Root-Mediated Gas Transport and Implications for Nitrous Oxide Dynamics in Wetlands

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirement for the Degree of

Master of Science

by

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August 2019

Abstract

Wetlands and other ecosystems at terrestrial-aquatic boundaries play an important role in controlling the release of reactive nitrogen from nonpoint sources into sensitive aquatic environments via microbial denitrification. Denitrification can lead to the accumulation and emission of nitrous oxide (N_2O), an important greenhouse gas (GHG) and ozone-depleting substance. Minimizing the potential tradeoff between water quality protection and GHG emissions, particularly in the context of constructed wetlands, requires process-level understanding of N_2O production, consumption, and the gas transfer processes mediating N_2O transfer from wetland soils to the atmosphere. The objective of this research was to explore N_2O transport through the root aerenchyma system of wetland macrophytes as a pathway for N_2O emissions from wetland soils. While plant-mediated transport is well-recognized as a critical emission pathway for methane, there has been little attention to the role of this process in N_2O fluxes from wetlands. This study addresses two questions:1) What are the kinetic constants for root-mediated N_2O transport and reaction rate constants for N_2O microbial reduction in the wetland rhizosphere; and 2) How significant is the root uptake pathway compared to the microbial reduction pathway of N_2O under environmentally relevant N_2O concentrations?

A set of laboratory vertical flow constructed wetland mesocosms were used to evaluate the role of wetland macrophytes on N_2O dynamics in denitrifying wetland buffer systems. Dissolved gas tracer push-pull tests (PPTs) [1][2] were used to probe the in situ behavior of N_2O in the wetland rhizosphere and determine the relative contribution of microbial N_2O reduction vs. N_2O transfer into roots in controlling the fate of N_2O produced in vegetated wetland soils. Two different modeling approaches were employed to interpret the experimental data and estimate kinetic constants for N_2O gas transfer based on the inert gas tracers helium and sulfur hexafluoride. The accuracy of these approaches was evaluated using a holdout cross-validation approach.

Results showed that plant uptake only accounts for 0.49% to 17.16% of total N_2O removed from the subsurface of the experimental mesocosms, indicating the root uptake pathway represents a relatively small N_2O sink. The relative importance of the root N_2O sink depends on the rates of microbial N_2O reduction, which can vary widely. Thus, N_2O emission via plant-mediated pathways is likely to represent a small fraction of the wetland N_2O budget, particularly in settings where microbial N_2O reduction is fast.

Biographical Sketch

Simiao Wang was born in Beijing, China in 1994. He graduated with a degree in Environment and Sustainable Development from Hong Kong Polytechnic University in 2017. His experience in environmental engineering includes a two-month internship as assistant technician in Acoustic and Air Testing Laboratory Co. Ltd. (Hong Kong), a six-month lab research as student assistant in the Guo research group at Hong Kong Polytechnic University. In August 2017, he entered Cornell University and started his study towards the M.S. Degree in Environmental Engineering working with Professor Matthew C. Reid.

Acknowledgement

First of all, I would like to thank the School of Civil and Environmental Engineering at Cornell University for giving me a precious chance to be there as a graduate student.

I also would like to express my sincere gratitude to my advisor Dr. Reid for his continuous support of my study and research during the past two years, for his patience, his motivations and his immense knowledge. It is my great honor to work with Dr. Reid. Without his guidance, I won't be able to finish my research and writing this thesis. The two-year experience in Reid research group has helped me to become a better person and would continuously influencing me after my graduate. When I joined the Reid research group, I was trusted with the chance to work on a novel project focusing on wetland plants' effect on N_2O emission, on which few studies have been conducted. During the research, he presents set up of mesocosm bioreactors and sampling skills to me step-by-step with great patience; every time I came up with experimental difficulties, he is always willing to discuss with me and guide me to further develop this field. Benefiting from the guidance of Dr. Reid, not only can I master the operations of many advanced lab instruments and data processing and graphing methods, I also learned how to set up plans for a new research project from planning to preparation and finally operation, which makes me better prepared

for pursuing a PhD. degree in the future. In the near future, I will work as a research assistant back in Hong Kong and I believe that the experience and skills I accumulated in Dr. Reid's research group will help me to grow into a qualified environmental engineer.

In the second place, I would like to give my sincere thanks to Dr. Gu for serving as my minor advisor. Her experienced suggestions helped me a lot during the research and defense. Also, I would like to express my sincere thanks to Dr. Helbling for generously serving as the proxy for Dr. Gu in my thesis defense examination.

In the third place, I would like to give my sincere thanks to every member of Dr. Reid's research group, especially Philip, Lena and Scott. With their generous help, I was able to learn various operational skills of most advance lab instruments in our lab.

I also gratefully acknowledge the funding received towards my research from National Science Foundation Grant # 1804975.

Last but not least, I would like to give my sincere thanks to my family and friends for their selfless support, unconditional love and care, which becomes my motivation to further develop myself in academic field and shelters me when times are rough. I truly thank them for sticking by my side.

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Chapter 1 Introduction

1.1 Nonpoint source surface water pollution with nitrogen

The large amount of nutrients being discharged into natural water bodies has made eutrophication one of the most important environmental problems [3][4]. Excess nitrogen, which works as the most limiting element for algal biomass synthesis and is most responsible for eutrophication in marine environments, results in various detrimental effects to human and environment health [3][5][6][7]. Excess reactive nitrogen in the environment can also increase emission of nitrous oxide, an important greenhouse gas and ozone depleting substance [5][8]. Nonpoint source surface water pollution, as one of the most significant and difficult-to-address contributors to water pollution, is responsible for a large fraction of total nitrogen being released into water in the form of agricultural runoff with excess fertilization and livestock manure [3][4][9][10][11]. It is necessary to treat nonpoint source polluted surface water and remove excess nutrient before discharging it into natural water bodies. However, nonpoint source surface water pollution varies spatiotemporally due to weather variations and human activities, which makes it hard to control and regulate [3][5]. Moreover, the cost of treating nonpoint source pollution would be too high if centralized treatment systems have been applied [10]. Thus, a low-cost treatment system which is able to deal with various nitrogen loads, flow rates and

environments is needed to prevent eutrophication from happening. Natural and constructed wetlands are popular low-cost tools to meet these requirements [10].

1.2 Nitrous oxide

It is widely known that global climate change is an important threat to the environment, with an observed increasing of 0.87℃ from 2006 to 2015 and a predicted increasing of 2.5 to 7.8℃ in 2100 compared to the baseline scenario of average temperature from 1850 to 1900 [12][13]. Greenhouse gases, which are known for their abilities to absorb infrared radiation, are the causes of global climate change [14][15]. Among those greenhouse gases, nitrous oxide (N_2O), though less than 1/1000 the concentration of carbon dioxide, serves as the third most significant contributor to global warming due to its residence time as long as 114 years and its global warming potential as high as 298 times that of carbon dioxide [14][16][17][18][19]. Moreover, N_2O is also thought to be the dominant ozone depleting substance throughout 21st century, as nitrous oxide, when being emitted into stratosphere, would produce nitrogen oxides (NO_x: $NO+NO_2$), which catalyzes the destruction of ozone layer [20][21][8][22].

Given its detrimental effect, actions are needed to cut its emission from sources. Natural sources account for up to 64% of N_2O emission, including emissions from upland

soil, riparian zone, oceans, rivers and estuaries [23]. In terms of the anthropogenic sources, agricultural source is the most significant anthropogenic sources, followed by cycling of anthropogenic N in rivers, estuaries and coastal source due to application of fertilizers [23]. These anthropogenic sources drew our research interests for reducing N_2O emission.

1.3 Constructed wetland

Wetland is the ecosystem that being flooded periodically or continuously, it serves as an intermediate between terrestrial system and aquatic system and dominated by anaerobic processes [24][25][26]. Wetlands provide a range of valuable ecosystem services, including flood alleviation, water purification and rich biodiversity [26]. Its high water purification ability, making wetland the "Earth's kidney", could also cause less impact to environment when removing nitrogen from water. Also, it has the least N_2O yields (defined as N_2O-N/(N_2-N + N_2O-N)) compared to agricultural and vegetated soils [27], making it an attractive option for treating N-rich wastewater.

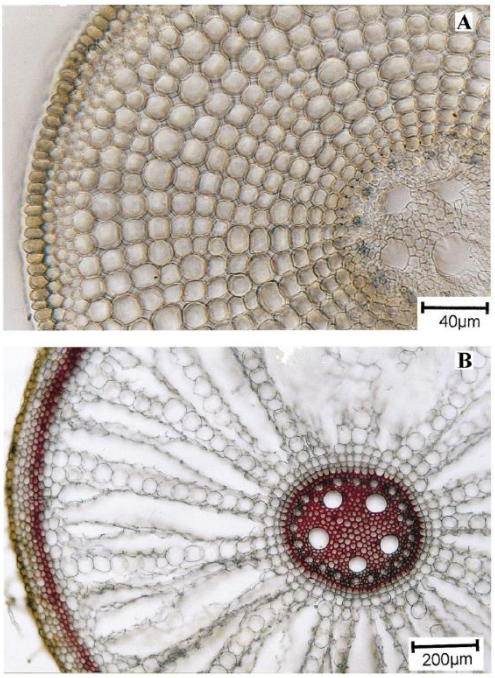
However, there is often a need to build wetland systems in critical points in the landscape, rather than relying on existing wetland ecosystems, to achieve the water quality benefits conferred by wetland environments. [10][26]. Constructed wetlands are engineered wetland systems that imitate natural wetland processes [10][26][28]. The

environmental conditions of constructed wetlands, such as hydraulics, growth media, and vegetation types, can be purposefully designed to maximize their benefit to human society [26][29]. The outstanding water purification abilities, combined with low cost and technology required and high ecological values, have made constructed wetland an attractive option for treating nonpoint source pollution [10][28].

Though they have relatively low N_2O yields, wetlands still serve as a globally significant source of anthropogenic N_2O due to the large amount of reactive nitrogen intercepted and transformed by wetlands. It is estimated that one-quarter of nitrogen applied to agricultural systems crosses the land-water boundary into surface waters [27] often passing through anaerobic wetland soils/sediments. As a result, wetlands account for approximately 29% of anthropogenic N_2O emission. This includes emission from estuarine and coastal wetland as well as indirect N_2O emission from surface runoff and leaching from agricultural soils that would also be further emitted by wetland [17][30]. Thus, it is necessary to investigate the mechanism of N_2O production, consumption, and transport in constructed wetland to further reduce its emissions and minimize the tradeoff between improved water quality and greenhouse N_2O emissions.

1.4 Wetland plants

Wetland plants, as an important part of wetland system, serves as the provider of organic carbon and resources for obligate root-associated organisms [31]. The interaction of wetland plants with the surrounding rhizosphere environment enable them to modify the amount, composition and diversity of microbial communities in soil [31][32][33][34]. Certain criteria has to be met for plants to be able to survive and further achieve better pollutant removal performance in constructed wetland system, including adaptation to extreme weather, tolerance to saturated soil and eutrophication conditions, and ability to absorb pollutant [28][35]. To meet these requirements, certain strategies have been applied, one of them is the development of extensive aerenchyma, tissue containing expanded gas space that is characteristic of vascular wetland plants as shown in **Figure 1** [18][36].



g. 2. Transverse sections of a *Phragmites* root stained with phloroglucinol and concentrated hydrochloric acid to ow lignification (red): (a) 5 mm from apex (note, absence of stelar and hypodermal lignification and of cortical enchyma; (b) 12 cm from apex (note, stelar and hypodermal lignification and presence of cortical aerenchyma).

Figure 1 *Layout of plant aerenchyma* [37]

Aerenchyma are of significance for plant survival in saturated soil, since it works as a low-resistance pathway for atmospheric oxygen entering roots from its aboveground part and further oxidize its rhizosphere [36][38][39]. This strategy, called Radial Oxygen Loss (ROL), could increase roots' tolerance of flood, salinity and heavy metals [40]. However, this pathway can also be used to release GHGs produced in the subsurface, such as methane (CH_4) and N_2O [36][41]. Transport through aerenchyma is a major conduit for CH_4 fluxes from wetland environments [42][43], which as much as 28~90% of wetland CH_4 emissions mediated by this pathway [44][45], but there has been little attention on the role of

aerenchyma as a transport pathway for N_2O [46]. Moreover, the oxygen introduced by aerenchyma will also inhibit the microbial reduction processes from nitrous oxide to nitrogen gases [47] since O_2 is a noncompetitive inhibitor of microbial nitrous oxide reductase, nosZ [47].

N_2O is produced in saturated soils through a variety of microbial processes, including denitrification and nitrifier denitrification [48]. Once N_2O has been produced in wetland soils, there are two potential sinks: First, N_2O can be enzymatically reduced to dinitrogen gas (N_2) in a reaction that can be described using Michaelis-Menten kinetics. Second, N_2O can released to the atmosphere via diffusion through the saturated soil column, mass transfer into roots and transport via the root aerenchyma pathway, or via bubble ebullition. The relative contribution of different N_2O emission pathways are not well-understood, and, in particular, the role of the aerenchyma transport pathway has received little attention. According to the IPCC's 2013 wetland supplement to its guidelines for National Greenhouse Gas Inventories, no consensus has been reached on the effect of aerenchmatous wetland plant on nitrous oxide emission, since both increasing and decreasing effects have been reported [49][50][51][52]. Thus it is important to understand the exact effect of wetland plants' root-mediated gas transport on nitrous oxide emission to help wetland plant selection.

1.5 Previous studies on wetland plants' effects on nitrous oxide emission

Constructed wetland can be an effective way for removing excess nutrients from nonpoint source surface water pollution [10][28]. However, due to our limitations in our fundamental knowledge of interactions among wetland plants, microbes, and dissolved gases, the effects of wetland plants on microbial communities and N_2O emissions still remain like a black box [50]. This makes it hard for us to select plants for constructed wetland systems that can not only show high nutrient removal abilities but also less N_2O emission ratios.

The effects of wetland plants on N_2O emission is investigated in the laboratory using pilot-scale mesocosms and previous studies have drawn contradictory conclusions on the effects of wetland plants on N_2O emission from wetland. Some of the studies have concluded that N_2O emission from wetland systems have been increased due to the effects of root uptake N_2O and release through aerenchyma, the inhibition effects of oxygen and the alteration of abundance and composition of bacteria communities in wetland systems caused by ROL [47][52][2]. While other studies concluded that wetland plants result in a decreasing N_2O emission due to competition for nitrate between wetland plant uptake and microbes [50]. Moreover, some mesocosm tests also indicate a lower N_2O emission from wetland planted with certain species of plants compared with unplanted mesocosms [52]. This also suggests understanding different contribution of different kinds of species to the

emission of N_2O is of significance for constructed wetland plants selection. However, few studies have systematically explored the contribution of common wetland plants for N_2O emissions, like their contribution of nitrate removal and conversion ratio of N_2O over total nitrogen removed.

1.6 Research questions

The experiments, data analysis, and modelling work described in this thesis were aimed at improving the fundamental understanding of the effects of root-mediated gas transport on N_2O fate in the wetland rhizosphere. A series of experiments were designed to investigate the kinetics of root uptake and microbial consumption to address the following research questions:

1. What are the kinetic constants for root-mediated N_2O transport and reaction rate constants for N_2O microbial reduction?
2. How significant is the root uptake pathway compared to the microbial reduction pathway of N_2O under environmentally relevant N_2O concentrations?

Chapter 2 Materials and Methods

2.1 Reactor design and monitoring

2.1.1 Reactor design

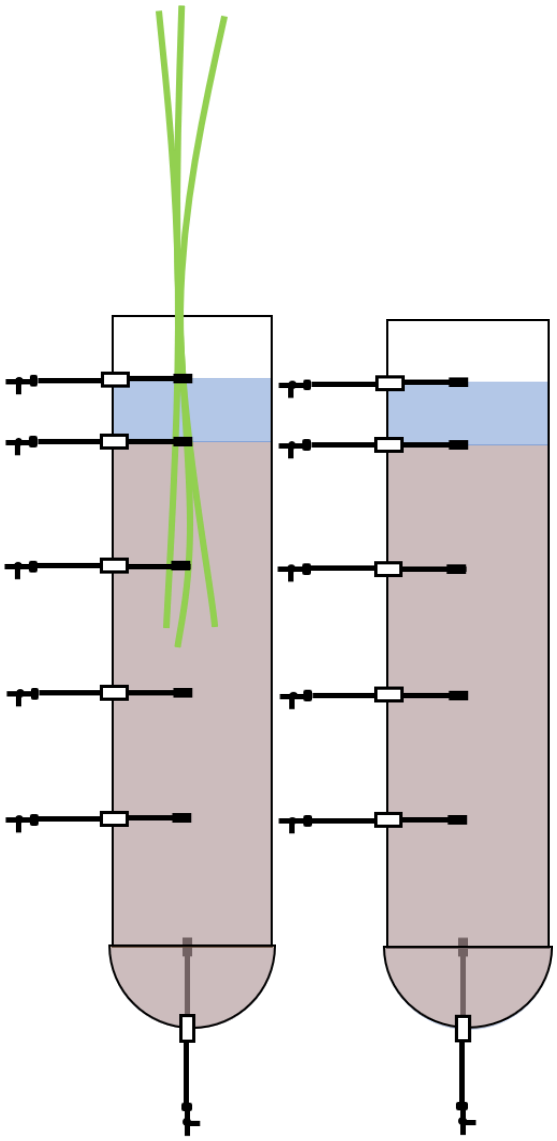


Figure 2 *Reactor design layout*

A mesocosm method was used that allowed imitation of constructed wetland system and the design is shown in **Figure 2**. Mesocosm bioreactors were built based on 50 cm PVC pipes. Four 0.64 cm diameter holes were drilled around a 15.24 cm diameter PVC pipe in 10 cm increments with the lowest hole 10 cm above the lower end of PVC pipe. A 0.64 cm hole was drilled 5 cm above the hole in the top to control surface water table. The bottom of the PVC pipe is sealed with a PVC cap, where a 0.64 cm diameter hole is drilled at the bottom. During the connection processes between PVC pipe and PVC cap, Purple PVC primer (Oatey) and Clear PVC cement (Oatey) were applied to the surface of the connection parts to make sure no leakage happens through the connection of the PVC pipe and cap. Screw thread was carved inside the holes and filled with Push-to-Connect Fitting for Drinking Water Adapter (for 1/4-inch tube outer diameter X 1/4 NPTF Male, McMaster-Carr Supply Company). Soft PVC pipe was inserted into the reactor through the Push-to-Connect adapter with air stone connected inside the reactor and Large-Bore Luer Stopcocks (Three-way, Cole-Parmer) outside the reactor and serves as sampling ports. The reactors were continuously exposed to 12 hours of light and 12 hours of darkness cycle at ambient temperature of 76°F and humidity of 50% RH.

