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CONSTRUCTION OF A SOIL CORE INCUBATION METHOD FOR MEASURING NITROGEN REMOVAL IN WETLANDS AND AN APPLICATION IN THE ELKHORN SLOUGH, CA, USA

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by

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Abstract

CONSTRUCTION OF A SOIL CORE INCUBATION METHOD FOR MEASURING NITROGEN REMOVAL IN WETLANDS AND AN APPLICATION IN THE ELKHORN SLOUGH, CA, USA

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Wetlands play a critical role in removing nitrogen pollution from the surrounding landscape by acting as a reactive zone for nitrogen. In anaerobic wetland soil, microorganisms convert nitrogen to inert di-nitrogen gas. Quantifying nitrogen removal in wetland ecosystems remains challenging, especially for wetlands experiencing regular cycles of wetting and drying. I present a laboratory-based soil core incubation method paired with membrane inlet mass spectrometry to measure total nitrogen removal by denitrification and anammox in wetlands. I applied this method to soils taken from a common pickleweed (*Salicornia pacifica*) dominated salt marsh delineated into wetland positions by degree of tidal inundation. Results suggest that the salt marsh acted as a total nitrogen sink during winter, with the wetland position inundated most often having the greatest nitrogen removal potential. The dense rhizosphere of *S. pacifica* may have facilitated nitrogen removal by soil microorganisms, even during plant dormancy.

To those who believe in the true magic of marsh mud.

Acknowledgments

Thank you to Margaret Zimmer, who let me be her first burnt pancake. Being a brand new professor cannot be easy, but you certainly made it look that way. I will always cherish the times you listened to my concerns and responded selflessly, giving me guidance and structure as any mentor should. We were a successful team, and navigated many incredible opportunities while constructing two field sites at the Elkhorn Slough.

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CHAPTER 1: **A Method for Measuring Nitrogen Removal Across Intertidal Wetlands**

Abstract

Methods used to measure subsurface nitrogen removal in coastal environments are subject to active refinement and development. Wetting and drying cycles induced by the tide poses difficulty to *in-situ* measurement. Yet, daily inundation of intertidal wetlands contributes to anaerobically-induced nitrogen removal via denitrification and anammox. We present adaptations to a laboratory-based soil core incubation method, paired with membrane inlet mass spectrometry, to measure nitrogen removal from wetland soils. To capture the wetland elevation gradient, we apply the method to three wetland positions— upper, middle, and lower— delineated by inundation duration. Overall cost reduction to the method is made by simplifying design and reducing dependence on machined materials and labor. Our methodological development and testing improves upon an essential method for quantifying nitrogen removal from soils across wetlands.

1.1. Introduction

Wetlands act as nitrogen sinks because of their role in anaerobically induced nitrogen removal (Mitsch & Gosselink, 2015). Two major pathways— denitrification and anammox— are utilized by anaerobic bacteria and archaea to convert bioavailable nitrogen to atmospheric di-nitrogen gas (N_2; Capone et al., 2008). Denitrification is the microbial reduction of nitrate (NO_3^-) to N_2, while anammox is the microbial oxidation of ammonium (NH_4^+) using NO_3^- as the electron acceptor in the production of N_2 (Maier, 2015). Controls over anaerobically-induced denitrification and anammox are

still being explored in the coastal landscape, with estuaries expressing either clear dominance in one process or shared nitrogen removal by both processes (see Table 1 Devol, 2015). To enhance understanding of coastal wetland ecosystems as nitrogen sinks, continued point measurements of nitrogen removal in a diverse array of wetlands worldwide are needed.

Coastal landscapes experience a range of hydrologic regimes, such as non-tidal, intertidal, and subtidal inundation. *In-situ* and laboratory methods have been developed to measure nitrogen removal across these regimes. *In-situ* benthic chambers measure solute flux at the sediment-water interface (Devol, 1987). They were first deployed in subtidal marine settings (Devol, 1987; Devol, 1991; Jahnke & Christiansen, 1989; Nielsen & Glud, 1996) and required a team of experts to install sophisticated chambers (see Figure 2 Devol, 1987) in deep-water environments. Later applications of *in-situ* benthic chambers took place in intertidal estuarine settings during low tide or under full atmospheric exposure (i.e. aerobic; Middelburg et al., 1995) and more recently have been adapted to non-tidal settings or under full inundation of surface water (i.e. anaerobic; O'Brien, Hamilton, & Podzikowski, 2012; Qin et al., 2017; Smith, Karl, Repert, & Hart, 2009). While *in-situ* benthic chamber design has become more simple (see Figure 2 Smith et al., 2009) and cost-effective, there remains a lack of application in settings that experience both inundation and exposure on diel timescales. Qin et al. (2017) described a method where *in-situ* chambers (water-filled or water-free) are flushed with an artificial atmosphere to measure nitrogen production, but the method requires further application in intertidal environments.

