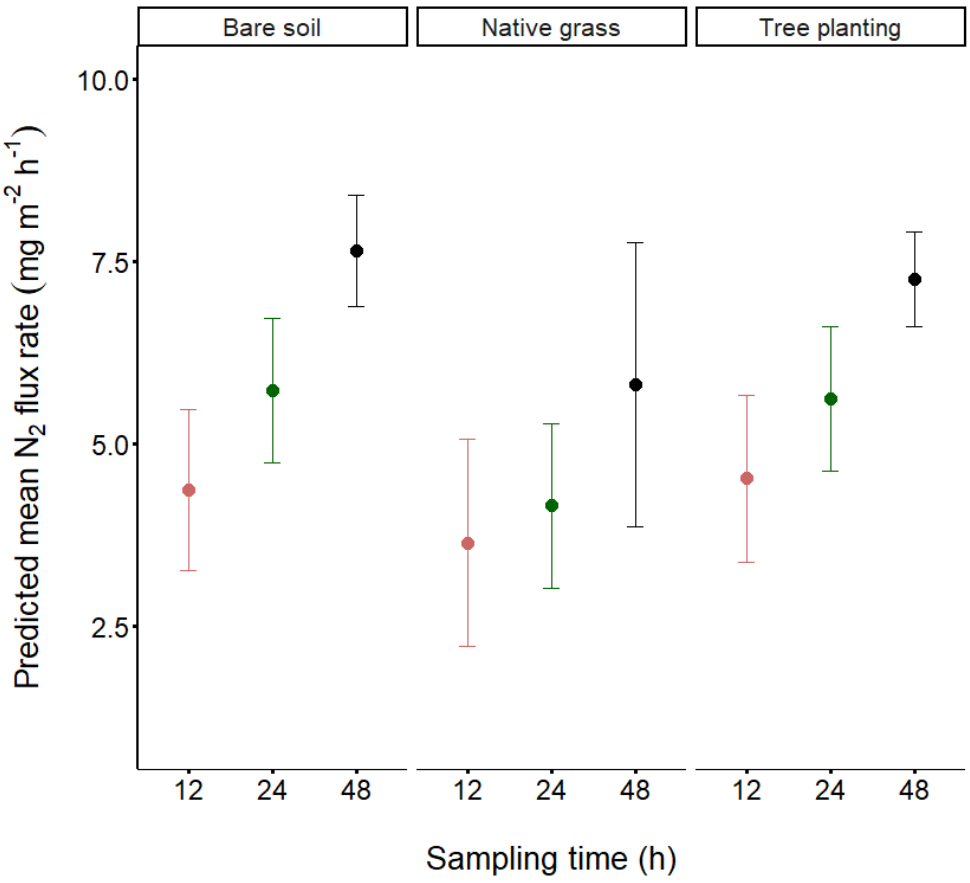

Figure 3.20.

Predicted mean N_2 flux rate at 12, 24, and 48 h sampling timepoint by habitat. Habitat types were bare soil, native grass, and tree planting. Error bars represent the 95% confidence interval.

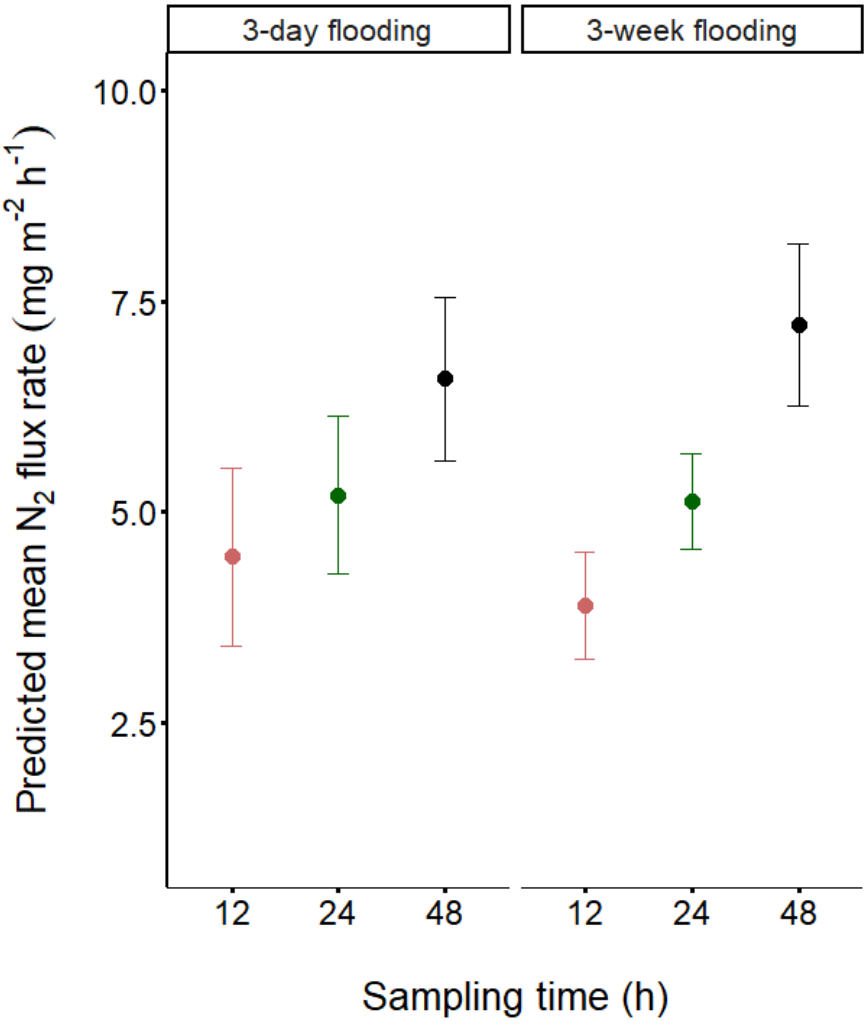

Figure 3.21.

Predicted mean N_2 flux rate at 12, 24, and 48 h sampling timepoint by hydrology. Hydrology levels were 3-day flooding and 3-week flooding. Error bars represent the 95% confidence interval.

Factors affecting N_2 flux

At the 12 h sampling timepoint, the mean N_2 production was significantly correlated with SOD (Chi-squared $_{(1,26)} = 16.48$, $P<0.0001$) and soil TN (Chi-squared $_{(1,26)} = 4.47$, $P = 0.0345$) (Table 3.7). Specifically, an increase in soil TN by one mg g^{-1} corresponded to an increase of 6 mg m^{-2} h^{-1} of N_2 production. The mean N_2 production rate was significantly influenced solely by SOD at 24 h (Chi-squared $_{(1,28)} = 49.46$, $P<0.0001$) and 48 h (Chi-squared $_{(1,28)} = 80.79$, $P<0.0001$) (Table 3.7). No significant effects of habitat or hydrology were observed across all sampling timepoints (Table 3.7). The native grass habitat displayed the lowest production rate at all sampling timepoints. The 3-week flooding showed lower production rate than 3-day flooding, except at 48 h sampling timepoint (Table 3.8).

Table 3.7.

Chi-squared statistics from ANCOVA for habitat, hydrology, interaction between habitat and hydrology, and soil properties at 12, 24, and 48 h for nitrogen (N_2) flux rate.

12 h

Source	df	Chi-squared	*P*
Intercept	1	2.97	0.0845
Habitat	2	0.62	0.7338
Hydrology	1	0.09	0.7583
Habitat: Hydrology	2	1.09	0.5776
SOD	1	16.48	<0.0001
Soil TN	1	4.47	0.0345

24 h

Source	df	Chi-squared	*P*
Intercept	1	0.63	0.4264
Habitat	2	0.56	0.7548
Hydrology	1	1.09	0.2954
Habitat: Hydrology	2	2.93	0.2315
SOD	1	49.46	<0.0001

48 h

Source	df	Chi-squared	*P*
Intercept	1	0.36	0.5464
Habitat	2	2.81	0.2454
Hydrology	1	0.20	0.6565
Habitat: Hydrology	2	0.81	0.6677
SOD	1	80.79	<0.0001

Table 3.8.

Predicted N_2 production rate for habitats and hydrology at 12 h, 24 h, and 48 h sampling timepoints. Habitat types includes bare soil, native grass, and tree planting. Hydrology levels include 3-day flooding and 3-week flooding.

	Sampling time		
	12 h	**24 h**	**48 h**
Habitat			
Bare soil	4.4 ± 0.5	5.7 ± 0.5	7.6 ± 0.3
Native grass	3.6 ± 0.7	4.2 ± 0.5	5.8 ± 0.9
Tree planting	4.5 ± 0.6	5.6 ± 0.5	7.3 ± 0.3
Hydrology			
3-day flooding	4.5 ± 0.5	5.2 ± 0.4	6.6 ± 0.5
3-week flooding	3.9 ± 0.3	5.1 ± 0.3	7.2 ± 0.5

3.6.6. Soil nutrients

Paired t-test was conducted to assess the differences between soil nutrients before and after the experiment. Soil mean TC levels before and after dosing the mesocosms were 7.18 and 6.73 mg g^{-1}, respectively, while soil mean TN levels were 0.61 and 0.90 mg g^{-1}, respectively. Soil TC decreased significantly (6.27%) following dosing ($t_{(34)} = -2.2737$, $P = 0.0294$) (Figure 3.22A). On the contrary, soil TN increased significantly (47.54%) post-dosing ($t_{(34)} = 19.697$, $P < 0.001$) (Figure 3.22B). No significant difference in soil P was found before and after dosing ($t_{(34)} = 0.2957$, $P = 0.7693$), suggesting that P levels remained relatively stable at 0.15 mg g^{-1}.

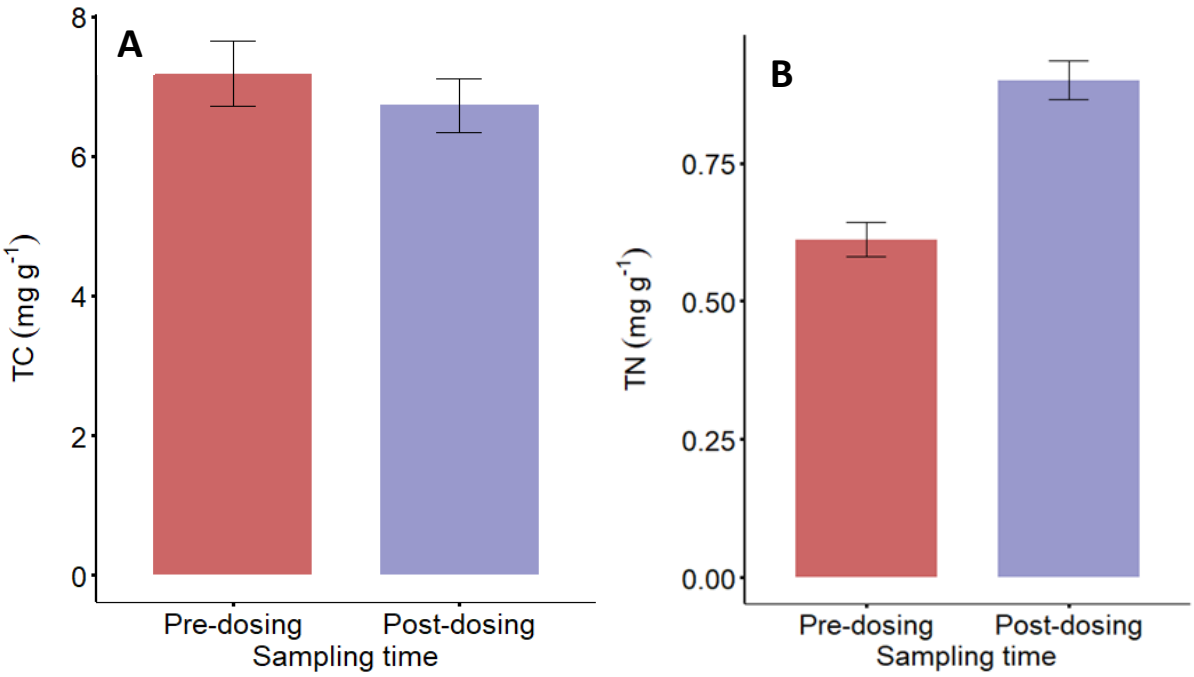

Figure 3.22.

Mean A) soil total carbon (TC), and B) total nitrogen (TN) before and after dosing. Error bars represent 95% confidence interval.

When examining individual habitat types, the soil TC in pre-dosing and post-dosing samples exhibited distinct patterns. In the bare soil habitat, there was a slight increase from 6.45 to 6.57 mg g^{-1} ($t_{(10)}$=0.42). In contrast, the native grass and tree planting habitats showed a significant decrease from 8.31 to 7.33 mg g^{-1} ($t_{(11)}$= -2.21), and 6.72 to 6.23 mg g^{-1} ($t_{(11)}$= -2.25), respectively. The largest difference in soil TC between pre-dosing and post-dosing samples was found in the native grass habitat in which soil TC decreased by 0.98 mg g^{-1} (P = 0.049) (Table 3.9). When examining the hydrology, there was a modest decrease in soil TC from 7.05 to 6.73 mg g^{-1} ($t_{(16)}$= -1.07) in 3-day flooding treatment. In the 3-week flooding treatment, the soil TC decreased significantly from 7.30 to 6.73 mg g^{-1} ($t_{(17)}$= -2.17, P=0.045) (Table 3.9).

For soil TN, significant differences between pre-dosing and post-dosing samples were evident for both habitat and hydrology treatments (P<0.001) (Table 3.9). In the bare soil habitat,

soil TN increased substantially from 0.57 to 0.9 mg g^{-1} ($t_{(10)} = 12$). In native grass and tree planting habitats, soil TN increased from 0.68 to 0.93 mg g^{-1} ($t_{(11)} = 17.4$) and 0.58 to 0.87 mg g^{-1} ($t_{(11)} = 9.53$), respectively. The largest difference in soil TN between pre-dosing and post-dosing samples was found in the bare soil habitat in which soil TN increased by 0.33 mg g^{-1} (Table 3.9). In terms of hydrology, both the 3-day flooding and 3-week flooding treatments showed an increase in soil TN. In the 3-day wet flooding, soil TN increased from 0.61 to 0.88 mg g^{-1} ($t_{(16)} = 13.1$), and in the 3-week wet treatment, it increased from 0.61 to 0.92 mg g^{-1} ($t_{(17)} = 14.9$).

Table 3.9.

P-value from Wilcoxon Ranked-Sign paired t-test results, differences in soil total carbon (TC) and soil total nitrogen (TN) before and after dosing, and the corresponding percent change.

	Soil TC			Soil TN		
Vegetation	*P*	Post-Pre	% Change	*P*	Diff.	% Change
Bare soil	0.683	0.12	1.8	<0.001	0.33	57.9
Native grass	0.049	-0.98	-11.8	<0.001	0.26	39.4
Tree planting	0.046	-0.44	-6.6	<0.001	0.28	48.3
Hydrology						
3-day wet	0.302	-0.32	-4.6	<0.001	0.27	44.4
3-week wet	0.045	-0.57	-7.8	<0.001	0.31	50.1

3.6.7. Greenhouse gas

N_2O flux at 12h, 24 h, and 48 h

Nitrous oxide production rate for bare soil, native grass, and tree planting habitats were 0.055, 0.031, and 0.040 mg m^{-2} h^{-1} at 12 h, 0.025, 0.014, and 0.021 mg m^{-2} h^{-1} at 24 h, and -0.002, 0.014, and 0.002 mg m^{-2} h^{-1} at 48 h sampling timepoint, respectively. Nitrous oxide was produced in all habitats at 12h and 24 h sampling timepoint, except for a few soil cores from bare soil habitat. At 48 h, some soil cores from bare soil and tree planting habitats were consuming N_2O (Figure 3.23). Nitrous oxide production generally exhibited a downward trend throughout the incubation period. However, a significant decrease in N_2O production was found only between 12 h and 48 h sampling timepoints in tree planting habitat, which exhibited the highest percentage decrease in N_2O production by 95% (Figure 3.23). The bare soil habitat displayed the highest production rate at 12 h and 24 h, while the production rate was highest from native grass habitat at 48 h (Figure 3.23). At 12 h and 24 h, native grass habitat showed the lowest production, while at 48 h, bare soil habitat had the lowest production (Figure 3.23). Notably, N_2O production at 48 h remained the same as at 24 h in native grass habitat.

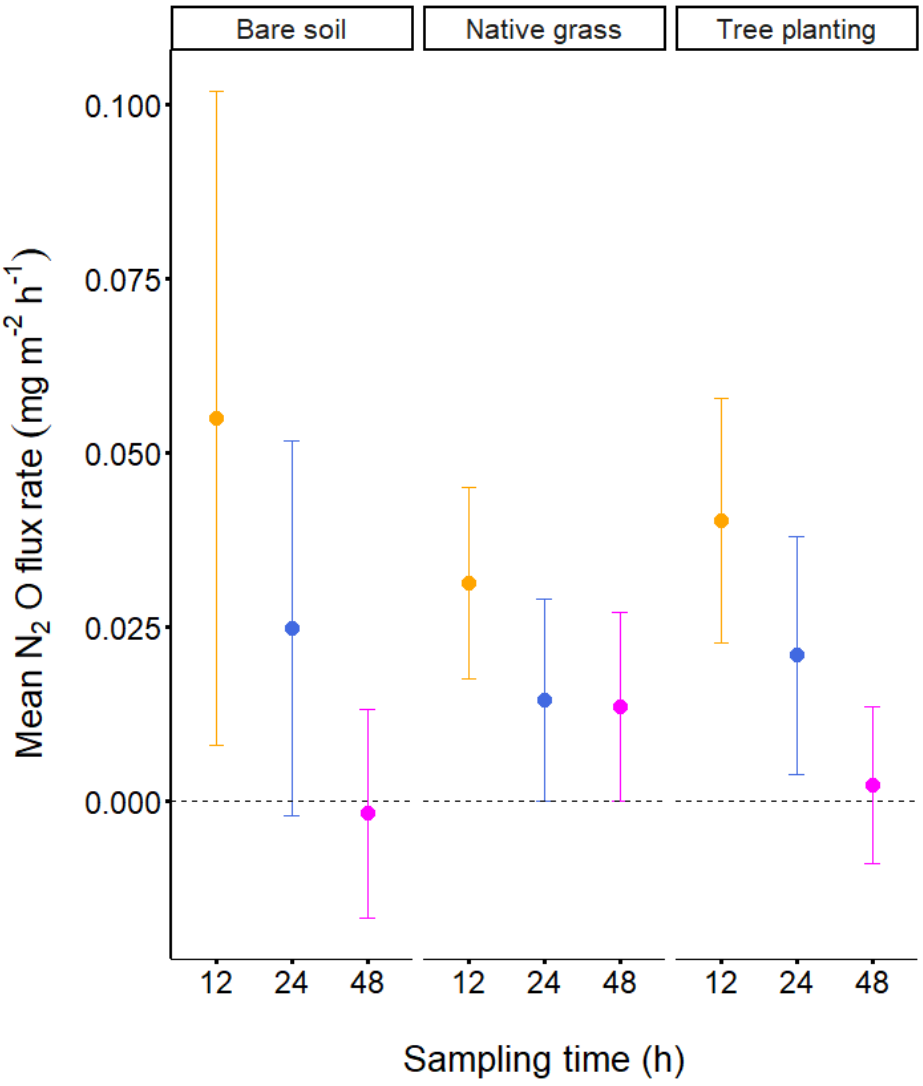

Figure 3.23.

Mean N_2O flux rate at 12, 24, and 48 h sampling timepoint by habitat. Habitat types were bare soil, native grass, and tree planting. Error bars represent the 95% confidence interval.

Nitrous oxide production rates for the 3-day flooding and 3-week flooding treatments were 0.049 and 0.035 mg m^{-2} h^{-1} at 12 h, 0.024 and 0.017 mg m^{-2} h^{-1} at 24 h, and 0.007 and 0.003 mg m^{-2} h^{-1} at 48 h, respectively. Nitrous oxide was produced in both 3-day and 3-week flooding treatments at 12 h, 24 h, and 48 h, with the exception of a few soil cores at the 48 h (Figure 3.24). There were no significant differences in N_2O production between the 3-day and 3-week flooding treatments at each sampling time. However, at 48 h, N_2O production was notably reduced compared to the 12 h, showing a decrease by 85.7% and 91.4% for the 3-day and 3-week flooding treatments, respectively (Figure 3.24). Nitrous oxide production rate decreased throughout the 48 h sampling period in all treatments.

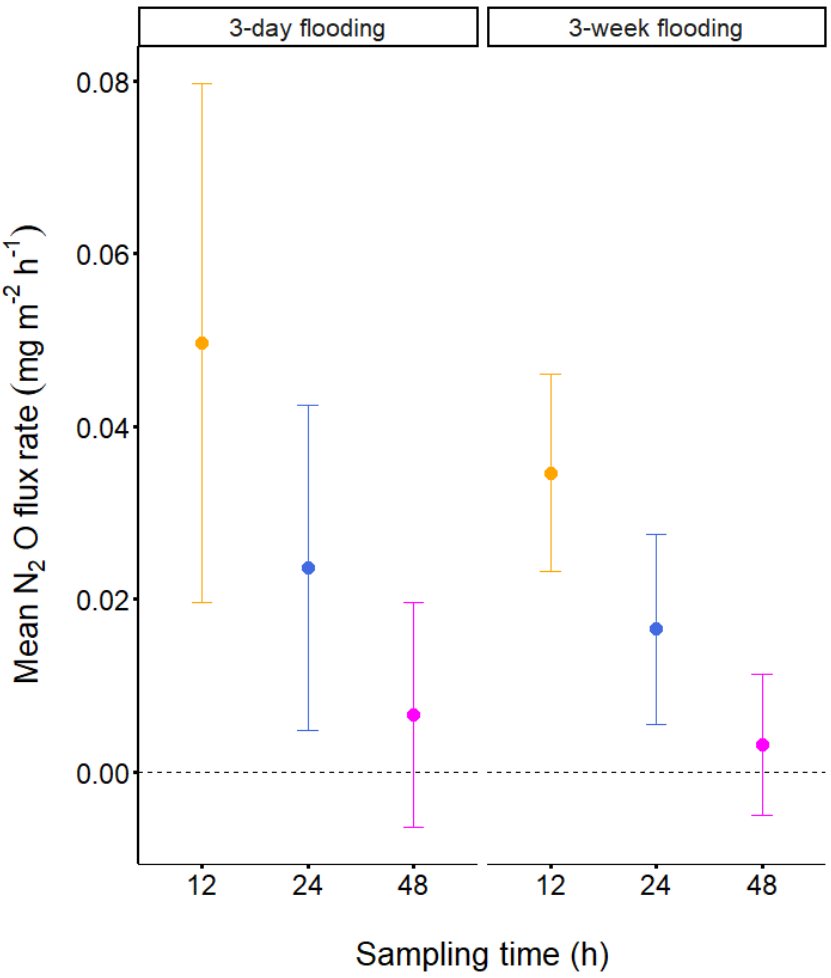

Figure 3.24.

Mean N_2O flux rate at 12, 24, and 48 h sampling timepoint by hydrology. Hydrology levels were 3-day flooding and 3-week flooding. Error bars represent the 95% confidence interval.

CH_4 flux at 12h, 24 h, and 48 h

Methane production rate for bare soil, native grass, and tree planting habitats were 0.004, 0.019, and 0.003 mg m^{-2} h^{-1} at 12 h, 0.009, 0.079, and 0.004 mg m^{-2} h^{-1} at 24 h, and 0.012, 0.232, and 0.009 mg m^{-2} h^{-1} at 48 h, respectively. In the bare soil and tree planting habitats, mean CH_4 production rate was close to zero at all sampling timepoints. However, CH_4 production showed an upward trend throughout the incubation period in the native grass habitat (Figure 3.25). Most variability in CH_4 production was observed from the native grass habitat at 48 h.

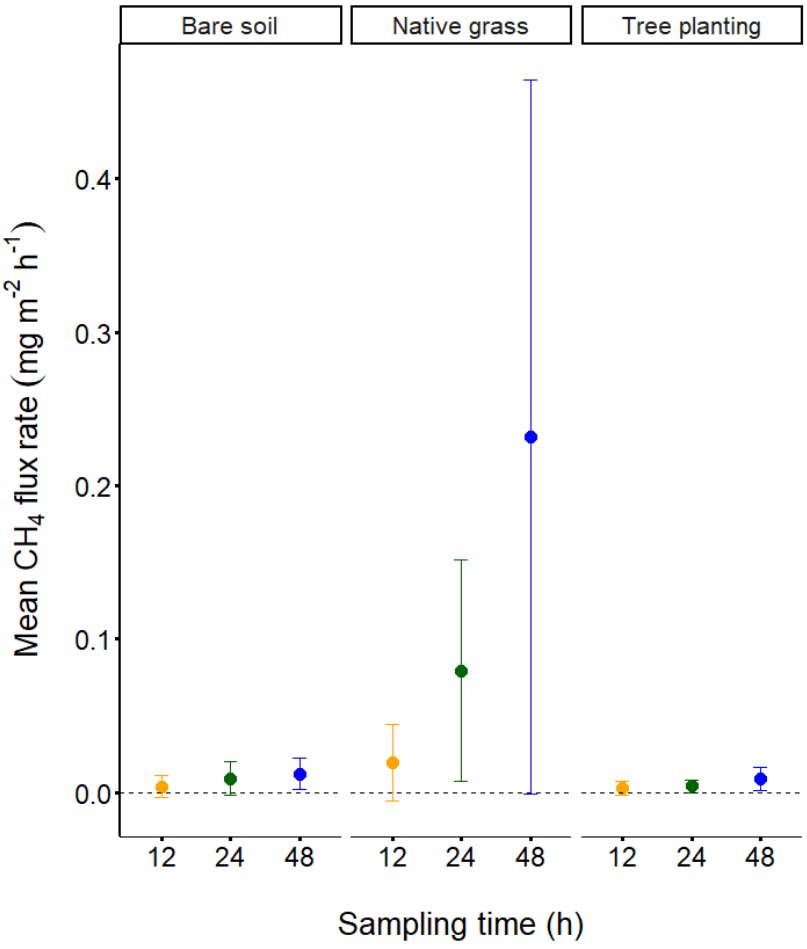

Figure 3.25.

Mean CH_4 flux rate at 12, 24, and 48 h sampling timepoint by habitat. Habitat types were bare soil, native grass, and tree planting. Error bars represent the 95% confidence interval.

Methane production rates for the 3-day flooding and 3-week flooding treatments were 0.011 and 0.006 mg m^{-2} h^{-1} at 12 h, 0.046 and 0.017 mg m^{-2} h^{-1} at 24 h, and 0.139 and 0.036 mg m^{-2} h^{-1} at 48 h sampling timepoints, respectively. More CH_4 was produced from the 3-day flooding treatment than the 3-week flooding treatment at all at sampling timepoints (Figure 3.26).

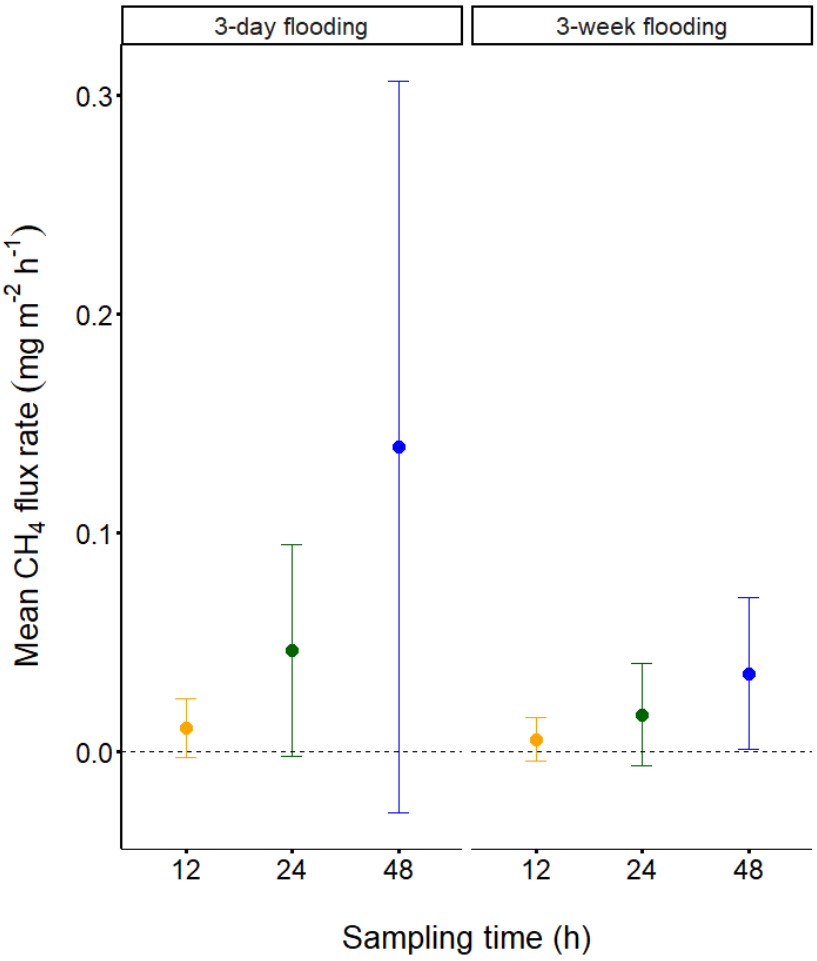

Figure 3.26.

Mean CH_4 flux rate at 12, 24, and 48 h sampling timepoint by hydrology. Hydrology levels were 3-day flooding and 3-week flooding. Error bars represent the 95% confidence interval.

3.7. Discussion

3.7.1. Hypotheses evaluation

Hypothesis i: Nitrogen and P retention in the porewater and N_2, N_2O, and CH_4 production will be affected by habitat types.

This hypothesis held true for both N and P retention. Furthermore, the effect of habitat on P retention also depended on hydrology for 2-3 days. While my hypothesis suggesting that N and P retention would be most pronounced in native grass habitat and least in bare soil habitat was generally confirmed. In terms of N_2 production, habitat types did not exhibit a significant influence, indicating that the hypothesis proposing that native grass habitat would produce the highest amounts of N_2 and N_2O was not corroborated, although the native grass treatment did produce the most CH_4. Furthermore, my hypothesis that N_2O production would be lowest in tree planting habitat was generally confirmed.

Hypothesis ii: Nitrogen and P retention in the porewater and N_2 production will be affected by flooding durations.

This hypothesis was not supported for N retention and P retention at first 24 h. Hydrology exhibited an interactive effect with habitat types for P retention at 2-3 days period. My hypothesis that retention would be higher for the 3-week flooding treatment held true for the bare soil and native grass habitats. For the tree planting habitat, P retention was higher in the 3-day flooding compared to the 3-week flooding. Hydrology had no significant effect on N_2 production at all sampling time points.

Hypothesis iii: Nitrogen and P retention in the porewater and N_2 production will be affected by soil properties.