2.1.2 Soil composition and feeding solution

Sediments were collected from the bank of Beebe lake (Ithaca, New York, USA) on December, 2018 and used as inoculant and organic carbon source in reactors. 1.7 kg of sediments were well-mixed with 58.1 kg of tube sand (Sakrete) and evenly filled into reactors to reach the second highest sampling port. Four reactors were divided into two groups: vegetated and non-vegetated. Each group has duplicated reactors. The vegetated group reactors were planted with 7 plugs of 5.08-cm *Sagittaria latifolia* and the non-vegetated group reactors were covered with soil from the same kind of plugs, which could also serve as organic carbon source. All reactors were fed with nutrient solution containing: 54.11 mg/L KNO_3, 3.10 mg/L Na_2HPO_4, 4.75 mg/L Cellobiose, 58.50 mg/L NaCl, 84.00 mg/L $NaHCO_3$, 18.05 mg/L $MgSO_4$, 16.65 mg/L $CaCl_2$, 0.29 mg/L H_3BO_3, 0.10 mg/L $MnCl_2{\cdot}4H_2O$, 0.06 mg/L $ZnSO_4{\cdot}7H_2O$, 0.09 mg/L $H_2MoO_4{\cdot}H2O$, 0.05 mg/L $CuSO_4{\cdot}5H_2O$. In the beginning stage, bioreactors were first flushed with pure CO_2 for 20 minutes from bottom and feeding solution was injected from bottom port at 1.5 mL/min to exclude bubble entrapment. Once water table reaches 5 cm above soil surface, feeding solution was injected from the second highest sampling port and extracted from the bottom sampling port at flow rate of 6 mL/min.

To further check whether this method could be applied to other plants or not, another planted bioreactor with different plants, *Schoenoplectus acutus*, was set up and similar

experiments were conducted on it to investigate the root uptake rate constant for *Schoenoplectus acutus.*

Schoenoplectus acutus were planted in the same bioreactor as shown in Figure 1, however, the soil and feeding solution recipe were changed. Soil recipe used was: 93.75% tube sand (Sakrete), 6% sediments from Beebe lake and 0.25% chopped dry leaves, which was used to enhance the organic carbon contents inside bioreactor and test the effect of organic matter amendments on N cycling. Feeding solution recipe was changed in the organic carbon aspect, 2.5mg/L Cellobiose was used instead of 2.5mg-C/L as Cellobiose.

Figure 3 below demonstrate a conceptual model for the operation processes of bioreactors.

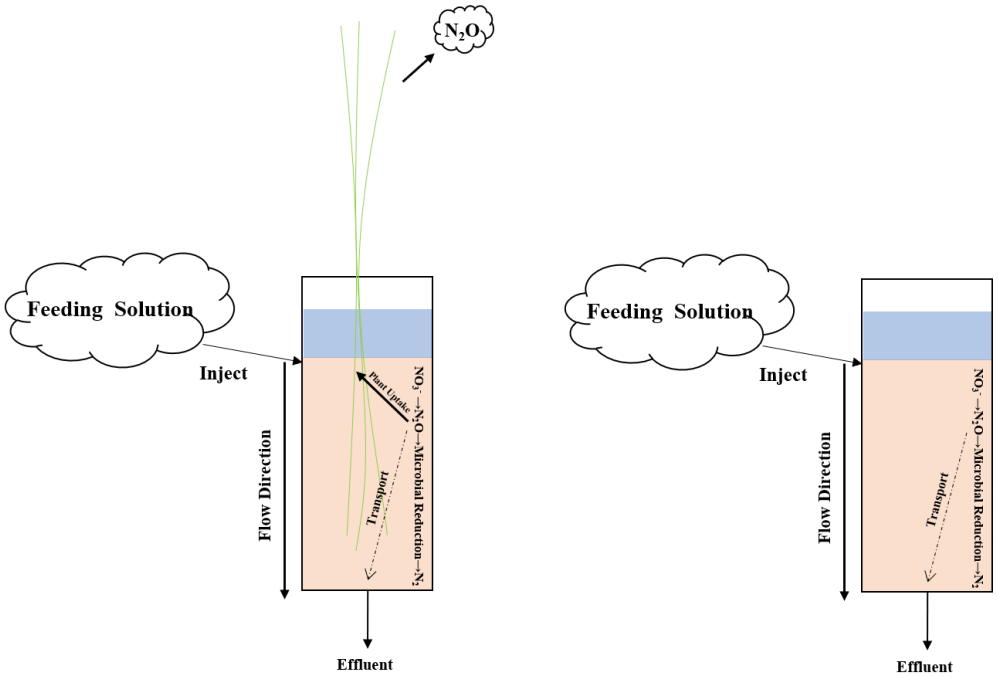


Figure 3 *Bioreactors concept model*

2.1.3 Sagittaria latifolia and Schoenoplectus acutus

To investigate the impact of root aerenchyma systems on nitrous oxide gas exchange and microbial cycling in wetland environments, a widely distributed emergent aquatic macrophyte in North American wetland habitats, *Sagittaria latifolia*, is chosen for this experiment [53][54]. This species is well suited to the studies of gas exchange of wetland plants because it shows relatively high radial oxygen loss compared with other wetland plants species, which is 0.0012~0.0014 υmol O_2 d^{-1} compare to 0.0002υmol O_2 d^{-1} for *Scirpus fluviatilis* [55].

Schoenoplectus acutus is a common native wetland plant in North America and is characterized by its high root density [56], which was thought to be an advantage for maximizing its gas root uptake ability.

These characteristics indicating the gas exchange ability of *Sagittaria latifolia* and *Schoenoplectus acutus* are outstanding among other common wetland plants and the impact of gas exchange of *Sagittaria latifolia* and *Schoenoplectus acutus* on nitrous oxide reduction and transport would be more obvious, which would magnify this impact.

2.1.4 Monitoring

To investigate the detailed biogeochemical conditions inside bioreactors, the following parameters are monitored: NO_3^- concentration, NO_2^- concentration, dissolved N_2O concentration, dissolved organic carbon (DOC) and total nitrogen (TN). At the end of the experiments, the microbial biomass density and root density are also measured.

To monitor N_2O concentration, 20mL solution are extracted from each sampling port by glass syringes, then, 10mL N_2 is added to the glass syringe. After shaking for 3 minutes to make N_2O in liquid phase and gas phase achieve equilibrium, the gas in syringe is injected into Gas Chromatography (GC) to measure the N_2O concentration.

To monitor other parameters, liquid samples are collected from all sampling ports and filtered by 0.22 μm filters for NO_3^- and NO_2^- concentration and 0.45 μm filters for DOC and TN. NO_3^- and NO_2^- concentration are measured by Ion Chromatography (IC) and DOC and TN are measured by Total Organic Carbon Analysis (TOC).

At the end of the experiments, all of the four bioreactors will be sacrificed and soil samples will be collected from 5cm to 15cm below the soil surface. Part of the soil samples will be used to measure the biomass density by extracting protein from them and use ultraviolet spectrum (UV) to measure the biomass density and the rest of the soil samples from planted reactors will be baked in oven for 72h hour at 105℃ and weighed the mass of soil and root for root density. Moreover, the dried soil, after separating from roots, was

also used to measure the porosity of medium. 100 mL of soil was put in three graduate cylinders, then water was added to these cylinders to make them saturated. Mass of cylinders was measured before and after the addition of water.

2.2 Push-Pull test

2.2.1 Introduction and definition

To meet the need of in situ quantitative information on N_2O reaction rates and mass transfer into roots, direct in situ measurement method becomes necessary to help develop and manage related environmental applications due to the following reasons. In particular, making quantitative measurements of N_2O transfer into roots in the subsurface is a challenge. First, simple batch-type experiments are unable to obtain accurate quantitative information about the processes of interest in subsurface environment due to samples being unable to reflect the complex environment of the wetland rhizosphere [57]. Second, according to previous experience, in situ measurements have been recognized by most professionals to be superior to laboratory testing [57]. Thus, an in situ and field-appropriate technique of investigating quantitative information about subsurface environmental processes, especially in situ rates of root uptake, potential volatilization, chemical and microbial reactions, has been developed, which is called push-pull test [57][2].

Push-pull test involves the injection of solution containing reactive and nonreactive chemicals, called “tracers”, into subsurface environment from a single injection point, which is the “push” part, and the extraction of tracer solution and porewater mixture from the same sampling point, which is the “pull” part [57][2][58]. The nonreactive tracer bromide is used to investigate the effect of dilution of tracer solution with porewater due to physical processes, such as dispersion, diffusion and advection while biologically reactive and/or volatile tracers, after the normalization by nonreactive tracers, reflect the rate of biogeochemical and/or gas transfer processes of interest, such as biotransformation and mass transfer into roots, by interpreting their concentration changes over time with mathematical models [57].

In this project, we want to investigate the root uptake rate and microbial reduction rate of N_2O in wetland environment by using push-pull test, which means the chemical of our interest is N_2O. N_2O is a sparingly soluble gas, which means a traditional push-pull test design [1][59][60][61] must be modified to account for gas transfer processes in the subsurface. Two specific gas transfer processes that must be considered in the context of vegetated wetland soils are: (1) bubble entrapment in porous media caused by trapping of residual gas phases when water levels fluctuate or biogenic gas formation in subsurface environment, into which dissolved gases can transfer and be sequestered; and (2) Gas transfer into the air-filled root aerenchyma system. Thus, a modified push-pull test method

that employs dissolved gas tracers to quantify rates of mass transfer processes is used to constrain N_2O reaction and transport kinetics in the wetland rhizosphere [2][62][63]. Volatilization push-pull test uses volatile chemicals as both reactive and nonreactive tracer so that the effect of partitioning for reactive tracers could be normalized by partitioning effects of nonreactive tracers.

2.2.2 Procedure

To investigate the impact of gas exchange of wetland plants on N_2O reduction and transport, N_2O is chosen as the reactive tracer. Helium (He) and sulfur hexafluoride (SF_6) are chosen as nonreactive volatile tracers because they are chemically and biologically inactive in groundwater [64][65]. Bromide (Br^-) also chosen as nonreactive tracer to normalize the dilution effect of diffusion since it is unlikely to lost due to adsorption or absorption and unaffected by microbial actions [66].

The physical and chemical properties, including K_{aw} values of volatile tracers used in this experiment as well as other following experiments are listed in **Table 1** below.

Chemical	Molecular Weight (g/mol)	Air-water partitioning constant (K_{aw})[a] (dimensionless)	Molecular Diameter (σ)[b] (Å)
He[c]	4	118.7	3.12
N_2O	44	1.5	3.71
SF_6[c]	140	163.4	4.71
Ethane	30	21	3.99

Table 1 *Physiochemical properties of gas tracers* [a] [67][68][69][70]*;* [b] $\sigma=0.809V_c^{1/3}$*, where* V_c *is the critical molar volume* [71]*;* [c] *At temperature of 20℃ and salinity of 8 ppt*

One day before the push-pull test, feeding solution is modified by removing NO_3^- from it so that no N_2O will be produced during the push-pull test. During the push-pull test, feeding solution injection and effluent extraction are stopped and the bioreactors are functioned as batch reactors during push-pull test so that the effects of physical processes, such as advection and hydrodynamic dispersion, on tracer concentration changes are minimized so that the effect of root uptake and biotransformation is more dominant in push-pull test.

To prepare the tracer solution, 9.48L deionized water is filled in a 10L tracer bag, 2.06g sodium bromide (NaBr) has been added to the tracer bag, then bubble the tracer bag

with pure helium for 30 minutes; at the same, two 500mL bottles are filled with deionized water and bubbled with nitrogen for 20 minutes; after 20 minutes, 120 mL pure N_2O is added to one of the bottles (Bottle 1) and vigorously shaken, while 60mL 100ppm SF_6 is added to the other bottle (Bottle 2) and vigorously shaken. Once the helium bubbling in the tracer bag ends, 20mL solution is extracted from Bottle 1 and added into the tracer bag and all solution in Bottle 2 is also added into the tracer bag. After excluding all the bubbles in the tracer bag, it is well sealed with teflon tape and cap. Then, leave the tracer bag sited still for 30 minutes for tracers to become well mixed in the tracer bag.

In experiments performed with *S. acutus*, the preparation of the tracer solution differed slightly. On test day, tracer solution was made by following procedures: 9.495L deionized water is filled in a 10L tracer bag, 2.06g sodium bromide (NaBr) has been added to the tracer bag, then bubble the tracer bag with pure helium for 30 minutes; at the same, two 500mL bottles are filled with deionized water and bubbled with nitrogen for 20 minutes; after 20 minutes, pure N_2O was added to one of the bottles (Bottle 1) to achieve 1 atmospheric pressure of N_2O inside the bottle and vigorously shaken, while 30mL 100ppm SF_6 is added to the other bottle (Bottle 2) and vigorously shaken. Once the helium bubbling in the tracer bag ends, 5mL solution is extracted from Bottle 1 and added into the tracer bag and all solution in Bottle 2 is also added into the tracer bag. After excluding all the bubbles in the tracer bag, it is well sealed with teflon tape and cap. Then, leave the tracer

bag sited still for 30 minutes for tracers to become well mixed in the tracer bag.

To investigate the rate of root uptake and biotransformation of N_2O in subsurface environment, tracer solution is injected into bioreactors from the sampling port 10cm below soil surface at flow rate of 20 mL/min for 10 minutes. Tracer solution samples are collected before and after the injection and 10mL reactors samples are collected before injection and 0.5, 1, 2, 3, 4, 6, 7, 9, 11 hours after injection to make sure the time is long enough for the processes of interest can be detected [57]. Samples are analyzed by IC for Br^- concentration and analyzed by GC for volatile tracers' concentration. **Figure 4** below provide a concept illustration of push-pull test.

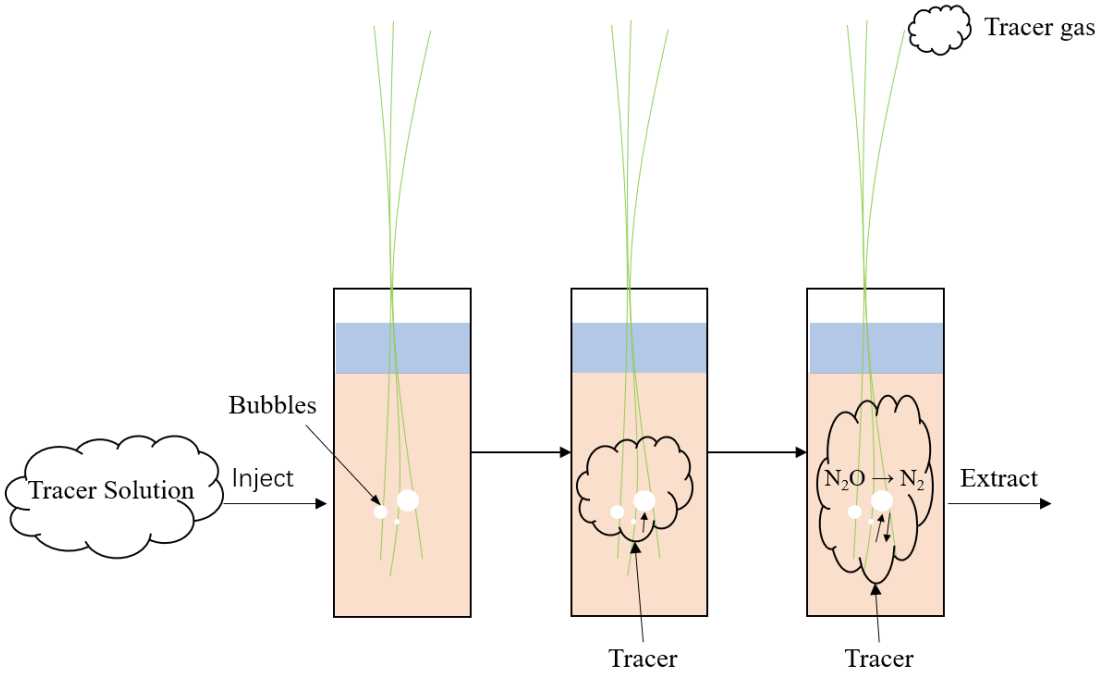


Figure 4 *Push-Pull test concept model*

2.2.3 Model for interpretation Push-Pull test results

Once the concentration profile of the tracers injected into bioreactors has been collected, we need mathematical models to interpret the data and estimate the rates of the processes of interest.

Concentration changes of solutes injected in the tracer plume are due to the following processes: 1) Hydrodynamic mixing with ambient porewater 2) Biotransformation (in the case of N_2O, reduction via enzymztic reaction mediated by the NosZ enzyme) 3) Mass transfer into root aerenchyma (Root uptake) and 4) Partitioning between liquid phase and trapped gas phases in the reactor porous media. For completeness, we can describe one-dimensional advective-dispersive transport for a non-sorbing, non-reactive, volatile tracer using the equation:

$$\frac{dC}{dt} = D\frac{d^2C}{dx^2} - \mathrm{v}\frac{dC}{dx} - k_v C - \left.\frac{dC}{dt}\right|_{gw}$$

Equation (1)

Where,

$\frac{dC}{dt} =$ The changes of aqueous concentration of tracers over time ($mg/L \cdot hr$);

$C =$ The aqueous concentration of tracers (mg/L);

$D\frac{d^2C}{dx^2} =$ The dispersion term of dissolved tracers ($mg/L \cdot hr$);

$D =$ Dispersion coefficient (m^2/hr);

$k_v C =$ The removal rate of dissolved tracers due to partitioning into roots ($mg/L \cdot hr$);

$k_v =$ The removal rate constant [T^{-1}], consist of root uptake term and biotransformation term;

$v =$ Velocity (m/hr);

$\left.\frac{dC}{dt}\right|_{gw} =$ The changes of aqueous concentration of tracers due to partitioning between liquid phase and trapped gas phase ($mg/L \cdot hr$);

In push-pull tests, effects of hydrodynamic mixing (i.e., advection and dispersion) are accounted for via tracer normalization. Standard push-pull approaches utilize Br- normalization for this purpose. To further simplify the *Equation (1)*, the retardation factor, R, is introduced into this equation to account for the source/sink term due to partitioning between liquid phase and gas phase. By assuming the partitioning equilibrium between gas phase and liquid is achieved in the early stage of the push-pull test and justified by fact that the partitioning rate is a order of magnitude faster than the root uptake rate for SF_6 [63]. This reduces the equation above to:

$$R\frac{dC}{dt} = -k_v C$$

Equation (2)

And the retardation factor can be calculated based on the following equation:

$$R = 1 + K_{aw}\frac{V_g}{V_w}$$

Equation (3)

Where,

$K_{aw} =$ Air-water partitioning coefficient (dimensionless);

$\frac{V_g}{V_w} =$ Ratio between gas-filled and water-filled pore volume[2] (dimensionless);

However, it is hard to directly measure the V_g/V_w value in the bioreactors, thus it is estimated using a mass balance approach based on the change in volatile tracer concentrations immediately after the tracer injection step, as presented by Reid et al [2]:

$$\frac{V_g}{V_w} = \frac{C_{t=0} - C_{t=i}}{C_{t=i}K_{aw}}$$

Equation (4)

Where,

$C_{t=0} =$ Initial aqueous tracer concentration at t=0 hour (mg/L);

$C_{t=1} =$ The aqueous tracer concentration at sample withdrawn at t =*1* hour (mg/L);

In unplanted mesocosms, the reactive tracer N_2O undergoes dispersion and biotransformation processes after the partitioning equilibrium is achieved, while the nonreactive tracer Br^- undergoes dispersion processes only, thus it is reasonable to use Br^-

normalization for data from unplanted group. However, for planted group, the partitioning processes continues even the equilibrium is once achieved, the root uptake processes will decrease the gas phase N_2O concentration and cause gases in trapped bubbles to re-partition into the aqueous phase to maintain equilibrium. Thus, in planted bioreactors, N_2O undergoes dispersion, biotransformation, root uptake and partitioning processes (both an initial partitioning into trapped bubbles followed by slower partitioning back into the aqueous phase). Because of the effect of bubble partitioning, using Br^- normalization would not well account for the processes of our interest as Br is not affected by bubble partitioning processes. Theoretically, this leads to underestimation of tracers removal rate, due to the effect of gas tracers partition back into water hasn't been accounted by Br^- normalization method [2]. Moreover, the fact that bromide may also be uptake by plants adds to the uncertainty of using bromide normalization method to account for the hydrodynamic mixing in planted bioreactors [72]. Thus, another normalization method developed for volatile tracers is applied to interpret the data, which is presented in Reid et al [2]. Tracer gases used in this method (He and SF_6) have similar air-water partitioning constant and thus similar bubble effects happened on both tracers and the uncertainties of trapped bubbles on removal rate constant were reduced and the calculated root uptake rate constants were assumed to be more accurate [2]. The detailed explanation of the two normalization methods are described in the following parts:

Bromide Normalization Method

Bromide is used as nonreactive tracer to account for hydrodynamic mixing of tracer solution injected with porewater [58][1]. Thus, these effects on reactive tracer is canceled after normalization:

$$C^{t,i}_{Br^- normalized} = \frac{\left(C^i_t - C^i_{bkgd}\right)/\left(C^i_{inj} - C^i_{bkgd}\right)}{\left(C^{Br^-}_t - C^{Br^-}_{bkgd}\right)/\left(C^{Br^-}_{inj} - C^{Br^-}_{bkgd}\right)}$$

Equation (5)

Where,

$C^{t,i}_{Br^- normalized} =$ The bromide normalized concentration of tracer *i* at time *t* (dimensionless)

$C^i_t =$ The aqueous concentration of tracer *i* at time *t* (mg/L);

$C^i_{inj} =$ The aqueous concentration of tracer *i* in tracer solution injected (mg/L);

$C^i_{bkgd} =$ The aqueous concentration of tracer *i* in bioreactors before push-pull test (mg/L);

$C^{Br^-}_t =$ The aqueous concentration of bromide at time *t* (mg/L);

$C^{Br^-}_{inj} =$ The aqueous concentration of bromide in tracer solution injected (mg/L);

$C^{Br^-}_{bkgd} =$ The aqueous concentration of bromide in bioreactors before push-pull test (mg/L);

Once the hydrodynamic mixing of reactive tracers is accounted by bromide

normalization, the concentration changes of reactive tracers can be described by the following equations:

$$R\frac{dC'}{dt} = -k_v C'$$

Equation (6)

Where,

$C' =$ The bromide normalized concentration of tracer (dimensionless);

Thus, the removal rate constant k_v can be calculated based on the first order relationship between the bromide normalized tracer removal rate and bromide normalized tracer concentration. This first order relationship is based on the two main processes dominate concentration changes of volatile tracers: root uptake and microbial reduction. Molecular diffusion across the porewater-root interface contributes significantly to root uptake processes of volatile tracers [73][74], thus its removal rate is dependent on the concentration of gas at water-root interface and thus it is dependent on dissolved gas concentrations at that interface (i.e. first order). In terms of microbial reduction of N_2O, as N_2O concentration in tracer solution is not significantly greater than half-saturation constant measured from previous research [47], thus its reaction rate could also be considered as first order. N_2O concentration in the tracer solution injected ranges from 0.38 to 0.55 mg/L and the half saturation constant (k_m) of N_2O in N_2O reduction ranges from

0.014 to 1.56 mg/L [47][75] and, thus it is hard to directly judge whether the N_2O concentration injected will lead to first-order reaction simply based on this. The Br^- normalized N_2O concentrations and their natural log values also performs linearly. For the simplification of calculation, we assumed the N_2O reduction to be first-order. Thus, the following equation could describe Br^- normalized concentration changes in push-pull test.

$$lnC_t' = -\frac{k_v}{R}t$$

Equation (7)

Dual Volatile Tracers Normalization Methods

Dual volatile tracers normalization method originates from estimation method for gas transfer in oceans and estuaries [76][77], and is modified by Reid et al [2] to be used for interpreting push-pull test results to determined root-mediated gas transfer rates in the wetland rhizosphere. Based on the relationship between root uptake rate and molecular diameter, an accurate estimation method for removal rate constant in planted bioreactors has been developed.

$$\frac{k_{v,i}}{k_{v,j}} = \left(\frac{\sigma_i}{\sigma_j}\right)^n$$

Equation (8)

Where,

$k_v =$ Root uptake rate constant [T^{-1}]

$\sigma =$ Molecular diameter of volatile tracers $[\text{Å}]$

n = Correction factor between root uptake rate constant and molecular diameter, the empirical value of *n* equals -8.85 ± 1 [2]

Based on *Equation (8)* and the advection-diffusion equation developed by Wanninkhof et al [76], the normalization method is able to include continuous partitioning between liquid phase and gas phase into consideration.

$$k_{v,j} = \frac{d}{dt}\left(\frac{R_j lnC_j - R_i lnC_i}{1 - (\sigma_i/\sigma_j)^{-8.85}}\right)$$

Equation (9)

Where,

$k_{v,j} =$ Removal rate constant of tracer j, which is the root uptake rate constant

With the help of dual tracer normalization method, more accurate estimation of root uptake rate constant can be made for volatile tracers whose uptake rate constant is not much larger than partitioning rate constant.

2.3 Isotope experiments

To further investigate the ratio of emitted N_2O over the total N_2 emitted by *Sagittaria latifolia*, $N_2O_{emitted}/(N_{2,emitted} + N_2O_{emitted})$, a modified push-pull test with $^{15}N_2O$ in the tracer solution and flux chambers is applied to bioreactors to achieve this objective. This value serves as an important parameter to assess the N_2O emission potential of soil ecosystem.

2.3.1 Flux chamber method

Flux chamber method is the in situ measurement method of gas fluxes from the reactor to the atmosphere by enclosing the soil and aboveground biomass and measuring the accumulation of trace gases [78]. An 8x8x12 inch (Unplanted) chamber and an 8x8x13 inch (Planted) chamber are constructed with PVC frame and plastic film with only one opening at the bottom. The plastic film is connected by hot seal and achieve airtightness and a sampling port with stop has been added to each flux chamber for in situ gas sampling during experiments. Since the flux chamber is made of plastic film, its shape is changeable within certain range and pressure inside could be kept as 1 atmospheric pressure after sample extraction from it. Then, flux chambers are applied to the top of a planted and an unplanted bioreactor and sealed with tape. The airtightness of each flux chamber is tested by injecting certain amount of air into each chamber to make it a little over-pressurized and

test whether its shape changes after 30 minutes. Once no change is observed, open the stops to reduce its pressure to 1 atmosphere and reclose them. After this procedure, the flux chamber is ready to be used in the isotope test. How often do you sample? How do you determine fluxes?

2.3.2 Isotope test procedure

To assess the ratio of N_2O emitted over total N_2 emitted by *Sagittaria latifolia*, the isotope experiments are conducted for planted and unplanted reactors. Since it is a modified push-pull test, most of its procedure is the same as push-pull test, except for the following:

(1) The 120mL pure N_2O added into bottle 1 is replaced with 90mL pure N_2O and 30mL $^{15}N_2O$.

(2) The liquid sample collected for N_2O analysis is not analyzed by GC. 5mL liquid sample is injected into a 60mL well-sealed vacuum vial and He is added to make up for the rest part of space. After vigorously shaking, 12mL gas sample is collected from the vial and injected into a 12mL well-sealed vacuum soda glass exetainer.

(3) At the same time of the liquid sample extraction, gas samples are collected from the sampling port on flux chambers. 12mL of the gas sample is also injected into a 12mL well-sealed vacuum soda glass exetainer.

(4) All the exetainers are sent to Stable Isotope Facility at University of California Davis for isotope analysis.

2.4 Model validation test

2.4.1 Motivation and definition

To check the accuracy of the estimation of root uptake rate constant obtained from push-pull test, in other words, the accuracy of root uptake rate constant of N_2O predicted based on He and SF_6 by empirical relationship between uptake rate constant and molecular diameter, ethane, of which the molecular diameter is between He and SF_6, just like N_2O, is used as an extra nonreactive tracer in push-pull test. Root uptake rate constant of ethane is estimated based on the root uptake rate constant of He and SF_6 and the concentration of ethane is predicted by the estimated root uptake rate constant, initial ethane concentration and the concentrations of another volatile nonreactive tracer (He or SF_6). By plotting the estimation and measurement of ethane concentration changes in push-pull test, the accuracy of the estimation could be justified.

Another reason to apply this method is that we failed to directly measure the root uptake rate constant for N_2O either by sterilizing the bioreactor with sodium azide or block N_2O reduction with acetylene. The former could also hurt the plant root and disturb plant

uptake rate measured while the latter required over pressurized acetylene solution to be added into tracer solution and also disturb plant uptake processes according to our experiment results. Thus, we need to predict the uptake rate constant of N_2O based on plant uptake rate constants of other volatile tracers. Thus, we need to know which method could best predict N_2O uptake rate constant.

However, to use ethane as a nonreactive tracer in push-pull test, it should be chemically and biologically inactive in groundwater. To test this property, tracer solution should also be injected into unplanted bioreactor. If ethane remains inactive in groundwater during push-pull test, its concentration changes, after normalized by Br^- concentration, should be negligible after partitioning equilibrium is achieved between gas phase and liquid phase in unplanted bioreactors.

2.4.2 Test procedure

To test the concentration changes of ethane in push-pull test and achieve the goal to test the accuracy of estimated results from push-pull test, ethane, He, SF_6 and Br^- are used for making tracer solution. 8.5 L deionized water is filled in a 10L tracer bag, 2.06g sodium bromide (NaBr) has been added to the tracer bag, then bubble the tracer bag with pure helium for 30 minutes; at the same, a 500mL bottle was filled with deionized water and bubbled with nitrogen for 20 minutes; after 20 minutes, 60mL 100ppm SF_6 was added to

the this bottle and vigorously shaken. 1L pure ethane was mixed with 1L deionized water and vigorously shaken to make it well mixed. Once the helium bubbling in the tracer bag ends, 500mL solution containing SF_6 and 1L solution containing ethane were added into the tracer bag. After excluding all the bubbles in the tracer bag, it is well sealed with teflon tape and cap. Then, leave the tracer bag sited still for 30 minutes for tracers to become well mixed in the tracer bag.

Tracer solution is injected into both planted and unplanted bioreactors from the sampling port 10cm below soil surface at flow rate of 20 mL/min for 10 minutes. Tracer solution samples are collected before and after the injection and 10mL reactors samples are collected before injection and 1, 2.5, 4, 5.5, 7, 8.5, 10, 11 hours after injection to make sure the time is long enough for the processes of interest can be detected [57]. Samples are analyzed by IC for Br^- concentration and analyzed by GC for volatile tracers' concentration.

2.5 Analytical Methods

2.5.1 Ion Chromatography (IC)

Quantification of analyte of NO_3^- from reactor monitoring and Br^- from push-pull test and isotope experiments was performed by Ion Chromatography (IC) (ThermoFisher

Scientific) with Dionex ICS-2100 model.

During the preparation of the test, samples were filtered by 0.22μm filters and injected into 0.8mL IC vials and placed in the Dionex AS-AP Autosampler (ThermoFisher Scientific). Then, samples were injected at 25μL volumes and were loaded onto Dionex™ CR-TC Continuously Regenerated Trap Columns (ThermoFisher Scientific). Elution from trap column and onto Dionex™ IonPac™ AS18 IC Columns (ThermoFisher Scientific) were performed using a gradient pump with 1mL/min flow rate of 14mM (for Br^- detection) and 17.5 mM (for NO_3^- and NO_2^- detection) KOH solution. Sample components will be separated inside the Dionex™ IonPac™ AS18 IC Columns (ThermoFisher Scientific) through all types of separation method by using conductivity detection and moves at different rate, thus, arrives at the conductivity detector at different times [79][80]. The arrival time of each component at the detector is related to the distribution ratio of the component, which is the ratio of the component in mobile phase and stationary phase and is unique for each of them, thus the arrival time, called retention time, is characteristic for components. After neutralized by suppressor, eluent with low background conductivity will bring solutes to the conductivity detector, and form positive peaks in the conductivity monitoring recorder for their higher conductivity [81][82]. Analytes were quantified from external calibration standards diluted from Multiple Anions Standards (ThermoFisher Scientific) and the calibration curve was based on the peak areas by linear least-squares

regression.

2.5.2 Gas Chromatography

Quantification of analyte of N_2O from bioreactors monitoring and volatile tracers from push-pull test and isotope experiments was performed by Gas Chromatography (GC, GC-2014, Shimadzu).

During the GC analysis, gas phase samples were injected into GC from injector and was brought through the analytical column by carrier gas (Argon and 10% Methane Balance Argon Certified Standard Gas Mixture, Airgas) mobile phase. Column were heated at high temperature and components in gas samples, due to stronger interaction with stationary phase, which is immobilized liquid phase material coating the solid support that packing the column, are separated with each other and reaches the detectors at their unique retention time [83][84]. The three detectors in GC are Flame Ionization Detector (FID, Supplied with H_2 and High Purity Air as fuel), Electron Capture Detector (ECD) and Thermal Conductivity Detector (TCD) [84]. Different detectors is able to recognize various gas phase components and the analogue signal from detectors was digitalized by the data system to form “Chromatogram”, which is the record of chromatographic separation [84]. Quantifications of analyte were performed by external calibration standards diluted from

Standard Gas Mixture, pure Helium and 100ppm SF_6 gas (Airgas) and the calibration curve was based on the peak areas by linear least-squares regression.

2.5.3 Total Organic Carbon Analyzer

Quantification of analyte of total organic carbon and total nitrogen from bioreactors' monitoring were performed by TOC-L, Laboratory Total Organic Carbon Analyzers (TOC, Shimadzu).

During the preparation, samples were filtered by 0.45μm filters and injected into 24mL TOC vials. After acidified by 2mM hydrochloric acid to pH value lower than 4, the TOC samples were placed in ASI-Autosampler (Shimadzu). For non-purgeable organic carbon (NPOC) parameter of our interest, which is usually referred as Dissolved Organic Carbon (DOC), samples were injected at 50μL volume and passing through total carbon furnace and inorganic carbon vessel, the organic carbon is converted into CO_2 with the help of catalytic oxidation combustion technique at high temperature and the amount of CO_2 produced is measure by Non-dispersive Infra-Red (NDIR) sensor [85]. In terms of total nitrogen, the total nitrogen module (TNM) is able to measure the amount nitrogen in sample by first combusted the nitrogen in sample into NO and NO_2 and then converted them into excited state of nitrogen dioxide by reacting with ozone, finally using chemiluminescence detector to measure the total nitrogen as light energy would be emitted

once the excited state returns to ground state [86]. Quantifications of analyte were performed by external calibration standards and the calibration curve was based on the peak areas by linear least-squares regression.

2.5.4 Protein measurement

Quantification of analyte of total protein from soil samples extracted at the end of the experiments were performed by Pierce^{TM} BCA Protein Assay Kit (ThermoFisher Scientific) and UV-2600 UV-Vis Spectrophotometers (Shimadzu).

Before the measurements, soil samples were mixed with cell lysis buffer (containing 9g/L NaCl, 1% Triton X100, 0.1% SDS, 10mM EDTA and 25mM Tris-HCl, adjusted pH=7.6). The mixture was vortexed for 2 minutes and put in ice bath for 2 minutes and repeat this procedure for 5 times. After vortex, soil/cell lysis buffer mixture were centrifuged for 15 minutes at 4700rpm and after this step, the supernatant could be used for protein measurements. Reagent A (containing Na_2CO_3, $NaHCO_3$, bicinchoninic acid and sodium tartrate in 0.1M NaOH) and Reagent B (containing 4% $CuSO_4$) were mixed at the ratio of 50:1[87]. 2mL reagent mixture was then mixed with 0.1mL supernatant from soil/cell lysis buffer mixture and incubated at 37℃ for 30 minutes [87]. Once samples returned to room temperature, read the samples at 562nm wavelength by using UV-Vis Spectrophotometers. The theoretical consideration behind this is that the protein is able to convert Cu^{2+} to Cu^{+} in alkaline medium and the chelation product of Cu^{+} and bicinchoninic

acid shows strong absorbance at 562nm wavelength, which is nearly linearly increasing with protein concentration increasing within the range of interest [87]. Quantifications of analyte were performed by external calibration standards of dilution from Albumin Standard Ampules (ThermoFisher Scientific) and the calibration curve was based on the wavelength absorbance by linear least-squares regression.

Chapter 3 Results

3.1 Reactor parameters

Several biogeochemical parameters of the wetland mesocosms planted with *Sagittaria Latifolia* were monitored during or after the experiments to 1) evaluate NO3- removal and N2O accumulation within wetland mesocosms and 2) collect data on root density to normalize kinetic constants obtained from push-pull tests to enable comparison with other results in the literature. For bioreactors planted with *Schoenoplectus Acutus*, only root density was measured for normalization of root uptake rate constants.

Two kinds of parameters could be used to justify the function of bioreactors: 1) N species concentrations along the depth of the bioreactor and 2) dissolved organic carbon, the electron donor required for denitrification.

N-concentration could directly reflect the denitrification ability of bioreactors, since the only N source injected into bioreactors are NO_3^-. According to the denitrification processes of $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$, as well as the conversion of NO_3^- into organic nitrogen due to plant assimilation [88], the concentrations of related substrates along the depth of the bioreactors were examined by IC and GC at 3, 4, 11 and 13 weeks after mesocosms were planted for NO_3^- and 11 and 12 weeks after mesocosms were planted for N_2O. However, due to the fact that the concentration of NO_2^- and NH_4^+ is negligible as

shown in the IC results and the concentration of N_2 is easily affected by the atmospheric environment, only the concentrations of NO_3^- and N_2O were measured to justify the denitrification ability of bioreactors. **Figure 5** and **Figure 6** illustrate the concentration of NO_3^- and N_2O measured from all sampling ports of bioreactors at four time points throughout the experiment. **Figure 7** listed the concentration of organic nitrogen, which is shown in *Equation (10)*.

$$Organic\ N = Total\ N - (NO_3^- - N + N_2O - N)$$

Equation (10)

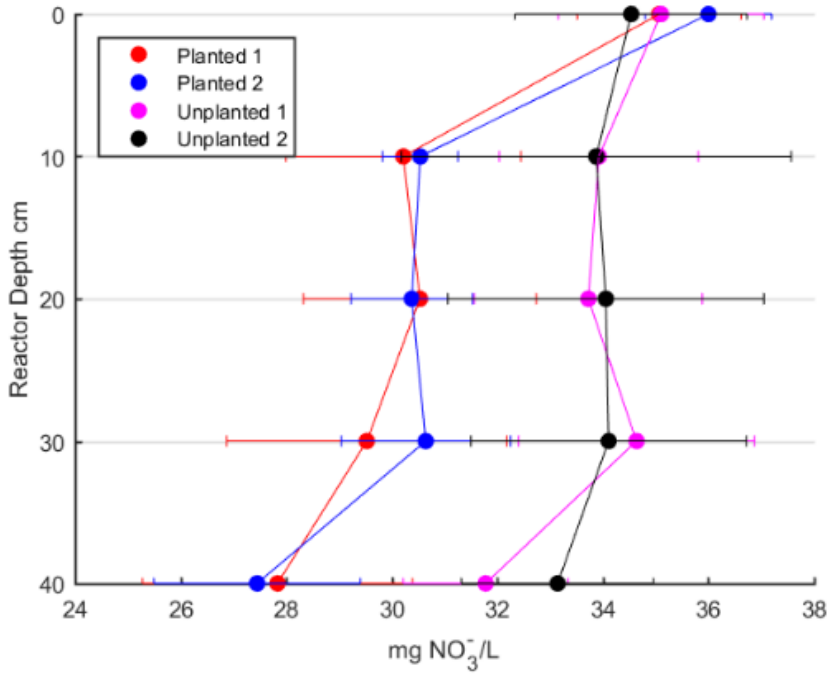


Figure 5 *Average NO_3^- concentration along the depth of bioreactors, error bars represent standard deviation among samples collected at different time points, y-axis refers to the depth of sampling point from soil surface*

According to **Figure 5**, with similar initial NO_3^- concentration injected at soil surface, more NO_3^- is removed in planted bioreactors compared to unplanted reactors, which suggests higher NO_3^- removal ability of planted group compared with unplanted group. In both planted and unplanted groups, significant NO_3^- removal happens within the first 10 cm below soil surface and the last 10 cm above the effluent port.