My hypothesis that soil TC and TN would be the most influential soil properties for NO_3^- retention and N_2 production, and that soil P would the most influential soil property be PO_4^{3-} retention was not supported. This hypothesis was only partially supported when examining the influence of soil TN on N_2 production at 12 h. However, this effect was not evident at the 24 h and 48 h sampling time points.

3.7.2. Dissolved nutrients

Dissolved organic carbon (DOC) release

After 5 days of inundation, all treatments released DOC into the soil pores. The release was lowest from the native grass habitat and highest from the bare soil habitat. Water residence time had positive linear relationship with DOC release, but the effect was most pronounced for bare soil habitat. The release of DOC was significantly influenced by hydrology, with notably higher levels observed during 3-week flooding in comparison to 3-day flooding. Upon further examination within different habitats, this difference in hydrology was only evident in the bare soil habitat. This observation can be attributed to two main factors: Firstly, during the extended 8-week flooding cycle, mesocosms subjected to 3-week flooding experienced prolonged saturation, which likely led to a higher influx of organic compounds into the soil matrix. Waterlogged conditions can facilitate the release of organic compounds into the soil solution, resulting in elevated DOC concentrations (Liu et al., 2022; Yang et al., 2016). Secondly, the extended flooding period may have created anaerobic conditions, thereby shifting organic matter decomposition towards anaerobic processes, such as fermentation. This, in turn, could contribute

to the production of higher amount of organic carbon (Filep & Rékási, 2011; Oeurng et al., 2011).

When analyzed for each hydrology separately, the significant difference in DOC release among habitats was present in 3-week flooding hydrology only, where the release was significantly greater in bare soil habitat compared to native grass habitat. This disparity can be attributed to the role of vegetation in promoting microbial activity and nutrient uptake, including the uptake of organic C, from soil pores. The increased nutrient uptake by plants can reduce the availability of organic C (Roiha et al., 2011; Rubbo et al., 2006), leading to lower DOC accumulation. This uptake can reduce the concentration of DOC in the porewater. Furthermore, grasses can create microenvironments around their roots, which can be favorable for the growth of microorganisms. These microorganisms can play a crucial role in utilizing DOC for their metabolic processes, leading to less DOC accumulation in porewater. Bare soil habitat, on the other hand, lacked shading effects and exhibited a noticeable proliferation of algae (Figure 3.4). Algae are known for releasing DOC into the water during photosynthesis (Hall et al., 2022). Hence, the higher presence of algae implies a greater contribution to the DOC levels in the bare soil habitat.

The release of DOC displayed a negative correlation with soil P. Elevated soil P content can promote the growth of P-accumulating microbes, which have the ability to immobilize P in the soil (Zhao et al., 2022). Consequently, this immobilization process may reduce the availability of P for other microorganisms involved in the decomposition of organic matter. This, in turn, can lead to a decrease in organic matter decomposition, ultimately resulting in lower levels of DOC release.

Nitrate (NO_3^-) retention

After 5 days of inundation, NO_3^- concentration significantly decreased, approaching nearly zero, with > 95% decrease in most treatments. Native grass habitat was most effective in reducing NO_3^-, with > 95% decrease in NO_3^- concentration achieved by day 3 suggesting that NO_3^- retention was more influenced by habitat types. Water residence time had positive linear relationship with NO_3^- retention in bare soil and tree planting habitats. However, this linear relationship was observed only up to day 3 in the native grass habitat, primarily due to the fact that by that point, most NO_3^- had already been effectively reduced.

Nitrate retention was significantly high in native grass habitat and lowest in bare soil habitat. This observation might be attributed to the presence of extensive and dense root system in grasses that can improve soil structure and increase water infiltration, ultimately facilitating nutrient absorption. These factors collectively create conditions favorable for retaining nitrate in the soil (Galdos et al., 2020). Additionally, labile C from root exudates and rapid organic matter decomposition can fuel microbial communities (Jordan et al., 1993; Taylor et al., 2015), potentially enhancing their activity and leading to higher N removal. A positive correlation was observed between initial porewater NO_3^- concentration of mesocosms and NO_3^- retention. This relationship can be attributed to the greater efficiency of microbial denitrification at elevated NO_3^- concentrations (Albina et al., 2019).

Phosphate (PO_4^{3-}) retention

After 5 days of inundation, there was a significant decrease in PO_4^{3-} concentration compared to the initial dosing concentration, approaching nearly zero. Native grass habitat proved most effective in decreasing PO_4^{3-}, achieving over 85% decrease in concentration by day

3, indicating a strong influence of habitat types on PO_4^{3-} retention. In the first 24 h after dosing, bare soil habitat (both hydrology levels) and tree planting with 3-day flooding exhibited a mean PO_4^{3-} release in the porewater. During day 2-3, water residence time demonstrated a positive linear relationship with PO_4^{3-} retention in all treatments. There was no further increase in the uptake rate beyond day 3.

During the initial 24 h after dosing, PO_4^{3-} retention was highest in native grass habitat and lowest in bare soil habitat. This difference could be attributed to the effects of rice cutgrass litter and its root system in improving soil structure and water infiltration into the soil pores (Song et al., 2020). Furthermore, well-developed root system in rice cutgrass most likely facilitated rapid PO_4^{3-} uptake, thereby contributing to the observed higher PO_4^{3-} retention.

During days 2-3, retention rates decreased across all treatments, potentially due to a reduction in PO_4^{3-} in the porewater, rendering the differences less ecologically significant. Despite the overall decline in retention rates during this later period, bare soil habitat continued to retain significantly lower PO_4^{3-} compared to native grass and tree planting habitats. In contrast to the initial 24 h, no significant difference was observed between native grass and tree planting habitats during days 2-3 for both hydrology levels. This lack of distinction can be attributed to the rapid uptake and retention by native grass during the first 24 h, potentially reaching a point of retention equilibrium. As a result, PO_4^{3-} retention between the native grass and tree planting habitats may become relatively similar during this later period.

3.7.3. Nitrogen gas (N_2) production

All treatments experienced net N_2 production at each time point in the incubation experiment. At 12h, N_2 production was positively correlated with soil TN and supported previous research which found that high soil N content enhanced denitrification (Ma et al., 2020; Zhang et al., 2012). These results suggest that cores with high soil TN potentially supported greater microbial biomass and activity at 12 h, leading to an increase in N_2 production. Beyond 12 h, N_2 production was not affected by soil TN. The strong correlation between N_2 and SOD was observed throughout the incubation period, which can be attributed to the promotion of anoxic conditions in the cores. This correlation between N_2 production and SOD has been identified in previous studies (Baldwin & Mitchell, 2000; Taylor et al., 2015). Higher SOD leads to rapid O_2 consumption in the sediment, creating an anoxic low redox environment suitable for optimal denitrification (Cornwell et al., 1999; Rohe et al., 2021).

3.7.4. Soil nutrients

The relatively stable levels of soil TC and soil P between pre and post-dosing samples suggest that the predominant mechanisms responsible for the release of DOC into the porewater and the reduction of PO_4^{3-} were most likely biological assimilatory and/or dissimilatory processes. However, there was a notable increase in soil TN post-dosing by approximately 48 %. Besides biological assimilation, this increase in TN may be attributed to the retention of NO_3^- in the soil particles. This increased TN level could have contributed to the significance of soil TN in the N_2 model at 12 h. However, beyond 12 h, no correlation was observed between soil TN and N_2 production.

3.7.5. Nitrous oxide (N_2O) production

Nitrous oxide production showed a decreasing trend as incubation time increased, suggesting that anoxic conditions does not favor N_2O production. Under reduced conditions, oxygen diffusion into the soil is limited, creating hypoxic conditions. Under these conditions, complete denitrification is more likely to occur, resulting in the reduction of N_2O to N_2 (Butterbach-Bahl et al., 2013) and decreasing N_2O production. The highest N_2O production was observed in bare soil habitat rather than in vegetated ones, potentially due to the depletion of oxygen in the root zone. The active respiratory activities of the microbial community at the soil-water interface, in conjunction with root metabolism and diminished oxygen diffusivity in water, rapidly deplete oxygen in the root zone, fostering an anaerobic environment (Balakhnina et al., 2012; Lüdemann et al., 2000). Nitrous oxide production was higher in mesocosms that received 3-day flooding. A longer dry cycle is found to favor incomplete denitrification to N_2O (Ciarlo et al., 2008; Robertson & Tiedje, 1987). Therefore, longer dry period between flooding may have contributed to this outcome in 3-day flooded mesocosms.

3.7.6. Methane (CH_4) production

Methane production in bare soil and tree planting habitat stayed near zero throughout the experiment. However, in native grass habitat, CH_4 production showed an increasing trend as incubation time increased, suggesting that anoxic conditions favored CH_4 production in this habitat. Prior studies have identified saturated soils as significant sources of CH_4 (Van Thao et al., 2022; Wang et al., 2018). The observed higher CH_4 production in the native grass habitat can be attributed to root zone metabolism, potentially creating an anaerobic environment at the soil-water interface. Additionally, the higher influx of organic matter from roots and decomposing

plant materials serves as a substrate for methanogens to produce CH_4. According Nahlik & Mitsch (2010), vegetation mostly influences CH_4 production by sequestering soil C. Conversely, bare soil lacking roots and tree planting habitat with potentially deeper roots may not have produced enough labile C in the top 15 cm of soil as efficiently as native grass habitat did. Additionally, microbial utilization of labile C may have contributed to higher methane production in native grass habitat. Native grass habitat exhibited lowest DOC release rates while it was highest in the bare soil habitat followed by tree planting habitat. Native gras habitat may have supported microbial communities that are more efficient in methanogenesis and potentially used available DOC to fuel both denitrification and ultimately CH_4 production.

3.8. Conclusion

Following 5 days of inundation, all treatments in the wetland mesocosm experiment effectively reduced porewater NO_3^- and PO_4^{3-} - concentrations to near-zero levels. This implies that holding floodwater for at least 5 days may be necessary to achieve optimal nutrient retention in restored wetlands. Notably, the native grass habitat demonstrated the highest efficiency in NO_3^- and PO_4^{3-} reduction and lowest DOC release. There was a significant release of DOC in the bare soil habitat. The interactions between habitat and hydrology influenced both DOC release and PO_4^{3-} retention, whereas NO_3^- retention was affected by habitat only. Furthermore, the initial concentrations of NO_3^- in the porewater immediately after nutrient dosing showed a positive correlation with subsequent NO_3^- retention. It's important to note that neither habitat nor hydrology had an effect on N_2 production. Instead, soil redox proved to be the most influential factor affecting N_2 production. Additionally, production of greenhouse gases (N_2O, CH_4) remained minimal in all treatments. The only significant increase in soil chemical properties

observed after inundation was for soil TN. This study emphasizes the significance of considering water residence time, habitat type, hydrology, and the interactions between habitat and hydrology to maximize nutrient retention in restored wetlands.

CHAPTER 4: GREENHOUSE GAS EMISSIONS FROM RESTORED WETLANDS UNDER INCREASED TEMPERATURE CONDITIONS

4.1. Abstract

The importance of increasing organic matter input and extending the residence time of carbon (C) pools in wetland restoration efforts is well recognized. However, in the context of rapidly changing climate, there is a concern that C sequestration may decrease, potentially leading to increased greenhouse gas emissions in various ecosystems worldwide. This study conducted an incubation experiment to evaluate the impact of elevated temperature on the fluxes of methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O) from restored wetlands. The experiment included various habitat types, including crop field, remnant forest, shallow water-dry, shallow water-wet, tree planting, and natural wetland. Intact soil cores were subjected to incubation at two different temperatures, 24°C and 29°C, with subsequent collection of gas samples to analyze greenhouse gas flux rates. Following this, the cores were processed to determine soil properties, including soil moisture (SM), bulk density (BD), pH, total carbon (TC), total nitrogen (TN), extractable phosphorus (soil P), and extractable iron (soil Fe). All soil properties varied significantly among habitat types. The mean SM, pH, soil TC, TN, and Fe were highest in natural wetland, while mean BD and soil P were highest in crop field. Greenhouse gas fluxes were significantly affected by habitat types. Mean CH_4 production was highest from the shallow water-wet habitat and lowest from the crop field. Methane flux was correlated positively with SM and pH. The increment in mean CH_4 production ranged from 1.5% in the crop field to 300% in the remnant forest habitat as the temperature was increased. Mean CO_2 production was highest from the remnant forest habitat and lowest from the shallow water-wet habitat. Carbon

dioxide flux was correlated positively with soil TC and TN. The increase in mean CO_2 production ranged from 5.5% in the natural wetland to 40% in the shallow water-dry habitat as the temperature increased. Crop field displayed the highest mean N_2O production, while natural wetland exhibited the lowest. Nitrous oxide flux was positively correlated with BD and soil P, and negatively with soil Fe. The increment in mean N_2O production ranged from 32% in the crop field to 360% in tree planting habitat as the temperature was increased. These findings emphasize how different habitats within restored wetlands respond differently to temperature increase and soil properties in the context of greenhouse gas emissions.

4.2. Introduction

Only six percent of the earth's land surface is covered by wetlands (Mitsch & Gosselink, 2015), but their significance cannot be understated. Wetlands provide numerous beneficial services for people and wildlife that may include environmental, economic, and recreational benefits (TN Department of Environment and Conservation, 2021). They are essential for biodiversity conservation, acting as hotspots for a wide range of plant and animal species (Balmford et al., 2002; Palay, 2021).Wetlands support diverse ecosystems, providing habitats for numerous species and contributing to the overall health and resilience of ecosystems (Amoros & Bornette, 2002). One of the key benefits of wetlands is their ability to sequester C and their importance as C sinks is recognized globally (Chmura et al., 2003). Wetlands are important for reducing emissions and regulating, capturing, and storing greenhouse gases (Bonetti et al., 2019; Palay, 2021). Freshwater wetlands hold significant potential for climate change mitigation owing to their large capacity to sequester atmospheric CO_2 (Dayathilake et al., 2020).

Wetlands also play a vital role in improving water quality. They function as natural filters, capturing sediments and contaminants, thus aiding in the purification of water (Ghermandi et al., 2010). Wetlands can intercept runoff before it reaches open water and remove excess nutrients by biological, chemical, and physical processes (TN Department of Environment and Conservation, 2021). Moreover, wetland vegetation is instrumental in flood regulation and minimizing soil erosion, as their root systems anchor soil and reduce the velocity of streams and runoff (Visser et al., 1996). Wetlands can also contribute to flooding reduction by acting as natural buffers and soaking up and storing floodwater (TN Department of Environment and Conservation, 2021).

Wetlands are, however, threatened by climate change and could change forever (Palay, 2021). The global shift in climate patterns have been disrupting natural systems worldwide, either by altering hydrological regimes or biological parameters. Therefore, it is likely that many of the world's wetland systems will become vulnerable and may be profoundly affected by climate change over the coming decades (Green et al., 2017). Some wetland complexes showing pronounced effects of climate change include the Mekong River Delta in Vietnam, southern Ontario in Canada, and the Sundarban in Bangladesh and India. Under such circumstances, the efforts to restore and manage wetlands are likely to become more challenging in the future (Erwin, 2009).

While wetland restoration initiatives aim to increase the organic matter input and residence time of C pools (Were et al., 2019), warmer temperatures have accelerated organic matter decomposition, resulting in the loss of the C stored in the wetland soils into the atmosphere (Bridgham et al., 2006; Inglett et al., 2012; Moomaw et al., 2018). Global warming could mean the loss of water from wetlands due to increased evaporation. As a result, previously

inundated organic matter can be oxidized and lead to CO_2 emissions, which will further contribute to global warming (Hicks et al., 1999). One such example is the degradation of Queensland's wetlands, particularly mangroves and melaleuca wetlands, which are now becoming sources of greenhouse gas (GHG) emissions (Commonwealth of Australia, 2012). Additionally, warming of wetland soils can aggravate CH_4 emissions, a gas that is 25 times more potent than CO_2 in terms of greenhouse effects (Palay, 2021). Consequently, higher temperatures in the future may shift the role of wetlands from C sinks to C source (Salimi et al., 2021). Wetland restoration efforts also focus on nutrient removal through denitrification, plant utilization, and soil adsorption. However, warmer temperatures followed by drought can create aerobic conditions and increase the production of N_2O relative to nitrogen gas (N_2) during denitrification. This elevated N_2O production can contribute to global warming (Seo & DeLaune, 2010).

Research needs on effects of future climate change on restored wetland function (greenhouse gas emissions)

According to the National Oceanic and Atmospheric Administration (NOAA), the average global temperature is projected to increase up to 5.4°C by the year 2100, mostly owing to human-induced GHG emissions (Herring, 2012). Recent climate research has adopted novel scenarios to model the future climate of earth, particularly in the aftermath of COVID-19 pandemic in 2020 (Tollefson, 2020). During this crisis, many countries turned to inexpensive fossil fuels as means of mitigating the economic challenges stemming from the pandemic. Unfortunately, this response led to increased C emissions and subsequent temperature elevations, setting the stage for 5^{o}C of warming by the end of the 21st century (Tollefson, 2020). Under such

circumstances, the successful restoration of wetlands in the future may depend on how we choose to respond to the effects of global warming.

More research is needed to determine how rising temperatures affect GHG emissions from different restored wetland habitats. However, to derive the influence of a single climate parameter, such as temperature, in the field is challenging due to co-variation or interaction of various environmental factors (Davidson et al., 2000; Pilegaard et al., 2006). Given that climate-related factors exhibit variability across different sites, comparing soil properties and their potential impact on GHG flux becomes a complex undertaking. To address these interactions, a practical approach is to incubate soil cores in a controlled laboratory setting. This methodology allows the independent manipulation of temperature, thereby eliminating the possibility of confounding co-variations.

Evaluations of greenhouse gas emissions from restored riparian wetlands are lacking globally. While some studies have investigated C sequestration and CH_4 fluxes in restored prairie potholes (Badiou et al., 2011) and peatlands (Hemes et al., 2018), others have primarily focused on one or two gas species under constant temperature conditions (del Prado et al., 2006; Ruser et al., 2006). In many laboratory incubation studies, researchers have used sieved soils (Bandibas et al., 1994) for the advantage of sample homogenization. However, this approach has a significant drawback as it breaks soil aggregates and artificially aerate the soils, thereby affecting gas fluxes substantially (Schaufler et al., 2010). Notably, laboratory simulation experiment using intact soil cores to assess the impact of realistic future temperature conditions on three different GHG species (CH_4, CO_2, and N_2O) across a wide range of habitat and soil types of restored riparian wetlands are so far not known. Gaining insights into how different habitat types, each associated with distinct restoration strategies, respond to temperature increase is crucial. This understanding

will help land managers to make informed decisions regarding optimal habitat management aimed at minimizing GHG emissions from restored wetlands.

4.3. Objectives

i) examine the variation in soil properties and GHG fluxes among habitat types and the relationship between the two

ii) examine if there is a predictable change in GHG fluxes with increased temperature

4.4. Hypotheses

i) Soil properties and fluxes of CH_4, CO_2, and N_2O will differ among habitats. Habitats that are permanently flooded or have high moisture in the soil (i.e., shallow water-wet and natural wetland habitats) will be dominated by CH_4 production. Newly restored easements experiencing legacy effects from soil cultivation and tillage will produce higher CH_4 compared to older easements due to the destruction of niches for methanotrophs which inhibits CH_4 oxidation. Methane flux (both uptake and release) will decrease with the increase in soil BD. Methane uptake will be highest in the habitat with a dense rhizosphere and lower BD (i.e., remnant forest) because forest soil is coarse-textured due to the presence of high organic matter, which supports higher microbial activity and is conducive to the rapid oxidation of CH_4 by methanotrophs. Because the optimum pH for CH_4 production is usually near neutrality (Wang et al., 1993), habitats with a soil pH close to 7 will produce more CH_4.

Drier habitats (i.e., crop field) will be dominated by CO_2 production due to easier oxidation and microbial decomposition of organic matter. Production of CO_2 will be

lowest from shallow water-wet and natural wetland habitats due to high SM, which inhibits oxidation of organic matter. Higher soil TC with correlate positively with CO_2 production because of the presence of adequate substrate for microbial respiration. Additionally, high soil TN will stimulate microbial decomposition of organic matter, resulting in an increase in CO_2 production.

The presence of a dense rhizosphere will promote a high amount of N_2O production from densely vegetated habitats due to the formation of anaerobic microsites that promote high denitrifiers activity. Nitrous oxide production will also be higher in habitats with high TN due to the presence of more N for denitrification. Habitats that are intermittently flooded or have moderate soil moisture (i.e., remnant forest) will be dominated by N_2O production due to incomplete denitrification. Nitrous oxide production will increase with decreasing soil pH because of the effect on the activity of incomplete denitrifiers (Liu & Greaver, 2009). The production of N_2O will be lowest in shallow water-wet and natural wetland habitats due to the production of a relatively higher amount of N_2 compared to N_2O during denitrification because of high moisture conditions.

ii) An increase in temperature will influence CO_2, CH_4, and N_2O fluxes from restored wetland habitats. Three major factors that limit decomposition in the wetlands are temperature, chemical and physical forms of organic matter, and soil saturation (Moomaw et al., 2018). An increase in temperature will accelerate microbial decomposition and oxidation of organic matter in all habitats leading to increased CO_2 production. Also, an increase in temperature will promote the oxidation of CH_4 by methanotrophs and decrease CH_4 production. However, the temperature increase will also

stimulate microbial activity that can decompose recalcitrant organic matter making C sources available for methanogens(Jenkinson et al., 1991; Tveit et al., 2015). Therefore, CH_4 production will increase in shallow water-wet and natural wetland habitats in response to increased temperature. Nitrous oxide production will also increase with an increase in temperate due to incomplete denitrification to N_2O. However, increasing temperature for the short term will not increase N_2O emissions from habitats with severe N limitations.

4.5. Methods

4.5.1. Study site and habitat types

For this study, four WRP/WREP easements located in Kentucky and Tennessee were selected (Figure 4.1). The easements included different habitats, namely crop field, remnant forest, shallow water-dry, shallow water-wet, tree planting, and natural wetland. The habitats were representative of the restoration practices. Crop fields were those areas in the easement which were actively farmed and recently entered the WRP program (Figure 4.2A). Remnant forest habitat included areas with native tree species that has not been in recent agricultural production, as determined by areal images dating back to the 1980s and 1990s. The hydrology in shallow water areas is often actively managed using water control structures (USDA NRCS, 2012). Thus, shallow water habitat was determined as dry or wet based on the absence or presence of water at the time of sampling. Sediment cores collected from the dry edge of shallow water-wet habitat were also classified as shallow water-dry cores. Tree planting habitat included areas where trees/shrubs were planted to supplement forest stand regeneration in locations where natural regeneration of desired species was not possible (USDA NRCS, 2016). Natural wetland

habitats are permanently or seasonally saturated, have wetland vegetations (Figure 4.2B) and were not intervened by WRP program (UNEP-DHI Partnership, 2017). However, all habitats were not present in every easement.

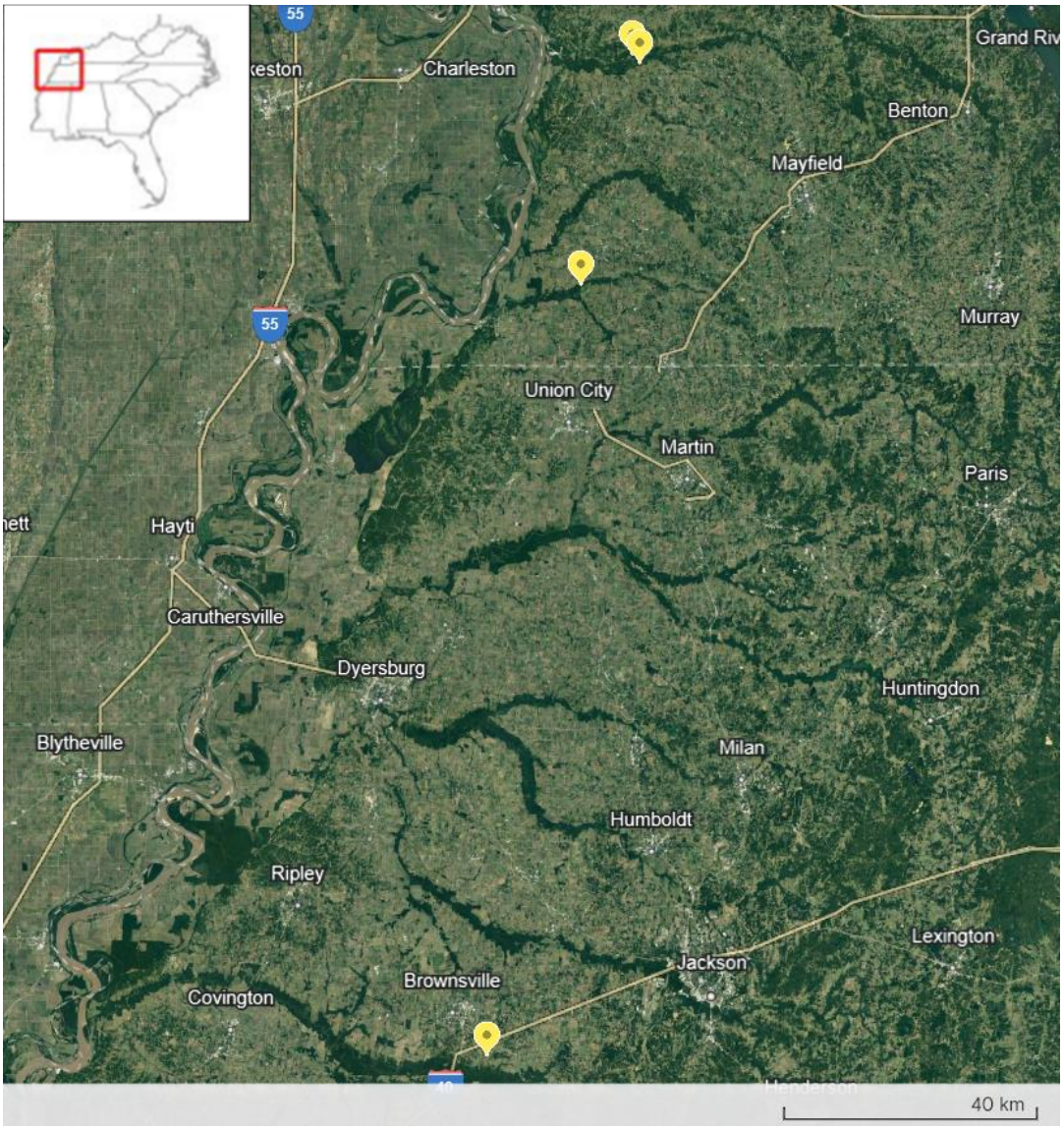

Figure 4.1.

Map of study sites in Kentucky and Tennessee. Four WREP easements where samples were collected are represented by yellow pins.

Figure 4.2.

A) Crop field and B) Natural wetland habitats.

4.5.2. Core collection

Intact soil cores were collected from June through August to ensure comparable phenological conditions. Intact soil cores were used in this study to avoid disturbance effects caused by soil drying, grinding, and sieving (Reichstein et al., 2005). Acrylic tubes were used for soil cores collection and incubation because they are inert to the gases being sampled (Collier et al., 2014). Thirty 10 cm deep soil cores were collected from representative habitats at each easement as described in Chapter 2.

Figure 4.3.

Sediment core from shallow water-wet habitat.

4.5.3. Core incubation

Soil cores were incubated in an environmental chamber (Figure 4.4). The incubation took place in the dark to restrict CO_2 utilization during photosynthesis. A temperature of 24^{o}C was maintained during the first incubation round to simulate average summer regional air temperature during the core collection period. Water from aquatic cores was siphoned out carefully, and the outside of acrylic tubes was wiped clean. The litter and vegetation layer were removed from cores collected from vegetated habitats. Each core was weighed without the top (lid) and adjusted in the chamber for 9 hours. This adjustment period was expected to help in the adaptation of the soil to the change in temperature from when the cores were transported to the lab on ice. It also allowed headspace gas to equilibrate with the environmental chamber atmosphere. To maintain constant soil weight throughout the incubation period, the cores were

re-weighed, and ultra-pure water was added to the soil surface as needed to replace evaporation loss. Next, the cores were capped with acrylic lids equipped with a stopcock regulated gas sampling port (i.d. 1.25 mm) and secured with pipe straps. Immediately after sealing the lid, time 0 (T0) gas samples were collected from each core. Another round of samples was collected after 3 hours (T3h) of incubation. After the first incubation cycle, the acrylic lids from the cores were removed, and the temperature of environmental chamber was increased by 5^{o}C (to 29^{o}C) to simulate the average global temperature projected by 2100. Once, the chamber reached 29^{o}C, cores were adjusted to this increase in temperature for 9 hours. Water was added as needed to maintain the soil moisture before re-capping the cores. Gas samples were collected at T0 and T3h after second incubation round.

Figure 4.4.

Incubation setup in an environment chamber. Acrylic lids were equipped with a stopcock regulated gas sampling port.

4.5.4. Gas sampling

Gas samples were collected in 20 mL glass vials fitted with aluminum crimp caps (Agilent Technologies, Inc. Santa Clara, California). The glass vials were flushed with high purity nitrogen gas (N_2). Gas samples were collected following the protocols of (Collier et al., 2014). Time 0 gas samples at each incubation temperature were collected immediately after sealing each soil core. Time 0 gas samples represented the initial gas concentration in each core. Another gas sample from the soil core were collected after 3 hours of incubation. Nguyen et al.

(2014) determined that gas sampling after sealing the chamber for 3 hours is the optimal choice for estimating GHG emissions in small chamber incubation experiments.

A 100 mL polypropylene gas-tight syringe fitted with a stopcock was attached to the sampling port present on the acrylic top (Figure 4.4). After drawing a 70 mL gas sample from the core headspace, the stop cocks on both syringe and the sampling port were closed, and the syringe was detached from the core. The syringe was then fitted with a needle. The flushing method was used while transferring the gas samples to vials. According to the flushing method, an extra needle was inserted near the edge of the vial's cap septum. After injecting approximately 40 mL sample into the vial, the extra needle was removed smoothly while continuing to inject the remaining 30 mL sample while slightly over pressurizing the vial. Initial sample injection helped to clear the previous content from the vial through the extra needle, and then when the extra needle was removed, the vial was filled to positive pressure with the gas sample. The stopcock was closed, and the syringe needle was withdrawn from the septum. The gas-filled vials were turned upside down to distinguish them from unfilled vials. Any delayed times were recorded and corrected during data analysis by adjusting the time associated with a certain sample. Chamber temperature and atmospheric pressure were also measured at the time of gas sampling. Each time 70 mL gas sample taken from the core headspace was replaced with high purity 70 mL N_2.

4.5.5. Greenhouse gas (GHG) analysis

Greenhouse gases (CH_4, CO_2, and N_2O) were analyzed simultaneously from a single sample immediately after collection using gas chromatography (Agilent 8890 GC System, Agilent Technologies, Inc. Santa Clara, California). The GC was coupled to a PAL3 Series II auto sampling system (Pal System, Zwingen, Switzerland) equipped with a custom sampling tray using Agilent OpenLab ChemStation (Figure 4.5). The GC was fitted with a ^{63}Ni electron capture detector (ECD) for N_2O and a flame ionization detector (FID) for CH_4 and CO_2 (after passing CO_2 through the methanizer) analysis. The detector temperature for ECD was 300^oC, and the makeup flow rate of carrier gas was set to 2 mL min^{-1}. The detector temperature for FID was 250^oC, and the makeup flow rate of carrier gas was set to 25 mL min^{-1}. The oven and syringe temperatures were set to 60^oC and 40^oC, respectively. The channel was maintained at a static pressure of 150 kPa.

Calibration was done using 100, 250, 500, 750, and 1000 μL L^{-1} CO_2, 0.5, 1.25, 2.5, 3.75, and 5 μL L^{-1} CH_4, and 0.1, 0.25, 0.5, 0.75, and 1 μL L^{-1} N_2O. The lowest detection limits were performed on ambient air samples. Fifteen outdoor air samples were collected and analyzed in the GC with a suite of standards. The CV of each GHG was calculated. The percent CV is the percent of the normal ambient air concentration of each gas. The GC was expected to detect changes in the concentration down to the lowest detection limit.

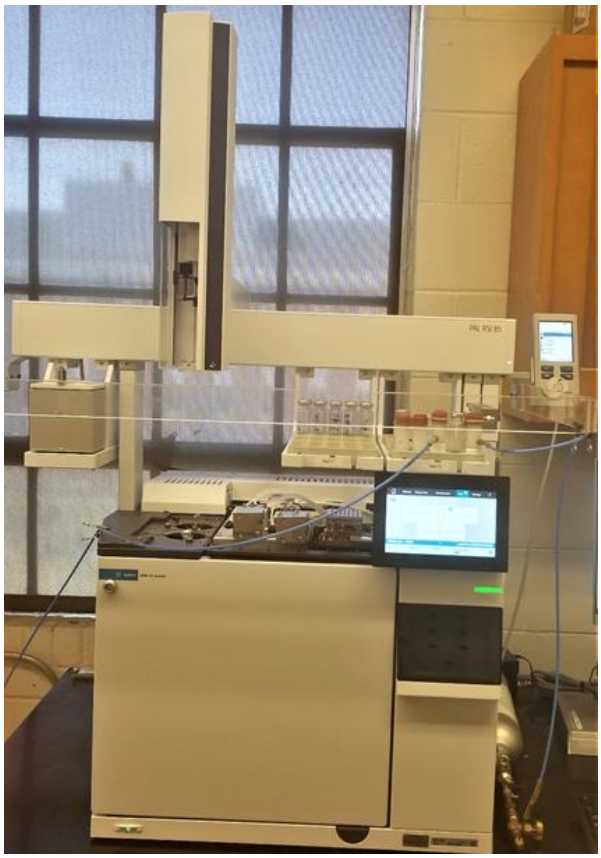

Figure 4.5.

Gas chromatograph.

Greenhouse gas concentrations of samples were calculated based on the peak areas measured by the detectors. Gas fluxes were determined by headspace concentration change in the incubated core from T0 to T3h and expressed as mg C-CH_4, mg C-CO_2, and mg N-N_2O m^{-2} h^{-1}. Gas fluxes were calculated using i) linear slope between T0 and T3h gas concentrations (Romero et al., 2021), ii) volume of incubation core headspace, iii) incubation core area, iv) the Ideal Gas Law, v) atmospheric pressure in the environmental chamber during sample collection (the higher the pressure the more gas molecules in the core headspace), and vi) temperature in the environmental chamber (higher temperatures decrease the number of gas molecules per volume) and then converted to mass per volume flux using the equation below (Zaman et al., 2021).

Emissions from the soil were represented by positive flux while uptake by the soil were represented by negative flux.

GHG flux (mg m^{-2} h^{-1}) = $C_t \times M \times 10^{-6} \times (V_{in}/A_{in}) \times P/ (R \times T)$

where,

C_t = slope derived from the linear regression for CH_4, CO_2, and N_2O (ppm h^{-1}) at t°C,

M = molar mass (g mol^{-1}) (N = 28 for N_2O and C = 12 for CH_4 and CO_2),

V_{in} = volume of the incubation core headspace (0.000912 m^3),

A_{in} = area of incubation core (0.00456 m^2),

P = environmental chamber pressure at the time of sampling (Pa),

R = gas constant (8.314 J mol^{-1} K^{-1}), and

T = environmental chamber temperature (K) (273.15 + t°C)

4.5.6. Core processing and soil properties analysis

After the completion of incubation, cores were removed from the environmental chamber and processed to determine SM, BD, pH, soil TC, soil TN, and extractable P (soil P) as described in Chapter 2. The Mehlich-3 extraction procedure was used to determine "plant-available" Fe using colorimetry (Mehlich, 1984). The Mehlich-3 extractant is a mixture of acetic acid (CH_3COOH), ammonium nitrate (NH_4NO_3), ammonium fluoride (NH_4F), nitric acid (HNO_3) and ethylenediaminetetraacetic acid (EDTA) at pH 2.5. The Mechlich-3 extractant mixture works by breaking down the chemical bond between the soil particles and adsorbed Fe, releasing the Fe into the solution. The solution is then filtered and extracted Fe is quantified using colorimetry. Soil nutrient concentrations were expressed in dry weight equivalent. The limitation of the incubation process is that the amount of nutrient left in the soil will be lower after the completion

of the process. However, application of similar incubation scheme to all soil cores was expected to make the potential bias equal for all soil cores, therefore making the soil properties data comparable.

Missing data

Data from core #3 at site #2 (shallow water-wet habitat) were not available due to issues with soil processing. The resulting sampling size was: n = 119 for soil properties (SM, BD, pH, soil TC, soil TN, soil P, and soil Fe) and GHG (CH_4, CO_2, N_2O) flux analyses.

4.5.7. Statistical Analysis

Statistical analysis was performed using R statistical software from the R Core Team (2022). Data were tested for normal distribution and variance homogeneity. For multiple comparisons of soil properties and gas fluxes data, Analysis of Variance (ANOVA) was used when the variance was homogeneous and Kruskal Wallis test was used when variances were inhomogeneous. Because the data were not normally distributed, Spearman rank correlation method was used for relating gas fluxes to site data (SM, BD, pH, soil TC, soil TN, soil P, and soil Fe).

4.6. Results

4.6.1. Soil properties

Habitat types exhibited significant differences across multiple soil properties. These differences were observed in SM (Kruskal-Wallis χ^2 (5) = 58.136, P<0.0001), BD ($\chi^2_{(5)}$ = 54.037, P<0.0001), pH (ANOVA $F_{(5)}$ = 5.451, P = 0.0002), soil TC ($\chi^2_{(5)}$ = 24.915, P = 0.0001), soil TN ($\chi^2_{(5)}$ = 18.096, P = 0.0028), soil P ($\chi^2_{(5)}$ = 25.578, P = 0.0001), and soil Fe ($\chi^2_{(5)}$ = 48.953, P<0.0001). The mean SM, pH, soil TC, soil TN, and soil Fe were highest in natural wetland habitat (Figures 4.6 A & C, Figures 4.7 A, B, & D, Table 4.1). Crop field exhibited the highest mean BD and soil P (Figures 4.6 B, Figure 4.7 C, Table 4.1). The mean SM and soil Fe was lowest in crop field (Figure 4.6 A, Figure 4.7 D, Table 4.1). Mean BD was lowest in natural wetland habitat (Figure 4.6 B, Table 4.1). Furthermore, shallow water-dry habitat had the lowest mean pH and soil P, (Figure 4.6 C, Figure 4.7 C, Table 4.1), while shallow water-wet habitat displayed the lowest mean soil TC and TN (Figures 4.7 A & B, Table 4.1).

Soil properties exhibited considerable variability, both within and among habitat types (Figures 4.6 & 4.7, Table 4.1). Soil P displayed the least variation within and among the habitats followed by soil Fe (Figures 4.7 C & D, Table 4.1). The variability in SM, BD, soil TC, and TN were most pronounced in the natural wetland habitat (Figures 4.6 A & B, Figures 4.7 A & B, Table 4.1). The variation in soil pH was highest in shallow water-dry habitat (Figure 4.6 C). However, the mean soil pH was nearly similar across all habitats (Table 4.1). Both soil P and Fe showed highest variability in shallow water-dry habitat (Figures 4.7 C & D).

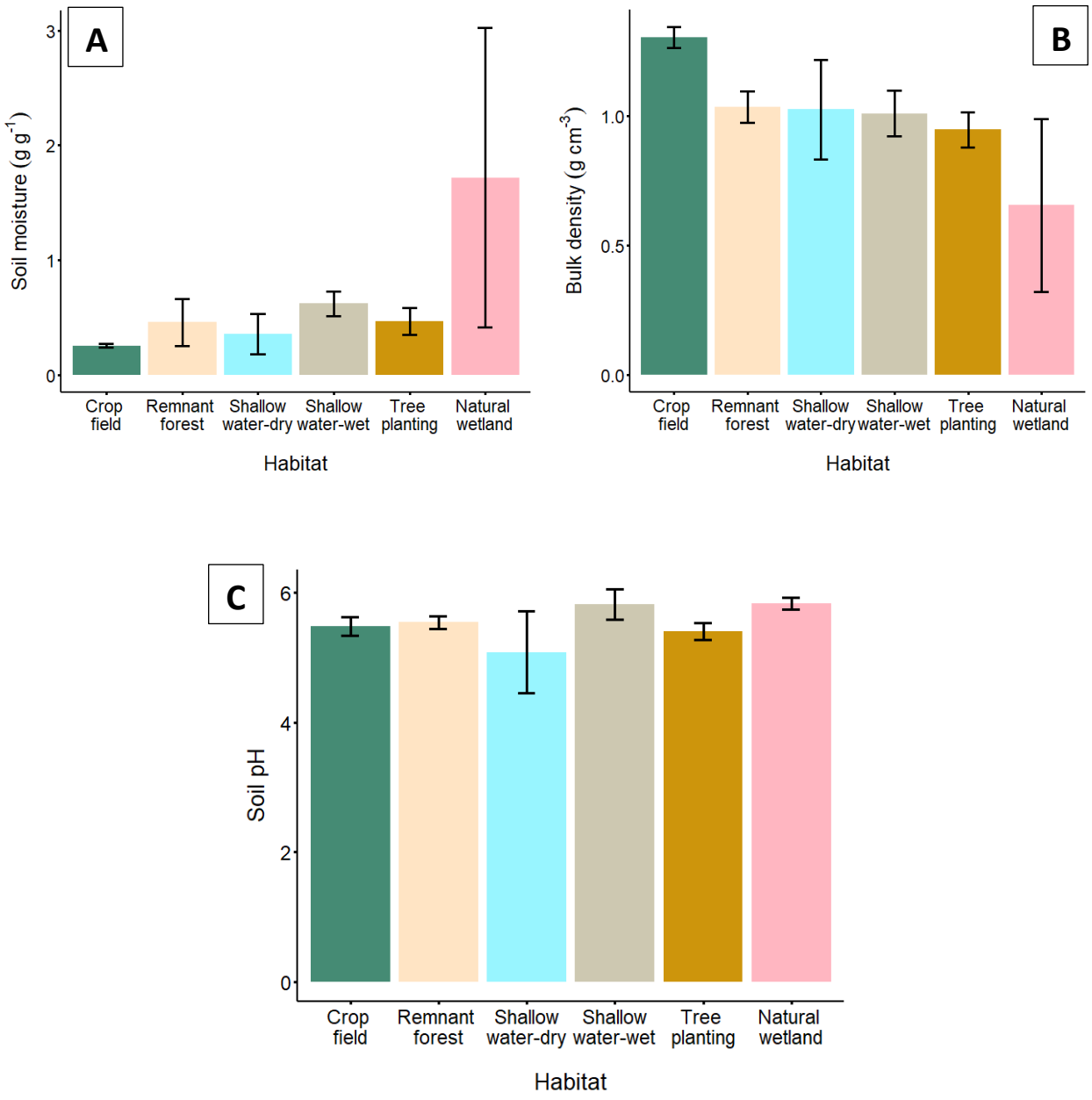

Figure 4.6.

Mean A) soil moisture, B) bulk density, and C) soil pH. Habitat types were crop field, remnant forest, shallow water-dry, shallow water-wet, tree planting, and natural wetland. Error bars represent 95% confidence interval.

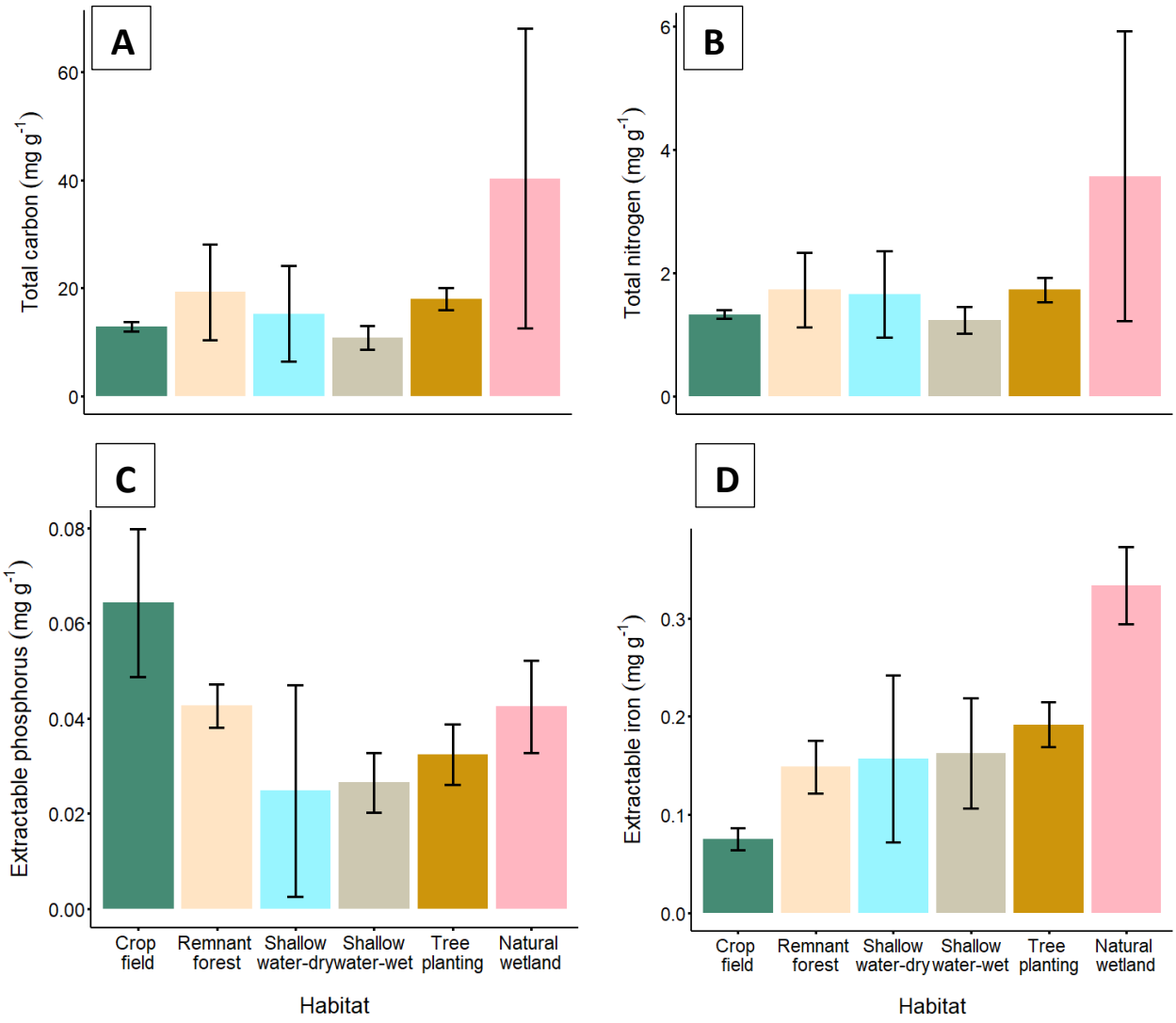

Figure 4.7.

Mean soil A) total carbon, B) total nitrogen, C) extractable phosphorus, and D) extractable iron. Habitat types were crop field, remnant forest, shallow water-dry, shallow water-wet, tree planting, and natural wetland. Error bars represent 95% confidence interval.

Table 4.1.

Soil properties means ± SE for 4 easements and 6 habitat types. The soil properties include soil moisture, bulk density, pH, total carbon, total nitrogen, extractable phosphorus, and extractable iron.

Habitat	n	Soil moisture ($g\ g^{-1}$)	Bulk density ($g\ cm^{-3}$)	pH	Total carbon ($mg\ g^{-1}$)	Total nitrogen ($mg\ g^{-1}$)	Extractable phosphorus ($mg\ g^{-1}$)	Extractable iron ($mg\ g^{-1}$)
Crop field	24	0.26 ± 0.01	1.30 ± 0.02	5.48 ± 0.07	12.88 ± 0.44	1.33 ± 0.04	0.06 ± 0.007	0.08 ± 0.01
Remnant forest	46	0.46 ± 0.10	1.04 ± 0.03	5.54 ± 0.05	19.29 ± 4.41	1.73 ± 0.30	0.04 ± 0.002	0.15 ± 0.01
Shallow water-dry	5	0.36 ± 0.06	1.03 ± 0.07	5.08 ± 0.23	15.28 ± 3.21	1.66 ± 0.25	0.02 ± 0.008	0.16 ± 0.03
Shallow water-wet	14	0.62 ± 0.05	1.01 ± 0.04	5.82 ± 0.11	10.84 ± 1.00	1.24 ± 0.10	0.03 ± 0.003	0.16 ± 0.03
Tree planting	24	0.47 ± 0.06	0.95 ± 0.03	5.40 ± 0.06	17.95 ± 0.99	1.73 ± 0.09	0.03 ± 0.003	0.19 ± 0.01
Natural wetland	6	1.72 ± 0.51	0.66 ± 0.13	5.84 ± 0.04	40.32 ± 10.80	3.58 ± 0.92	0.04 ± 0.004	0.33 ± 0.02

4.6.2. Methane (CH_4) flux

CH_4 flux at 24°C at 29°C and relation with soil properties

Methane fluxes were significantly different among habitat types at both 24°C (Kruskal-Wallis $\chi^2_{(5)} = 46.151$, $P<0.0001$) and 29°C ($\chi^2_{(5)} = 45.268$, $P<0.0001$). Methane production remained negligible in crop field, remnant forest, and shallow water- dry habitats under both temperature conditions (Figure 4.8) and the mean production rates ranged from 0.01-0.02 mg m^{-2} h^{-1} at 24°C and 0.01-0.04 mg m^{-2} h^{-1} at 29°C (Table 4.2). The highest production rates were measured for shallow water-wet habitat followed by natural wetland and tree planting habitats. (Figure 4.8, Table 4.2). Specifically, the mean CH_4 production rates at 24°C for the shallow water-wet, tree planting, and natural wetland habitats were 2.67, 0.39, and 1.28 mg m^{-2} h^{-1}, respectively. At 29°C, these rates increased to 6.46, 1.14, and 2.48 mg m^{-2} h^{-1} (Table 4.2). Shallow water-wet habitat exhibited the highest variability in CH_4 production rates (Figure 4.8). Few soil cores from tree planting (both temperatures) and natural wetland (24°C) habitats were observed to consume CH_4. However, at 29°C, CH_4 production replaced consumption in the natural wetland habitat (Figure 4.8).

Methane production exhibited a consistent upward trend as SM levels increased, reaching highest production when SM exceeded 80%, both at 24°C and 29°C. (Figure 4.9). Notably, at both temperatures, some soil cores exhibited CH_4 consumption within SM levels ranging from 60% to 80%. The highest variability in CH_4 production rates occurred when the SM was within the 60-80% range (Figure 4.9). Additionally, at 24°C, CH_4 fluxes were positively correlated (Spearman correlation) with SM ($r = 0.42$, $P < 0.0001$), and pH ($r = 0.31$, $P = 0.0006$). Again, at 29°C, CH_4 fluxes displayed positive correlations with SM ($r = 0.41$, $P < 0.0001$), and pH ($r = 0.44$, $P < 0.0001$) (Table 4.3).

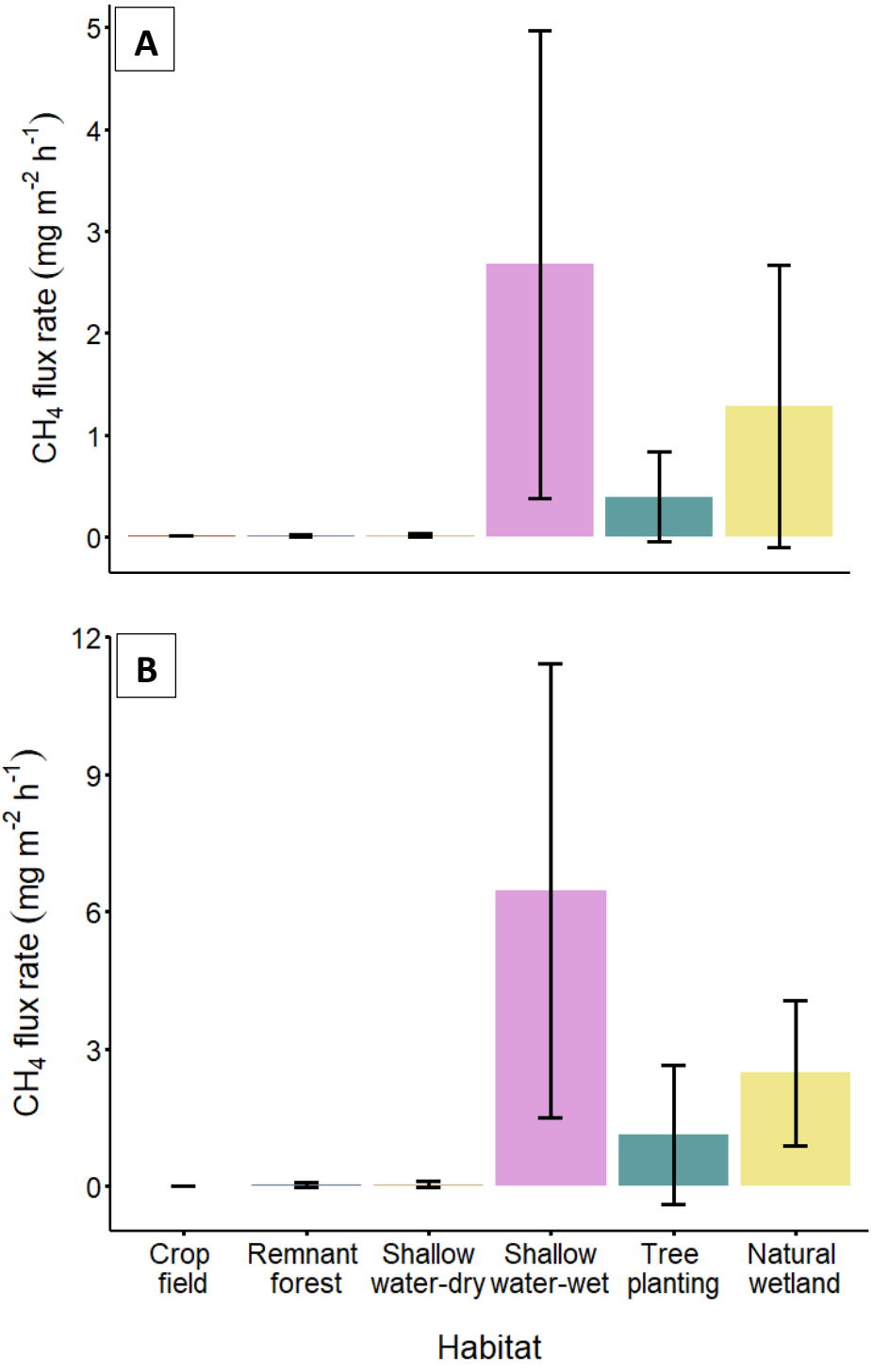

Figure 4.8.

Mean CH_4 flux rate at A) 24°C and B) 29°C. Habitat types were crop field, remnant forest, shallow water-dry, shallow water-wet, tree planting, and natural wetland. Error bars represent 95% confidence interval.

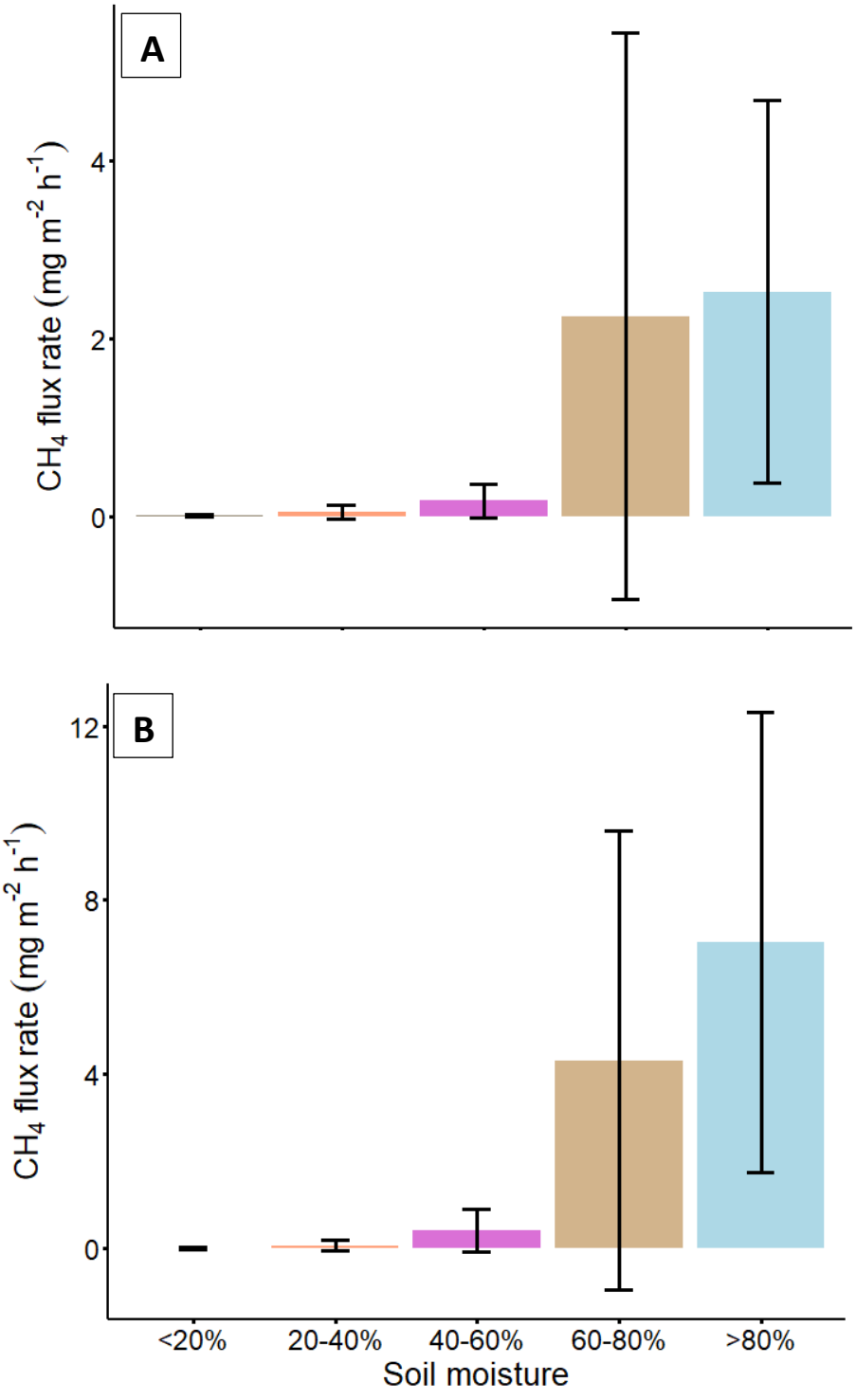

Figure 4.9.

Mean CH_4 flux rate at A) 24°C and B) 29°C. Soil moisture categories were < 20 %, 20-40%, 40-60%, 60-80%, and >80%. Error bars represent 95% confidence interval.

CH_4 flux between 24°C and 29°C

As the temperature increased from 24°C to 29°C, there was no noticeable increase in the mean CH_4 production rate within the crop field (1.54% increase). Remarkably, although remnant forest exhibited one of the lowest levels of CH_4 production compared to other habitats, it experienced the highest percentage increase in the mean CH_4 production rate when temperature was increased, with a remarkable 298% rise (Figure 4.10). Shallow water-dry, shallow water-wet, and tree planting habitats displayed mean CH_4 production rate increases by 168%, 142%, and 188%, respectively. The natural wetland habitat exhibited the smallest percentage increase in the mean CH_4 production rate after the crop field, at 93% (Figure 4.10).

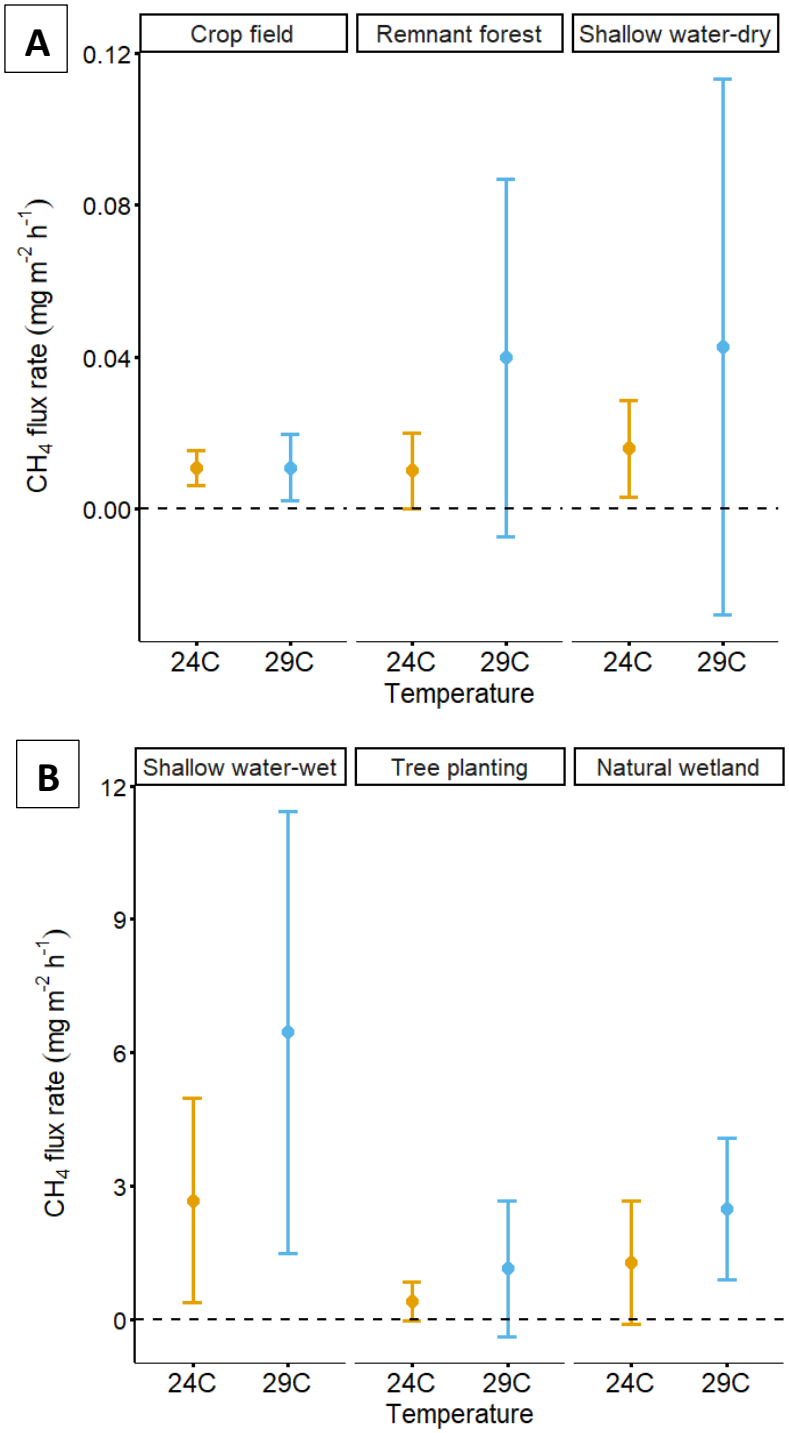

Figure 4.10.