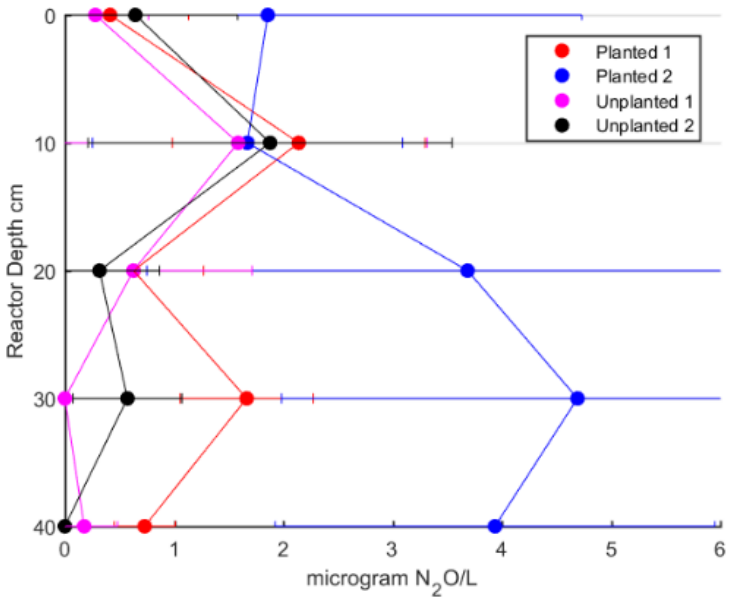


Figure 6 *Average N_2O concentration along the depth of bioreactors, error bars represent standard deviation among samples collected at different time points, y-axis refers to the depth of sampling point from soil surface*

According to **Figure 6**, N_2O was produced and consumed along the flow pathway from soil surface to effluent ports. Planted group showed higher concentration of N_2O compared with unplanted group. In unplanted group, N_2O concentration achieves maximum at 10 cm below soil surface while in planted group, N_2O concentration peaks at

10 cm or 30 cm below soil surface.

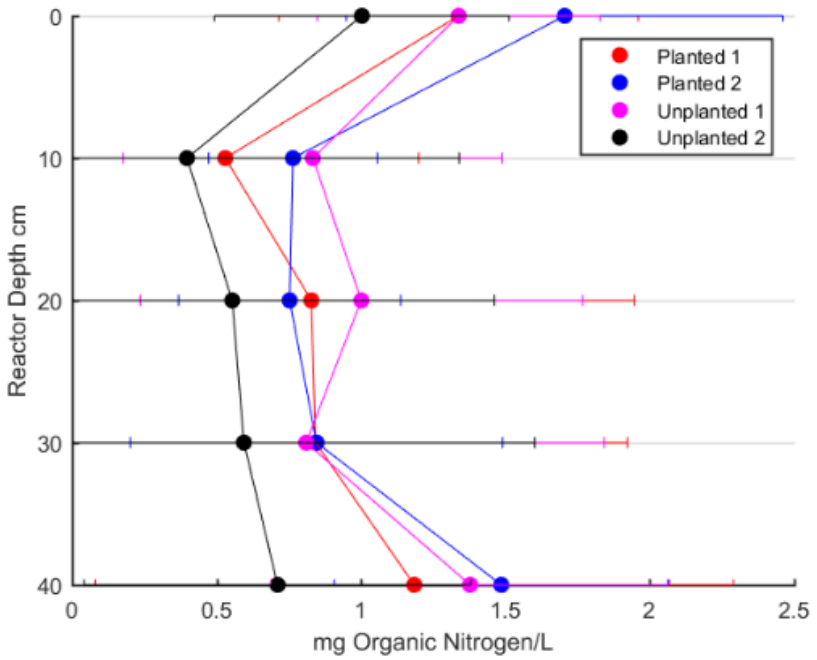


Figure 7 *Average organic nitrogen concentration along the depth of bioreactors, error bars represent standard deviation among samples collected at different time points, y-axis refers to the depth of sampling point from soil surface*

According to **Figure 7**, all bioreactors shows similar trend and amount of dissolved organic nitrogen concentration. Organic nitrogen tends to be reduced within the first 10 cm below soil surface and remains relatively constant from 10 to 30 cm below soil surface and increase again at the end 10 cm. Their similar property suggests they are not significantly affected by the presence of plant or NO_3^- removal ability.

To further investigate the effects of the presence of plants on N_2O production and consumption, the N_2O yield was calculated based *Equation (11)* below.

$$N_2O\ yield = \frac{(C^{j}_{N_2O} - C^{o}_{N_2O})}{(C^{o}_{NO_3^-} - C^{j}_{NO_3^-})}$$

Equation (11)

Where,

$C^{o}_{N_2O}$ = Aqueous concentration of N_2O in surface water $(\mu g/L)$

$C^{j}_{N_2O}$ = Aqueous concentration of N_2O at sampling port j $(\mu g/L)$

$C^{o}_{NO_3^-}$ = NO_3^- concentration in surface water (mg/L)

$C^{o}_{NO_3^-}$ = NO_3^- concentration at sampling port j (mg/L)

The results are shown in **Figure 8** below.

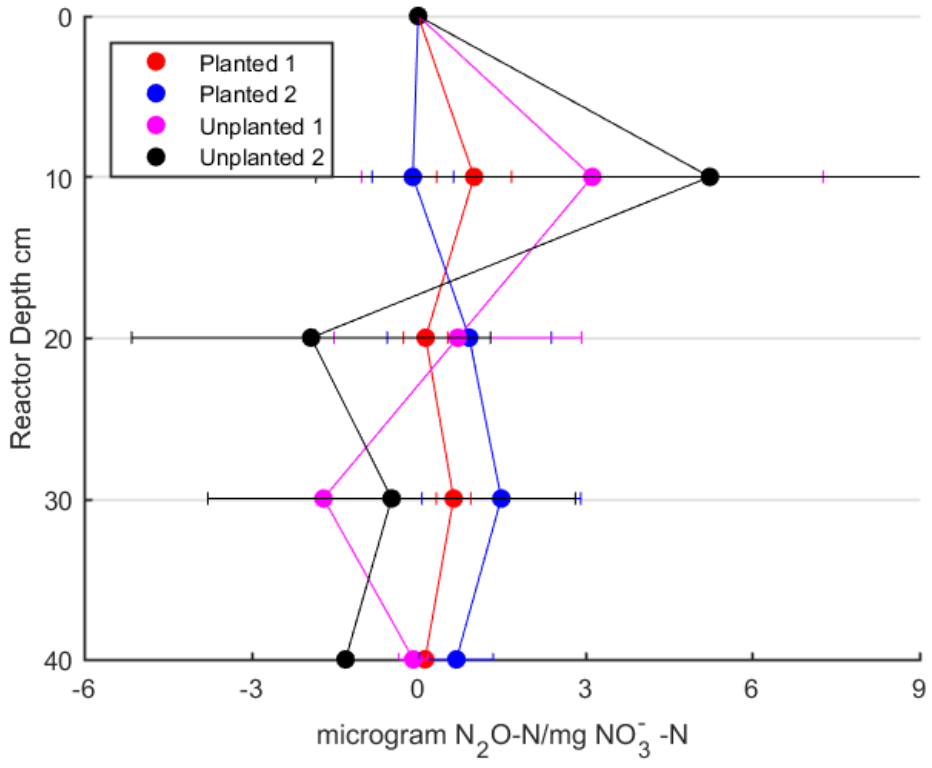


Figure 8 *N_2O yield in different bioreactors*

Dissolved organic carbon (DOC) concentration was investigated to prove our assumption that the organic carbon source in bioreactors was in excess and was not the limiting factor for denitrification rate. **Figure 9** listed the concentration of DOC from all sampling ports from both planted and unplanted groups. The DOC concentrations measured in bioreactors were at least twice the median amount of half-saturation constant of DOC for denitrification from literature review [89], which provide another evidence supporting our assumption that DOC concentrations were in excess for denitrification.

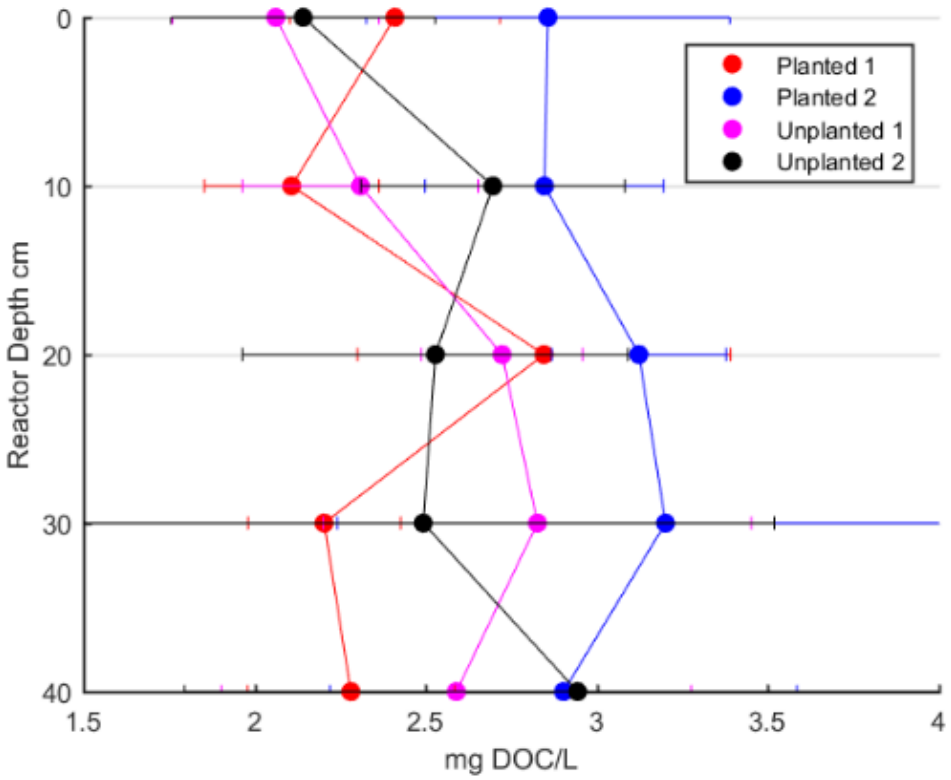


Figure 9 *Average dissolved organic carbon concentration along the depth of bioreactors, error bars represent standard deviation among samples collected at different time points, y-axis refers to the depth of sampling point from soil surface*

In order to normalize the N_2O reduction rates by biomass density, the concentration of protein in soil collected from both planted and unplanted groups from 5 cm to 15 cm below soil surface was measured. **Table 2** showed the concentration of protein and an estimation of biomass density based on the empirical relationship of 55% of cell dry weight as protein [90].

The gas-permeable surface area of aerenchymal roots is an important control on root uptake of dissolved gases. The root density serves as an approximate proxy for this term [91], thus, it is necessary to normalize the root uptake rate constant by root density for planted bioreactors to allow for intercomparison between different studies and plant types.

Bioreactors	**Planted 1[a]**	**Planted 2[a]**	**Planted 3[b]**	**Unplanted 1**	**Unplanted 2**
Protein Concentration (µg/g)	1.40 ± 0.38	0.94 ± 0.14	N.A.	1.40 ± 0.58	1.19 ± 0.30
Biomass Density (µg/g)	2.54 ± 0.69	1.70 ± 0.25	N.A.	2.54 ± 1.05	2.16 ± 0.55
Root Density (mg/g)	0.154	0.149	0.740	N.A.	N.A.

Table 2 *Protein concentration (mg/L), biomass density (mg/L) and root density (mg/kg) of bioreactors*

Moreover, the porosity of the medium was measured based on mass differences between dried soil and saturated soil as shown below.

Soil Volume (mL)	Soil Mass (g)	Saturated Soil Mass (g)	Porosity (dimensionless)	Average Porosity (dimensionless)
100	179.07	213.1	0.3403	0.3402 ± 0.0142
100	189.46	224.9	0.3544	
100	183.77	216.37	0.3260	

Table 3 *Porosity of medium*

Based on the porosity measured, the average hydraulic retention time (HRT) was calculated based on *Equation (12)*.

$$HRT = \frac{porosity \times V}{Q}$$

Equation (12)

Where,

$V =$ Total volume of bioreactors (mL)

$Q =$ Flow rate (mL/min)

Thus, the calculated hydraulic retention time of bioreactors is: $6.90 \pm 0.29\ hours$.

3.2 Push-Pull test results

Tracer concentration changes reflect the rapid partitioning processes of volatile tracers between liquid phase and gas phase, dispersion processes of all tracers, root uptake processes of volatile tracers and microbial reduction of N_2O. First, **Figure 10** to **Figure 12** will list the concentration changes of all tracers under various normalization methods.

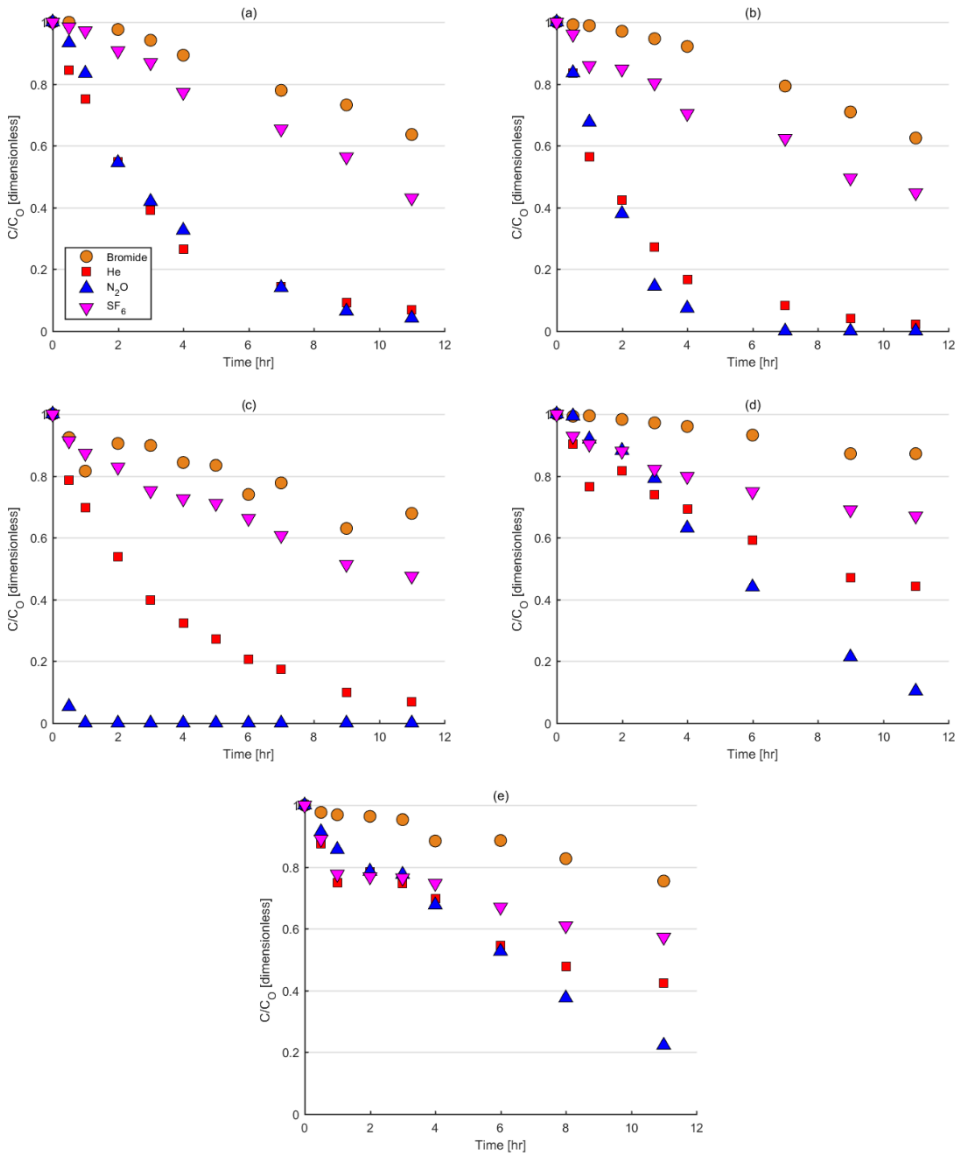


Figure 10 *Tracer concentrations normalized by initial concentration of the tracer solution for porewater samples collected during push-pull test. (a) and (b) are planted group with Sagittaria Latifolia; (c) is planted group with Schoenoplectus Acutus; (d) and (e) are unplanted group;*

As shown in **Figure 10**, C/C_o for all dissolved gas tracers decreased faster than C/C_o for the conservative Br^- tracer, indicating additional physiochemical and/or biological removal processes beyond the hydrodynamic mixing that affects Br^- concentrations. The change of C/C_o for the dissolved gases in both vegetated and unvegetated mesocosms during the 11 hours experiment increased in the order SF_6 < He < N_2O. For bioreactor planted with *Schoenoplectus acutus*, its N_2O removal rate is so fast that nearly all N_2O injected was removed within the first hour, thus makes it hard to calculate N_2O removal rate constant in *Schoenoplectus acutus* planted bioreactor.