Mean CH_4 flux rate at 24°C and 29°C by habitat. Habitat types were A) crop field, remnant forest, and shallow water-dry and B) shallow water-wet, tree planting, and natural wetland. Error bars represent the 95% confidence interval.

4.6.3. Carbon dioxide (CO_2) flux

CO_2 flux at 24°C at 29°C and relation with soil properties

Significant differences in CO_2 fluxes were observed across habitat types at both 24°C ($\chi^2_{(5)} = 45.237$, $P<0.0001$) and 29°C ($\chi^2_{(5)} = 47.555$, $P<0.0001$). The highest production rate was measured for remnant forest habitat followed by tree planting, crop field, shallow water-dry, and natural wetland habitats. The lowest production rate was measured for shallow water-wet habitat. (Figure 4.11, Table 4.2). Mean CO_2 production rate for crop field, remnant forest, shallow water-dry, shallow water-wet, tree planting, and natural wetland habitats were 92.55, 118.98, 86.28, 37.89, 113.94, and 47 mg m^{-2} h^{-1} at 24°C and 129.44, 162.44, 120.92, 45.55, 157.72, and 49.97 mg m^{-2} h^{-1} at 29°C, respectively (Table 4.2). Carbon dioxide production was observed in all habitats at both temperatures, except for some soil cores from shallow water-dry habitat. The highest variability in CO_2 production rate was also seen within shallow water-dry habitat (Figure 4.11). The optimum SM range for CO_2 production was found to be between 20 and 60% at both 24°C and 29°C (Figure 4.12).

The lowest CO_2 production rate was observed when the SM ranged from 60% to 80%. At both temperatures, some soil cores with SM levels < 20% were consuming CO_2. The highest variability in CO2 production rate was also observed when the SM was < 20% (Figure 4.12). At 24°C, CO_2 fluxes correlated (Spearman correlation) positively with soil TC (r = 0.39, $P <$ 0.0001), and soil TN (r = 0.26, $P = 0.0038$). At 29°C, CO_2 fluxes correlated negatively with BD (r = -0.18, $P = 0.0451$) and soil pH (r = -0.18, $P = 0.0497$) and positively with soil TC (r = 0.45, $P < 0.0001$), soil TN (r = 0.34, $P = 0.0002$), and soil P (r=0.20, $P = 0.0297$) (Table 4.3).

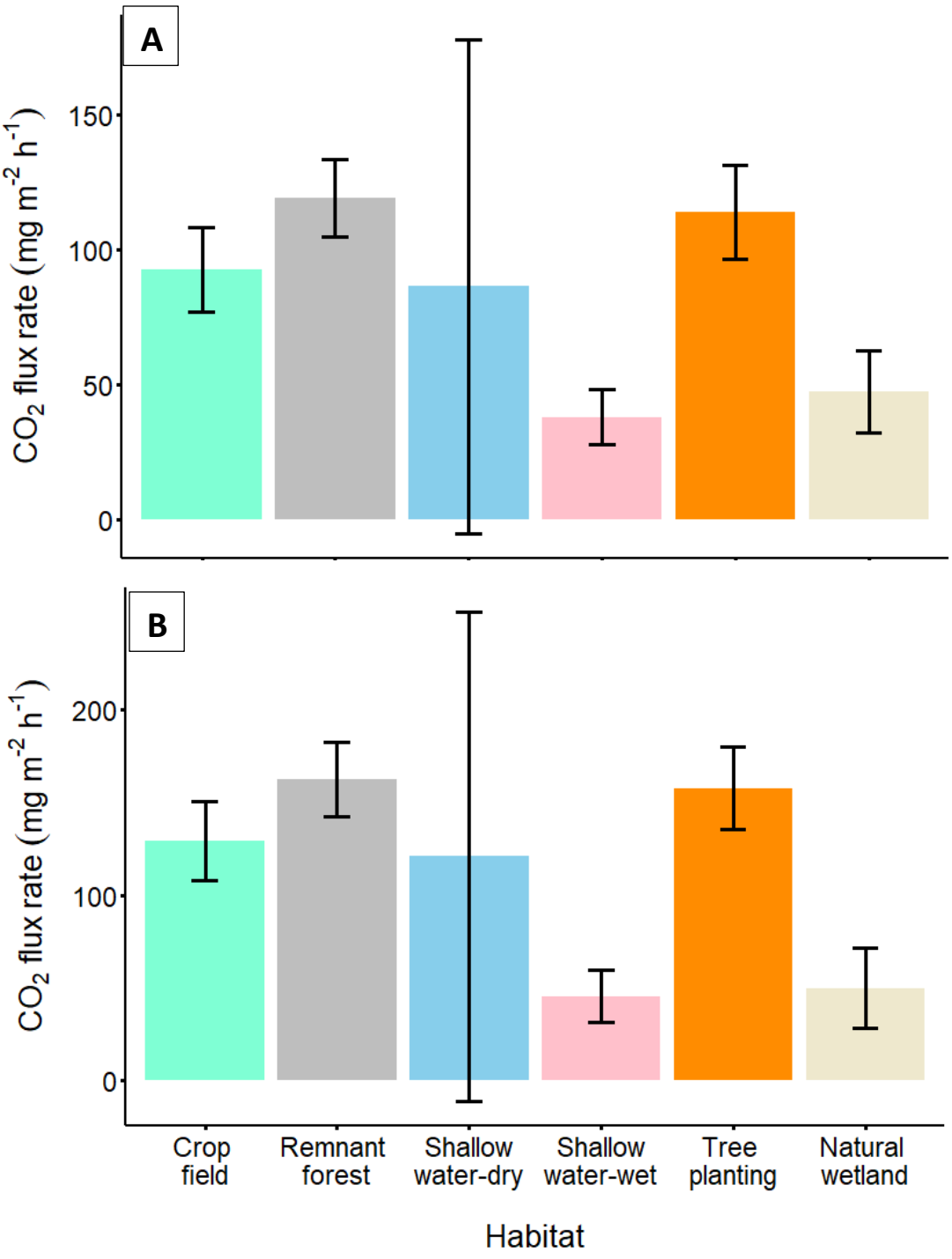

Figure 4.11.

Mean CO_2 flux rate at A) 24°C and B) 29°C. Habitat types were crop field, remnant forest, shallow water-dry, shallow water-wet, tree planting, and natural wetland. Error bars represent 95% confidence interval.

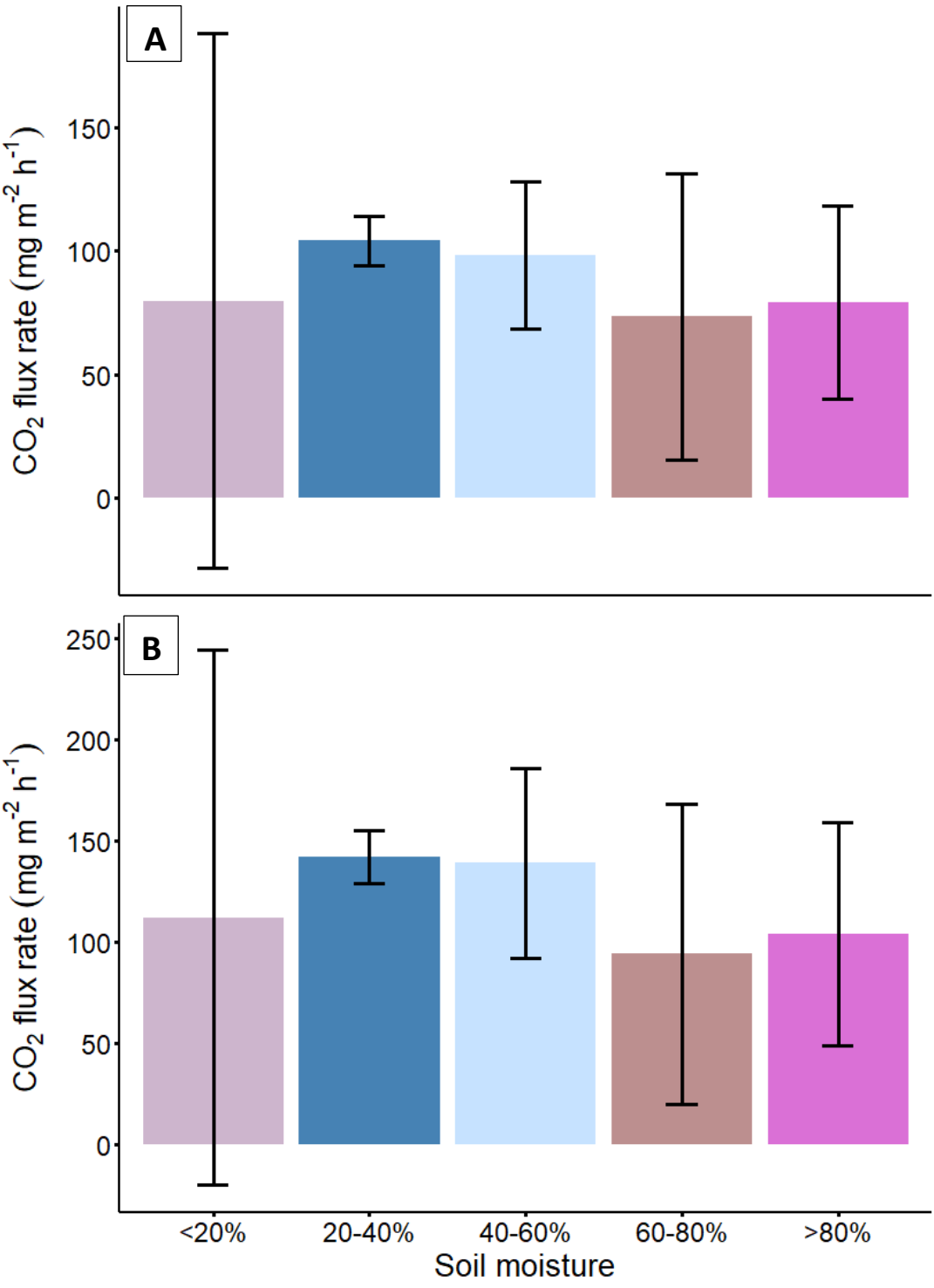

Figure 4.12.

Mean CO_2 flux rate at A) 24°C and B) 29°C. Soil moisture categories were < 20 %, 20-40%, 40-60%, 60-80%, and >80%. Error bars represent 95% confidence interval.

CO_2 flux between 24°C and 29°C

With the increase in temperature from 24°C to 29°C, mean CO_2 production rate increased in all habitat types. The highest percent increase in the mean CO_2 production rate occurred in shallow water-dry habitat (40.1%) (Figure 4.13). Close behind, followed the crop field, tree planting, and remnant forest habitats with mean production rate increase by 39.9%, 38.4%, and 36.5%, respectively. Shallow water-wet habitat experienced more modest increases, with mean production rate increase by 20.2%. The natural wetland habitat showed the smallest percentage increase in the mean CO_2 production rate (5.6%) (Figure 4.13).

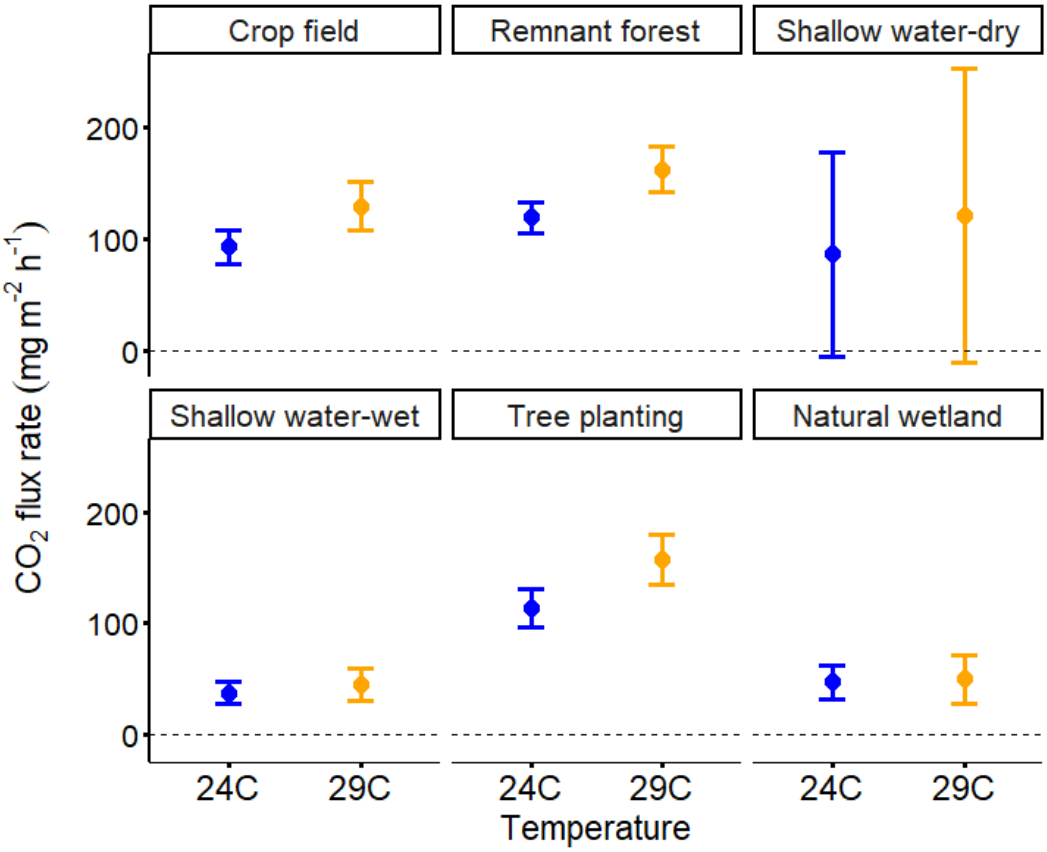

Figure 4.13.

Mean CO_2 flux rate at 24°C and 29°C by habitat. Habitat types were crop field, remnant forest, shallow water-dry, shallow water-wet, tree planting, and natural wetland. Error bars represent the 95% confidence interval.

4.6.4. Nitrous oxide (N_2O) flux

N_2O flux at 24°C at 29°C and relation with soil properties

Nitrous oxide fluxes exhibited significant variations across habitat types at both 24°C ($\chi^2_{(5)} = 27.023$, $P<0.0001$) and 29°C ($\chi^2_{(5)} = 27.998$, $P<0.0001$). The production of N_2O remained minimal in natural wetland, regardless of temperature (Figure 4.14), with mean rates of 0.002 mg m^{-2} h^{-1} at 24°C and 0.004 mg m^{-2} h^{-1} at 29°C (Table 4.2). At 24°C, crop field showed the highest N_2O production rates, followed by remnant forest, shallow water-wet, shallow water-dry, and tree planting habitats. At 29°C, the highest production rates were observed in the crop field, followed by shallow water-dry, remnant forest, shallow water-wet, and tree planting habitats (Figure 4.14, Table 4.2). The mean N_2O production rate for crop field, remnant forest, shallow water-dry, shallow water-wet, tree planting, and natural wetland habitats were 0.12, 0.10, 0.07, 0.09, 0.01, and 0.002 mg m^{-2} h^{-1} at 24°C and 0.16, 0.09, 0.15, 0.08, 0.03, and 0.004 mg m^{-2} h^{-1} at 29°C, respectively (Table 4.2). Among these habitats, shallow water-dry habitat displayed the highest variability in N_2O production rates (Figure 4.14). Few soil cores from shallow water-dry (at both temperatures) and shallow water-wet (24°C) habitats exhibited N_2O consumption. However, at 29°C, N_2O production replaced consumption in most soil cores from shallow water-wet habitat (Figure 4.14).

Nitrous oxide production consistently increased as the SM levels reached 40-60% and then declined, at both 24°C and 29°C. (Figure 4.15). The greatest variability in N_2O production rates also occurred when the soil moisture fell within the 40-60% range. Furthermore, at both temperatures, some soil cores displayed N_2O consumption when the soil moisture was below 20% and exceeded 60% (Figure 4.15). At 24°C, N_2O fluxes were positively correlated (Spearman correlation) with BD ($r = 0.32$, $P = 0.0004$) and soil P ($r = 0.26$, $P = 0.0041$), but

negatively with soil Fe (r = -0.33, *P* = 0.0003). Again, at 29°C, N_2O fluxes were positively correlated with BD (r = 0.22, *P* = 0.0153), and soil P (r = 0.38, *P* < 0.0001), but negatively with soil Fe (r = -0.31, *P* = 0.0006) (Table 4.3).

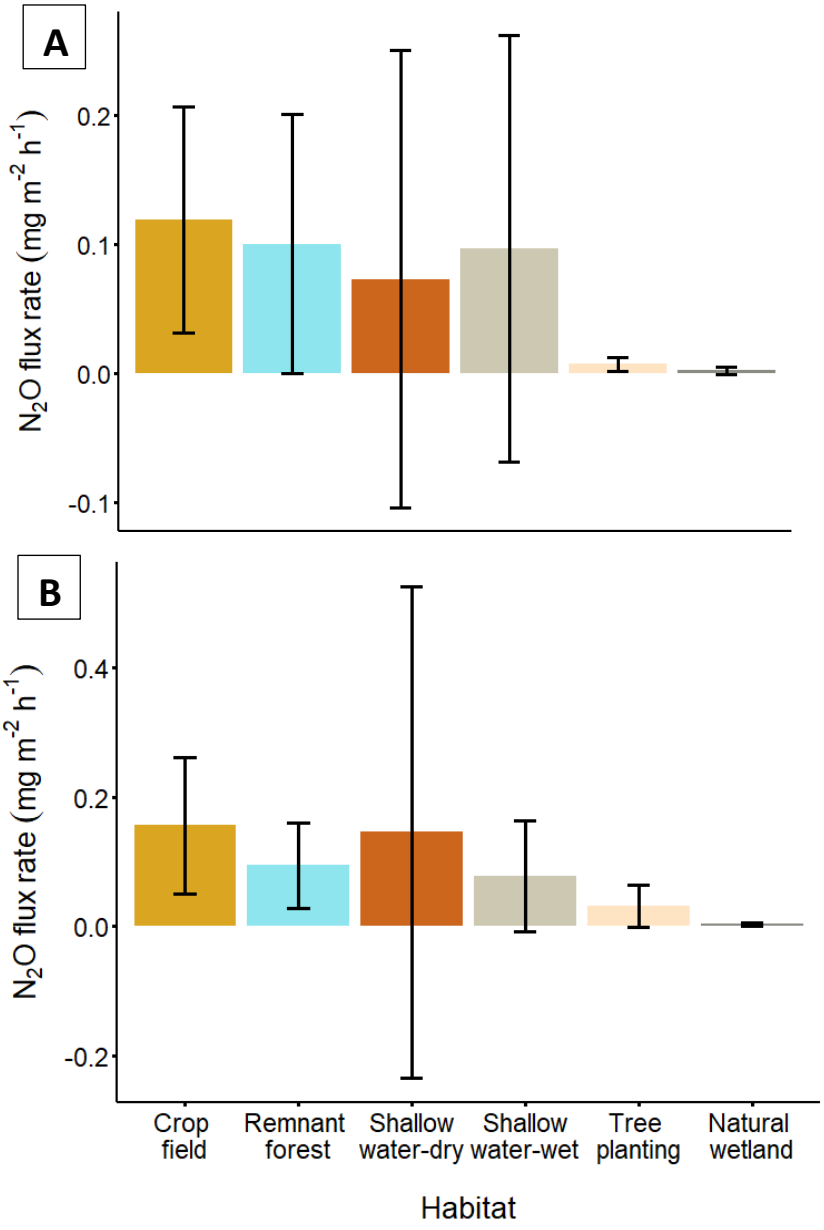

Figure 4.14.

Mean N_2O flux rate at A) 24°C and B) 29°C. Habitat types were crop field, remnant forest, shallow water-dry, shallow water-wet, tree planting, and natural wetland. Error bars represent 95% confidence interval.

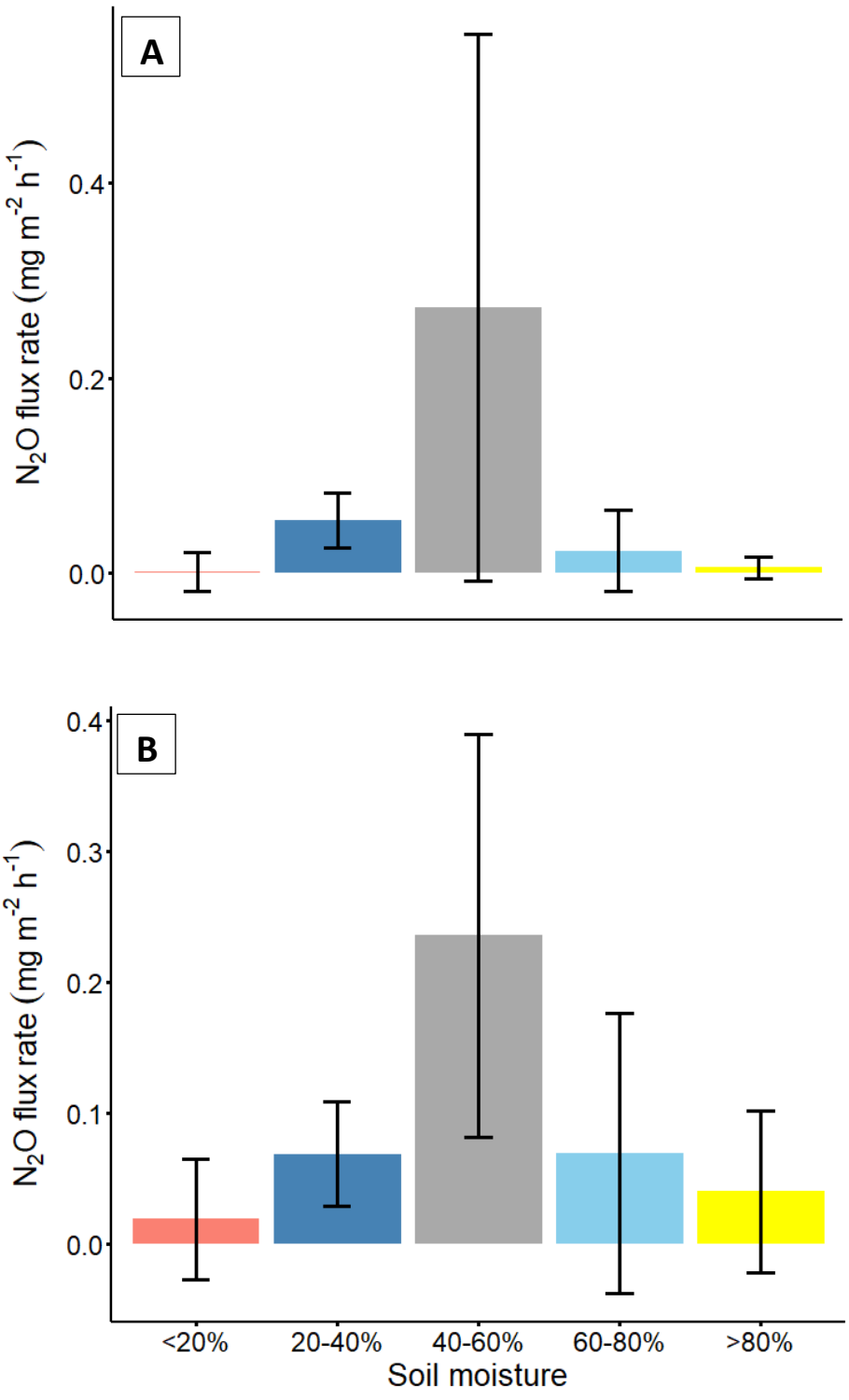

Figure 4.15.

Mean N_2O flux rate at a) 24°C and b) 29°C. Soil moisture categories were < 20 %, 20-40%, 40-60%, 60-80%, and >80%. Error bars represent 95% confidence interval.

N_2O flux between 24°C and 29°C

As the temperature increased from 24°C to 29°C, the mean N_2O production rate increased in most habitats. However, in shallow water-wet and remnant forest habitats, there was a decrease by 19% and 6%, respectively (Figure 4.16, Table 4.2). The highest percent increase in the mean N_2O production rate was observed in tree planting habitat, surging by 360%. Following this were shallow water-dry habitat with a 100% increase and the natural wetland with a 73% increase. Crop field displayed the smallest percentage increase in the mean N_2O production rate by 32% (Figure 4.16).

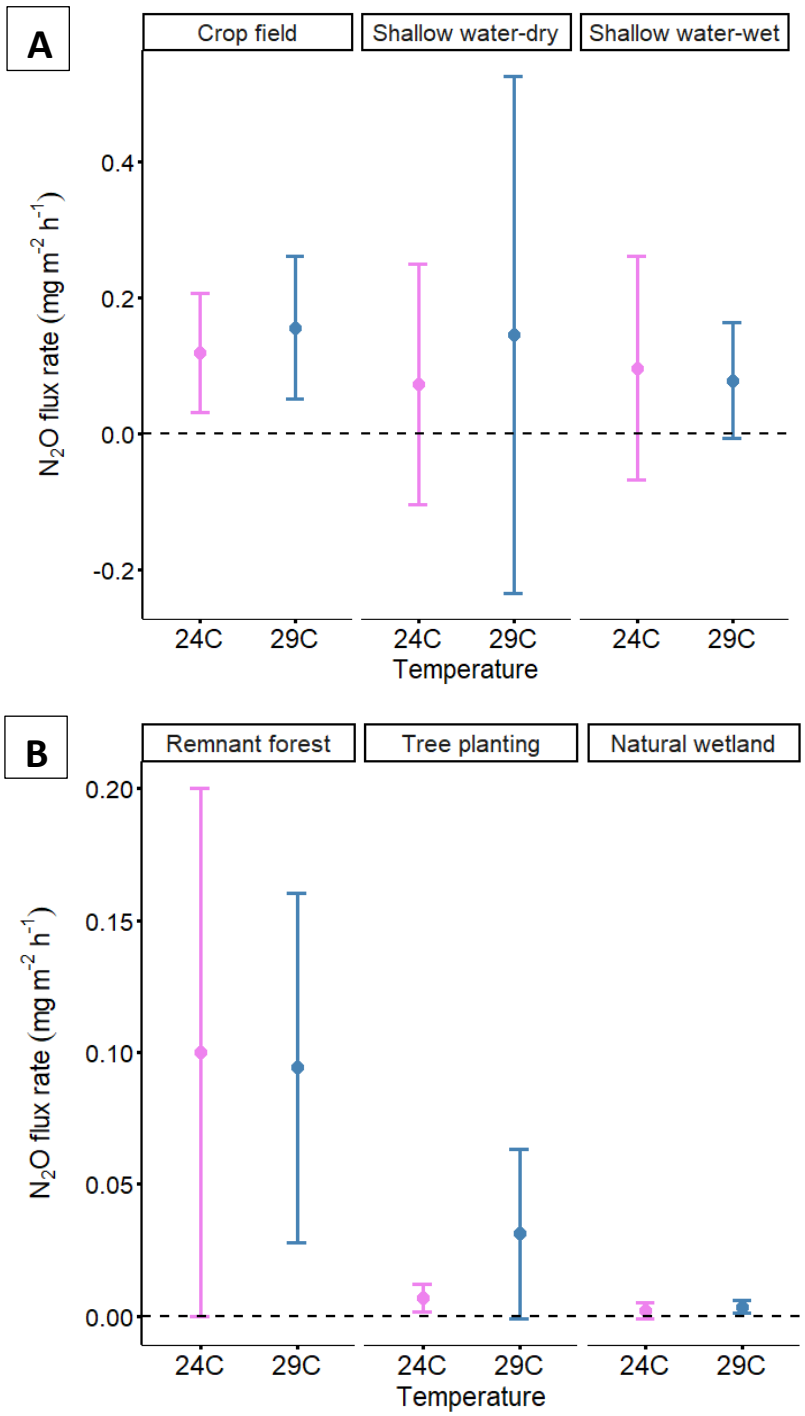

Figure 4.16.

Mean N_2O flux rate at 24°C and 29°C by habitat. Habitat types were a) crop field, remnant forest, and shallow water-dry and b) shallow water-wet, tree planting, and natural wetland. Error bars represent the 95% confidence interval.

Table 4.2.

Means ± SE flux rates of methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O) observed at 24°C and 29°C for 4 easements and 6 habitat types.

Habitat	n	CH_4 (mg m^{-2} h^{-1})		CO_2 (mg m^{-2} h^{-1})		N_2O (mg m^{-2} h^{-1})	
		24°C	29°C	24°C	29°C	24°C	29°C
Crop field	24	0.01 ± 0.002	0.01 ± 0.004	92.55 ± 7.61	129.44 ± 10.33	0.12 ± 0.042	0.16 ± 0.051
Remnant forest	46	0.01 ± 0.005	0.04 ± 0.023	118.98 ± 7.17	162.44 ± 9.95	0.10 ± 0.050	0.09 ± 0.033
Shallow water-dry	5	0.02 ± 0.005	0.04 ± 0.025	86.28 ± 32.95	120.92 ± 47.61	0.07 ± 0.064	0.15 ± 0.137
Shallow water-wet	14	2.67 ± 1.063	6.46 ± 2.299	37.89 ± 4.73	45.55 ± 6.61	0.09 ± 0.076	0.08 ± 0.040
Tree planting	24	0.39 ± 0.213	1.14 ± 0.738	113.94 ± 8.40	157.72 ± 10.77	0.01 ± 0.003	0.03 ± 0.016
Natural wetland	6	1.28 ± 0.539	2.48 ± 0.619	47.32 ± 5.96	49.97 ± 8.33	0.002 ± 0.001	0.004 ± 0.001

Table 4.3.