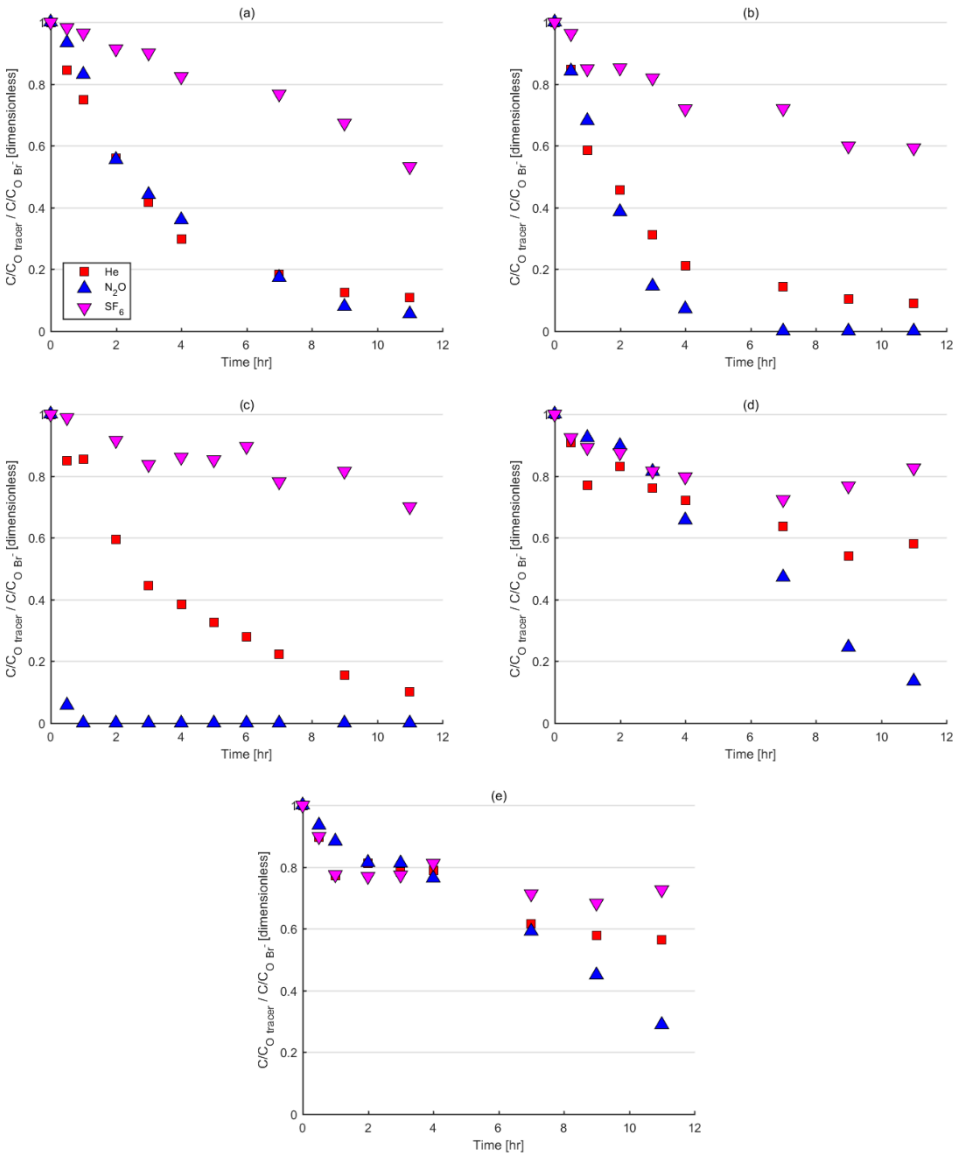


Figure 11 *Bromide normalized tracer concentration for porewater samples collected during push-pull test. (a) and (b) are planted group with Sagittaria Latifolia; (c) is planted group with Schoenoplectus Acutus; (c) and (d) are unplanted group*

After normalization by Br^-, the effect of concentration changes due to dispersion is accounted for. As shown in **Figure 11**, in planted reactors all three dissolved gases show

clear evidence of a sink that removes the compounds from the soil solution. Removal of SF_6 is slower than removal of He and N_2O, with approximately 40% of SF_6 mass removed from solution while ≥90% of He and N_2O mass is removed. In contrast, in unplanted reactors only N_2O shows sustained removal throughout the 11 hours experiment, with between 70 and 85% of the N_2O mass removed. The normalized concentrations of He and SF_6 decreased in the first phase of the experiment and then increased after 7 to 11 hours.

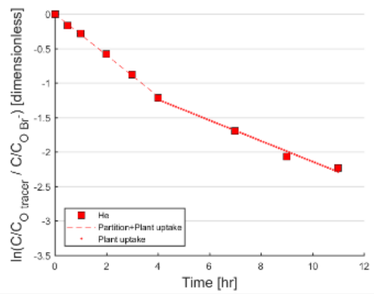
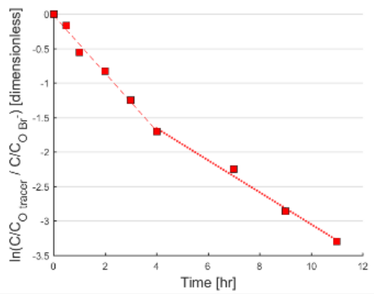
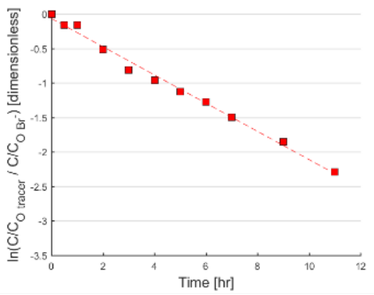
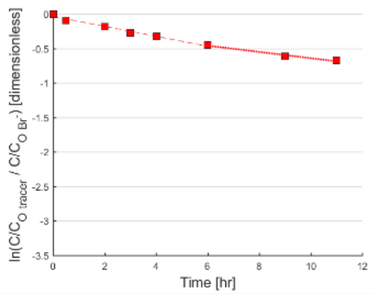
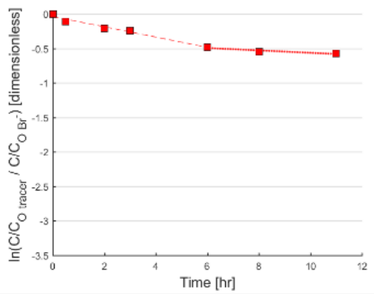
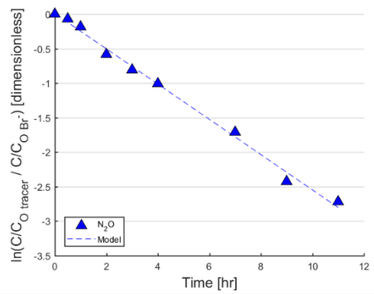
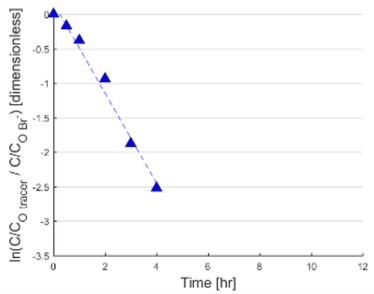
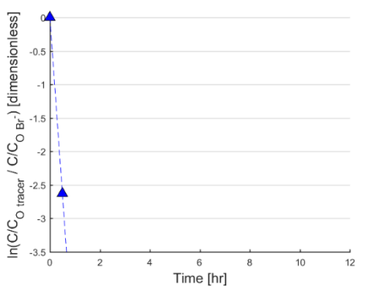
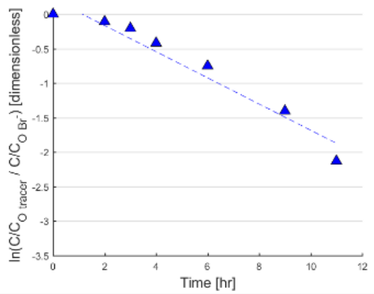
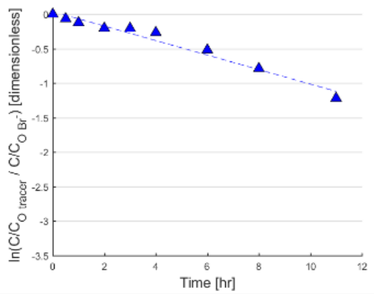
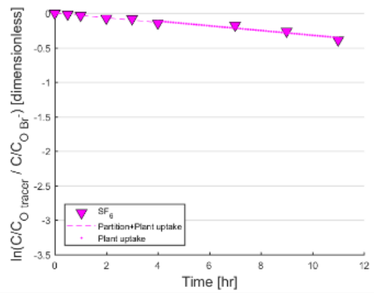
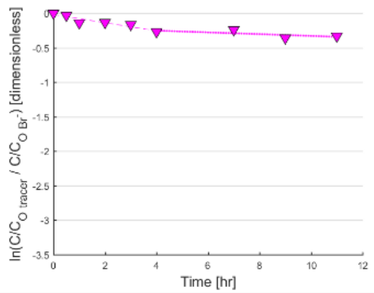
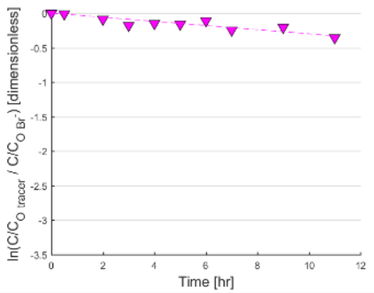
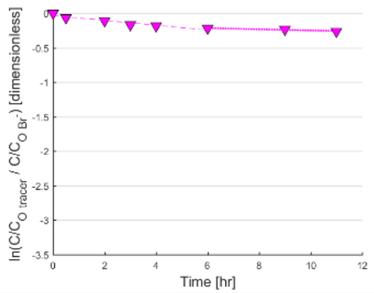
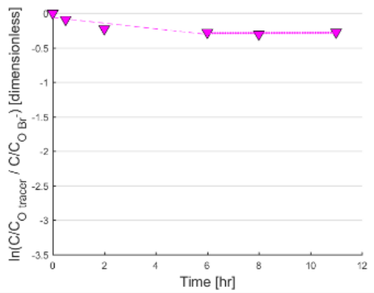
Tracers	Planted 1[a]	Planted 2[a]	Planted 3[b]	Unplanted 1	Unplanted 2
He					
N_2O					
SF_6					

Figure 12 *Natural log of Bromide normalized push-pull test results [a] refers to planted with Sagittaria Latifolia, [b] refers to planted with Schoenoplectus Acutus, used to determine first-order rate constants*

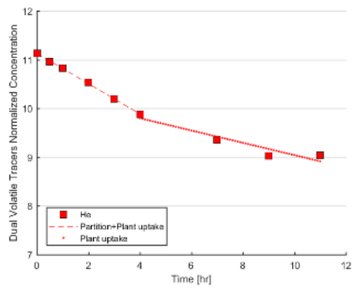
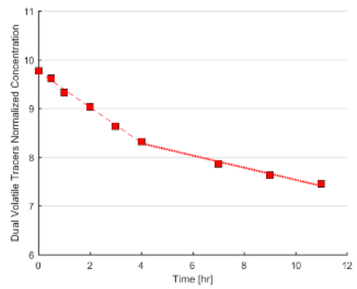
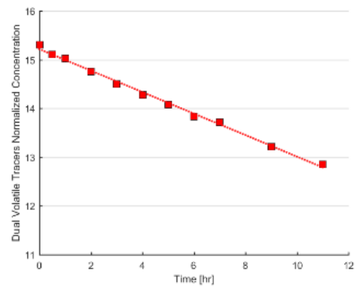
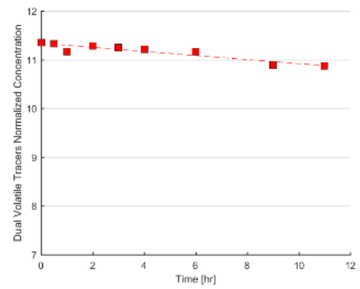
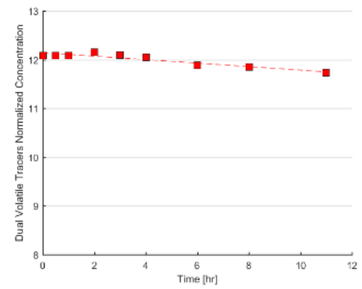
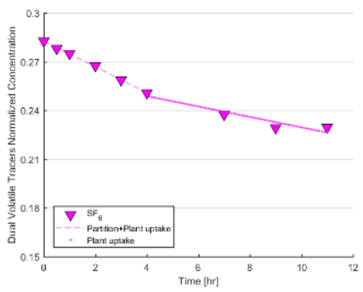
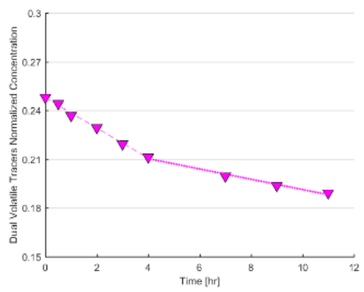
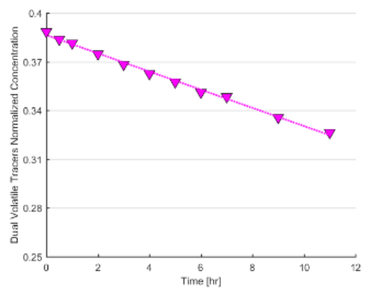
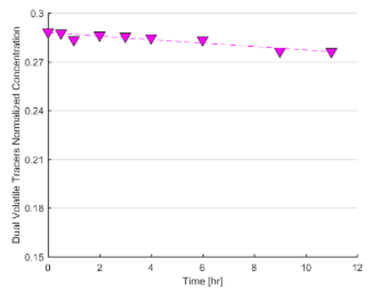
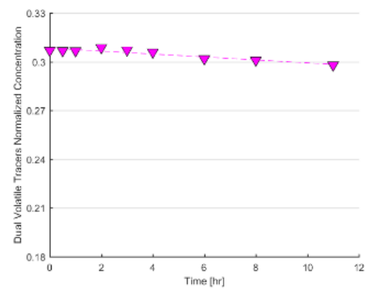
Tracers	Planted 1[a]	Planted 2[a]	Planted 3[b]	Unplanted 1	Unplanted 2
He					
SF_6					

Figure 13 *Dual volatile tracers normalized push-pull test result a refers to planted with Sagittaria latifolia, b refers to planted with Schoenoplectus acutus*

Dual volatile tracer normalization method and Bromide normalization method were applied to tracer concentration obtained from push-pull test for planted and unplanted groups and the results are shown in **Figure 12** and **Figure 13**. He and SF_6 in both groups showed faster decreasing trend in the first 4 hours after the injection of tracer solution, which is thought to be the effect of partitioning between liquid phase and trapped gas phases (i.e., bubbles) in the porous media though the bubble volume was relatively small according to V_g/V_w value determined from initial tracer concentration changes in the first hour. In planted group, after 4 hours, the processes that dominated concentration decreasing is thought to be plant root uptake for He and SF_6, while in unplanted group, after 4 hours, the concentration of He and SF_6 remains relatively constant, which were in agreement with the fact that they are nonreactive tracers in this test. N_2O continuously decreases due to both plant uptake (in planted group) and microbial reduction (in both groups), however we cannot split them without prediction of root mediated gas transport rate of N_2O. As the removal rate of *Schoenoplectus acutus* planted bioreactor is so fast, which may be related to the higher initial organic carbon content in soil recipe of this bioreactor, that all N_2O was reduced within the first hour after injection and its normalized concentration curve of N_2O only consists of two data points. Thus, removal rate constant calculated based on this may have a large uncertainty. The development and assessment of prediction based on empirical relationships is in Chapter 4.3. The removal rate constant of each tracers in all bioreactors

by Br^- normalization methods, predicted N_2O uptake rate constants and microbial reduction rate constants of N_2O are shown in **Table 4**.

Parameters	Planted 1[a]	Planted 2[a]	Planted 3[b]	Unplanted 1	Unplanted 2
V_g/V_w ($\times 10^{-3}$)	1.48 ± 1.84	3.57 ± 3.59	2.27 ± 1.96	1.68 ± 1.27	2.43 ± 0.57
Retardation Factor	**Planted 1[a]**	**Planted 2[a]**	**Planted 3[b]**	**Unplanted 1**	**Unplanted 2**
He	1.17 ± 0.22	1.12 ± 0.12	1.27 ± 0.23	1.20 ± 0.15	1.29 ± 0.07
N_2O	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
SF_6	1.24 ± 0.30	1.17 ± 0.17	1.37 ± 0.32	1.27 ± 0.21	1.40 ± 0.09
Bromide Normalization Method: Removal rate constant, predicted N_2O uptake constant and reaction rate constant (hr^{-1})					
He	0.1762 ± 0.0369	0.2603 ± 0.0422	0.2596 ± 0.0182	0.0544 ± 0.0497	0.0223 ± 0.0514
N_2O	0.2561 ± 0.0087	0.6537 ± 0.0626	5.2729 ± 0.0000	0.1902 ± 0.0221	0.1056 ± 0.0090
SF_6	0.0426 ± 0.0252	0.0150 ± 0.0241	0.0412 ± 0.0159	0.0108 ± 0.0224	-0.0020 ± 0.0051
N_2O_{uptake}	0.0943 ± 0.0367	0.0774 ± 0.0494	0.1172 ± 0.0347	0.0274 ± 0.0366	N.A.
$N_2O_{reaction}$	0.1618 ± 0.0377	0.5763 ± 0.0797	5.1557 ± 0.0347	0.1628 ± 0.0428	0.1056 ± 0.0090

Scaling Factor *n*	3.4209	6.8773	4.4365	3.8896	N.A.
Dual Volatile Tracers Normalization Method: Removal rate constant, predicted N_2O uptake constant and reaction rate constant (hr^{-1})					
He	0.1263 ± 0.0641	0.1248 ± 0.0241	0.2204 ± 0.0105	0.0418 ± 0.0074	0.0357 ± 0.0062
SF_6	0.0032 ± 0.0016	0.0032 ± 0.0006	0.0056 ± 0.0003	0.0011 ± 0.0002	0.0009 ± 0.0002
N_2O_{uptake}	0.0265 ± 0.0134	0.0262 ± 0.0051	0.0262 ± 0.0051	0.0088 ± 0.0016	0.0075 ± 0.0013
$N_2O_{reaction}$	0.2296 ± 0.0160	0.6275 ± 0.0628	5.2467 ± 0.0051	0.1814 ± 0.0222	0.0981 ± 0.0091
Scaling Factor *n*	8.8507	8.8507	8.6690	8.5080	8.4990

Table 4 *Push-Pull test results based one different normalization methods, a refers to planted with Sagittaria Latifolia, b refers to planted with Schoenoplectus Acutus*

In planted reactors, removal rate constants for dissolved gases increased in the order SF_6 < He < N_2O (***Table 4***). Faster mass transfer of He than SF_6 into root aerenchyma has been shown previously and is due to the higher molecular diffusion coefficient of He for mass transfer from the porewater to the gas-filled root aerenchyma [2][58]. The observed overall removal rate of N_2O is due to the combination of N_2O mass transfer into root aerenchyma and microbial N_2O reduction. During this processes, N_2O production was expected to be negligible as the bioreactor was flushed with feeding solution without NO_3^- for a whole day before push-pull test and no NO_3^- and N_2O was detected before the injection of tracer solution. In the unplanted reactors, removal rates of He and SF_6 were not significantly different than zero. This is consistent with the fact that the only removal mechanism for these biologically nonreactive tracers in the subsurface is mass transfer into root aerenchyma, which are not present in the unplanted reactors. However, N_2O removal rates in the unplanted reactors were significantly greater than zero. This is due to the activity of N_2O-reducing microorganisms in the subsurface of the unplanted reactors. Compared between planted and unplanted groups, the former showed higher denitrification rate, which may be related with the effect of plant root exudates or other residues as organic carbon sources.

I next sought to separate the observed removal rate constant of N_2O into a biological component (i.e., what is the rate of microbial reduction of N_2O to N_2?) vs. a physiochemical

component (i.e., what is the rate of N_2O mass transfer into root aerenchyma?). To our knowledge, the kinetics of N_2O mass transfer into root aerenchyma have not been measured or estimated previously. The root uptake rate constants for N_2O in planted mesocosms were estimated by scaling the root uptake rate constants of He and SF_6, using a scaling relationship based on the molecular diameters of the gases which was developed in Reid et al. [2]. A scaling factor, *n*, was estimated based on the plant uptake rate constant of He and SF_6. And then, based on the relationship between molecular diameters and plant uptake rate constants, the plant uptake rate constant for N_2O was predicted as shown in **Table 4**. However, this estimation method does not work for Unplanted 2 bioreactors, as its removal rate constant of SF_6 being negative and not significantly different from zero.

The scaling factor, *n,* which was initially assumed to be 8.85 ± 1 for the application of dual volatile tracer normalization method [2] and results calculated from dual volatile tracer normalization method support this assumption. For dual volatile tracer normalized results, both planted and unplanted bioreactors' scaling factors are 8.85 ± 1, which support the initial assumption for scaling factor. The scaling factors in unplanted bioreactors are also 8.85 ± 1, which is caused by molecular diffusion acts as the main sink pathway for He and SF_6 in unplanted bioreactors after partitioning equilibrium was achieved and is same processes that dominate plant uptake [74]. Thus, it is reasonable for scaling factor from unplanted bioreactors to be the same as planted bioreactors. However,

for Br^- normalized results, the scaling factors are much smaller, which may indicate the overestimation of uptake rate constants for certain volatile tracers [2].

Combined with the root density and biomass density measured in previous parts, the normalized root uptake rate constants and normalized microbial reduction rate constant were shown in the **Table 5** below. The root uptake rate constant for N_2O was estimated to be intermediate between the directly-measured rate constants for He and SF_6. This is because the molecular diffusion coefficient for N_2O is between those of SF_6 and He.

Tracers	Planted 1[a]	Planted 2[a]	Planted 3[b]	Unplanted 1	Unplanted 2
Bromide Normalization Method: Normalized root uptake *(g soil/mg root · hr)* and microbial reduction rate ($g\ soil/\mu g\ biomass \cdot hr$)					
He	1.1442 ± 0.2396	1.7470 ± 0.2832	0.3508 ± 0.0246	N.A.	N.A.
SF_6	0.2766 ± 0.1636	0.1001 ± 0.1617	0.0557 ± 0.0215	N.A.	N.A.
N_2O_{uptake}	0.6123 ± 0.2383	0.5195 ± 0.3315	0.1584 ± 0.0469	N.A.	N.A.
$N_2O_{reaction}$	0.0637 ± 0.0148	0.3390 ± 0.0469	N.A.	0.0641 ± 0.0169	0.0489 ± 0.0042
Dual Volatile Tracers Normalization Method: Normalized root uptake *(g soil/mg root · hr)* and microbial reduction rate constants ($g\ soil/\mu g\ biomass \cdot hr$)					
He	0.8201 ± 0.4162	0.8376 ± 0.1617	0.2978 ± 0.0142	N.A.	N.A.
SF_6	0.0208 ± 0.0104	0.0208 ± 0.0040	0.0076 ± 0.0004	N.A.	N.A.

N_2O_{uptake}	0.1721 ± 0.0870	0.1758 ± 0.0342	0.0354 ± 0.0069	N.A.	N.A.
$N_2O_{reaction}$	0.0904 ± 0.0063	0.3691 ± 0.0369	N.A.	0.0714 ± 0.0087	0.0454 ± 0.0042

Table 5 *Normalized root uptake rate constant and microbial reduction rate constant*

Though the root uptake rate constant for *Schoenoplectus acutus* was higher than *Sagittaria latifolia*, after normalized by root density, the normalized root uptake rate constants for *Schoenoplectus acutus* were far smaller than those of *Sagittaria latifolia*, which may suggests factors other than root density would also control root uptake rate constant of wetland plants, which will be further discussed in Chapter 4.5

3.3 Model validation test

A cross-validation technique using a holdout method was used to test the accuracy of the scaling method used to estimate root uptake rate constants based on directly-measured rate constant for He and SF_6. I used measurements of ethane during a push-pull test as a testing set. To confirm the chemical and biological inactivity of ethane in the bioreactor subsurface, ethane was also co-injected with Br^-, He, and SF_6 in a push-pull test conducted in an unplanted reactor. The concentrations of ethane and Br^- in unplanted bioreactor is shown in **Figure 14**.

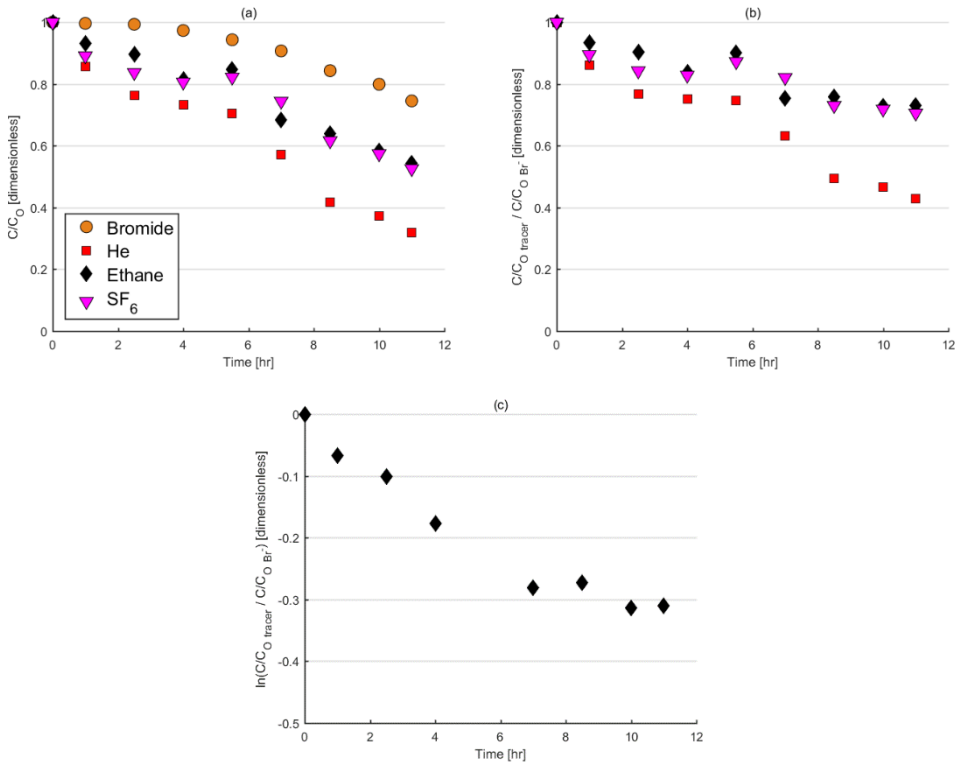


Figure 14 *(a) Tracers' concentration normalized by initial concentrations in planted bioreactor; (b) Bromide normalized tracers' concentrations in unplanted bioreactor; (c) Natural log value of bromide normalized ethane concentration in unplanted bioreactor*

After the first four hours of the push-pull test, Br^- normalized ethane concentrations are stable, indicating the lack of any biological sink for ethane in the unplanted reactors. The decrease of ethane relative to Br in the first four hr may be attributed to ethane partitioning into trapped gas bubbles in the reactor porous media. The stability of ethane in the unplanted subsurface stands in contrast to the behavior of ethane in the planted reactor, where it decreases continuously over the course of the 11hr experiment.

The model validation test is conducted on planted bioreactor and the results are shown in **Figure 15**.

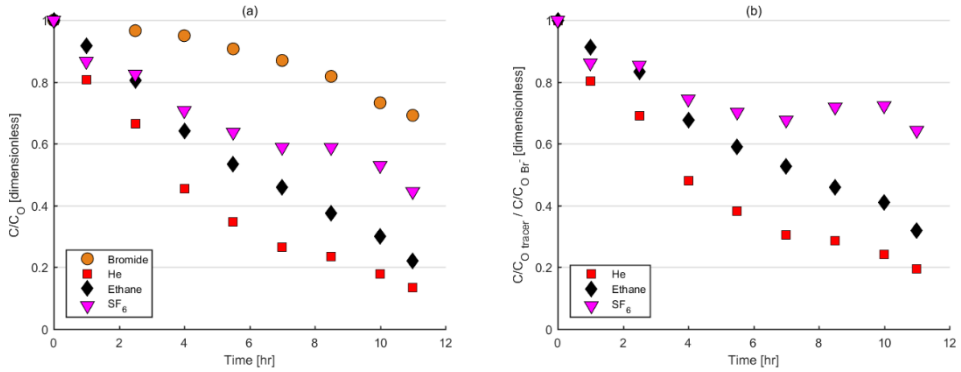


Figure 15 *Model validation test result. (a) Tracer concentrations normalized by their initial concentration; (b) Br^- normalized volatile tracer concentrations*

V_g/V_w ratio and retardation factors for each tracers were calculated for further uses. The results are shown in **Table 6**.

V_g/V_w	1.51 ± 0.81
Tracers	**Retardation Factor**
He	1.18 ± 0.10
Ethane	1.03 ± 0.02
SF_6	1.25 ± 0.13

Table 6 *V_g/V_w ratio and retardation factors*

Volatile tracer concentrations were modeled by both normalization method and both the normalized concentrations and their scaling factors (with and without ethane) are shown in **Figure 16** and **Figure 17** below.

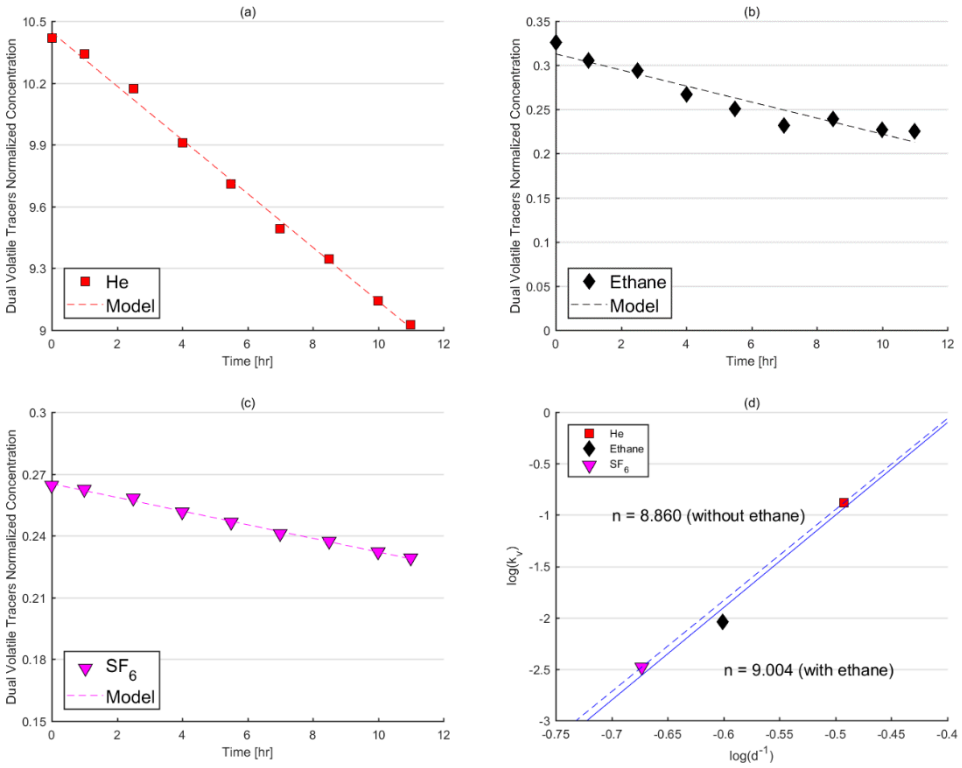


Figure 16 *Dual volatile tracer normalized results; (a)~(c) Dual volatile tracer normalized He, ethane and SF_6; (d) Scaling factor with and without ethane*

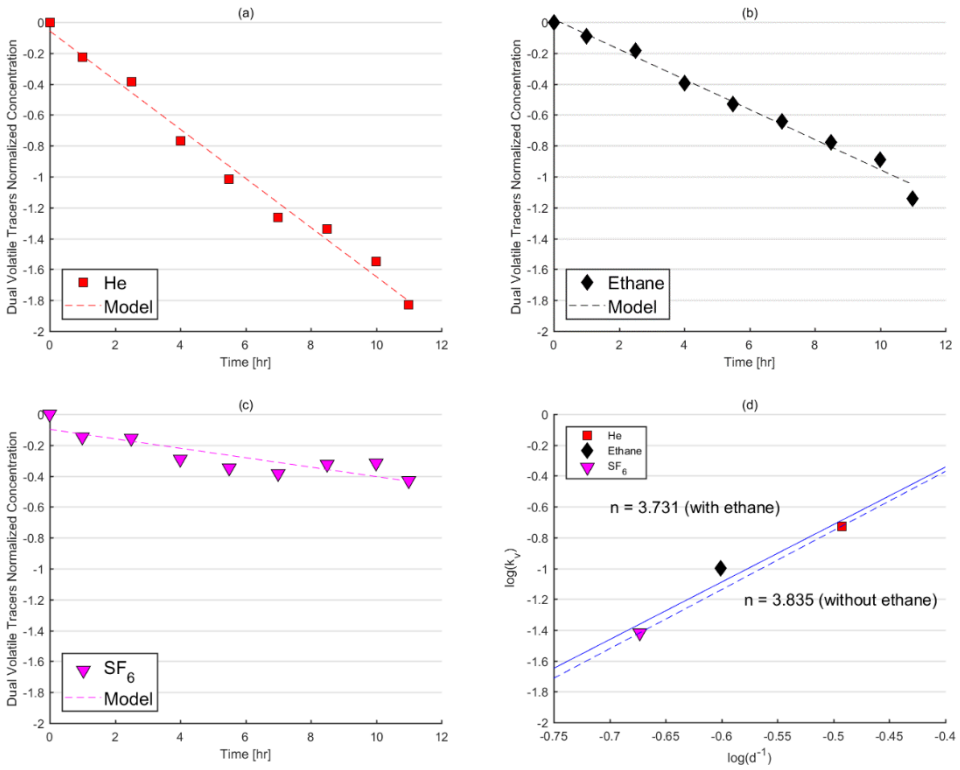


Figure 17 *Bromide normalized results; (a)~(c) Dual volatile tracer normalized He, ethane and SF_6; (d) Scaling factor with and without ethane*

According to **Figure 16**, scaling factors from dual volatile tracer normalization method were still around 8.85 ± 1 with or without ethane, which proves to support the reliability of this normalization method and its assumptions. Results calculated from both normalization methods were shown in **Table 7** below for further prediction of ethane plant root uptake rate constant.

Tracers	Dual Volatile Tracer Normalization	Bromide Normalization
He	0.131 ± 0.003	0.187 ± 0.010
Ethane	0.009 ± 0.001	0.100 ± 0.005
SF_6	0.003 ± 0.000	0.038 ± 0.010

Table 7 *Plant uptake rate constants (hr^{-1}) for different tracers normalized by bromide and dual volatile tracer normalization methods*

Plant root uptake rate constant for ethane was calculated based on both normalization methods and also predicted based on root uptake rate constants of He and scaling factors from two normalization methods to determine which normalization method would lead to the most accurate prediction. The results are shown in **Table 8**.

Scaling Factors	Dual Volatile Tracer Normalization	Bromide Normalization
Bromide Normalization (n=3.835)	0.050 ± 0.001	0.072 ± 0.004
Dual Volatile Tracer Normalization (n = 8.860)	0.014 ± 0.000	0.021 ± 0.001

Calculation Results	0.009 ± 0.001	0.100 ± 0.005

Table 8 *Ethane plant uptake rate prediction*

Though predictions were made for different normalization methods, all the estimation were based models which have their assumptions and limitations associated with them. It is hard to judge which normalization method and scaling factor works better for root uptake rate constant prediction simply based on this only. Thus, another judgment method was used, which is to simulate ethane concentration profiles based on prediction of ethane root uptake rate constant and two normalization methods. The one with the least residual sum of squares of simulation (RSS) value was supposed to be the best-fit prediction method.

For dual volatile tracer normalization model, ethane concentration was simulated by using a root uptake rate constant that was scaled from the rate constants directly measured with He or SF_6, according to *Equation (13),* which is derived from *Equation (9)*.

$$C_{ethane}^{t} = e^{\left(\frac{R_i ln C_i^t + \left(1 - \left(\frac{\sigma_i}{\sigma_{ethane}}\right)^{-8.85}(-k_v t + b)\right)}{R_{ethane}}\right)}$$

Equation (13)

Where,

$C_{ethane}^{t} =$ Aqueous concentration of ethane at time point *t* (mg/L)

$C_i^t =$ Aqueous concentration of volatile tracer *i* at time point *t* (mg/L)

$R_{ethane} =$ Retardation factor of ethane

$R_i =$ Retardation factor of volatile tracer i

$\sigma_{ethane} =$ Molecular diameter of ethane

$\sigma_i =$ Molecular diameter of volatile tracer i

$k_v =$ Estimated root uptake rate constant of ethane (hr^{-1})

$b =$ Constant estimated based on initial ethane concentration and *Equation (13)*

While for Br^- normalized data, ethane concentrations can be simulated by *Equation (14)*, which is derived from *Equation (5)* and *(7)*.

$$C_{ethane}^t = \frac{C_{ethane}^o C_{Br^-}^t}{C_{Br^-}^o} e^{-k_v t}$$

Equation (13)

Where,

$C_{ethane}^o =$ Aqueous concentration of ethane in tracer solution (mg/L)

$C_{Br^-}^o =$ Bromide concentration in tracer solution (mg/L)

$C_{Br^-}^t =$ Bromide concentration at time point *t* (mg/L)

To judge which model prediction has better fit the measured concentrations, the discrepancy between measured ethane concentration and model estimation simulation

was measure by RSS value and the simulation with both models was shown in **Figure 18**.

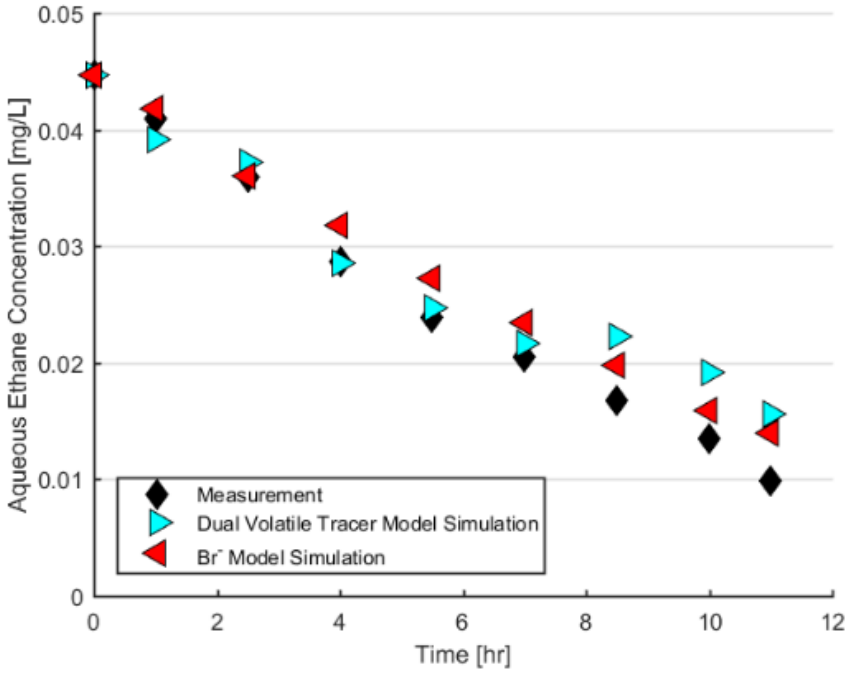


Figure 18 *Ethane concentration simulation with both normalization models*

Dual volatile tracer normalization model proves to be better simulating ethane concentrations from 0 to 7 hours after injection of tracer solution with its RSS value of 6.912×10^{-6} compared to Br^- normalization model's RSS value of 3.079×10^{-5}. However, in the final 4 h of the experiment, the simulation overestimated the measured concentration by approximately 50% due to the concentration of the other volatile tracer used as reference being so low that errors become easily magnified. Thus, throughout the whole processes, Br^- normalization model has lower RSS value (6.279×10^{-5}) compared to dual volatile tracer normalization model (1.028×10^{-4}).