Sperman correlation coefficient (r) and significance level (P) between methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O) fluxes and soil properties.

	CH_4				**CO_2**				**N_2O**			
	24ºC		**29ºC**		**24ºC**		**29ºC**		**24ºC**		**29ºC**	
Soil properties	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*
Soil moisture	**0.42**	**0.0000**	**0.41**	**0.0000**	-0.03	0.7566	-0.06	0.5099	-0.09	0.3287	0.01	0.8777
Bulk density	-0.10	0.3012	-0.15	0.1039	-0.15	0.1112	**-0.18**	**0.0451**	**0.32**	**0.0004**	**0.22**	**0.0153**
pH	**0.31**	**0.0006**	**0.44**	**0.0000**	-0.16	0.0854	**-0.18**	**0.0497**	-0.05	0.5539	0.09	0.3333
Total C	-0.05	0.5525	-0.03	0.7382	**0.39**	**0.0000**	**0.45**	**0.0000**	-0.05	0.5637	-0.04	0.6989
Total N	-0.02	0.8195	0.03	0.7473	**0.26**	**0.0038**	**0.34**	**0.0002**	-0.07	0.4289	-0.03	0.7769
Soil P	-0.15	0.1067	0.01	0.9130	0.16	0.0880	**0.20**	**0.0297**	**0.26**	**0.0041**	**0.38**	**0.0000**
Soil Fe	0.17	0.0596	0.13	0.1657	0.10	0.2657	0.10	0.2606	**-0.33**	**0.0003**	**-0.31**	**0.0006**

4.7. Discussion

4.7.1. Hypotheses evaluation

Hypothesis i: Soil properties and fluxes of CH_4, CO_2, and N_2O will differ among habitats.

My hypothesis that soil properties and CH_4, CO_2, and N_2O fluxes will differ among habitats was supported. As hypothesized, habitats that had high SM, such as shallow water-wet and natural wetland habitats, were dominated by CH_4 production. However, the hypothesis that newly restored habitats experiencing legacy effects from soil cultivation and tillage would produce higher CH_4 compared to older habitats did not hold as crop field produced negligible CH_4. Contrary to the expectation, CH_4 flux did not decrease with the increase in BD and there was no observed relationship between BD and CH_4 flux. Finally, the hypothesis that CH_4 production would be highest from habitats with near neutral soil pH was confirmed.

Contrary to my hypothesis that drier habitats (i.e., crop field) will be dominated by CO_2 production, the production of CO_2 was highest from habitats with moderate SM (i.e., remnant forest and tree planting). My expectation that CO_2 production would be lowest in shallow water-wet and natural wetland habitats was confirmed. Furthermore, my hypotheses that soil TC and TN would correlate positively with CO_2 production, were both confirmed.

My hypothesis that N_2O production will be higher in habitats with high N content was not supported as no relationship was found between soil TN and N_2O production. Instead, N_2O production was highest from the crop field. My hypothesis that habitats with moderate SM, such as remnant forest, would be dominated by N_2O production was supported only at 24°C. However, the expectation that N_2O production would increase with decreasing soil pH was not supported as no relationship between N_2O production and soil pH was observed. My hypothesis

that N_2O production would be lowest in shallow water-wet and natural wetland habitats was partially supported, with evidence found only in natural wetland.

Hypothesis ii: An increase in temperature influences CO_2, CH_4, and N_2O fluxes from restored wetland habitats

My hypothesis regarding increased CO_2 production in all habitats with the increase in temperature from 24°C to 29°C was confirmed. However, the anticipated decrease in CH_4 production with a temperature increase was not observed. On the contrary, the results corroborated my opposing hypothesis, showing an increase in CH_4 production in shallow water-wet and natural wetland habitats when the temperature rose from 24°C to 29°C. My hypothesis regarding the increase in N_2O production with rising temperatures was partially supported, as there was a 19% and 6% decrease in N_2O production in shallow water-wet and remnant forest habitats, respectively.

4.7.2. Soil properties

The SM was highest in the natural wetland habitat. Natural wetlands usually have high water tables that contribute to saturated or near-saturated soil conditions (Miao et al., 2013). Furthermore, the presence of abundant vegetation and high amount of organic matter most likely contributed to higher water retention (Dabrowska-Zielinska et al., 2016) and consequential elevated SM levels in natural wetland habitat. The higher BD in crop field, in comparison to other habitat types, may be attributed to the intensive tillage practices employed during the previous cropping season, which could have led to soil compaction and the subsequent increase in BD (Alam et al., 2014; Reichert et al., 2009). Additionally, the absence of enough organic

matter that typically fosters soil structure improvement and a reduction in BD in the crop field could be another contributing factor to higher BD.

On the contrary, low BD in natural wetland habitat can be attributed to high SM, which reduces soil compaction and promotes the accumulation of organic matter (Craft, 2007; Wang et al., 2016). Moreover, the anaerobic conditions inherent to natural wetlands play a vital role in preserving organic materials, further promoting the accumulation of C and N (Craft et al., 2002; Hossler & Bouchard, 2010). Alternately, slow organic matter decomposition results in the addition of a lower number of acidic cations to the soil, likely contributing to higher soil pH in natural wetland compared to other habitat types. Higher soil P in the crop field may be attributed to the legacy effect of applying P-containing fertilizers in the previous cropping season (Christianson et al., 2021; Liu et al., 2019). The higher amount of soil Fe in natural wetland compared to other habitat types may be linked to Fe retention facilitated by saturated conditions and its interactions with organic matter (Bai et al., 2005; Sundareshwar et al., 2003).

4.7.3. Methane (CH_4)

Methane production among habitat types and relation with soil properties

The two primary habitats with highest CH_4 production were shallow water-wet habitat, followed by natural wetland at both incubation temperatures. Subsequent analysis revealed a substantial amount of CH_4 production when SM levels exceeded 60%, indicating that CH_4 production is favored in wet conditions. Furthermore, a positive correlation between CH_4 flux and SM was found at both incubation temperatures. One plausible explanation for this association is that elevated SM levels may have inhibited aerobic soil respiration, thereby creating a favorable anaerobic environment for methanogens, which are responsible for CH_4

production (Wang et al., 2018; Zhang et al., 2021). Methanogens, which thrive in oxygen-depleted, wet environments, play a pivotal role in converting organic matter into CH_4 through the process of methanogenesis (Hanson & Hanson, 1996). Consequently, higher SM levels can promote the activity and abundance of methanogens, resulting in elevated CH_4 production rates (Zhang et al., 2017; Zhao et al., 2019).

Shallow water-wet and natural wetland habitats exhibited higher soil pH levels compared to other habitats. Also, a positive correlation was found between soil pH and CH_4 flux at both incubation temperatures. This relationship can be explained by considering how soil pH can impact CH_4 production by influencing the composition and activity of microbial communities involved in methanogenesis (Wagner et al., 2017). Notably, methanogens are known to be sensitive to pH levels, with their activity being most favorable at neutral to slightly acidic soil pH conditions (Wagner et al., 2017). Therefore, soils with near neutral pH levels in shallow water-wet and natural wetland habitats compared to other habitats most likely provided an environment conducive to methanogens, resulting in higher CH_4 production.

Effect of the increase in temperature in CH_4 production

The increase in CH_4 production with the increase in temperature in this study is consistent with the literature (Chen et al., 2008; Koch et al., 2007; Koh et al., 2009). According to Dean et al. (2018) methanogens are highly sensitive to fluctuations in temperature, and the short term experimental temperature increments, such as observed in this study, can lead to substantial increases in CH_4 production. The increase in CH_4 production is the consequence of increase in the metabolic activity of methanogens (Walter & Heimann, 2000). However, it is noteworthy that the response of CH_4 production to temperature varied across habitat types,

highlighting the intricate interplay of environmental factors in regulating CH_4 production in each distinct habitat. The varying responses observed across these habitats, ranging from 1.5% increase in the crop field to approximately 300% increase in the remnant forest habitat emphasize the habitat-specific nature of CH_4 production.

The minimal (1.5%) increase in CH_4 production in the crop field may be attributed to management practices such as fertilization and tillage that took place in the prior cropping season, which can influence soil conditions and microbial activity. These practices can limit substrate availability or create less favorable conditions for methanogens, resulting in negligible increase in CH_4 production with the rise in temperature(Zou et al., 2005). On the contrary, the remnant forest habitat, in spite of producing lowest levels of CH_4 compared to other habitats at both temperatures, exhibited a remarkable 298% increase in CH_4 production when the temperature increased. Several factors may have contributed to this result. First, the methanogens in remnant forest habitat may be less adapted to CH_4 production at milder temperatures. The increase in temperature most likely accelerated their activity, resulting in a more significant percentage change in CH_4 production (Davidson & Janssens, 2006). Second, the temperature increase may have activated dormant methanogens in remnant forest habitat. These previously inactive microbial populations may become more dominant or active with temperature increase, thus accounting for a substantial percentage increase in CH_4 production (Butterbach-Bahl et al., 2013; McDaniel et al., 2021).

Despite being the habitat with the smallest percentage increase in mean CH_4 production rate after the crop field, the natural wetland displayed a 93% increase as temperatures rose from 24°C to 29°C. This noticeable increase may be attributed to the enhanced decomposition of recalcitrant organic matter, such as peat or lignin-rich plant material often found in natural

wetlands. As the temperature increases, the decomposition of this recalcitrant organic matter can accelerate, leading to the greater supply of substrates for methanogens. This, in turn, may result in a substantial increase in CH_4 production (Kirwan et al., 2014). Additionally, temperature increases can elevate the availability of labile organic matter. Labile organic matter serves as a vital nutrient source for methanogens, promoting their growth and ultimately increasing CH_4 production (Zhu et al., 2020).

4.7.4. Carbon dioxide (CO_2)

Carbon dioxide production among habitat types and relation with soil properties

Carbon dioxide production was highest from remnant forest habitat, closely followed by tree planting habitat, under both incubation temperatures. This higher CO_2 production from remnant forest and tree planting habitats can be attributed to several factors. Primarily, these habitats may have C sources that are more readily decomposable, such as leaf litter incorporated into the soil profile, resulting in increased substrate availability. This abundance of substrate may promote microbial respiration (Luyssaert et al., 2008), leading to higher CO_2 production. Additionally, remnant and tree planting habitats can support a greater plant root density, contributing to elevated soil respiration rates. Carbon dioxide is produced as the byproduct of their metabolic processes, resulting in higher CO_2 production from remnant forests (Lu et al., 2016). Furthermore, the remnant forest and tree planting habitats may accommodate more microbial biomass and activity compared to other habitats. The decomposition of organic matter by these microbes through heterotrophic respiration releases CO_2 (Sjögersten et al., 2014), leading to higher CO_2 production from these habitats.

Carbon dioxide productions were lowest from shallow water-wet and natural wetland habitats. Lower CO_2 production from these habitats may be due to the competition of CO_2 production with methanogenesis. According to Sjögersten et al. (2014), the high levels of methanogenic activity in the saturated soil can divert C away from CO_2 production. Additionally, C sequestration process occurring in wetlands, attributed to the slow decomposition of organic matter under saturated conditions, allows for long-term C storage, thereby contributing to lower CO_2 production (Yang et al., 2022).

Under further analysis, it was evident that a substantial amount of CO_2 was produced when SM levels ranged from 20% to 60%. However, both below 20% and above 60%, there was a noticeable decline in CO_2 production, indicating that excessively wet or dry conditions are not conducive to CO_2 production. This outcome can be attributed to two key factors. Firstly, when SM falls below 20%, water scarcity can limit microbial activity and the decomposition of organic matter. Microbes rely on sufficient SM for their metabolic processes, and the lack of SM can hinder their activity (Xu et al., 2004), resulting in reduced CO_2 production. Conversely, when SM exceeds 60%, excess water can fill the soil pore spaces, reducing oxygen availability. This, in turn, inhibits aerobic respiration and promotes anaerobic processes, such as methanogenesis, which generates CH_4 rather than CO_2 (Xu & Ye, 2001). Furthermore, high SM levels can impact gas diffusion by reducing the exchange of gas between the soil and the atmosphere (Schwendenmann & Veldkamp, 2006), which may result in lower CO_2 production.

A positive correlation of CO_2 flux with soil TC and TN was observed at both incubation temperatures. This correlation can be explained by the vital role of these nutrients in supporting microbial growth, activity, and organic matter decomposition. The availability of C and N significantly influences the activity and abundance of microbial communities involved in

decomposing organic matter (Wang et al., 2020; Xu & Ye, 2001), ultimately resulting in increased CO_2 production. Furthermore, higher levels of soil C provide more substrate for microbial metabolism and energy production (Korkanç et al., 2022), which, in turn, leads to more CO_2 production. At 29°C, CO_2 production exhibited a negative correlation with BD and soil pH and positive correlation with soil P. The lower CO_2 production at higher pH levels can be attributed to the inhibition of enzyme activity and the alteration of microbial community composition responsible for organic matter decomposition (Korkanç et al., 2022). Additionally, higher BD can compact the soil and limit the movement of air and water within the soil, thereby affecting optimal microbial activity, and slowing organic matter decomposition (Xu & Ye, 2001), which may result in lower CO_2 production. Phosphorus, being another essential nutrient for microbial growth and activity, can enhance microbial decomposition rates and, consequently, CO_2 production (Liu et al., 2016; Wang et al., 2020).

Effect of the increase in temperature in CO_2 production

The increase in temperature from 24°C to 29°C led to a rise in CO_2 production across all habitats. This temperature-induced increase may be attributed to the stimulation of metabolic activity in soil microorganisms and the accelerated decomposition of organic matter. The habitats that exhibited the highest percentage increase in CO_2 production were shallow water-dry habitat, followed by crop field. This outcome may be explained by the relatively low SM availability in these habitats compared to others. Under conditions of low moisture, more oxygen (O_2) is accessible for aerobic decomposition, which can further enhance CO_2 production as temperature rise. Conversely, natural wetland habitat displayed the smallest percentage increase in CO_2 production. Wetlands are known for their capacity to sequester substantial amounts of C

in the form of organic matter, rendering them significant C sinks (Lu et al., 2016; Melton et al., 2013). This high C storage capability of wetlands can limit the release of CO_2 into the atmosphere, making them more resilient to temperature increases in terms of CO_2 production. Additionally, saturated conditions in wetlands create anaerobic environments, which can promote the production of CH_4 rather than CO_2 (Turetsky et al., 2014). Consequently, during conditions of temperature increase, CH_4 production may be favored over CO_2 production in natural wetlands.

4.7.5. Nitrous oxide (N_2O)

Nitrous oxide production among habitat types and relation with soil properties

Nitrous oxide production was lowest from natural wetland habitat and highest from crop field. This is likely due to the high SM in natural wetlands, which facilitates complete denitrification and a subsequent reduction of N_2O to N_2 (Tangen & Bansal, 2019). Moreover, natural wetland contains higher levels of soil TC compared to the crop field. The higher organic matter content in wetlands serves as C source for denitrifying microbes, which can consume N_2O and reduce its emissions. In contrast, the lower organic matter content in crop field may limit N_2O reduction (Euliss et al., 2006), resulting in higher N_2O production. Additionally, soil management practice, such as tillage in previous cropping season, may have created favorable conditions for N_2O production in crop field (Sanz-Cobena et al., 2017). Furthermore, crop field may have higher O_2 levels, which may subsequently promote N_2O production during denitrification (Ju et al., 2019; Song et al., 2019b).

Despite having the highest mean SM, shallow water-wet habitat exhibited higher N_2O production compared to natural wetland. This difference can be attributed to the soil disturbances

that occurred during restoration processes, which have the potential to influence microbial processes and promote N_2O production (Moreno-Mateos et al., 2012). Additionally, shallow water-wet habitat may have received different organic matter inputs and higher decomposition rates when compared to natural wetlands. Changes in organic matter availability and decomposition processes can influence microbial activity, resulting in increased N_2O production rates (Taylor & Middleton, 2004).

Nitrous oxide production followed a distinct pattern in response to varying SM levels. It showed an increasing trend until SM reached the range of 40% to 60%, after which it declined. This pattern suggests that N_2O production is influenced by specific SM thresholds, with notable shifts in production dynamics across different moisture levels. At both extremely low SM levels (below 20%) and excessively high levels (above 80%), N_2O production was low, indicating that extremely wet or dry conditions are not conducive to N_2O production. The plausible reasons for this are twofold. At extremely low moisture levels, microbial activity, particularly the denitrification process responsible for N_2O production, is limited. Microbes require adequate moisture for their metabolic processes, and in very dry conditions, their activity is restricted (Butterbach-Bahl et al., 2013). Conversely, when SM exceeds 80%, O_2 diffusion into the soil is limited, creating anaerobic conditions. Under these conditions, complete denitrification is more likely to occur, resulting in the reduction of N_2O to N_2 (Butterbach-Bahl et al., 2013). This shift toward N_2 production can explain the decrease in N_2O production beyond 60% SM.

In the 40-60% moisture range, there is often an optimal balance between oxygen and water content in the soil. This balance is conducive to N_2O production due to several key factors. At moderate SM levels there is sufficient O_2 available for nitrification, the microbial process responsible for producing nitrate (NO_3^-), which is a vital precursor for denitrification. However,

the SM is not too high to cause waterlogging and limited O_2 diffusion (Butterbach-Bahl et al., 2013) nor too low to restrict microbial activity (Manzoni et al., 2012). This equilibrium can foster incomplete denitrification, consequently leading to higher N_2O production at 40-60% moisture levels.

A positive correlation of N_2O flux with BD and soil P, and negative correlation with soil Fe was observed at both incubation temperatures. The positive correlation with BD may be explained by two key mechanisms: soil compaction and restricted oxygen availability. Soil compaction can cause higher BD, reduce pore space, and limit O_2 diffusion. This, in turn, can create anaerobic microsites within the soil, providing favorable conditions for denitrification and subsequent N_2O production (Chapuis-Lardy et al., 2007; Sutka et al., 2008). Furthermore, high BD can restrict the movement of water and nutrients, including NO_3^-, within the soil profile. This restricted mobility can lead to the accumulation of NO_3^- in localized areas, which in turn can promote denitrification and N_2O production (ŠImek & Cooper, 2002). The positive correlation observed with soil P can be attributed to the potential of higher soil P levels to enhance microbial activity and nutrient availability. This, in turn, can lead to increased N cycling and stimulation of denitrification processes, potentially resulting in elevated N_2O production (Butterbach-Bahl et al., 2013). Furthermore, higher soil P levels may enhance N mineralization and nitrification, thereby supplying more substrates for denitrification and, consequently, producing more N_2O (Timilsina et al., 2020). Conversely, the negative correlation observed with soil Fe can be explained by the fact that availability of Fe in the soil can catalyze the conversion of N_2O to N_2 during denitrification and result in lower N_2O production (Zhu et al., 2013).

Effect of the increase in temperature in N_2O production

The increase in temperature from 24°C to 29°C led to a rise in N_2O production across all habitats, except shallow water-wet and remnant forest habitats. This increase in N_2O production can be attributed to several underlying factors. Firstly, the increase in temperature can increase microbial activity and enzymatic reactions (Butterbach-Bahl et al., 2013; Yvon-Durocher et al., 2010). Secondly, the temperature increase can enhance substrate availability through the decomposition of organic matter and increased nutrient mineralization, both of which contribute to denitrification and subsequent N_2O production (Hu et al., 2015). Lastly, the increase in temperature can enhance gas diffusion rates, thereby facilitating the increase in transportation of N_2O from microbial hotspots to the atmosphere (Peng et al., 2015). Interestingly, despite exhibiting the highest N_2O production rate at both 24^{o}C and 29^{o}C, crop field displayed the lowest increase in N_2O production rate (32%) when the temperature rose. This may be attributed to lower temperature sensitivity of microbes responsible for N_2O production in the crop field compared to other habitats (Davidson & Janssens, 2006). Furthermore, it is plausible that the enzymatic activity of microbes in the crop field was already optimized at 24°C (Alster et al., 2016), resulting in a less pronounced response to the temperature increase compared to other habitats. It is important to note that, despite the 73% increase in N_2O production observed in the natural wetland with the increase in temperature, the production rate remained notably low compared to other habitats at both temperatures.

4.8. Conclusion

When the temperature was increased from 24^{o}C to 29^{o}C, an increase in CH_4, CO_2, and N_2O was observed from most habitats. In general, natural wetlands appear to be least affected by temperature increase compared to other restored habitats. In agreement with our hypothesis, we found the effect of habitat types in GHG production. Although CH_4 production was highest from shallow water-wet habitat, CO_2 and N_2O production was lowest from this habitat. Carbon dioxide production was highest from the remnant forest and tree planting habitat, while N_2O production was highest from the crop field. Understanding these variations in GHG production highlights the importance of considering different habitats during wetland restoration.

Methane production peaked under wetter conditions (>80%). Maximum CO_2 and N_2O were produced when the SM was 20-60%, and 40-60%, respectively. Methane production responded positively to SM and pH, while CO_2 production responded positively to soil TC and TN. Nitrous oxide responded positively to BD and soil P, and negatively to soil Fe. The relationships provide insights into the complex interplay of environmental conditions and microbial processes that affect GHG emissions in restored wetland ecosystems. This knowledge is crucial for understanding the role of restored wetlands in global GHG budgets and their responses to changing environmental conditions.

CHAPTER 5: SUMMARY AND CONCLUSIONS

In this dissertation, I investigated the influence of environmental factors on nutrient retention and greenhouse gas (GHG) emissions within restored agricultural floodplain wetlands. My study focused on characterizing soil structural properties across diverse wetland habitats and assessing their influence on nitrogen gas (N_2) production (Chapter 2). Additionally, I examined how vegetation species and flooding duration affect nitrogen (N) and phosphorus (P) retention in the porewater (Chapter 3). Finally, I compared GHG production among restored wetland habitats, analyzed the relationships between GHG production and soil properties, and examined how these habitats would respond to the rise in temperature. My study showed that soil properties, hydrology, vegetation, and climate (i.e., temperature) affect nutrient retention and GHG production differently across various habitats and over time, highlighting the complexity of wetland restoration and diverse interactions within these ecosystems.

5.1. Chapter 2 summary

In a study across 23 restored floodplain wetlands in western Kentucky and Tennessee, I investigated the maximum potential denitrification rates during a 2-day simulated flood event. The five distinct restoration habitats examined included natural regeneration, remnant forest, shallow water-dry, shallow water-wet, and tree planting. The mean N_2 gas production was highest in natural regeneration habitat and least in shallow water-wet habitat. All habitats produced N_2 throughout the incubation period. Additionally, varying degrees of influence from several soil properties on N_2 production was observed. These findings suggest that while all habitats efficiently remove N over a 48 h flood period, achieving the highest removal rates may

depend on the duration of flooding in a specific habitat and the soil characteristics within it. Notably, after flooding for 24 h, the influence of soil properties on N_2 production became less prominent.

5.2. Chapter 3 summary

In a wetland mesocosm experiment, I investigated how habitat types and hydrological conditions influence nitrate (NO_3^-) and phosphate (PO_4^{3-}) retention and dissolved organic carbon (DOC) release in soil porewater, and the production of nitrogen gas (N_2), nitrous oxide (N_2O), and methane (CH_4) during simulated flooding. The habitats comprised bare soil as a control, native grass represented by rice cutgrass (*Leersia oryzoides* L.), and tree plantings represented by bald cypress (*Taxodium distichum* (L.) Rich) and river birch (*Betula nigra* L.). Hydrological treatments included 3-day and 3-week inundation flood regimes. After 5 days of inundation, there was a substantial reduction in NO_3^- and PO_4^{3-} concentrations, nearing zero, with native grass habitat proving most effective in decreasing both, emphasizing habitat-dependent differences. However, DOC release significantly increased, and the release rate was lowest for native grass habitat irrespective of hydrology. Bare soil mesocosms under the 3-week flooding treatment exhibited the highest percentage increase in DOC release. In the first 24 hours, bare soil habitat and tree planting with 3-day flooding showed a mean PO_4^{3-} release, while all other treatments retained PO_4^{3-} until day 3. Beyond day 3, there was no further increase in the uptake rate for all treatments. Levels of soil total carbon (TC) and soil phosphorus (P) remained stable, while soil total nitrogen (TN) increased post-dosing, potentially contributing to the significance of soil TN in N_2 production at 12 h. Subsequently, N_2 production beyond 12 h was solely affected by sediment oxygen demand (SOD), highlighting water residence time as the primary

regulator. The production of N_2O and CH_4 was minimal across the vegetation types and hydrology levels.

5.3. Chapter 4 summary

In research across 4 restored floodplain wetlands in western Kentucky and Tennessee, I analyzed greenhouse gas (GHG) emissions using a simulated experiment. The restoration habitats examined included crop field, remnant forest, shallow water-dry, shallow water-wet, tree planting, and natural wetland. The fluxes of methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O) were affected by habitat types and displayed varying relationships with soil properties. While shallow water-wet habitat showed the highest CH_4 production, it concurrently had the lowest CO_2 and N_2O production. Conversely, remnant forest and tree planting habitats showed the highest CO_2 production, and the crop field had the highest N_2O production. These variations in greenhouse gas (GHG) production emphasize the need for an approach to wetland restoration that accommodate the unique characteristics of each habitat type, taking into account their distinct impact on GHG emissions. Managers and policymakers should evaluate the specific traits of each habitat type, acknowledging that optimizing for the reduction of one GHG may lead to an increase in another. This understanding is crucial for developing effective wetland management strategies that balance wetland restoration goals with minimizing overall GHG emissions. Furthermore, this study revealed that as the temperature was increased from 24°C to 29°C, there was a notable rise in CH_4, CO_2, and N_2O emissions across most habitats. The finding that the natural wetland habitat showed the least percentage increase in emissions in response to temperature change highlights its resilience, signifying its potential as a stable refuge in future conservation efforts amid rapid climate change. Protecting such habitats is imperative for

maintaining ecological balance, particularly in the context of ongoing climate variability, emphasizing the need to prioritize their conservation.

5.4. Management implications and future work

In this study, the natural regeneration habitat showed the highest N_2 production, and this production increased with water residence time. Furthermore, native grass habitat (represented by rice cutgrass) showed greater effectiveness in retaining nitrogen (N) and phosphorus (P), with retention levels increasing with prolonged water residence time. Given that native grasses are usually low-maintenance and adaptable to various environmental conditions, there is potential for natural regeneration through seedbanks, emphasizing the ecosystem's self-sustaining qualities. To optimize the observed benefits in these habitats, it is recommended to prioritize natural regeneration practices that support the existing ecosystem. Instead of investing labor and capital in planting different species, fostering natural regeneration during wetland restoration, with a focus on increasing water residence, may offer a more economical and ecologically sensitive approach when nutrient reduction is a primary restoration objective.

Moreover, considering water residence time optimization as a management strategy can further enhance nutrient retention in restored wetlands, aligning with the natural dynamics of the ecosystem and supporting its ecological functions. While increased water residence time may enhance nutrient retention, it is essential to acknowledge potential trade-offs. Longer water residence may lead to elevated CH_4 production, necessitating balanced wetland management strategies. Despite these challenges, higher resilience of natural wetland to the temperature increase observed in this study highlights the importance of conserving existing natural wetlands for the future .

The variability in soil properties, N_2 production, and greenhouse gas (GHG) emissions observed both within and among habitats in this study suggests the inherent heterogeneity of wetland ecosystems. A more detailed analysis of environmental factors and vegetation dynamics within each habitat could provide deeper insights into the key factors influencing nutrient retention and GHG emissions. Additionally, long-term monitoring may be valuable for capturing seasonal and interannual variations and improving the overall understanding of the complex relationships between soil properties and biogeochemical processes. Furthermore, it is imperative to conduct additional studies in other locations to ascertain the generalizability of the relationships observed in this dissertation to broader contexts. Chapter 3 emphasized the primary influence of vegetation types on nutrient retention rates. To further improve our understanding, future studies could analyze both soil and plant nutrient levels before and after nutrient dosing experiments. This would help to understand the extent of nutrient retention in the soil and the incorporation of nutrients within the vegetation.

APPENDIX A: CHAPTER 2 DATA

Supplemental table 2.1.

Top 10 cm soil properties data for 23 easements. SM=soil moisture (g g^{-1}), BD=bulk density (g cm^{-3}), pH=soil pH, TC=soil total carbon (mg g^{-1}), TN=soil total nitrogen (mg g^{-1}), P=soil extractable phosphorus (mg g^{-1}).