3.4 Isotope test

To further test the effect of plants on N_2O yield ($N-N_2O/N-N_2O+N-N_2$) in constructed wetland, a push-pull test with ^{15}N-N_2O was conducted on both planted and unplanted bioreactors. However, it is hard to tell the N_2O yield in planted bioreactor as its $^{15}N_2$ concentration has a decreasing trend, thus, only the N_2O yield for unplanted bioreactor was calculated. The isotope test results of dissolved tracers in porewater and gas phase tracers in flux chambers are listed in **Table 9** and **Table 10** below

Time (hr)	**Planted $^{15}N_2O$ (mg/L)**	**Planted $^{15}N_2$ (mg/L)**	**Unplanted $^{15}N_2O$ (mg/L)**	**Unplanted $^{15}N_2$ (mg/L)**
0	0.068	9.470	0.068	9.470
1	0.041	14.723	0.059	10.466
2	0.023	9.810	0.037	10.526
4	0.027	10.683	0.027	10.525
6	0.008	9.776	0.025	9.547
11	0.001	12.164	0.001	10.840
11	0.003	11.520		

Table 9 *Isotope Test results of porewater samples*

Time (hr)	Planted $^{15}N_2O$ (mg/L)	Planted $^{15}N_2$ (mg/L)	Unplanted $^{15}N_2O$ (mg/L)	Unplanted $^{15}N_2$ (mg/L)
0	2.032×10^{-6}	3.535	1.692×10^{-6}	3.138
1	5.722×10^{-5}	3.535	5.727×10^{-6}	3.007
2		3.227	3.337×10^{-5}	3.193
4	1.774×10^{-5}	3.136	1.666×10^{-5}	3.193
6	1.754×10^{-5}	3.208	1.340×10^{-5}	3.302
11	9.220×10^{-6}	3.256	3.051×10^{-6}	3.314
11	7.605×10^{-6}	3.106		

Table 10 *Isotope Test results of flux chamber samples*

Based on **Table 10**, the average N_2O yield for unplanted bioreactors is $(1.48 \pm 1.63) \times 10^{-4}$ based on *Equation (14) below*.

$$N_2O\ Yield = \frac{^{15}N_2O - N}{^{15}N_2O - N + ^{15}N_2 - N}$$

Equation (14)

To further compare the removal rate of N_2O between planted and unplanted bioreactors during isotope test, the Br^- normalized N_2O concentrations are shown in **Figure 19** below.

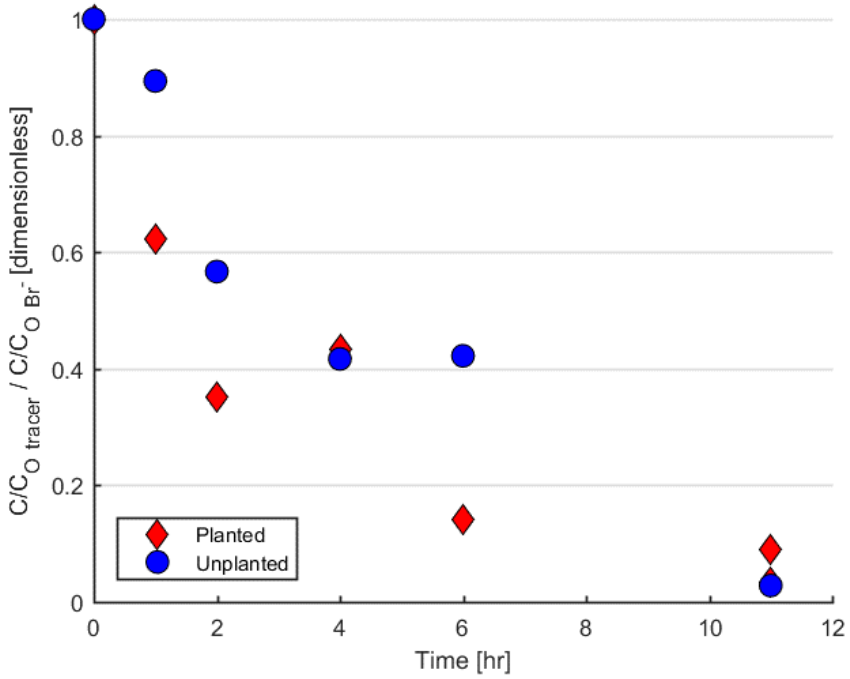


Figure 19 *Br^- normalized $^{15}N_2O$ concentrations*

According to **Figure 19**, planted bioreactor still shows faster removal rate compared with unplanted bioreactor after Br^- normalization.

Chapter 4 Discussion

4.1 Bioreactors monitoring

The reduction of NO_3^- and detection of N_2O in the porewater of both planted and unplanted bioreactors indicated the presence of NO_3^- reduction in both groups, which was used as evidence to support the existence of denitrifiers in both planted and unplanted groups. Compared between planted and unplanted bioreactors, planted bioreactors showed more NO_3^- removal, which agreed with results from previous research that the presence of macrophyte significantly increases NO_3^- removal [92]. Reasons for such phenomenon were reported to be addition of extra carbon produced by plants to porewater as well as plant uptake and assimilation of NO_3^- [92]. However, combined with the DOC profile and biomass density data, the differences between DOC and biomass density in planted and unplanted groups did not vary significantly, which was in contrast to the claim by Lin et al. that high NO_3^- removal in planted wetland was related to organic carbon produced and plant uptake only contributes to 4~11% of total nitrogen removed while the rest part were due to denitrification [92]. In this case, since DOC and biomass density does not vary a lot, vegetation uptake may be a more significant sink pathway for NO_3^-.

In terms of other products from NO_3^- reduction, which includes denitrification products and dissimilatory reduction of NO_3^- to NH_4^+, NO_2^- and NH_4^+ were reported to be

below detection limits of analytical instruments. Though NO was not measured, it was reasonable to assume low or negligible levels of NO, as NO production rate was reported to be far lower than N_2O production rate during denitrification from previous research, which would suggest NO produced would be readily converted into N_2O and no accumulation of NO would be formed to make it detectable [93][24]. Moreover, it was also reasonable for negligible level of NH_4^+ as bioreactors being submerged all the time during the experiments, which makes an anaerobic environment that favors denitrification over dissimilatory reduction of NO_3^- [24]. In terms of NO_2^-, the low concentrations of it may be related to relatively low amount of NO_3^- being converted between each depth, the depth between each sampling port being long enough for NO_2^- to be reduced into NO, N_2O and N_2 and the fact that denitrification rate of NO_2^- being 1.5 to 2 times higher than NO_3^- [94], which could ALSO be supported by the NO_3^- and N_2O profile in **Figure 5** and **Figure 6**.

In terms of N_2O, which was of our research interest, planted bioreactors showed higher peak concentrations compared to unplanted bioreactors, which can be positively related to two factors given the assumption that DOC was enough for denitrification during experiments and NO_2^- and NO were readily converted into N_2O: amount of NO_3^- removal and disturbance of oxygen introduced by root transport.

According to the NO_3^- profile shown in **Figure 5**, in unplanted bioreactors, NO_3^- removal mainly happens in the 30~40 cm below soil surface, however the N_2O production

peaked at 10 cm below soil surface, which was hypothesized to be caused by oxygen diffusion from atmosphere that inhibit N_2O reduction and though more N_2O would be produced in 30~40 cm below soil surface for unplanted bioreactors as indicated by NO_3^- removal, the lack of oxygen made the conversion from N_2O to N_2 not being inhibited and thus less N_2O was measured from 30~40 cm below soil surface.

While in planted bioreactors, NO_3^- removal mainly concentrated in 0~10 cm and 30~40 cm below soil surface, however, N_2O remained relatively high from 10 to 30 cm below soil surface. If NO_3^- removal played the dominant role in determining N_2O concentration, it was unlikely that N_2O at 40 cm in planted bioreactors were lower than it at 30 cm, since more NO_3^- were removal through 30~40 cm soil below surface compared with 20~30 cm below surface, thus, the NO_3^- removal would not be the most significant factor in determining N_2O concentration changes. So the hypothesis become that the presence of oxygen could play the determining role for N_2O concentration. The presence of wetland plants transport oxygen from atmosphere to submerged soil, which affected 0~30 cm soil below surface. Thus, N_2O concentration remains relatively high in 10~30 cm part and from 30~40 cm, the transport of oxygen was reduced and thus the accumulation of N_2O started to decrease.

Another evidence could also support the effect of wetland plants on N_2O production and consumption is related to introducing oxygen into bioreactors is that the N_2O yield

(**Figure**) in both planted and unplanted bioreactors. Between planted and unplanted bioreactors, N_2O yield does vary between two groups, especially for soil more than 20 cm below surface, where planted group shows positive N_2O yield compared to unplanted group, of which N_2O yield is positive in surface layer of soil and changes into negative when it comes to deeper layer of it. That would also imply that O_2 released from ROL plays a role in determining N_2O concentrations. Otherwise, if NO_3^- dominate this processes, N_2O yield would be almost the same for both planted and unplanted bioreactors.

So, our hypothesis for oxygen transport and inhibition being the most significant factor in determining N_2O profile could explain the measured N_2O concentrations.

Though this hypothesis could explain the N_2O concentrations measured, a direct measurement of oxygen concentration or oxygen flux to each sampling port depth would be necessary to support it, which was not conducted in this experiment as measurement techniques in our lab cannot achieve in situ oxygen level measurement in wetland soil. Further experiments could be conducted to support this hypothesis.

The DOC profile was measured to not only detect the production of organic carbon from plant root decay but also to support the assumption that DOC concentrations were high enough to not be the rate limiting factor for denitrification processes in regular operation of the bioreactors and push-pull tests. During regular operation of bioreactors, the concentrations of DOC remains relatively constant along the depth of bioreactors,

which indicated that the consumption of dissolved organic carbon due to denitrification was offset by DOC sources within the mesocosm. Also compared with the N_2O level injected for push-pull test, the DOC levels were far higher than it, which could also support the assumption that DOC concentrations were not the rate limiting factor in denitrification. Another supporting evidence for this assumption is that the DOC concentrations in feeding solution were at least twice of the median of half saturation constant of DOC for NO_3^- reduction [89], which also convince us that DOC won't be the rate limiting factor in denitrification.

4.2 Push-Pull test results interpretation

As shown in **Figure 10**, the tracers' concentration changes were relatively small in the first hour compared with **Figure S4 A** in Appendices, which illustrating the situation in an unplanted mesocosm when significant volume of bubbles affecting volatile tracers' concentrations (more than 40% tracer concentrations removed from the aqueous phase) after the first hour of tracer solution injection due to gas partitioning into bubbles. The relatively small reduction of tracer concentrations in **Figure 10** suggests the bubble volume was very small, which was further proved by the small V_g/V_w ratio calculated. The small bubble volume may be caused by the preparation procedure that bioreactors were first

flushed with CO_2 to increase the solubility of the pore gas and then slowly filled with water from the bottom.

In **Figure 11**, the Br^- normalized concentrations of He and SF_6 showed a trend of decreasing and then increasing back, which should be attributed to the volatile tracers in bubbles that partitioned back into liquid phase as the concentration of dissolved tracer decreases due to dispersion. This leads to an increase in the Br-normalized concentrations, since there is no source of Br^- in immobile bubble phases to "buffer" the aqueous phase concentration. This trend is also in agreement with **Figure S4 B**.

The tracer removal rate constants, k_v, were estimated from push-pull test by bromide normalization method and dual volatile tracer normalization method were supposed to be root uptake rate constants for He and SF_6 and a combination of root uptake and microbial reduction rate constants for N_2O.

For unplanted bioreactors, after normalization, nonreactive tracers will first decrease due to hydrodynamic mixing and partitioning and then remains relatively constant ($k_v \approx 0$) after partitioning equilibrium is achieved, as the only effect on solute concentrations is hydrodynamic dispersion, and this is accounted for by Br^- normalization. The reactive tracer, N_2O, in unplanted bioreactors was primarily affected by microbial reduction. Since the solubility of N_2O is relatively high ($2.875 \times 10^{-2}\, mol/L \cdot atm\ \ at$ 20℃) it is only negligibly affected by the presence of trapped gas bubbles. Because of this, the

concentration decreasing of N_2O during push-pull test in unplanted bioreactors performed linearly during push-pull test, reflecting the microbial reduction processes of N_2O [68].

In planted bioreactors, after normalized with another tracer, nonreactive tracers will first decrease fast due to hydrodynamic mixing, partitioning into trapped bubbles, and gas transfer into root aerenchyma. Once the partitioning equilibrium is achieved, the decreasing rates of nonreactive tracers' concentrations are smaller as only plant uptake contributes to its decreasing. In addition, tracer losses due to gas transfer into roots are partly offset by gas partitioning from trapped bubbles back into the aqueous phase to maintain equilibrium. This is why the k_v values are multiplied by the retardation factor R (*Equation (3)*), since apparent removal rates are in fact an underestimate. In terms of reactive tracer N_2O in planted bioreactors, due to the same reason in unplanted bioreactors, its decreasing trend still performed linearly, but reflect the combination of microbial reduction and root uptake processes of N_2O.

We also tried to separate the kinetic constant measurement of plant uptake of N_2O from total removal rate constant by adding acetylene into tracer solution for push-pull test to make its concentration higher than 0.1 atm, which is said to be able to achieve complete inhibition of N_2O reduction [95]. However, this added more disturbance to plant uptake measurements as its preparation procedure requires to add a large volume of over-pressurized acetylene solution into tracer solution, which was found to affect the behavior

of other gases.

Microbial N_2O reduction rate constants were faster in the planted reactors relative to the unplanted reactors, despite the fact that microbial biomass density was not different between these reactors. To explain this phenomena, further research is still in need.

The dual volatile tracer normalization method showed smaller uncertainties than Br- normalization methods, especially for volatile tracers with slow plant uptake rate, such as SF_6. Though mentioned in Chapter 2.2.3 that Br^- normalized results tend to underestimate the root uptake rate constants due to effect of tracer gas partition back from bubbles, Br^- normalized results showed much higher values compared with dual volatile tracer normalization method, which was assumed to be more accurate due to its performance in model validation test. This may be attributed to the fact that the root uptake rate constant of plant used to be around 90% smaller than the plant used by previous research [2], which add to the significance of errors introduced by partitioning effect to Br^- normalized results.

As shown in the Br^- normalized push-pull test results for planted bioreactors (**Table 4**), the estimated uptake rate constant for SF_6 is more than 10 times higher than the estimated results from dual volatile tracer method. For volatile tracers that have faster uptake rate, such as He, the uptake rate constants calculated from two normalization methods do not vary a lot. Since the uptake rate constant for He itself is relatively large, partitioning effects on He uptake rate constant is relatively small, thus the error does not

count much in it. This indicate dual volatile tracer normalization method to be a good alternative for Br^- normalization method when dealing with gas tracers with slow uptake rate.

In terms of scaling factors from both normalization method, dual volatile tracer normalized results strictly follow the empirical value of 8.85 ± 1, while Br^- normalized results showed much lower scaling factors, several reasons could attribute to this. First of all, overestimation of Br^- normalized plant uptake rate constant, especially for SF_6 could be one of the reasons cause low scaling factor values. Second, though the empirical value of scaling factor is 8.85 ± 1, it is not universal for all situations, root density as well as plant uptake rate constant would also contribute to the value of scaling factors as supported by Reid et. al [58], low root density and plant uptake rate constant would lead to scaling factors being smaller than empirical 8.85 ± 1 values. The root densities and plant uptake rate constants in all three planted bioreactors used are much lower compared to that of other planted sampling locations used in previous research, where both Br^- and dual volatile tracer normalized results could make a scaling factor of 8.85 ± 1 [2][58].

4.3 Reliability of dual volatile tracer normalization method

The tracer uptake rate constants calculated from push-pull test by dual volatile tracers

normalization method further proved the dependence of root uptake rate constants on reciprocal of molecular diameters as claimed by Reid et al. [2]. The *n* values estimated here match the values determined previously of 8.85 ± 1 and could serve as an evidence for the assumption presented by Reid et al. that root uptake rate constants have a dependence on mass diffusion coefficients that has the similar relationship to reciprocal of molecular diameters [2].

To further assess the reliability of using dual volatile tracer normalization method to predict the root uptake rate constant for reactive tracers, such as N_2O, the model validation test was conducted. Simulated ethane concentrations were predicted based on initial ethane concentration, predicted ethane root uptake rate constant and concentration profile of He or SF_6 and compared with measured ethane concentrations for both Br^- and dual volatile tracer normalization method. Though lowest RSS value of simulation is found with for Br^- normalization method, which is as low as 6.279×10^{-5}, the RSS value of simulation from dual volatile tracer normalization method is similar to that value. Moreover, when the concentrations of other volatile tracers being not too low, dual volatile tracer normalization model performs much better compared to Br^- normalization model. This still proves the simulation and predicted root uptake rate constant for dual volatile tracer normalization method are reliable.

Reasons for dual volatile tracer method to work better in early stage is because 1) in

early stage, gases are still in the processes of equilibrating with bubbles, which is not accounted by Br^- normalization method. But when it comes to later time points, the Br^- method becomes more advantageous in part because bubble partitioning processes are at equilibrium; 2) the simulation was based on the concentration profile of other tracers, (i.e. He), once the reference tracer was decreased to a relatively low concentration in later phase, there may be more experimental error which introduces uncertainty into the scaling estimates and is propagated to the ethane through the scaling models, causing inaccurate predictions. However, given these limits, dual volatile tracer normalization model still serves as a good alternative for Br^- normalization model, especially for situations where tracer gases' concentrations being not too low.

The accuracy of model validation test to predict ethane concentrations further added to the reliability of using this method to predict N_2O root uptake rate constant, since ethane and N_2O shared the similarity that their molecular diameters being between He and SF_6. Though both normalization models prove to be reliable based on their accuracy of simulation, we choose dual volatile tracer normalization method for predicting N_2O uptake rate constant as it introduces less uncertainties compared to Br^- normalization model.

4.4 Effects of the presence of wetland plants on N_2O removal

Based on the empirical relationships, the root uptake rate constants for N_2O were predicted for planted bioreactors and the microbial reduction rate constants were also calculated by using overall N_2O removal rate minus predicted root uptake rate constant for N_2O. Planted bioreactors showed higher N_2O removal ability compared with unplanted bioreactors, which indicate the presence of plants can contribute to faster N_2O removal rate. Though plants decay can serve as organic carbon source for denitrifiers, DOC concentrations and biomass density did not vary significantly between planted and unplanted bioreactors, it is unlikely that the presence of plants augment the biomass density that lead to faster N_2O removal rate. A hypothesis would be the presence of plants alters the structure of biomass community in planted bioreactors, however, no directly evidence could prove this hypothesis in this experiment. Further research would be needed to analyze the biomass community composition before and after being planted by using qPCR techniques.

In terms of wetland plant's role as transport pathway for N_2O to be emitted into atmosphere, based on dual volatile tracer normalized results, the predicted root uptake rate constant shows that root uptake of N_2O only takes up 2.71%~17.16% of overall N_2O removal for *Sagittaria latifolia* planted bioreactors and 0.49% for *Schoenoplectus acutus* planted bioreactor due the higher initial organic carbon source inside bioreactors,

enzymatic N2O reduction is the dominant N_2O removal pathway in both planted and unplanted bioreactors. Compared with methane, of which plant uptake serves as an important emission pathway and molecular diameter is about the same as N_2O, N_2O cannot remain stable in mostly anaerobic wetland soil and microbial reduction becomes another sink for N_2O, while methane is stable in anaerobic environment and plant transport becomes the only important loss mechanism for it [58]. Rates of microbial N_2O reduction are faster than root uptake, so root uptake acts as a relatively small sink for N_2O that is produced in wetland soils and if the DOC level in feeding solution was further increased, it is predictable that the contribution of root uptake for N_2O removal would be further decreased.

The calculated fraction of N_2O being removed by plant uptake in this experiment may not be generally applied to other situations, as there are too many factors that will affect plant uptake and microbial reduction rate constant, such as root density, soil porosity, biomass density and types of denitrifiers. Thus, directly applying results to other situations may lead to inaccurate predictions. However, this experiment could provide a framework for solving such problems, which could be generally applied for solving such problems.

4.5 Root uptake rate comparison between different plants

Compared the push-pull test results from *Sagittaria latifolia* and *Schoenoplectus acutus*, though *Schoenoplectus acutus* showed higher volatile tracer uptake rate constant, after normalized by root density, it turns out to be much lower than *Sagittaria latifolia*, which indicating root density is not the only factor that determining the ability of plant root uptake rate for volatile tracers. It is worth noting that though with relative low root densities, *Sagittaria latifolia* showed much higher radial oxygen loss compared with *Scirpus atrovirens* and *Scirpus fluviatilis*, which are of the same genus as *Schoenoplectus acutus* [55].

Our hypothesis is that the root uptake rate of dissolved gases is related to radial oxygen loss since both processes make use of the aerenchyma as a low resistance pathway to transport gas. Thus, plant factors affecting radial oxygen loss may also affect root uptake rate. Thus, plant structural characteristics like aerenchyma porosity, which was reported to be positively related to transport of oxygen and other gases in plants [38], permeability of the root epidermis through which gases diffuse between aerenchyma and soil [37], and the ability of plant to generate pressure gradient by either generating positive pressure in living shoots or reducing pressure in dead culms that cause venturi-induced suction [96][97][98]. However, for ROL, a positive pressure in above ground part is necessary for active transport of oxygen but for dissolved gases in wetland to escape from it, they required a

low pressure in above ground part for them to escape from soil by active transport, which is against the functions of those plant tissues that generate pressure gradient. However, according to previous studies, for dissolved gases in wetland, such as methane, active transport is not the main emission pathway of them, molecular diffusion or effusion turns out to be the main emission pathway for them [74][73]. Thus, the porosity of plants would be an interesting research focus to be investigated for determining root uptake rate constants.

Another factor that could also contributed various root uptake rate constant for different gases is the permeability of root surface. Since root wall permeability is one of the determination factors for oxygen diffusion rate through root surface and the fact that molecular diffusion and effusion act as the main gas transport pathway from soil to atmosphere, it is reasonable to hypothesize that root surface permeability could also affect the diffusion processes of dissolved gas from submerged soil through root surface [99]. The permeability of plants varies from not only different types of plants but also the distance from root tips, where the highest permeability of root surface would be observed [99]. Thus, the difference in permeability between *Sagittaria latifolia* and *Schoenoplectus acutus*, such as variations in permeability at tip of root, permeability changes along the distance from the tip of root or even the depth and amount of root tips of root in each bioreactors could cause the root uptake rate constant for *Sagittaria latifolia* being higher

after normalized by root density. The root structural properties that control root-mediated exchanges of O_2 from atmosphere to soil, or CH_4 and N_2O from soil to atmosphere, are thus complex and difficult to parameterize into models. The root uptake rates measured via push-pull tests in this study provide an aggregated measurement of root-mediated gas exchange that integrates all of these factors.

To make decision for wetland plants that emitted the least N_2O into atmosphere, these traits of plants may be used to better understand the root's N_2O uptake ability of plant and further build up a more environmental-friendly constructed wetland. However, future work is still necessary to quantify the effect of each factor and set up mathematical models for root uptake ability of plant.

4.6 Comparison of isotope test results between planted and unplanted bioreactors

N_2O yield from unplanted bioreactors was estimated by isotope test, however, the result shows no significantly different from zero. However, based on the atom percentage of both planted and unplanted flux chambers, $^{15}N_2O$ has been released into both flux chambers (See Appendix) by various transport pathways. Though originally thought tracers being unable to reach surface water, there may still be a little N_2O reaches surface (below detection limit) and diffuse into atmosphere. Also, though being unable to tell the N_2O

yield from planted bioreactor and compare it with unplanted bioreactor, we can still, based on atom percentage of $^{15}N_2O$ in flux chamber, tell that planted reactor emitted more $^{15}N_2O$ into flux chamber by the effect of plant uptake. However, the N_2O emission was really small compared with the total amount of $^{15}N_2O$ injected into bioreactors, which could also support our conclusion that the contribution of plant root uptake to overall N_2O removal and emission is limited.

The different trend of $^{15}N_2$ concentration in flux chamber between planted (decreasing) and unplanted (increasing) bioreactors may also attributed to the effect of plant as gas transport pathway. Since the concentration of N_2 in tracer solution may be lower than air saturated concentration of N_2 due to the tracer solution preparation procedure. With plant as gas pathway, N_2 in flux chamber would be driven by concentration gradient to enter submerged soil and thus cause a decreasing trend in $^{15}N_2O$ in flux chamber.

Another interesting point about the isotope test results is that the N_2O yield calculated by unplanted bioreactors is far lower than the N_2O yield ratio calculated by Schlesinger et al. [27]. Reasons attribute to this are hypothesized to be 1) Lack of plant as gas transport pathway; 2) Different nitrogen source species; 3) Different incubation environment. For previous research, which is based on natural or N-fertilized conditions, the nitrogen source for denitrification would be different in composition and varied in amount compared to the N_2O used in tracer solution. Moreover, as shown by push-pull tests results of different

plants, factors like type of plants or internal organic carbon source could also affect the denitrification rate and further affect N_2O yield.

Chapter 5 Conclusions

- The presence of wetland plant will increase NO_3^- removal, N_2O yield and N_2O reduction rate constant in submerged ecosystem.
- Given excess NO_3^- and DOC, N_2O concentrations in planted wetland environment are mainly affected by oxygen introduced by ROL from wetland plant root.
- When plant uptake rate constants are relatively small, Br^- normalization method tends to overestimate uptake rate constants, especially for tracers with slow uptake rate. Dual volatile tracer normalization method proves to be a good alternative for Br^- normalization when dealing with slow plant uptake rate situations. However, it becomes more easily affected by errors in reference tracer gases when reference gas's concentrations being low.
- Overall N_2O removal was calculated from push-pull test results and plant uptake rate constant for N_2O was predicted based on that of He and SF_6. Plant uptake proves to only be a small sink for N_2O as microbial reduction rate is much faster.
- Both plant uptake rate and microbial reduction rate play roles in determining fraction of N_2O being removed by plant uptake pathway. However, in our experiments, plant uptake rate constant does not vary a lot between different types

of plants, microbial reduction matters much in determining the fraction of N_2O emitted by plant uptake pathway. In reality, it may hard to adjust plant uptake rate constant, but by increasing microbial reduction rate, such as providing organic carbon, it would be a more effective pathway to decrease N_2O emission.

- Though *Schoenoplectus acutus* showed faster uptake rate constant for dissolved tracers compared to *Sagittaria latifolia*, after normalized by dry root mass, *Sagittaria latifolia* performs faster uptake rate constant. This indicate factors other than root density, such as aerenchyma porosity and gas permeability of root surface, will also affect plant uptake rate for dissolved gases.

Chapter 6 Future Work

All data presented in this thesis demonstrate that plant uptake serves only as a small sink for dissolved N_2O in subsurface environment. However, direct evidence is still necessary for supporting this hypothesis, such as N_2O flux or measuring N_2O plant uptake rate constant by separating it from microbial reduction.

Though not varied in biomass density and DOC concentrations, planted bioreactors still showed faster N_2O reduction ability compared to unplanted bioreactors, reasons behind this phenomenon would also be an interesting direction to discover. Whether it is due to certain chemicals from plant contributes to faster N_2O removal chemically or gene expression of denitrifiers was affected by the presence of plant that cause faster removal rate, or maybe some reasons still unknown. Understanding this could also help reducing N_2O emission from constructed wetland.

Finally, in terms of plant uptake rate normalized by root density, it still varies a lot between different plant species. Plant structural traits that will affect its uptake rate constant also worth further research to investigate what traits would affect uptake rate constants, to what extent do they matter and how they functioned to affect plant uptake rate constants.

Appendices

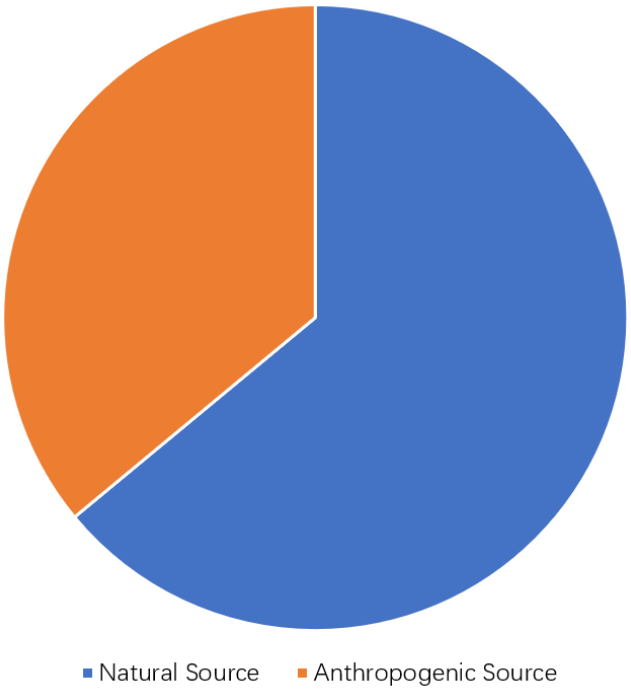


Figure S1 *N_2O emissions from all sources to the atmosphere* [23]

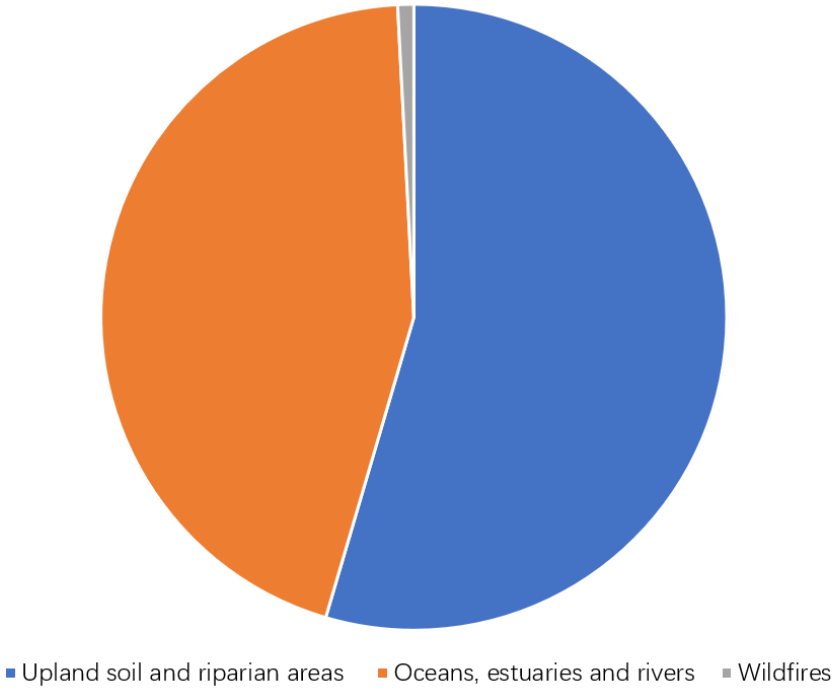


Figure S2 *N_2O emission from natural sources* [23]

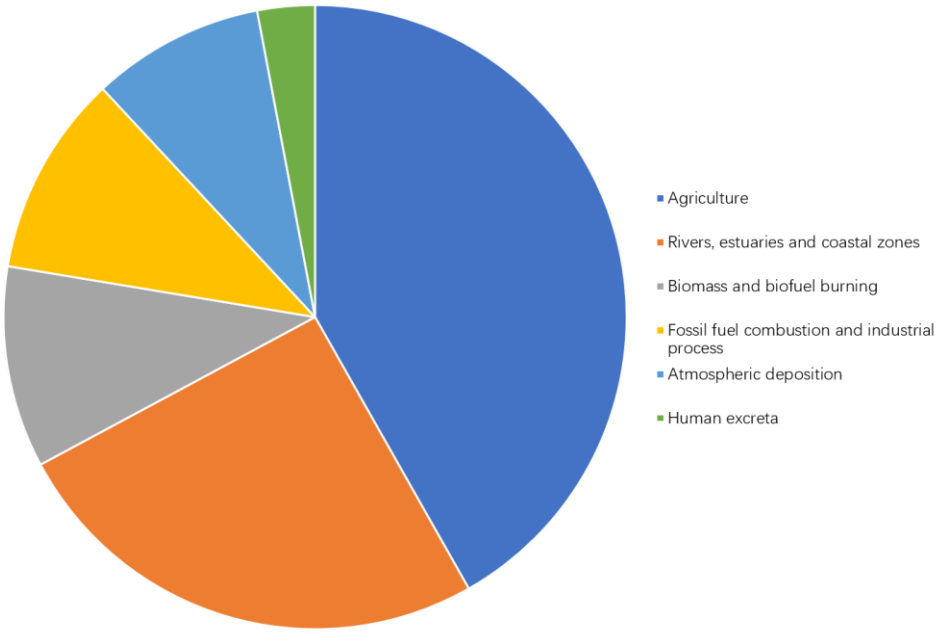


Figure S3 *N_2O emission from anthropogenic sources* [23]

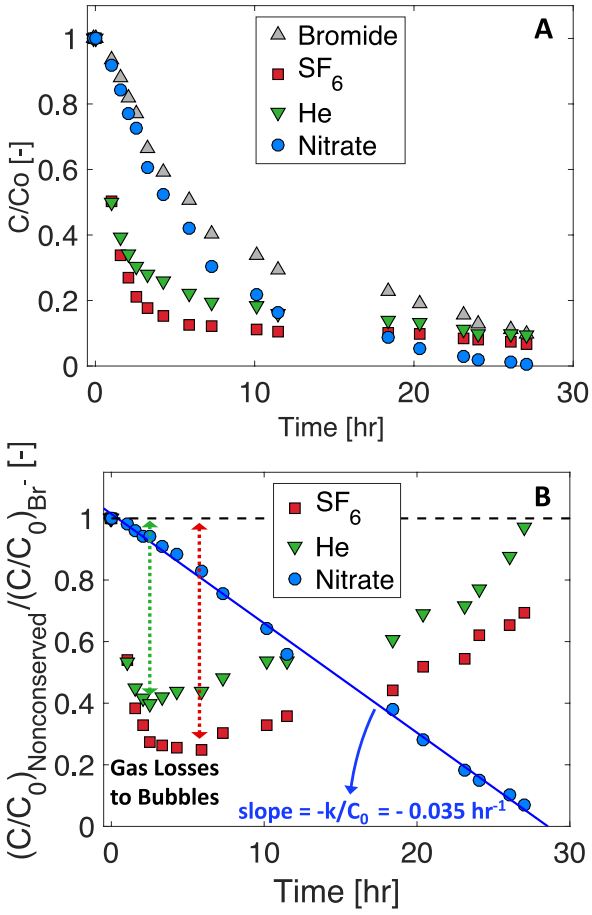


Figure S4 *Another push-pull test results in unplanted bioreactors from unpublished work of*

Matthew Reid

Time	Br^- concentration (mg/L)	He concentration (mg/L)	N_2O concentration (mg/L)	SF_6 concentration (mg/L)
0	162.38	0.5675	0.4629	8.9E-05
0.5	162.30	0.4792	0.4317	8.73E-05
1	162.83	0.4266	0.3856	8.61E-05
2	158.53	0.3105	0.2509	7.94E-05
3	152.84	0.2224	0.1924	7.54E-05
4	145.02	0.1506	0.1489	6.54E-05
7	126.52	0.0809	0.0625	5.32E-05
9	118.89	0.0524	0.0271	4.39E-05
11	103.31	0.0389	0.0164	3.01E-05

Table S1 *Push-Pull test raw data Planted 1*

Time	Br^- concentration (mg/L)	He concentration (mg/L)	N_2O concentration (mg/L)	SF_6 concentration (mg/L)
0	161.56	0.4581	0.3686	0.000134
0.5	160.24	0.3852	0.3076	0.000128
1	159.78	0.2656	0.2483	0.000112
2	156.81	0.2029	0.1382	0.000111
3	152.95	0.1357	0.0508	0.000104
4	148.87	0.0894	0.0244	8.86E-05
7	128.13	0.0525	0.0000	7.63E-05
9	114.61	0.0335	0.0000	5.68E-05
11	100.99	0.0256	0.0000	4.96E-05

Table S2 *Push-Pull test raw data Planted 2*

Time	Br^- concentration (mg/L)	He concentration (mg/L)	N_2O concentration (mg/L)	SF_6 concentration (mg/L)
0	162.38	0.5675	0.4629	8.9E-05
0.5	161.24	0.5119	0.4600	8.16E-05
1	161.52	0.4348	0.4252	7.89E-05
2	159.60	0.4632	0.4080	7.66E-05
3	157.81	0.4195	0.3657	7.05E-05
4	155.94	0.3933	0.2910	6.81E-05
6	151.38	0.3363	0.2022	6E-05
9	141.61	0.2676	0.0967	5.96E-05
11	124.05	0.2517	0.0452	5.61E-05

Table S3 *Push-Pull test raw data Unplanted 1*

Time	Br^- concentration (mg/L)	He concentration (mg/L)	N_2O concentration (mg/L)	SF_6 concentration (mg/L)
0	175.32	0.6276	0.5577	0.000125
0.5	171.22	0.5486	0.5092	0.000109
1	169.85	0.4698	0.4771	9.36E-05
2	168.96	0.4915	0.4376	9.24E-05
3	167.10	0.4684	0.4318	9.19E-05
4	154.98	0.4378	0.3765	8.94E-05
6	155.25	0.3426	0.2924	7.87E-05
8	144.98	0.3004	0.2077	7.04E-05
11	132.23	0.2666	0.1218	6.82E-05

Table S4 *Push-Pull test raw data Unplanted 2*

Time	Br^- concentration (mg/L)	He concentration (mg/L)	N_2O concentration (mg/L)	SF_6 concentration (mg/L)
0	185.85	0.6057	0.0447	0.000175
1	178.50	0.4889	0.0410	0.000151
2.5	175.53	0.4033	0.0360	0.000144
4	167.65	0.2758	0.0287	0.000124
5.5	160.72	0.2096	0.0239	0.000111
7	151.19	0.1611	0.0205	0.000103
8.5	135.39	0.1424	0.0168	0.000103
10	127.89	0.1077	0.0135	9.25E-05
11	185.85	0.0815	0.0099	7.78E-05

Table S5 *Model validation test raw data for planted bioreactor*

Time	Br^- concentration (mg/L)	He concentration (mg/L)	N_2O concentration (mg/L)	SF_6 concentration (mg/L)
0	182.53	0.6057	0.0447	0.000175
1	181.82	0.5217	0.0416	0.000156
2.5	181.27	0.4652	0.0401	0.000146
4	177.68	0.4476	0.0364	0.000141
5.5	172.07	0.4312	0.0379	0.000143
7	165.52	0.3534	0.0306	0.00013
8.5	153.94	0.2619	0.0287	0.000107
10	145.78	0.2357	0.0261	9.96E-05
11	136.07	0.2045	0.0244	9.12E-05

Table S6 *Model validation test raw data for unplanted bioreactor*

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