Site ID.	Habitat	Latitude	Longitude	SM	BD	pH	TC	TN	P
1	Remnant forest	35.99754	-89.36053	0.48	0.92	5.02	19.82	2.17	0.041
1	Shallow water-dry	35.99600	-89.35680	1.17	0.59	5.85	18.90	2.00	0.039
1	Shallow water-wet	35.99600	-89.35682	1.03	0.82	6.26	18.72	1.80	0.027
1	Tree planting	35.99533	-89.35886	0.32	1.07	5.23	16.23	1.73	0.033
2	Natural regeneration	36.96115	-88.74852	0.12	1.15	5.94	16.30	1.38	0.058
2	Remnant forest	36.96222	-88.75305	0.25	1.05	5.54	19.59	1.73	0.060
2	Shallow water-dry	36.96465	-88.74935	0.25	1.29	5.64	8.44	0.86	0.024
2	Shallow water-wet	36.96467	-88.74918	1.02	0.99	5.77	16.73	1.56	0.037
2	Tree planting	36.96223	-88.75027	0.17	1.08	5.65	18.18	1.65	0.046
3	Remnant forest	36.42591	-88.96340	0.41	0.96	5.47	27.08	2.53	0.072
3	Shallow water-dry	36.42352	-88.96815	0.50	1.04	5.33	11.68	1.57	0.045
3	Shallow water-wet	36.42335	-88.96823	0.61	1.08	5.57	10.54	1.48	0.048

Supplemental table 2.1 (continued)

Site ID.	Habitat	Latitude	Longitude	SM	BD	pH	TC	TN	P
3	Tree planting	36.42198	-88.96612	0.28	0.96	5.57	18.52	1.89	0.059
4	Remnant forest	36.16800	-89.39293	0.48	1.05	5.13	19.27	2.17	0.040
4	Shallow water-dry	36.16580	-89.39275	0.41	1.29	5.73	11.38	1.13	0.044
4	Shallow water-wet	36.16595	-89.39323	0.52	1.19	5.69	10.28	1.03	0.033
4	Tree planting	36.16734	-89.38920	0.40	1.28	5.41	11.05	1.10	0.046
5	Remnant forest	36.61240	-89.11285	0.92	0.66	5.62	46.40	3.98	0.040
5	Shallow water-dry	36.61064	-89.12026	0.69	0.96	5.35	25.96	2.52	0.021
5	Shallow water-wet	36.60895	-89.12015	0.91	0.82	5.50	24.05	2.50	0.019
5	Tree planting	36.61045	-89.11977	0.40	0.98	5.65	26.69	2.57	0.033
6	Shallow water-dry	36.93837	-89.03424	0.50	1.16	5.52	14.46	1.59	0.029
6	Shallow water-wet	36.93795	-89.03401	0.70	0.97	5.40	16.79	1.77	0.042
6	Tree planting	36.93877	-89.03594	0.45	1.15	5.62	15.62	1.56	0.059
7	Tree planting	35.29969	-89.00434	0.32	1.11	4.88	14.15	1.28	0.028
8	Natural regeneration	35.96528	-89.15392	0.71	0.86	5.18	24.34	2.43	0.067

Supplemental table 2.1 (continued)

Site ID.	Habitat	Latitude	Longitude	SM	BD	pH	TC	TN	P
8	Shallow water-wet	35.96857	-89.15709	0.44	1.19	6.17	6.78	0.99	0.042
9	Remnant forest	36.90479	-89.08402	0.81	0.81	6.06	38.39	3.05	0.046
9	Shallow water-dry	36.90893	-89.08222	0.38	1.08	6.16	18.61	1.89	0.069
9	Shallow water-wet	36.90802	-89.08293	0.69	1.00	7.07	16.41	1.75	0.059
10	Natural regeneration	36.69797	-88.80540	0.28	1.21	5.70	13.71	1.54	0.028
10	Shallow water-wet	36.69909	-88.80353	0.68	0.99	6.36	12.02	1.43	0.023
10	Tree planting	36.69770	-88.80521	0.90	0.67	5.20	42.75	3.28	0.030
11	Natural regeneration	35.53486	-89.42208	0.25	1.00	5.27	17.42	1.73	0.046
11	Remnant forest	35.53077	-89.42373	0.29	0.91	5.03	19.11	1.84	0.031
11	Shallow water-dry	35.53940	-89.42093	0.50	1.06	4.84	15.78	1.62	0.089
11	Shallow water-wet	35.53891	-89.42094	0.51	1.16	5.11	10.63	1.10	0.065
11	Tree planting	35.53915	-89.42344	0.36	0.99	5.09	19.02	1.96	0.048
12	Remnant forest	35.96398	-89.14117	0.29	1.07	5.03	19.15	2.03	0.045
12	Shallow water-wet	35.96252	-89.14192	0.96	0.78	5.22	28.27	2.43	0.038

Supplemental table 2.1 (continued)

Site ID.	Habitat	Latitude	Longitude	SM	BD	pH	TC	TN	P
12	Tree planting	35.96353	-89.14356	0.23	1.09	4.91	15.88	1.34	0.040
13	Remnant forest	36.53534	-89.00155	0.31	0.99	5.78	31.85	2.86	0.074
13	Shallow water-dry	36.53390	-89.00340	0.37	1.17	5.70	14.30	1.30	0.025
13	Shallow water-wet	36.53509	-89.00062	0.59	1.03	5.35	12.07	1.33	0.019
13	Tree planting	36.53472	-89.00200	0.39	1.59	5.57	13.41	1.39	0.032
14	Remnant forest	36.62967	-88.95040	0.41	1.01	5.31	20.48	2.19	0.037
14	Shallow water-wet	36.62961	-88.94995	0.94	0.83	5.30	17.85	2.15	0.050
14	Tree planting	36.62844	-88.94902	0.32	1.08	5.65	16.68	1.86	0.060
15	Remnant forest	36.92597	-88.92983	1.18	0.80	5.82	53.59	4.29	0.051
16	Remnant forest	36.69216	-89.05516	0.32	1.18	6.26	11.43	1.09	0.065
16	Shallow water-dry	36.68936	-89.05566	0.70	0.82	5.56	26.50	2.37	0.069
16	Shallow water-wet	36.68906	-89.05602	0.85	0.95	6.69	19.84	2.02	0.042
16	Tree planting	36.68922	-89.05562	0.36	1.12	5.61	19.72	1.85	0.037
17	Natural regeneration	36.05238	-88.43636	0.53	0.85	4.20	23.58	2.39	0.051

Supplemental table 2.1 (continued)

Site ID.	Habitat	Latitude	Longitude	SM	BD	pH	TC	TN	P
17	Shallow water-dry	36.05482	-88.44362	0.22	1.22	4.71	8.09	0.89	0.014
17	Shallow water-wet	36.05505	-88.44353	0.55	1.10	6.04	10.40	1.09	0.022
17	Tree planting	36.05476	-88.43599	0.39	1.02	4.55	14.85	1.43	0.033
18	Remnant forest	36.94327	-88.86280	0.28	1.13	4.79	20.05	1.87	0.023
18	Shallow water-dry	36.94407	-88.86521	0.59	0.71	3.95	27.85	2.73	0.034
18	Tree planting	36.94283	-88.86419	0.26	1.13	4.93	15.08	1.49	0.022
19	Remnant forest	35.51913	-89.17007	0.18	0.97	5.06	18.20	1.79	0.038
19	Shallow water-dry	35.51678	-89.16445	0.65	1.02	5.24	12.20	1.43	0.014
19	Shallow water-wet	35.51740	-89.16801	0.62	1.00	5.45	8.25	1.28	0.026
19	Tree planting	35.51656	-89.16248	0.13	1.09	5.23	14.53	1.51	0.030
20	Remnant forest	35.51648	-72.98005	0.39	1.00	5.26	19.57	1.78	0.024
20	Shallow water-dry	35.51803	-89.20297	0.27	1.12	4.78	10.42	1.27	0.013
20	Shallow water-wet	35.51697	-89.20373	0.49	1.14	5.29	7.37	0.77	0.011
20	Tree planting	35.52088	-89.20026	0.32	1.13	5.29	12.60	1.06	0.010

Supplemental table 2.1 (continued)

Site ID.	Habitat	Latitude	Longitude	SM	BD	pH	TC	TN	P
21	Natural regeneration	35.93253	-88.87288	1.01	0.83	5.86	28.69	2.34	0.051
21	Tree planting	35.93263	-88.87434	0.31	1.09	5.70	14.11	1.30	0.023
22	Natural regeneration	36.72150	-88.87247	0.33	1.20	6.07	14.70	1.54	0.072
22	Remnant forest	36.72205	-88.87259	0.41	1.00	5.24	21.30	2.13	0.066
23	Remnant forest	36.93260	-88.93563	0.33	1.12	5.64	10.62	1.02	0.046
23	Shallow water-dry	36.93565	-88.94045	0.48	0.89	5.53	22.58	2.25	0.043
23	Shallow water-wet	36.93567	-88.94077	0.61	1.02	5.79	12.83	1.48	0.034

Supplemental table 2.2.

Nitrogen gas (N_2) and oxygen (O_2) flux data for 23 easements at 24 h and 48 h after incubation.

Site ID.	Sampling time (h)	Habitat	Latitude	Longitude	Average N_2 flux (mg m^2 h^{-1})	Average O_2 flux (mg m^2 h^{-1})
1	24	Remnant forest	35.99754	-89.36053	4.94	-44.11
1	24	Shallow water-dry	35.99600	-89.35680	0.87	-27.60
1	24	Shallow water-wet	35.99600	-89.35682	1.68	-38.41
1	24	Tree planting	35.99533	-89.35886	4.00	-47.37
2	24	Natural regeneration	36.96115	-88.74852	9.71	-92.13
2	24	Remnant forest	36.96222	-88.75305	6.82	-61.35
2	24	Shallow water-dry	36.96465	-88.74935	6.71	-66.21
2	24	Shallow water-wet	36.96467	-88.74918	3.37	-68.30
2	24	Tree planting	36.96223	-88.75027	7.78	-66.78
3	24	Remnant forest	36.42591	-88.96340	6.16	-50.58
3	24	Shallow water-dry	36.42352	-88.96815	5.68	-53.01
3	24	Shallow water-wet	36.42335	-88.96823	3.97	-65.14
3	24	Tree planting	36.42198	-88.96612	7.98	-64.96
4	24	Remnant forest	36.16800	-89.39293	5.37	-66.04
4	24	Shallow water-dry	36.16580	-89.39275	4.57	-72.69
4	24	Shallow water-wet	36.16595	-89.39323	5.63	-77.33
4	24	Tree planting	36.16734	-89.38920	5.04	-70.79
5	24	Remnant forest	36.61240	-89.11285	1.30	-35.10
5	24	Shallow water-dry	36.61064	-89.12026	2.94	-39.34
5	24	Shallow water-wet	36.60895	-89.12015	1.00	-42.45
5	24	Tree planting	36.61045	-89.11977	4.19	-51.94

Supplemental table 2.2 (continued)

Site ID.	Sampling time (h)	Habitat	Latitude	Longitude	Average N_2 flux (mg m^2 h^{-1})	Average O_2 flux (mg m^2 h^{-1})
6	24	Shallow water-dry	36.93837	-89.03424	3.27	-46.59
6	24	Shallow water-wet	36.93795	-89.03401	1.11	-48.39
6	24	Tree planting	36.93877	-89.03594	3.34	-52.53
7	24	Tree planting	35.29969	-89.00434	4.15	-56.93
8	24	Natural regeneration	35.96528	-89.15392	5.84	-81.76
8	24	Shallow water-wet	35.96857	-89.15709	4.06	-52.25
9	24	Remnant forest	36.90479	-89.08402	1.73	-40.51
9	24	Shallow water-dry	36.90893	-89.08222	2.75	-45.37
9	24	Shallow water-wet	36.90802	-89.08293	2.50	-50.75
10	24	Natural regeneration	36.69797	-88.80540	8.56	-69.14
10	24	Shallow water-wet	36.69909	-88.80353	2.85	-70.65
10	24	Tree planting	36.69770	-88.80521	5.74	-68.78
11	24	Natural regeneration	35.53486	-89.42208	7.36	-75.93
11	24	Remnant forest	35.53077	-89.42373	3.05	-40.97
11	24	Shallow water-dry	35.53940	-89.42093	7.83	-55.63
11	24	Shallow water-wet	35.53891	-89.42094	1.82	-77.82
11	24	Tree planting	35.53915	-89.42344	3.01	-39.98
12	24	Remnant forest	35.96398	-89.14117	4.23	-48.74
12	24	Shallow water-wet	35.96252	-89.14192	1.06	-53.71
12	24	Tree planting	35.96353	-89.14356	5.81	-71.11
13	24	Remnant forest	36.53534	-89.00155	2.87	-62.54

Supplemental table 2.2 (continued)

Site ID.	Sampling time (h)	Habitat	Latitude	Longitude	Average N_2 flux (mg m^2 h^{-1})	Average O_2 flux (mg m^2 h^{-1})
13	24	Shallow water-dry	36.53390	-89.00340	2.96	-51.05
13	24	Shallow water-wet	36.53509	-89.00062	2.18	-74.31
13	24	Tree planting	36.53472	-89.00200	4.59	-73.51
14	24	Remnant forest	36.62967	-88.95040	2.70	-41.17
14	24	Shallow water-wet	36.62961	-88.94995	3.03	-75.40
14	24	Tree planting	36.62844	-88.94902	5.99	-63.06
15	24	Remnant forest	36.92597	-88.92983	8.28	-72.65
16	24	Remnant forest	36.69216	-89.05516	5.09	-62.24
16	24	Shallow water-dry	36.68936	-89.05566	8.47	-85.48
16	24	Shallow water-wet	36.68906	-89.05602	2.59	-69.83
16	24	Tree planting	36.68922	-89.05562	6.60	-78.62
17	24	Natural regeneration	36.05238	-88.43636	6.58	-78.45
17	24	Shallow water-dry	36.05482	-88.44362	6.22	-63.75
17	24	Shallow water-wet	36.05505	-88.44353	2.91	-55.79
17	24	Tree planting	36.05476	-88.43599	5.45	-63.57
18	24	Remnant forest	36.94327	-88.86280	4.73	-44.80
18	24	Shallow water-dry	36.94407	-88.86521	7.73	-62.00
18	24	Tree planting	36.94283	-88.86419	6.82	-70.15
19	24	Remnant forest	35.51913	-89.17007	3.73	-52.84
19	24	Shallow water-dry	35.51678	-89.16445	4.36	-89.14
19	24	Shallow water-wet	35.51740	-89.16801	5.05	-77.91

Supplemental table 2.2 (continued)

Site ID.	Sampling time (h)	Habitat	Latitude	Longitude	Average N_2 flux (mg m^2 h^{-1})	Average O_2 flux (mg m^2 h^{-1})
19	24	Tree planting	35.51656	-89.16248	6.01	-85.73
20	24	Remnant forest	35.51648	-72.98005	4.98	-47.57
20	24	Shallow water-dry	35.51803	-89.20297	7.56	-41.75
20	24	Shallow water-wet	35.51697	-89.20373	4.27	-27.71
20	24	Tree planting	35.52088	-89.20026	7.20	-65.05
21	24	Natural regeneration	35.93253	-88.87288	5.10	-72.29
21	24	Tree planting	35.93263	-88.87434	4.27	-53.74
22	24	Natural regeneration	36.72150	-88.87247	5.26	-64.55
22	24	Remnant forest	36.72205	-88.87259	5.54	-64.77
23	24	Remnant forest	36.93260	-88.93563	2.73	-35.23
23	24	Shallow water-dry	36.93565	-88.94045	6.34	-53.46
23	24	Shallow water-wet	36.93567	-88.94077	4.10	-57.14
1	48	Remnant forest	35.99754	-89.36053	3.36	-88.72
1	48	Shallow water-wet	35.99600	-89.35682	1.56	-89.28
1	48	Tree planting	35.99533	-89.35886	2.58	-91.41
2	48	Natural regeneration	36.96108	-88.74815	12.52	-103.02
2	48	Remnant forest	36.96222	-88.75305	7.08	-90.20
2	48	Shallow water-dry	36.96465	-88.74935	7.45	-82.83
2	48	Shallow water-wet	36.96467	-88.74918	6.63	-80.12
2	48	Tree planting	36.96223	-88.75027	7.16	-86.53
3	48	Remnant forest	36.42591	-88.96340	5.27	-84.65

Supplemental table 2.2 (continued)

Site ID.	Sampling time (h)	Habitat	Latitude	Longitude	Average N_2 flux (mg m^2 h^{-1})	Average O_2 flux (mg m^2 h^{-1})
3	48	Shallow water-dry	36.42352	-88.96815	4.18	-91.37
3	48	Shallow water-wet	36.42335	-88.96823	3.29	-89.96
3	48	Tree planting	36.42198	-88.96612	5.21	-95.48
4	48	Remnant forest	36.16800	-89.39293	4.77	-75.54
4	48	Shallow water-dry	36.16580	-89.39275	4.61	-83.43
4	48	Shallow water-wet	36.16595	-89.39323	6.83	-97.07
4	48	Tree planting	36.16734	-89.38920	6.34	-83.58
5	48	Remnant forest	36.61240	-89.11285	2.51	-87.72
5	48	Shallow water-dry	36.61064	-89.12026	3.53	-79.58
5	48	Shallow water-wet	36.60895	-89.12015	2.65	-89.15
5	48	Tree planting	36.61045	-89.11977	4.46	-100.42
6	48	Shallow water-dry	36.93837	-89.03424	6.23	-95.14
6	48	Shallow water-wet	36.93795	-89.03401	3.09	-92.85
6	48	Tree planting	36.93877	-89.03594	6.50	-87.55
7	48	Tree planting	35.29969	-89.00434	3.95	-102.82
8	48	Natural regeneration	35.96528	-89.15392	5.74	-112.36
8	48	Shallow water-wet	35.96857	-89.15709	5.63	-100.05
9	48	Remnant forest	36.90479	-89.08402	3.26	-92.09
9	48	Shallow water-dry	36.90893	-89.08222	4.53	-96.19
9	48	Shallow water-wet	36.90751	-89.08322	4.29	-97.09
10	48	Natural regeneration	36.69797	-88.80540	6.46	-92.89

Supplemental table 2.2 (continued)

Site ID.	Sampling time (h)	Habitat	Latitude	Longitude	Average N_2 flux (mg m^2 h^{-1})	Average O_2 flux (mg m^2 h^{-1})
10	48	Shallow water-wet	36.69911	-88.80361	3.63	-104.91
10	48	Tree planting	36.69770	-88.80521	5.59	-108.83
11	48	Natural regeneration	35.53486	-89.42208	7.98	-112.55
11	48	Remnant forest	35.53077	-89.42373	4.32	-93.52
11	48	Shallow water-dry	35.53940	-89.42093	9.25	-113.26
11	48	Shallow water-wet	35.53891	-89.42094	4.95	-113.76
11	48	Tree planting	35.53915	-89.42344	3.41	-100.91
12	48	Remnant forest	35.96398	-89.14117	2.98	-88.09
12	48	Shallow water-wet	35.96252	-89.14192	2.91	-94.26
12	48	Tree planting	35.96353	-89.14356	5.14	-99.47
13	48	Remnant forest	36.53534	-89.00155	4.47	-103.72
13	48	Shallow water-dry	36.53390	-89.00340	3.55	-91.37
13	48	Shallow water-wet	36.53509	-89.00062	3.98	-97.81
13	48	Tree planting	36.53472	-89.00200	6.39	-107.98
14	48	Remnant forest	36.62967	-88.95040	2.63	-85.20
14	48	Shallow water-wet	36.62961	-88.94995	3.75	-99.76
14	48	Tree planting	36.62844	-88.94902	7.08	-100.04
15	48	Remnant forest	36.92597	-88.92983	8.71	-92.53
16	48	Remnant forest	36.69216	-89.05516	6.94	-92.99
16	48	Shallow water-dry	36.68936	-89.05566	9.29	-97.31
16	48	Shallow water-wet	36.68906	-89.05602	3.67	-82.06

Supplemental table 2.2 (continued)

Site ID.	Sampling time (h)	Habitat	Latitude	Longitude	Average N_2 flux (mg m^2 h^{-1})	Average O_2 flux (mg m^2 h^{-1})
16	48	Tree planting	36.68922	-89.05562	6.59	-92.32
17	48	Natural regeneration	36.05238	-88.43636	8.93	-97.22
17	48	Shallow water-dry	36.05482	-88.44362	6.12	-93.63
17	48	Shallow water-wet	36.05505	-88.44353	4.41	-94.72
17	48	Tree planting	36.05476	-88.43599	6.46	-86.89
18	48	Remnant forest	36.94327	-88.86280	3.63	-95.79
18	48	Shallow water-dry	36.94407	-88.86521	4.71	-91.69
18	48	Tree planting	36.94283	-88.86419	5.22	-101.20
19	48	Remnant forest	35.51913	-89.17007	3.22	-93.89
19	48	Shallow water-dry	35.51678	-89.16445	4.88	-109.97
19	48	Shallow water-wet	35.51740	-89.16801	5.57	-96.10
19	48	Tree planting	35.51656	-89.16248	7.58	-118.64
20	48	Remnant forest	35.51648	-72.98005	6.45	-90.51
20	48	Shallow water-dry	35.51803	-89.20297	5.87	-84.68
20	48	Shallow water-wet	35.51697	-89.20373	4.63	-75.90
20	48	Tree planting	35.52088	-89.20026	6.18	-103.96
21	48	Natural regeneration	35.93253	-88.87288	9.63	-117.50
21	48	Tree planting	35.93263	-88.87434	4.90	-98.20
22	48	Natural regeneration	36.72150	-88.87247	7.16	-98.14
22	48	Remnant forest	36.72205	-88.87259	7.50	-110.39
23	48	Remnant forest	36.93260	-88.93563	4.05	-74.25

Supplemental table 2.2 (continued)

Site ID.	Sampling time (h)	Habitat	Latitude	Longitude	Average N_2 flux (mg m^2 h^{-1})	Average O_2 flux (mg m^2 h^{-1})
23	48	Shallow water-dry	36.93565	-88.94045	8.50	-86.37
23	48	Shallow water-wet	36.93567	-88.94077	7.35	-83.16

APPENDIX B: CHAPTER 3 DATA

Supplemental table 3.1.

Dissolved organic carbon (DOC) concentrations on day 0, percentage increase in concentrations by day 1, 2, 3, 4, and 5, and days 1-5 release rate data for 36 mesocosms. Mes=mesocosm, Veg=vegetation, Hydro=Hydrology.

Mes. ID.	Veg.	Hydro	Day 0 DOC (mg L^{-1})	DOC increase by day 1 (%)	DOC increase by day 2 (%)	DOC increase by day 3 (%)	DOC increase by day 4 (%)	DOC increase by day 5 (%)	Days 1-5 DOC release rate (mg L^{-1} day^{-1})
1	Tree planting	3-day flooding	1.597	91.36	154.73	177.77	281.78	311.83	1.00
2	Tree planting	3-day flooding	1.305	92.87	126.44	142.68	306.67	447.28	1.17
3	Bare soil	3-day flooding	1.404	102.21	120.01	42.45	94.02	91.45	0.26
4	Bare soil	3-day flooding	2.251	-0.36	102.40	129.94	159.88	179.96	0.81
5	Bare soil	3-day flooding	3.521	-24.57	-0.80	-12.55	14.77	122.24	0.86
6	Native grass	3-day flooding	2.229	39.75	75.24	91.34	132.48	154.46	0.69
7	Native grass	3-day flooding	2.742	-3.06	29.47	64.84	95.15	114.33	0.63
8	Native grass	3-day flooding	2.002	23.78	116.58	144.26	203.35	254.45	1.02
9	Tree planting	3-day flooding	1.661	45.70	96.69	119.87	204.76	399.46	1.33
10	Native grass	3-week flooding	3.51	59.32	65.75	48.97	59.40	100.14	0.70
11	Tree planting	3-week flooding	2.391	88.50	153.79	266.25	280.68	322.00	1.54
12	Tree planting	3-week flooding	1.853	29.63	167.24	202.27	250.73	462.87	1.72
13	Bare soil	3-week flooding	2.11	36.82	97.01	250.90	424.64	428.44	1.81

Supplemental table 3.1 (continued)

Mes. ID.	Veg.	Hydro	Day 0 DOC (mg L^{-1})	DOC increase by day 1 (%)	DOC increase by day 2 (%)	DOC increase by day 3 (%)	DOC increase by day 4 (%)	DOC increase by day 5 (%)	Days 1-5 DOC release rate (mg L^{-1} day^{-1})
14	Bare soil	3-week flooding	1.385	113.65	171.70	284.84	359.13	490.76	1.36
15	Tree planting	3-week flooding	2.358	94.61	156.87	186.60	108.44	185.58	0.88
16	Bare soil	3-week flooding	2.176	120.63	135.52	231.89	191.87	320.13	1.39
17	Native grass	3-week flooding	2.475	77.98	83.23	93.54	120.08	139.19	0.69
18	Native grass	3-week flooding	2.253	54.06	119.13	101.78	116.60	188.37	0.85
19	Native grass	3-day flooding	2.551	25.05	48.14	96.24	62.68	132.18	0.67
20	Bare soil	3-day flooding	1.284	28.35	420.87	211.06	117.76	72.27	0.19
21	Tree planting	3-day flooding	1.425	92.84	163.02	197.19	183.30	236.21	0.67
22	Native grass	3-day flooding	2.164	70.43	99.21	138.86	172.18	188.12	0.81
23	Native grass	3-day flooding	1.821	84.46	98.68	108.02	162.33	236.68	0.86
24	Tree planting	3-day flooding	2.555	69.51	131.08	136.01	28.73	47.08	0.24
25	Tree planting	3-day flooding	3.739	13.72	20.27	17.73	53.54	32.42	0.24
26	Bare soil	3-day flooding	1.392	124.07	83.19	324.50	333.62	514.22	1.43
27	Bare soil	3-day flooding	1.78	79.38	67.70	61.80	206.91	270.90	0.96
28	Native grass	3-week flooding	1.797	54.76	73.96	103.39	144.91	207.96	0.75
29	Tree planting	3-week flooding	1.577	46.48	15.73	19.28	123.65	329.11	1.04

Supplemental table 3.1 (continued)

Mes. ID.	Veg.	Hydro	Day 0 DOC (mg L^{-1})	DOC increase by day 1 (%)	DOC increase by day 2 (%)	DOC increase by day 3 (%)	DOC increase by day 4 (%)	DOC increase by day 5 (%)	Days 1-5 DOC release rate (mg L^{-1} day^{-1})
30	Native grass	3-week flooding	2.072	55.07	63.66	66.70	108.40	142.86	0.59
31	Bare soil	3-week flooding	2.561	122.22	27.57	44.55	129.05	396.68	2.03
32	Bare soil	3-week flooding	1.757	182.19	161.01	289.87	409.50	501.02	1.76
33	Native grass	3-week flooding	1.741	69.39	82.94	118.04	166.34	188.23	0.66
34	Tree planting	3-week flooding	1.986	124.67	243.30	249.19	217.77	224.22	0.89
35	Tree planting	3-week flooding	1.74	88.33	115.23	36.55	82.47	63.22	0.22
36	Bare soil	3-week flooding	1.266	189.73	269.75	492.42	738.86	1133.02	2.87

Supplemental table 3.2.

Nitrate (NO_3^-) concentrations on day 0, percentage increase in concentrations by day 1, 2, 3, 4, and 5, and days 1-4 flux rate data for 36 mesocosms. Mes=mesocosm, Veg=vegetation, Hydro=Hydrology.

Mes. ID.	Veg.	Hydro	Day 0 NO_3^- (mg L^{-1})	NO_3^- increase by day 1 (%)	NO_3^- increase by day 2 (%)	NO_3^- increase by day 3 (%)	NO_3^- increase by day 4 (%)	NO_3^- increase by day 5 (%)	Days 1-4 NO_3^- flux rate (mg L^{-1} day^{-1})
1	Tree planting	3-day flooding	9.03	-43.12	-52.99	-74.97	-95.76	-100.00	-2.16
2	Tree planting	3-day flooding	6.10	-58.10	-71.77	-73.51	-94.37	-98.96	-1.44
3	Bare soil	3-day flooding	9.03	-23.41	-38.01	-75.07	-75.68	-79.13	-1.71
4	Bare soil	3-day flooding	8.54	-26.34	-54.90	-68.61	-88.21	-94.43	-1.88
5	Bare soil	3-day flooding	7.33	-23.21	-52.11	-78.11	-95.04	-98.16	-1.74
6	Native grass	3-day flooding	8.17	-85.72	-97.34	-99.57	-100.00	-99.92	-2.04
7	Native grass	3-day flooding	7.05	-69.91	-91.82	-99.61	-100.00	-100.00	-1.76
8	Native grass	3-day flooding	7.59	-65.91	-92.30	-99.58	-100.00	-99.96	-1.90
9	Tree planting	3-day flooding	6.26	-69.07	-67.16	-79.61	-91.97	-99.72	-1.44
10	Native grass	3-week flooding	8.39	-36.97	-77.37	-95.90	-100.00	-100.00	-2.10
11	Tree planting	3-week flooding	8.38	-12.16	-56.61	-79.38	-92.31	-99.70	-1.93
12	Tree planting	3-week flooding	7.48	-41.53	-45.61	-85.22	-96.18	-99.60	-1.80
13	Bare soil	3-week flooding	9.07	-11.43	-28.09	-79.35	-84.09	-97.75	-1.91
14	Bare soil	3-week flooding	8.36	8.81	-9.90	-74.56	-75.06	-87.31	-1.57

Supplemental table 3.2 (continued)

Mes. ID.	Veg.	Hydro	Day 0 NO_3^- (mg L^{-1})	NO_3^- increase by day 1 (%)	NO_3^- increase by day 2 (%)	NO_3^- increase by day 3 (%)	NO_3^- increase by day 4 (%)	NO_3^- increase by day 5 (%)	Days 1-4 NO_3^- flux rate (mg L^{-1} day^{-1})
15	Tree planting	3-week flooding	7.67	-32.98	-49.07	-78.65	-98.45	-99.85	-1.89
16	Bare soil	3-week flooding	9.85	-14.91	-30.84	-55.52	-83.80	-95.09	-2.06
17	Native grass	3-week flooding	7.56	-45.33	-68.23	-97.00	-100.00	-100.00	-1.89
18	Native grass	3-week flooding	7.49	-56.00	-72.91	-95.89	-100.00	-99.89	-1.87
19	Native grass	3-day flooding	7.30	-62.88	-91.03	-99.27	-100.00	-99.73	-1.83
20	Bare soil	3-day flooding	6.66	-11.11	-29.38	-43.70	-45.05	-51.46	-0.75
21	Tree planting	3-day flooding	8.44	-42.92	-65.51	-88.76	-96.12	-99.66	-2.03
22	Native grass	3-day flooding	8.17	-59.86	-94.80	-99.59	-100.00	-99.84	-2.04
23	Native grass	3-day flooding	7.63	-62.32	-88.29	-99.63	-100.00	-99.91	-1.91
24	Tree planting	3-day flooding	8.05	-14.23	-31.38	-83.01	-99.38	-100.00	-2.00
25	Tree planting	3-day flooding	6.45	-49.43	-66.24	-95.99	-100.00	-99.98	-1.61
26	Bare soil	3-day flooding	6.48	-18.42	-35.05	-79.01	-79.89	-93.34	-1.29
27	Bare soil	3-day flooding	8.17	-16.24	-42.00	-70.83	-81.06	-94.41	-1.66
28	Native grass	3-week flooding	8.31	-62.97	-88.92	-98.45	-100.00	-99.91	-2.08
29	Tree planting	3-week flooding	6.57	-69.64	-84.96	-91.03	-89.88	-95.49	-1.48
30	Native grass	3-week flooding	8.08	-60.01	-85.47	-99.41	-100.00	-99.96	-2.02

Supplemental table 3.2 (continued)

Mes. ID.	Veg.	Hydro	Day 0 NO_3^- (mg L^{-1})	NO_3^- increase by day 1 (%)	NO_3^- increase by day 2 (%)	NO_3^- increase by day 3 (%)	NO_3^- increase by day 4 (%)	NO_3^- increase by day 5 (%)	Days 1-4 NO_3^- flux rate (mg L^{-1} day^{-1})
31	Bare soil	3-week flooding	9.82	-13.34	-34.91	-55.12	-79.74	-96.40	-1.96
32	Bare soil	3-week flooding	8.28	-2.50	-18.63	-61.44	-82.17	-95.93	-1.70
33	Native grass	3-week flooding	8.53	-47.03	-86.05	-87.19	-99.36	-100.00	-2.12
34	Tree planting	3-week flooding	7.94	-22.59	-59.46	-71.71	-90.58	-98.67	-1.80
35	Tree planting	3-week flooding	7.88	-29.06	-60.55	-89.13	-88.98	-97.37	-1.75
36	Bare soil	3-week flooding	8.07	2.77	-26.39	-55.95	-98.08	-99.83	-1.98

Supplemental table 3.3.

Phosphate (PO_4^{3-}) concentrations on day 0, percentage increase in concentrations by day 1, 2, 3, 4, and 5, and first 24 h and days 2-3 flux rate data for 36 mesocosms. Mes=mesocosm, Veg=vegetation, Hydro=Hydrology.

Mes. ID.	Veg	Hydro.	Day 0 PO_4^{3-} (mg L^{-1})	% PO_4^{3-} increase by					PO_4^{3-} flux (mg L^{-1} day^{-1})	
				Day 1	Day 2	Day 3	Day 4	Day 5	First 24 h	Days 2-3
1	Tree planting	3-day flooding	0.283	9.74	-55.31	-82.99	-74.83	-96.33	0.028	-0.131
2	Tree planting	3-day flooding	0.026	263.12	-17.11	-15.97	38.02	-38.40	0.069	-0.037
3	Bare soil	3-day flooding	0.031	21.61	-37.74	-39.03	-21.29	-43.55	0.007	-0.009
4	Bare soil	3-day flooding	0.105	-77.96	-48.38	-84.92	-80.92	-87.88	-0.082	-0.004
5	Bare soil	3-day flooding	0.057	-58.48	-73.67	-77.56	-22.26	-70.49	-0.033	-0.005
6	Native grass	3-day flooding	0.300	-80.37	-61.45	-95.16	-95.69	-84.18	-0.241	-0.022
7	Native grass	3-day flooding	0.316	-50.11	-71.71	-90.43	-92.37	-95.88	-0.158	-0.064
8	Native grass	3-day flooding	0.155	-82.74	-53.65	-87.98	-90.30	-89.72	-0.128	-0.004
9	Tree planting	3-day flooding	0.100	25.30	-69.38	-82.23	-76.51	-81.02	0.025	-0.054
10	Native grass	3-week flooding	0.484	-58.01	-79.34	-94.82	-96.12	-96.76	-0.281	-0.089
11	Tree planting	3-week flooding	0.481	-73.41	-92.58	-97.01	-97.57	-97.05	-0.353	-0.057
12	Tree planting	3-week flooding	0.087	-50.87	-3.01	-81.97	-75.38	-69.02	-0.044	-0.013
13	Bare soil	3-week flooding	0.099	-51.37	-52.07	-86.45	-73.21	-68.55	-0.051	-0.017
14	Bare soil	3-week flooding	0.026	35.66	-42.64	-57.36	-52.33	-21.32	0.009	-0.012

Supplemental table 3.3 (continued)

Mes. ID.	Veg	Hydro.	Day 0 PO_4^{3-} (mg L^{-1})	% PO_4^{3-} increase by					PO_4^{3-} flux (mg L^{-1} day^{-1})	
				Day 1	Day 2	Day 3	Day 4	Day 5	First 24 h	Days 2-3
15	Tree planting	3-week flooding	0.202	-78.04	-88.33	-94.26	-92.38	-93.52	-0.158	-0.016
16	Bare soil	3-week flooding	0.068	156.14	-70.91	-77.19	-88.01	-63.74	0.107	-0.080
17	Native grass	3-week flooding	0.405	-61.24	-87.29	-93.63	-97.65	-96.32	-0.248	-0.066
18	Native grass	3-week flooding	0.359	-55.12	-76.60	-89.40	-97.18	-96.23	-0.198	-0.061
19	Native grass	3-day flooding	0.196	-69.88	-75.09	-84.43	-92.29	-91.68	-0.137	-0.014
20	Bare soil	3-day flooding	0.020	120.10	50.25	80.90	-59.80	473.37	0.024	-0.004
21	Tree planting	3-day flooding	0.116	-46.05	-79.30	-88.83	-91.32	-90.46	-0.054	-0.025
22	Native grass	3-day flooding	0.332	-72.05	-88.57	-91.28	-95.79	-93.53	-0.240	-0.032
23	Native grass	3-day flooding	0.226	-38.58	-66.42	-88.91	-94.70	-87.23	-0.087	-0.057
24	Tree planting	3-day flooding	0.090	52.95	-11.90	-80.20	-91.21	-81.65	0.048	-0.060
25	Tree planting	3-day flooding	0.076	12.84	19.53	-79.42	-85.06	-74.31	0.010	-0.035
26	Bare soil	3-day flooding	0.014	112.41	87.59	23.36	125.55	124.82	0.015	-0.006
27	Bare soil	3-day flooding	0.081	-71.46	-74.07	-83.50	-84.24	-76.30	-0.058	-0.005
28	Native grass	3-week flooding	0.120	-8.97	-29.15	-86.13	-82.81	-84.14	-0.011	-0.046
29	Tree planting	3-week flooding	0.029	-24.91	-62.81	-74.39	32.98	-19.65	-0.007	-0.007
30	Native grass	3-week flooding	0.337	-70.84	-88.12	-96.59	-95.90	-95.72	-0.239	-0.043

Supplemental table 3.3 (continued)

Mes. ID.	Veg	Hydro.	Day 0 PO_4^{3-} (mg L^{-1})	% PO_4^{3-} increase by					PO_4^{3-} flux (mg L^{-1} day^{-1})	
				Day 1	Day 2	Day 3	Day 4	Day 5	First 24 h	Days 2-3
31	Bare soil	3-week flooding	0.151	-69.28	-87.59	-93.10	-83.61	-69.48	-0.104	-0.018
32	Bare soil	3-week flooding	0.047	-49.15	-61.28	-79.36	-58.30	-20.85	-0.023	-0.007
33	Native grass	3-week flooding	0.086	18.30	-34.50	-54.31	-70.63	-83.57	0.016	-0.031
34	Tree planting	3-week flooding	0.145	-33.54	-76.97	-89.63	-90.18	-87.21	-0.049	-0.041
35	Tree planting	3-week flooding	0.076	-37.48	-42.78	-80.66	-85.30	-78.15	-0.028	-0.016
36	Bare soil	3-week flooding	0.016	0.00	0.00	0.00	0.00	0.00	0.000	0.000

Supplemental table 3.4.

Nitrogen gas (N_2), oxygen (O_2), nitrous oxide (N_2O), and methane (CH_4) flux data for 36 mesocosms at 12 h, 24 h and 48 h after incubation. Mes=mesocosm.

Mes. ID.	Vegetation	Hydrology	Sampling time (h)	Gas flux (mg m^{-2} h^{-1})			
				N_2	O_2	N_2O	CH_4
1	Tree planting	3-day flooding	12	1.890	-20.357	0.075	0.005
2	Tree planting	3-day flooding	12	1.644	-4.719	0.010	0.019
3	Bare soil	3-day flooding	12	5.384	-22.289	0.198	0.001
4	Bare soil	3-day flooding	12	4.036	-17.006	0.004	0.003
5	Bare soil	3-day flooding	12	3.192	-15.427	0.026	-0.008
6	Native grass	3-day flooding	12	5.748	-41.475	0.008	0.007
7	Native grass	3-day flooding	12	11.680	-57.792	0.047	0.101
8	Native grass	3-day flooding	12	8.339	-53.866	0.007	0.038
9	Tree planting	3-day flooding	12	1.975	-9.419	0.013	0.002
10	Native grass	3-week flooding	12	4.567	-50.082	0.046	-0.001
11	Tree planting	3-week flooding	12	2.623	-14.308	0.058	-0.004
12	Tree planting	3-week flooding	12	4.964	-16.452	0.078	0.005
13	Bare soil	3-week flooding	12	6.402	-18.451	0.016	0.024
14	Bare soil	3-week flooding	12	2.562	-13.223	0.020	0.015
15	Tree planting	3-week flooding	12	3.589	-27.515	0.053	-0.006
16	Bare soil	3-week flooding	12	2.311	-14.796	0.015	-0.004
17	Native grass	3-week flooding	12	9.096	-58.380	0.017	-0.004
18	Native grass	3-week flooding	12	5.222	-33.281	0.072	0.074
19	Native grass	3-day flooding	12	2.016	-13.553	0.028	0.002

Supplemental table 3.4 (continued)

Mes. ID.	Vegetation	Hydrology	Sampling time (h)	Gas flux (mg m^{-2} h^{-1})			
				N_2	O_2	N_2O	CH_4
20	Bare soil	3-day flooding	12	2.743	-35.507	0.028	0.001
21	Tree planting	3-day flooding	12	3.284	-16.723	0.031	0.011
22	Native grass	3-day flooding	12	6.737	-49.561	0.023	0.014
23	Native grass	3-day flooding	12	3.882	-24.421	0.048	-0.013
24	Tree planting	3-day flooding	12	1.842	-10.473	0.060	-0.003
25	Tree planting	3-day flooding	12	7.755	-9.173	-0.006	0.004
26	Bare soil	3-day flooding	12	3.977	-19.214	0.059	-0.009
27	Bare soil	3-day flooding	12	3.733	-31.505	0.190	0.016
28	Native grass	3-week flooding	12	2.948	-26.584	0.035	-0.003
29	Tree planting	3-week flooding	12	3.396	-17.777	0.014	-0.001
30	Native grass	3-week flooding	12	5.987	-43.907	0.013	-0.001
31	Bare soil	3-week flooding	12	2.822	-5.610	0.018	0.000
32	Bare soil	3-week flooding	12	3.898	-14.511	0.013	0.001
33	Native grass	3-week flooding	12	NA	NA	NA	NA
34	Tree planting	3-week flooding	12	1.720	-14.580	0.043	-0.005
35	Tree planting	3-week flooding	12	2.282	-16.670	0.057	0.003
36	Bare soil	3-week flooding	12	2.914	-15.748	0.022	0.006
1	Tree planting	3-day flooding	24	2.982	-29.325	0.045	0.005
2	Tree planting	3-day flooding	24	2.944	-15.992	-0.008	0.011
3	Bare soil	3-day flooding	24	7.304	-27.130	0.047	-0.001
4	Bare soil	3-day flooding	24	5.590	-31.388	-0.009	0.006

Supplemental table 3.4 (continued)

Mes. ID.	Vegetation	Hydrology	Sampling time (h)	Gas flux (mg m^{-2} h^{-1})			
				N_2	O_2	N_2O	CH_4
5	Bare soil	3-day flooding	24	3.921	-26.755	0.001	0.000
6	Native grass	3-day flooding	24	6.660	-50.141	0.001	0.029
7	Native grass	3-day flooding	24	11.764	-60.201	0.031	0.342
8	Native grass	3-day flooding	24	9.202	-62.620	-0.014	0.213
9	Tree planting	3-day flooding	24	2.845	-21.452	-0.016	-0.002
10	Native grass	3-week flooding	24	6.032	-57.852	0.027	0.015
11	Tree planting	3-week flooding	24	3.714	-22.551	0.032	0.001
12	Tree planting	3-week flooding	24	4.853	-26.958	0.028	0.005
13	Bare soil	3-week flooding	24	9.913	-45.901	0.027	0.030
14	Bare soil	3-week flooding	24	2.553	-19.519	-0.007	0.021
15	Tree planting	3-week flooding	24	5.667	-30.390	0.014	-0.004
16	Bare soil	3-week flooding	24	3.192	-25.377	0.004	-0.001
17	Native grass	3-week flooding	24	8.255	-62.008	-0.003	0.008
18	Native grass	3-week flooding	24	6.454	-40.202	0.042	0.200
19	Native grass	3-day flooding	24	4.809	-27.416	-0.003	0.000
20	Bare soil	3-day flooding	24	3.262	-38.062	0.032	-0.004
21	Tree planting	3-day flooding	24	7.635	-30.990	0.013	0.015
22	Native grass	3-day flooding	24	7.539	-61.089	0.017	0.112
23	Native grass	3-day flooding	24	4.189	-34.045	0.064	0.003
24	Tree planting	3-day flooding	24	6.361	-39.123	0.032	0.005
25	Tree planting	3-day flooding	24	2.252	-21.285	-0.006	0.017

Supplemental table 3.4 (continued)

Mes. ID.	Vegetation	Hydrology	Sampling time (h)	Gas flux (mg m^{-2} h^{-1})			
				N_2	O_2	N_2O	CH_4
26	Bare soil	3-day flooding	24	4.193	-28.932	0.031	0.000
27	Bare soil	3-day flooding	24	4.504	-34.362	0.133	0.046
28	Native grass	3-week flooding	24	4.121	-37.482	0.013	0.013
29	Tree planting	3-week flooding	24	4.451	-27.373	0.002	-0.003
30	Native grass	3-week flooding	24	5.604	-47.069	-0.007	0.025
31	Bare soil	3-week flooding	24	2.621	-14.377	-0.005	0.001
32	Bare soil	3-week flooding	24	7.455	-33.559	0.004	0.001
33	Native grass	3-week flooding	24	4.119	-36.489	0.006	-0.010
34	Tree planting	3-week flooding	24	2.928	-26.545	0.081	0.001
35	Tree planting	3-week flooding	24	2.395	-18.940	0.033	-0.001
36	Bare soil	3-week flooding	24	4.051	-26.233	0.006	0.007
1	Tree planting	3-day flooding	48	7.349	-72.694	-0.012	0.015
2	Tree planting	3-day flooding	48	5.752	-46.557	-0.018	0.019
3	Bare soil	3-day flooding	48	9.152	-65.016	-0.022	0.000
4	Bare soil	3-day flooding	48	6.522	-54.126	-0.012	0.028
5	Bare soil	3-day flooding	48	5.159	-39.077	-0.014	-0.001
6	Native grass	3-day flooding	48	6.530	-81.801	-0.015	0.057
7	Native grass	3-day flooding	48	13.552	-97.341	0.003	1.113
8	Native grass	3-day flooding	48	8.979	-81.250	-0.001	0.841
9	Tree planting	3-day flooding	48	4.421	-52.253	-0.021	-0.001
10	Native grass	3-week flooding	48	6.759	-88.194	0.029	0.042

Supplemental table 3.4 (continued)

Mes. ID.	Vegetation	Hydrology	Sampling time (h)	Gas flux (mg m^{-2} h^{-1})			
				N_2	O_2	N_2O	CH_4
11	Tree planting	3-week flooding	48	4.741	-44.288	0.005	-0.003
12	Tree planting	3-week flooding	48	7.859	-56.462	0.004	0.009
13	Bare soil	3-week flooding	48	12.506	-97.711	0.041	0.041
14	Bare soil	3-week flooding	48	2.965	-33.106	-0.005	0.036
15	Tree planting	3-week flooding	48	7.796	-59.262	0.001	0.007
16	Bare soil	3-week flooding	48	5.877	-48.186	-0.008	0.003
17	Native grass	3-week flooding	48	8.161	-89.433	0.005	0.029
18	Native grass	3-week flooding	48	6.465	-54.211	0.011	0.273
19	Native grass	3-day flooding	48	4.852	-61.353	0.049	-0.010
20	Bare soil	3-day flooding	48	3.718	-45.108	0.008	0.007
21	Tree planting	3-day flooding	48	7.980	-67.915	0.041	0.022
22	Native grass	3-day flooding	48	7.487	-79.418	0.010	0.259
23	Native grass	3-day flooding	48	5.038	-56.725	0.058	-0.013
24	Tree planting	3-day flooding	48	8.236	-69.697	0.009	0.001
25	Tree planting	3-day flooding	48	6.568	-50.546	0.004	0.034
26	Bare soil	3-day flooding	48	6.697	-55.194	-0.004	0.003
27	Bare soil	3-day flooding	48	6.868	-51.130	0.038	0.028
28	Native grass	3-week flooding	48	4.783	-50.770	0.003	0.021
29	Tree planting	3-week flooding	48	6.023	-37.248	-0.012	-0.006
30	Native grass	3-week flooding	48	11.043	-44.633	0.011	0.157
31	Bare soil	3-week flooding	48	6.179	-41.927	-0.019	-0.002

Supplemental table 3.4 (continued)

Mes. ID.	Vegetation	Hydrology	Sampling time (h)	Gas flux (mg m^{-2} h^{-1})			
				N_2	O_2	N_2O	CH_4
32	Bare soil	3-week flooding	48	10.751	-71.586	-0.014	0.006
33	Native grass	3-week flooding	48	5.549	-78.626	-0.001	0.011
34	Tree planting	3-week flooding	48	6.075	-44.894	0.026	0.001
35	Tree planting	3-week flooding	48	3.548	-37.516	0.000	0.010
36	Bare soil	3-week flooding	48	5.551	-45.506	-0.021	0.013

Supplemental table 3.5.

Top 10 cm soil properties data for 36 mesocosms. Mes=mesocosm, pre=pre-experiment, post=post-experiment, TC=soil total carbon (mg g^{-1}), TN=soil total nitrogen (mg g^{-1}), P=soil extractable phosphorus (mg g^{-1}), Chl-a=chlorophyll-a (mg m^{-2}), and AFDM=ash-free dry mass (mg g^{-1}).

Mes ID.	Vegetation	Hydrology	Pre TC	Pre TN	Pre P	Post TC	Post TN	Post P	Chl-*a*	AFDM
1	Tree planting	3-day flooding	5.3	0.5	0.016	5.5	0.9	0.014	123.63	29.7
2	Tree planting	3-day flooding	5.9	0.5	0.018	5.8	0.9	0.015	15.50	31.4
3	Bare soil	3-day flooding	6.9	0.6	0.018	7.1	0.9	0.018	129.26	29.7
4	Bare soil	3-day flooding	7.6	0.6	0.017	NA	NA	NA	NA	NA
5	Bare soil	3-day flooding	5.3	0.5	0.013	6.5	0.9	0.014	273.80	31.1
6	Native grass	3-day flooding	9.3	0.8	0.024	10.2	1.1	0.018	59.39	35.2
7	Native grass	3-day flooding	10.1	0.8	0.018	6.7	1.0	0.012	9.65	31.9
8	Native grass	3-day flooding	7.9	0.6	0.014	7.1	0.9	0.014	51.34	33.5
9	Tree planting	3-day flooding	5.7	0.5	0.017	6.0	0.8	0.015	44.30	29.5
10	Native grass	3-week flooding	7.2	0.6	0.016	6.5	0.9	0.018	26.19	29.1
11	Tree planting	3-week flooding	7.2	0.6	0.014	7.1	1.0	0.019	20.42	31.7
12	Tree planting	3-week flooding	6.7	0.6	0.014	6.5	1.0	0.012	20.05	31.2
13	Bare soil	3-week flooding	6.5	0.5	0.016	8.1	1.0	0.016	26.75	32.5

Supplemental table 3.5 (continued)

Mes ID.	Vegetation	Hydrology	Pre TC	Pre TN	Pre P	Post TC	Post TN	Post P	Chl-*a*	AFDM
14	Bare soil	3-week flooding	6.7	0.6	0.013	7.6	0.9	0.013	22.42	28.7
15	Tree planting	3-week flooding	8.6	0.7	0.015	8.1	1.0	0.017	23.77	32.1
16	Bare soil	3-week flooding	7.8	0.7	0.014	7.9	1.0	0.013	23.04	35.1
17	Native grass	3-week flooding	9.8	0.8	0.017	7.7	1.1	0.020	22.29	30.5
18	Native grass	3-week flooding	10.2	0.7	0.015	6.8	0.9	0.016	16.47	30.7
19	Native grass	3-day flooding	8.6	0.7	0.024	7.1	1.0	0.020	90.76	28.4
20	Bare soil	3-day flooding	6.3	0.6	0.016	6.4	0.8	0.016	29.96	26.8
21	Tree planting	3-day flooding	7.0	0.6	0.015	5.2	0.7	0.014	87.21	25.5
22	Native grass	3-day flooding	6.6	0.6	0.016	7.9	0.8	0.016	43.66	25.0
23	Native grass	3-day flooding	9.0	0.7	0.014	7.0	1.0	0.016	32.39	28.0
24	Tree planting	3-day flooding	7.1	0.6	0.012	6.4	0.8	0.013	128.70	30.8
25	Tree planting	3-day flooding	7.4	0.7	0.020	7.7	0.9	0.016	78.10	29.1
26	Bare soil	3-day flooding	5.8	0.6	0.018	5.5	0.8	0.018	46.03	30.9
27	Bare soil	3-day flooding	5.7	0.5	0.015	6.3	0.8	0.016	115.21	30.2
28	Native grass	3-week flooding	8.4	0.7	0.013	8.6	0.9	0.015	12.75	31.6

Supplemental table 3.5 (continued)

Mes ID.	Vegetation	Hydrology	Pre TC	Pre TN	Pre P	Post TC	Post TN	Post P	Chl-*a*	AFDM
29	Tree planting	3-week flooding	7.9	0.6	0.013	6.7	0.9	0.019	11.60	26.0
30	Native grass	3-week flooding	6.8	0.6	0.015	6.3	0.9	0.018	2.29	30.1
31	Bare soil	3-week flooding	6.0	0.5	0.015	5.1	0.9	0.018	7.75	30.8
32	Bare soil	3-week flooding	7.5	0.6	0.013	6.0	1.0	0.017	6.31	29.9
33	Native grass	3-week flooding	5.8	0.5	0.011	6.0	0.7	0.015	4.80	24.4
34	Tree planting	3-week flooding	6.0	0.6	0.015	5.8	0.8	0.014	7.43	25.9
35	Tree planting	3-week flooding	5.8	0.5	0.010	4.5	0.7	0.011	21.17	26.4
36	Bare soil	3-week flooding	6.5	0.6	0.010	5.8	0.9	0.010	30.21	27.7

APPENDIX C: CHAPTER 4 DATA

Supplemental table 4.1.

Top 10 cm soil properties data for 4 easements. SM=soil moisture (g g^{-1}), BD=bulk density (g cm^{-3}), pH=soil pH, TC=soil total carbon (mg g^{-1}), TN=soil total nitrogen (mg g^{-1}), P=soil extractable phosphorus (mg g^{-1}), and Fe=soil extractable iron (mg g^{-1}).

Site ID.	Core ID.	Habitat	Latitude	Longitude	SM	BD	pH	TC	TN	P	Fe
1	1	Crop field	35.5282	-89.2015	0.20	1.22	4.86	13.70	1.20	0.095	0.053
1	2	Crop field	35.5229	-89.202	0.23	1.29	4.81	18.50	1.20	0.161	0.080
1	3	Crop field	35.5226	-89.2027	0.20	1.40	5.09	10.70	1.05	0.106	0.050
1	4	Crop field	35.5242	-89.2017	0.29	1.24	5.35	12.70	1.25	0.083	0.098
1	5	Crop field	35.5346	-89.2018	0.22	1.44	5.17	11.50	1.20	0.079	0.099
1	6	Crop field	35.525	-89.2019	0.21	1.26	5.06	13.70	1.35	0.111	0.107
1	7	Shallow water-dry	35.5168	-89.2027	0.27	1.18	5.12	9.35	1.25	0.010	0.122
1	8	Shallow water-wet	35.5165	-89.2031	0.49	1.12	5.08	9.85	0.85	0.017	0.114
1	9	Shallow water-wet	35.5171	-89.2044	0.48	1.20	5.34	5.80	0.85	0.006	0.063
1	10	Shallow water-dry	35.5174	-89.2029	0.28	1.04	4.49	10.70	1.10	0.022	0.196
1	11	Shallow water-dry	35.5199	-89.2033	0.27	1.13	4.74	11.20	1.45	0.006	0.100
1	12	Shallow water-wet	35.5173	-89.2037	0.50	1.10	5.44	6.45	0.60	0.011	0.073
1	13	Tree planting	35.5199	-89.1995	0.32	1.04	5.36	10.50	1.00	0.014	0.146
1	14	Tree planting	35.5206	-89.2001	0.31	1.08	5.37	16.15	0.80	0.008	0.150
1	15	Tree planting	35.5214	-89.201	0.32	1.14	5.12	12.00	1.15	0.009	0.169

Supplemental table 4.1 (continued)

Site ID.	Core ID.	Habitat	Latitude	Longitude	SM	BD	pH	TC	TN	P	Fe
1	16	Tree planting	35.5202	-89.1996	0.38	0.90	5.14	16.55	1.40	0.025	0.264
1	17	Tree planting	35.5209	-89.1998	0.31	1.19	5.40	9.60	0.90	0.007	0.110
1	18	Tree planting	35.5213	-89.2008	0.28	1.33	5.41	8.70	1.05	0.004	0.104
1	19	Remnant forest	35.5152	-89.2015	0.34	0.92	4.97	19.60	1.95	0.018	0.181
1	20	Remnant forest	35.5168	-89.2015	0.34	0.91	5.08	12.55	1.55	0.025	0.184
1	21	Remnant forest	35.5184	-89.1982	0.39	1.04	5.41	19.60	1.75	0.027	0.259
1	22	Remnant forest	35.5179	-89.1989	0.36	0.97	5.17	17.65	1.90	0.014	0.146
1	23	Remnant forest	35.514	-89.1963	0.38	0.97	5.50	21.90	1.70	0.019	0.137
1	24	Remnant forest	35.5161	-89.1968	0.30	1.38	5.68	7.80	0.95	0.025	0.088
1	25	Remnant forest	35.5178	-89.1975	0.55	0.92	4.98	23.75	2.25	0.026	0.434
1	26	Remnant forest	35.5169	89.202	0.62	0.58	4.84	44.90	3.70	0.044	0.389
1	27	Remnant forest	35.5148	-89.196	0.27	1.24	5.53	9.40	0.80	0.021	0.081
1	28	Remnant forest	35.5157	-89.1976	0.34	1.01	5.01	16.50	1.35	0.018	0.210
1	29	Remnant forest	35.5177	-89.1982	0.37	1.05	5.11	15.20	1.65	0.021	0.169
1	30	Remnant forest	NA	NA	0.40	0.99	5.78	26.00	1.85	0.029	0.110
2	1	Shallow water-wet	36.6099	-89.0333	0.61	1.03	5.84	8.95	1.15	0.028	0.198

Supplemental table 4.1 (continued)

Site ID.	Core ID.	Habitat	Latitude	Longitude	SM	BD	pH	TC	TN	P	Fe
2	2	Shallow water-wet	36.6111	-89.0331	0.61	1.04	5.87	10.50	1.15	0.028	0.251
2	3	Shallow water-wet	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	4	Shallow water-wet	36.6096	-89.0333	0.54	0.92	6.30	9.50	1.20	0.024	0.242
2	5	Shallow water-wet	36.6105	-89.0332	0.81	0.86	6.40	9.30	1.00	0.021	0.189
2	6	Shallow water-wet	36.6119	-89.033	0.99	0.78	6.44	14.45	1.65	0.034	0.343
2	7	Tree planting	36.6106	-89.0323	0.81	0.82	5.92	21.85	1.90	0.045	0.199
2	8	Tree planting	36.6108	-89.0322	0.30	1.04	5.35	18.70	2.05	0.039	0.154
2	9	Tree planting	36.6113	-89.0317	0.27	1.01	5.74	20.10	1.95	0.036	0.134
2	10	Tree planting	36.6105	-89.0329	1.47	0.58	5.92	26.55	2.50	0.027	0.297
2	11	Tree planting	36.6108	-89.0329	0.75	0.77	6.08	12.40	1.35	0.028	0.199
2	12	Tree planting	36.6114	-89.0328	0.91	0.91	5.71	27.25	2.35	0.039	0.275
2	13	Tree planting	36.6117	-89.0327	0.61	1.04	5.61	18.15	1.75	0.030	0.189
2	14	Tree planting	36.612	-89.0327	0.56	1.03	5.68	22.05	2.00	0.043	0.221
2	15	Tree planting	36.6125	-89.0328	0.28	0.99	5.43	18.55	2.00	0.039	0.167
2	16	Tree planting	36.6116	-89.0323	0.39	0.92	5.25	17.95	2.10	0.037	0.150
2	17	Tree planting	36.6119	-89.0321	0.40	0.92	5.09	18.20	1.95	0.042	0.230

Supplemental table 4.1 (continued)

Site ID.	Core ID.	Habitat	Latitude	Longitude	SM	BD	pH	TC	TN	P	Fe
2	18	Tree planting	36.6124	-89.0322	0.44	0.75	5.19	24.65	2.30	0.048	0.256
2	19	Tree planting	36.6106	-89.0336	0.52	0.81	4.97	21.45	2.05	0.058	0.276
2	20	Tree planting	36.6109	-89.0338	0.32	0.88	5.02	20.00	1.85	0.041	0.187
2	21	Tree planting	36.6125	-89.0334	0.32	0.83	5.38	17.30	1.90	0.038	0.236
2	22	Tree planting	36.6103	-89.0337	0.49	0.82	5.12	17.75	1.80	0.056	0.175
2	23	Tree planting	36.6113	-89.0338	0.25	1.03	5.16	15.90	1.65	0.031	0.133
2	24	Tree planting	36.6119	-89.0335	0.25	0.92	5.17	18.45	1.70	0.039	0.190
2	25	Remnant forest	36.617	-89.0279	0.26	1.05	5.38	14.60	1.45	0.053	0.092
2	26	Remnant forest	36.6168	-89.0283	0.27	0.97	5.88	13.00	1.30	0.063	0.134
2	27	Remnant forest	36.6164	-89.0292	0.27	1.20	5.65	11.55	1.15	0.053	0.119
2	28	Remnant forest	36.6173	-89.0277	0.41	0.98	5.24	21.55	2.30	0.070	0.215
2	29	Remnant forest	36.6172	-89.0283	0.55	0.94	5.26	22.10	2.50	0.069	0.175
2	30	Remnant forest	36.6165	-89.0292	0.48	1.03	5.37	14.40	1.30	0.049	0.172
3	1	Crop field	36.9237	-88.9276	0.23	1.24	5.73	12.15	1.30	0.058	0.058
3	2	Crop field	36.9234	-88.9284	0.29	1.35	5.67	15.00	1.60	0.076	0.059
3	3	Crop field	36.9234	-88.9292	0.25	1.31	5.94	16.70	1.50	0.098	0.049
3	4	Crop field	36.9242	-88.9288	0.32	1.07	5.75	14.80	1.65	0.037	0.079
3	5	Crop field	36.9239	-88.9293	0.32	1.18	5.76	15.50	1.60	0.025	0.059

Supplemental table 4.1 (continued)

Site ID.	Core ID.	Habitat	Latitude	Longitude	SM	BD	pH	TC	TN	P	Fe
3	6	Crop field	36.9243	-88.9279	0.28	1.32	5.96	11.90	1.30	0.016	0.045
3	7	Crop field	36.9255	-88.9284	0.26	1.43	5.77	12.15	1.40	0.100	0.100
3	8	Crop field	36.9253	-88.9289	0.27	1.23	5.74	12.15	1.40	0.013	0.046
3	9	Crop field	36.9259	-88.9293	0.25	1.42	5.84	10.20	1.20	0.032	0.049
3	10	Crop field	36.925	-88.9303	0.27	1.29	5.68	10.95	1.40	0.058	0.127
3	11	Crop field	36.925	-88.9303	0.27	1.30	5.16	12.75	1.50	0.036	0.094
3	12	Crop field	36.9246	-88.9313	0.25	1.39	5.24	13.15	1.45	0.097	0.129
3	13	Crop field	36.9235	-88.9298	0.23	1.40	5.51	10.50	1.15	0.034	0.056
3	14	Crop field	36.9233	-88.9306	0.23	1.18	5.64	12.85	1.35	0.048	0.057
3	15	Crop field	36.9233	-88.9319	0.21	1.34	5.41	9.85	1.00	0.074	0.088
3	16	Crop field	36.9245	-88.9297	0.33	1.21	5.16	14.70	1.40	0.032	0.050
3	17	Crop field	36.924	-88.9308	0.29	1.32	5.53	12.90	1.45	0.039	0.099
3	18	Crop field	36.9237	-88.9319	0.23	1.46	5.73	10.00	1.05	0.041	0.090
3	19	Remnant forest	36.9256	-88.9309	0.38	0.87	5.79	20.30	2.10	0.063	0.186
3	20	Remnant forest	36.9254	-88.9315	0.29	1.12	5.80	14.80	1.55	0.034	0.166
3	21	Natural wetland	36.9263	-88.9309	1.21	0.76	5.85	31.60	2.90	0.048	0.389
3	22	Natural wetland	36.926	-88.9314	0.38	1.08	5.70	9.15	0.90	0.050	0.321
3	23	Remnant forest	36.9259	-88.9284	0.84	0.78	5.89	36.30	3.20	0.067	0.423
3	24	Remnant forest	36.9263	-88.9286	4.99	0.16	5.91	210.75	14.45	0.046	
3	25	Natural wetland	36.9268	-88.9295	0.65	0.90	5.96	24.15	2.10	0.026	0.318

Supplemental table 4.1 (continued)

Site ID.	Core ID.	Habitat	Latitude	Longitude	SM	BD	pH	TC	TN	P	Fe
3	26	Natural wetland	36.9269	-88.9293	2.26	0.39	5.78	58.15	5.15	0.049	0.289
3	27	Remnant forest	36.9262	-88.9299	0.32	0.77	5.73	23.65	2.65	0.060	0.139
3	28	Natural wetland	36.9267	-88.9301	2.06	0.57	5.85	35.50	3.25	0.037	0.317
3	29	Remnant forest	36.9264	-88.9297	0.28	1.11	5.81	15.75	1.80	0.036	0.137
3	30	Natural wetland	36.9267	-88.9298	3.75	0.23	5.87	83.35	7.15	0.047	0.369
4	1	Shallow water-wet	36.9361	-88.9405	0.42	1.20	5.61	9.65	1.25	0.026	0.080
4	2	Shallow water-wet	36.9361	-88.9406	0.48	1.09	5.43	10.00	1.15	0.031	0.137
4	3	Shallow water-wet	36.9348	-88.9409	0.87	0.80	5.88	17.10	1.80	0.032	0.244
4	4	Shallow water-dry	36.9357	-88.9406	0.36	1.00	5.81	18.95	2.10	0.041	0.109
4	5	Remnant forest	36.9327	-88.9325	0.27	1.24	5.84	4.45	0.60	0.027	0.077
4	6	Remnant forest	36.9328	-88.9327	0.20	1.25	5.25	2.55	0.30	0.046	0.040
4	7	Shallow water-wet	36.9361	-88.9408	0.40	1.21	5.72	8.45	1.15	0.049	0.038
4	8	Shallow water-wet	36.936	-88.9409	0.67	0.94	5.99	12.85	1.50	0.036	0.043
4	9	Shallow water-wet	36.9349	-88.9409	0.84	0.86	6.09	18.95	2.00	0.033	0.263
4	10	Shallow water-dry	36.9356	-88.9403	0.60	0.78	5.25	26.20	2.40	0.046	0.260
4	11	Remnant forest	36.9337	-88.9331	0.25	1.39	6.02	3.25	0.45	0.046	0.065

Supplemental table 4.1 (continued)

Site ID.	Core ID.	Habitat	Latitude	Longitude	SM	BD	pH	TC	TN	P	Fe
4	12	Remnant forest	36.9338	-88.9335	0.29	1.26	5.30	7.30	0.60	0.055	0.088
4	13	Remnant forest	36.9331	-88.9331	0.33	1.08	5.39	8.15	0.65	0.047	0.067
4	14	Remnant forest	36.9319	-88.9348	0.27	0.88	5.56	11.30	1.25	0.033	0.064
4	15	Remnant forest	36.9324	-88.9349	0.24	1.17	5.59	10.20	0.95	0.048	0.076
4	16	Remnant forest	36.9329	-88.9353	0.25	1.25	6.24	7.10	0.60	0.044	0.080
4	17	Remnant forest	36.9316	-88.9378	0.33	1.01	5.61	13.40	0.80	0.048	0.070
4	18	Remnant forest	36.9319	-88.9372	0.31	1.16	5.42	12.05	1.15	0.046	0.111
4	19	Remnant forest	36.9336	-88.9334	0.32	1.09	5.44	8.65	0.90	0.045	0.104
4	20	Remnant forest	36.9332	-88.9365	0.37	0.91	5.53	16.55	1.55	0.047	0.194
4	21	Remnant forest	36.9334	-88.9366	0.41	0.96	5.45	16.10	1.85	0.066	0.162
4	22	Remnant forest	36.9331	-88.9361	0.39	1.07	5.07	13.35	1.50	0.043	0.209
4	23	Remnant forest	36.9331	-88.9346	0.29	1.22	5.33	9.05	1.20	0.064	0.190
4	24	Remnant forest	36.9333	-88.9349	0.39	1.17	5.57	11.55	1.15	0.055	0.080
4	25	Remnant forest	36.9334	-88.9347	0.36	1.19	5.99	9.00	0.80	0.047	0.068
4	26	Remnant forest	36.9319	-88.9369	0.31	1.01	5.98	18.30	1.55	0.039	0.164
4	27	Remnant forest	36.9313	-88.9388	0.42	1.12	5.67	12.75	1.10	0.040	0.116

Supplemental table 4.1 (continued)

Site ID.	Core ID.	Habitat	Latitude	Longitude	SM	BD	pH	TC	TN	P	Fe
4	28	Remnant forest	36.9313	-88.9389	0.34	1.23	5.97	10.30	0.95	0.040	0.083
4	29	Remnant forest	36.9314	-88.9388	0.42	0.98	6.00	13.20	1.25	0.049	0.103
4	30	Remnant forest	36.9313	-88.9388	0.40	1.05	5.91	15.10	1.30	0.050	0.109

Supplemental table 4.2.

Methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O) flux data for 4 easements at 24°C and 29°C.

Site ID.	Core ID.	Temp. (°C)	Habitat	Latitude	Longitude	Greenhouse gas flux (mg m^2 h^{-1})		
						CH_4	CO_2	N_2O
1	1	24	Crop field	35.5282	-89.2015	0.0177	67.8836	-0.0059
1	2	24	Crop field	35.5229	-89.202	0.0109	92.0456	0.0163
1	3	24	Crop field	35.5226	-89.2027	0.0112	128.1408	0.0101
1	4	24	Crop field	35.5242	-89.2017	0.0115	80.8397	0.0377
1	5	24	Crop field	35.5346	-89.2018	0.0128	58.5990	0.0087
1	6	24	Crop field	35.525	-89.2019	0.0125	65.4891	0.0115
1	7	24	Shallow water-dry	35.5168	-89.2027	0.0129	44.4256	0.0085
1	8	24	Shallow water-wet	35.5165	-89.2031	0.1107	36.0239	0.0029
1	9	24	Shallow water-wet	35.5171	-89.2044	0.0327	19.0721	-0.0025
1	10	24	Shallow water-dry	35.5174	-89.2029	0.0037	32.5377	0.0029
1	11	24	Shallow water-dry	35.5199	-89.2033	0.0101	29.3076	0.0102
1	12	24	Shallow water-wet	35.5173	-89.2037	0.8531	46.3404	0.0112
1	13	24	Tree planting	35.5199	-89.1995	0.0121	143.8115	0.0092
1	14	24	Tree planting	35.5206	-89.2001	0.0078	158.7935	0.0043
1	15	24	Tree planting	35.5214	-89.201	0.0084	224.9506	-0.0022
1	16	24	Tree planting	35.5202	-89.1996	0.0084	126.8358	0.0123
1	17	24	Tree planting	35.5209	-89.1998	0.0085	109.6070	0.0083
1	18	24	Tree planting	35.5213	-89.2008	0.0188	99.9869	0.0096
1	19	24	Remnant forest	35.5152	-89.2015	-0.0101	112.7679	0.0074
1	20	24	Remnant forest	35.5168	-89.2015	0.0064	86.1610	0.0002

Supplemental table 4.2 (continued)

Site ID.	Core ID.	Temp. (ºC)	Habitat	Latitude	Longitude	Greenhouse gas flux (mg m^2 h^{-1})		
						CH_4	CO_2	N_2O
1	21	24	Remnant forest	35.5184	-89.1982	0.0033	200.4111	0.0116
1	22	24	Remnant forest	35.5179	-89.1989	0.0003	86.5892	0.0061
1	23	24	Remnant forest	35.514	-89.1963	0.0033	163.8634	0.0038
1	24	24	Remnant forest	35.5161	-89.1968	0.0067	84.1041	0.0309
1	25	24	Remnant forest	35.5178	-89.1975	0.0077	155.8094	0.0549
1	26	24	Remnant forest	35.5169	89.202	0.0064	205.6042	0.0261
1	27	24	Remnant forest	35.5148	-89.196	0.0245	186.2575	0.0688
1	28	24	Remnant forest	35.5157	-89.1976	-0.0040	104.1917	0.0109
1	29	24	Remnant forest	35.5177	-89.1982	0.0020	102.8933	0.0101
1	30	24	Remnant forest	NA	NA	0.0057	220.5605	0.1120
1	1	29	Crop field	35.5282	-89.2015	0.0008	102.9633	-0.0012
1	2	29	Crop field	35.5229	-89.202	0.0011	131.5344	0.0356
1	3	29	Crop field	35.5226	-89.2027	0.0031	168.9140	0.0240
1	4	29	Crop field	35.5242	-89.2017	0.0018	110.0926	0.0939
1	5	29	Crop field	35.5346	-89.2018	0.0038	69.5003	0.0070
1	6	29	Crop field	35.525	-89.2019	0.0015	86.5952	0.0101
1	7	29	Shallow water-dry	35.5168	-89.2027	0.0033	59.5071	0.0045
1	8	29	Shallow water-wet	35.5165	-89.2031	0.3397	41.6123	0.0060
1	9	29	Shallow water-wet	35.5171	-89.2044	0.0795	18.7496	0.0053
1	10	29	Shallow water-dry	35.5174	-89.2029	0.0037	52.9327	-0.0002
1	11	29	Shallow water-dry	35.5199	-89.2033	-0.0002	36.7910	0.0017
1	12	29	Shallow water-wet	35.5173	-89.2037	1.7437	51.2355	0.0037

Supplemental table 4.2 (continued)

Site ID.	Core ID.	Temp. (ºC)	Habitat	Latitude	Longitude	Greenhouse gas flux (mg m^2 h^{-1})		
						CH_4	CO_2	N_2O
1	13	29	Tree planting	35.5199	-89.1995	0.0001	157.3153	-0.0069
1	14	29	Tree planting	35.5206	-89.2001	-0.0006	191.2967	0.0037
1	15	29	Tree planting	35.5214	-89.201	0.0058	248.1421	0.0029
1	16	29	Tree planting	35.5202	-89.1996	-0.0030	153.3301	0.0027
1	17	29	Tree planting	35.5209	-89.1998	-0.0008	130.6570	0.0020
1	18	29	Tree planting	35.5213	-89.2008	0.0145	108.2129	0.0071
1	19	29	Remnant forest	35.5152	-89.2015	-0.0143	128.4699	0.0035
1	20	29	Remnant forest	35.5168	-89.2015	-0.0027	105.7660	0.0092
1	21	29	Remnant forest	35.5184	-89.1982	-0.0055	204.9120	0.0413
1	22	29	Remnant forest	35.5179	-89.1989	-0.0083	140.5852	0.0068
1	23	29	Remnant forest	35.514	-89.1963	0.0004	193.8208	0.0091
1	24	29	Remnant forest	35.5161	-89.1968	-0.0015	108.7421	0.0693
1	25	29	Remnant forest	35.5178	-89.1975	-0.0022	162.3762	0.0999
1	26	29	Remnant forest	35.5169	89.202	-0.0019	259.4036	0.0843
1	27	29	Remnant forest	35.5148	-89.196	0.0313	274.0887	0.0477
1	28	29	Remnant forest	35.5157	-89.1976	-0.0056	132.3807	0.0030
1	29	29	Remnant forest	35.5177	-89.1982	-0.0025	151.9029	0.0267
1	30	29	Remnant forest	NA	NA	-0.0019	294.0814	0.0562
2	1	24	Shallow water-wet	36.6099	-89.0333	9.2119	61.9150	-0.0041
2	2	24	Shallow water-wet	36.6111	-89.0331	4.3429	44.9451	-0.0017
2	3	24	Shallow water-wet	NA	NA	NA	NA	NA
2	4	24	Shallow water-wet	36.6096	-89.0333	1.1310	22.2389	-0.0035

Supplemental table 4.2 (continued)

Site ID.	Core ID.	Temp. (ºC)	Habitat	Latitude	Longitude	Greenhouse gas flux (mg m^2 h^{-1})		
						CH_4	CO_2	N_2O
2	5	24	Shallow water-wet	36.6105	-89.0332	1.7398	29.4926	-0.0063
2	6	24	Shallow water-wet	36.6119	-89.033	13.1903	82.7307	-0.0041
2	7	24	Tree planting	36.6106	-89.0323	0.0586	125.6994	0.0582
2	8	24	Tree planting	36.6108	-89.0322	0.0025	90.0374	0.0029
2	9	24	Tree planting	36.6113	-89.0317	0.0017	89.4765	0.0106
2	10	24	Tree planting	36.6105	-89.0329	4.7634	50.6928	-0.0042
2	11	24	Tree planting	36.6108	-89.0329	1.6235	71.9502	0.0037
2	12	24	Tree planting	36.6114	-89.0328	1.5188	35.3570	-0.0086
2	13	24	Tree planting	36.6117	-89.0327	0.4609	83.1114	0.0114
2	14	24	Tree planting	36.612	-89.0327	0.9599	146.2282	0.0076
2	15	24	Tree planting	36.6125	-89.0328	0.0063	89.2425	-0.0001
2	16	24	Tree planting	36.6116	-89.0323	0.0068	139.9381	0.0153
2	17	24	Tree planting	36.6119	-89.0321	-0.0005	109.6875	0.0090
2	18	24	Tree planting	36.6124	-89.0322	-0.0002	183.9998	0.0076
2	19	24	Tree planting	36.6106	-89.0336	0.0001	144.1235	0.0084
2	20	24	Tree planting	36.6109	-89.0338	0.0018	104.5036	0.0003
2	21	24	Tree planting	36.6125	-89.0334	-0.0030	110.4318	-0.0047
2	22	24	Tree planting	36.6103	-89.0337	-0.0021	89.1355	0.0071
2	23	24	Tree planting	36.6113	-89.0338	0.0010	103.2954	-0.0020
2	24	24	Tree planting	36.6119	-89.0335	0.0017	103.7676	-0.0006
2	25	24	Remnant forest	36.617	-89.0279	-0.0057	122.0581	0.0013
2	26	24	Remnant forest	36.6168	-89.0283	-0.0023	134.3898	0.0069

Supplemental table 4.2 (continued)

Site ID.	Core ID.	Temp. (ºC)	Habitat	Latitude	Longitude	Greenhouse gas flux (mg m^2 h^{-1})		
						CH_4	CO_2	N_2O
2	27	24	Remnant forest	36.6164	-89.0292	-0.0067	63.9634	0.0001
2	28	24	Remnant forest	36.6173	-89.0277	-0.0042	183.5613	0.0298
2	29	24	Remnant forest	36.6172	-89.0283	-0.0050	92.6910	0.2364
2	30	24	Remnant forest	36.6165	-89.0292	0.0326	73.6469	2.2363
2	1	29	Shallow water-wet	36.6099	-89.0333	10.3298	59.5758	-0.0029
2	2	29	Shallow water-wet	36.6111	-89.0331	14.4132	60.6682	0.0088
2	3	29	Shallow water-wet	NA	NA	NA	NA	NA
2	4	29	Shallow water-wet	36.6096	-89.0333	3.7469	23.6275	0.0105
2	5	29	Shallow water-wet	36.6105	-89.0332	2.5433	28.9240	0.0060
2	6	29	Shallow water-wet	36.6119	-89.033	11.4497	113.8937	0.0089
2	7	29	Tree planting	36.6106	-89.0323	0.1558	180.2282	0.3720
2	8	29	Tree planting	36.6108	-89.0322	0.0067	138.4477	0.0118
2	9	29	Tree planting	36.6113	-89.0317	0.0060	123.3730	0.0008
2	10	29	Tree planting	36.6105	-89.0329	17.3327	56.6351	0.0009
2	11	29	Tree planting	36.6108	-89.0329	2.7445	106.3154	0.0163
2	12	29	Tree planting	36.6114	-89.0328	4.4931	61.2411	0.0038
2	13	29	Tree planting	36.6117	-89.0327	1.0059	113.0789	0.0513
2	14	29	Tree planting	36.612	-89.0327	1.4944	195.7504	0.1006
2	15	29	Tree planting	36.6125	-89.0328	0.0118	156.0735	0.0094
2	16	29	Tree planting	36.6116	-89.0323	0.0053	196.5854	0.0373
2	17	29	Tree planting	36.6119	-89.0321	0.0017	160.1951	0.0244
2	18	29	Tree planting	36.6124	-89.0322	-0.0006	277.1020	0.0411

Supplemental table 4.2 (continued)

Site ID.	Core ID.	Temp. (ºC)	Habitat	Latitude	Longitude	Greenhouse gas flux (mg m^2 h^{-1})		
						CH_4	CO_2	N_2O
2	19	29	Tree planting	36.6106	-89.0336	0.0026	228.0521	0.0325
2	20	29	Tree planting	36.6109	-89.0338	0.0007	141.3064	0.0040
2	21	29	Tree planting	36.6125	-89.0334	0.0016	207.4328	0.0048
2	22	29	Tree planting	36.6103	-89.0337	0.0008	146.7164	0.0244
2	23	29	Tree planting	36.6113	-89.0338	0.0043	163.8558	0.0031
2	24	29	Tree planting	36.6119	-89.0335	0.0043	143.9351	0.0017
2	25	29	Remnant forest	36.617	-89.0279	0.0003	188.1200	0.0183
2	26	29	Remnant forest	36.6168	-89.0283	0.0002	212.4222	0.0278
2	27	29	Remnant forest	36.6164	-89.0292	-0.0051	109.0472	0.0002
2	28	29	Remnant forest	36.6173	-89.0277	0.0027	313.1097	0.0749
2	29	29	Remnant forest	36.6172	-89.0283	-0.0008	145.6307	0.6770
2	30	29	Remnant forest	36.6165	-89.0292	0.1089	98.3586	0.9396
3	1	24	Crop field	36.9237	-88.9276	0.0006	94.1649	0.0082
3	2	24	Crop field	36.9234	-88.9284	0.0378	189.0633	0.9131
3	3	24	Crop field	36.9234	-88.9292	0.0038	116.9158	0.2053
3	4	24	Crop field	36.9242	-88.9288	0.0337	174.1177	0.1174
3	5	24	Crop field	36.9239	-88.9293	0.0021	128.9969	0.1671
3	6	24	Crop field	36.9243	-88.9279	0.0018	99.7029	0.0242
3	7	24	Crop field	36.9255	-88.9284	0.0364	92.8808	0.2684
3	8	24	Crop field	36.9253	-88.9289	0.0041	53.0940	0.0081
3	9	24	Crop field	36.9259	-88.9293	0.0152	64.8221	0.0153
3	10	24	Crop field	36.925	-88.9303	0.0045	74.5546	0.0909

Supplemental table 4.2 (continued)

Site ID.	Core ID.	Temp. (ºC)	Habitat	Latitude	Longitude	Greenhouse gas flux (mg m^2 h^{-1})		
						CH_4	CO_2	N_2O
3	11	24	Crop field	36.925	-88.9303	0.0023	64.9554	0.0153
3	12	24	Crop field	36.9246	-88.9313	0.0043	139.5008	0.4556
3	13	24	Crop field	36.9235	-88.9298	0.0027	75.9291	0.0226
3	14	24	Crop field	36.9233	-88.9306	0.0043	72.5601	0.0555
3	15	24	Crop field	36.9233	-88.9319	0.0092	51.8299	0.0110
3	16	24	Crop field	36.9245	-88.9297	0.0073	80.3561	0.0483
3	17	24	Crop field	36.924	-88.9308	0.0027	105.3937	0.3342
3	18	24	Crop field	36.9237	-88.9319	0.0072	49.3509	0.0088
3	19	24	Remnant forest	36.9256	-88.9309	0.1081	82.8006	0.0225
3	20	24	Remnant forest	36.9254	-88.9315	-0.0379	96.2817	0.0048
3	21	24	Natural wetland	36.9263	-88.9309	2.5542	54.3432	0.0043
3	22	24	Natural wetland	36.926	-88.9314	3.0603	56.4964	-0.0007
3	23	24	Remnant forest	36.9259	-88.9284	0.1448	213.1684	0.0281
3	24	24	Remnant forest	36.9263	-88.9286	0.1078	212.7872	0.0057
3	25	24	Natural wetland	36.9268	-88.9295	0.0922	27.9972	0.0009
3	26	24	Natural wetland	36.9269	-88.9293	0.1628	38.1883	0.0065
3	27	24	Remnant forest	36.9262	-88.9299	-0.0099	212.0609	0.0209
3	28	24	Natural wetland	36.9267	-88.9301	1.6203	39.2969	0.0001
3	29	24	Remnant forest	36.9264	-88.9297	-0.0227	120.2923	0.0064
3	30	24	Natural wetland	36.9267	-88.9298	0.2060	67.5929	0.0015
3	1	29	Crop field	36.9237	-88.9276	0.0045	129.6528	0.0205
3	2	29	Crop field	36.9234	-88.9284	0.0549	213.0839	1.0889

Supplemental table 4.2 (continued)

Site ID.	Core ID.	Temp. (ºC)	Habitat	Latitude	Longitude	Greenhouse gas flux (mg m^2 h^{-1})		
						CH_4	CO_2	N_2O
3	3	29	Crop field	36.9234	-88.9292	0.0075	149.0525	0.1094
3	4	29	Crop field	36.9242	-88.9288	0.0788	249.1830	0.1286
3	5	29	Crop field	36.9239	-88.9293	0.0021	193.8005	0.3147
3	6	29	Crop field	36.9243	-88.9279	0.0007	152.9108	0.0505
3	7	29	Crop field	36.9255	-88.9284	0.0563	123.8872	0.3164
3	8	29	Crop field	36.9253	-88.9289	0.0021	78.9801	0.0198
3	9	29	Crop field	36.9259	-88.9293	0.0193	94.6014	0.0198
3	10	29	Crop field	36.925	-88.9303	0.0014	106.7111	0.0853
3	11	29	Crop field	36.925	-88.9303	0.0021	94.3276	0.0189
3	12	29	Crop field	36.9246	-88.9313	0.0025	225.0374	0.5861
3	13	29	Crop field	36.9235	-88.9298	0.0018	115.3378	0.0402
3	14	29	Crop field	36.9233	-88.9306	0.0031	100.6504	0.2137
3	15	29	Crop field	36.9233	-88.9319	0.0065	67.8018	0.0162
3	16	29	Crop field	36.9245	-88.9297	0.0045	117.1252	0.1223
3	17	29	Crop field	36.924	-88.9308	0.0014	158.0389	0.4053
3	18	29	Crop field	36.9237	-88.9319	-0.0009	66.8515	0.0181
3	19	29	Remnant forest	36.9256	-88.9309	0.0967	129.9453	0.0324
3	20	29	Remnant forest	36.9254	-88.9315	-0.0273	135.9902	-0.6452
3	21	29	Natural wetland	36.9263	-88.9309	3.7752	62.0132	0.0035
3	22	29	Natural wetland	36.926	-88.9314	4.8965	43.3068	0.0016
3	23	29	Remnant forest	36.9259	-88.9284	0.6016	280.3838	0.0906
3	24	29	Remnant forest	36.9263	-88.9286	0.9021	297.0546	0.0079

Supplemental table 4.2 (continued)

Site ID.	Core ID.	Temp. (ºC)	Habitat	Latitude	Longitude	Greenhouse gas flux (mg m^2 h^{-1})		
						CH_4	CO_2	N_2O
3	25	29	Natural wetland	36.9268	-88.9295	1.7118	34.7366	0.0039
3	26	29	Natural wetland	36.9269	-88.9293	0.9356	37.1152	0.0079
3	27	29	Remnant forest	36.9262	-88.9299	0.0002	319.9734	0.0258
3	28	29	Natural wetland	36.9267	-88.9301	1.9269	36.4660	0.0024
3	29	29	Remnant forest	36.9264	-88.9297	-0.0041	169.9880	0.0150
3	30	29	Natural wetland	36.9267	-88.9298	1.6320	86.1818	0.0024
4	1	24	Shallow water-wet	36.9361	-88.9405	0.0211	36.8412	1.0729
4	2	24	Shallow water-wet	36.9361	-88.9406	0.0053	28.2965	0.1672
4	3	24	Shallow water-wet	36.9348	-88.9409	3.7080	41.7922	0.0012
4	4	24	Shallow water-dry	36.9357	-88.9406	0.0243	129.4075	0.0143
4	5	24	Remnant forest	36.9327	-88.9325	0.0040	42.0377	-0.0012
4	6	24	Remnant forest	36.9328	-88.9327	0.0062	43.4072	0.0002
4	7	24	Shallow water-wet	36.9361	-88.9408	0.0247	22.5366	0.0081
4	8	24	Shallow water-wet	36.936	-88.9409	0.0125	19.2263	0.1231
4	9	24	Shallow water-wet	36.9349	-88.9409	3.0457	39.0498	-0.0131
4	10	24	Shallow water-dry	36.9356	-88.9403	0.0285	195.7009	0.3279
4	11	24	Remnant forest	36.9337	-88.9331	0.0097	42.0396	0.0320
4	12	24	Remnant forest	36.9338	-88.9335	0.0036	98.7158	0.0943
4	13	24	Remnant forest	36.9331	-88.9331	0.0057	97.9950	0.1815
4	14	24	Remnant forest	36.9319	-88.9348	-0.0215	116.9967	0.0065
4	15	24	Remnant forest	36.9324	-88.9349	0.0027	88.4842	-0.0020
4	16	24	Remnant forest	36.9329	-88.9353	-0.0102	45.7011	-0.0029

Supplemental table 4.2 (continued)

Site ID.	Core ID.	Temp. (ºC)	Habitat	Latitude	Longitude	Greenhouse gas flux (mg m^2 h^{-1})		
						CH_4	CO_2	N_2O
4	17	24	Remnant forest	36.9316	-88.9378	0.0004	126.5695	0.0473
4	18	24	Remnant forest	36.9319	-88.9372	-0.0021	93.4254	0.0404
4	19	24	Remnant forest	36.9336	-88.9334	0.0184	70.2121	0.1281
4	20	24	Remnant forest	36.9332	-88.9365	-0.0006	126.1519	0.0711
4	21	24	Remnant forest	36.9334	-88.9366	-0.0044	77.8830	-0.0088
4	22	24	Remnant forest	36.9331	-88.9361	0.0013	124.7278	0.0252
4	23	24	Remnant forest	36.9331	-88.9346	-0.0019	105.2005	0.0122
4	24	24	Remnant forest	36.9333	-88.9349	0.0048	92.3109	0.0119
4	25	24	Remnant forest	36.9334	-88.9347	0.0212	137.7568	0.0441
4	26	24	Remnant forest	36.9319	-88.9369	-0.0069	116.5936	-0.0067
4	27	24	Remnant forest	36.9313	-88.9388	0.0005	107.1988	0.6179
4	28	24	Remnant forest	36.9313	-88.9389	0.0796	143.2953	0.1232
4	29	24	Remnant forest	36.9314	-88.9388	-0.0007	129.2622	0.1331
4	30	24	Remnant forest	36.9313	-88.9388	-0.0015	130.3404	0.1112
4	1	29	Shallow water-wet	36.9361	-88.9405	0.0138	45.9585	0.4740
4	2	29	Shallow water-wet	36.9361	-88.9406	0.0058	34.3657	0.1988
4	3	29	Shallow water-wet	36.9348	-88.9409	26.8255	64.1655	0.0047
4	4	29	Shallow water-dry	36.9357	-88.9406	0.1216	168.2039	0.0294
4	5	29	Remnant forest	36.9327	-88.9325	0.0032	50.9288	0.0323
4	6	29	Remnant forest	36.9328	-88.9327	0.0128	63.5598	0.0350
4	7	29	Shallow water-wet	36.9361	-88.9408	0.0119	26.3063	0.0360
4	8	29	Shallow water-wet	36.936	-88.9409	0.0359	23.2056	0.3227

Supplemental table 4.2 (continued)

Site ID.	Core ID.	Temp. (ºC)	Habitat	Latitude	Longitude	Greenhouse gas flux (mg m^2 h^{-1})		
						CH_4	CO_2	N_2O
4	9	29	Shallow water-wet	36.9349	-88.9409	18.9190	45.3474	0.0088
4	10	29	Shallow water-dry	36.9356	-88.9403	0.0851	287.1429	0.6931
4	11	29	Remnant forest	36.9337	-88.9331	0.0090	47.9788	0.0485
4	12	29	Remnant forest	36.9338	-88.9335	0.0066	127.1782	0.1794
4	13	29	Remnant forest	36.9331	-88.9331	0.0065	132.0747	0.1579
4	14	29	Remnant forest	36.9319	-88.9348	-0.0198	184.3630	0.0417
4	15	29	Remnant forest	36.9324	-88.9349	0.0066	122.8930	0.0158
4	16	29	Remnant forest	36.9329	-88.9353	-0.0004	78.1009	0.0208
4	17	29	Remnant forest	36.9316	-88.9378	0.0017	187.7041	0.0981
4	18	29	Remnant forest	36.9319	-88.9372	0.0001	178.7351	0.0673
4	19	29	Remnant forest	36.9336	-88.9334	0.0335	105.3057	0.1164
4	20	29	Remnant forest	36.9332	-88.9365	0.0006	175.1192	0.0570
4	21	29	Remnant forest	36.9334	-88.9366	-0.0057	122.9346	0.0339
4	22	29	Remnant forest	36.9331	-88.9361	0.0021	174.1196	0.1374
4	23	29	Remnant forest	36.9331	-88.9346	-0.0033	131.5122	0.0202
4	24	29	Remnant forest	36.9333	-88.9349	0.0042	89.7287	0.0347
4	25	29	Remnant forest	36.9334	-88.9347	0.0361	128.2338	0.0437
4	26	29	Remnant forest	36.9319	-88.9369	-0.0025	171.6309	0.0061
4	27	29	Remnant forest	36.9313	-88.9388	0.0025	139.1144	0.7035
4	28	29	Remnant forest	36.9313	-88.9389	0.0738	141.5463	0.2883
4	29	29	Remnant forest	36.9314	-88.9388	0.0005	165.2483	0.1267
4	30	29	Remnant forest	36.9313	-88.9388	0.0015	197.7924	0.3454

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