Laboratory-based soil core experiments followed a similar history of development and application, with initial experiments taking place in sediments extracted from subtidal marine and estuarine settings. Flow-through experiments pump spiked seawater containing $^{15}N\text{-}NO_3^-$ at a constant rate across the mixed headspaces of sediment cores (Nishio, Koike, & Hattori, 1982), while static experiments rely on mixed core headspaces that are replenished by unspiked seawater during sample extraction (Kana, Cornwell, & Zhong, 2006; Nowicki, Requintina, Van Keuren, & Portnoy, 1999). Laboratory-based experiments mimic subtidal inundation through the collection of surface water and subsequent incubation of extracted sediment cores. Anaerobic nitrogen removal is induced and measured in a controlled environment intended to mimic that of *in-situ* tidal inundation and temperature.

Both the *in-situ* chamber and laboratory incubation methods utilize the same basic principle for measuring rates of nitrogen removal by measuring the accumulation of the end-product, N_2. Common methods to quantify N_2 production include the acetylene inhibition technique (Balderston, Sherr, & Payne, 1976; Knowles, 1990), ^{15}N isotope pairing technique (Nielsen, 1992; Steingruber & Friedrich, 2001), and direct measurement of nitrogen-argon ratio (N_2:Ar) by membrane inlet mass spectrometry (MIMS; Kana et al., 1998; Kana et al., 1994). Although acetylene inhibition was the most common method utilized in a recent review of 236 papers (Almaraz, Wong, & Yang, 2020), the method has a long-standing history of underestimating nitrogen removal measurements via adverse elemental interactions (e.g. low NO_3^- concentrations, presence of sulfide) and suppression of NO_3^--producing nitrification

(Knowles, 1990). Nitrification can fuel denitrification removal processes by supplying available NO_3^- in a coupled reaction. When acetylene was applied to estuarine sediments, a mixed response was observed in sulfur reducing bacteria (genus groups over and underrepresented) or nitrogen-fixers common to coastal wetlands (Fulweiler et al., 2015). The ^{15}N isotope pairing technique (IPT) requires a homogeneous distribution of $^{15}N\text{-}NO_3^-$, yet root-derived oxygen (O_2) diffusion by vascular plants— like vegetation ubiquitous to intertidal wetland platforms— leads to subsurface chemical heterogeneity (Groffman et al., 2006; Robertson et al., 2019). Furthermore, the IPT assumes denitrification is the only process producing N_2 (Robertson et al., 2019). MIMS quantifies direct N_2 or the product of complete nitrogen removal through the measurement of N_2:Ar. Optimized by Kana et al. (1994), this method allows for rapid and precise analysis of environmental water samples with minimal preparation and requires a small sample volume. Ar is measured as the reference to nitrogen, assuming Ar remains unaffected by changes in temperature and salinity (Eyre et al., 2002). Due to its properties as a noble gas, Ar is not influenced by biotic processes and is suitable in N_2 analysis using MIMS (Kana et al., 1994).

We present a version of the laboratory-based soil core incubation method and extend point measurements of nitrogen removal to positions across a coastal wetland delineated by tidal inundation. This approach utilizes simultaneous incubations of soil cores collected from wetland positions (upper, middle, lower) with different inundation durations. Intertidal inundation regimes present unique challenges to measuring nitrogen removal, as atmospherically-derived N_2 occupies the pore spaces of

unsaturated soils (e.g. oxygenation of soil at low tide; Groffman et al., 2006). Therefore, saturated conditions provided by soil core incubation proves compatible with MIMS analysis. Anammox removal pathways are increasingly explored in conjunction with the widely cited denitrification removal pathway (see Fozia et al., 2020 and Liu et al., 2020), highlighting that anammox cannot be neglected.

1.2. Methods

1.2.1. Overview

Prior to soil core incubation, we established upper, middle, and lower wetland positions across the wetland based on the length of time the wetland platform remained inundated by tidal surface water. The soil core experiment took place over a three-day period. We reserved day 1 for soil core extraction from the field and core equilibration, day 2 for soil core incubation and sampling of water and soil, and day 3 for MIMS analysis. Following the experiment, we analyzed water and soil samples for nutrients related to nitrogen cycling.

1.2.2. Wetland Position Delineation Based on Tidal Surface Water Inundation

We delineated wetland positions based on inundation duration, which we determined using water level data and a topographic elevation survey. Slight changes in microtopography alter plant and soil communities, lending to small-scale heterogeneity (Liu et al. 2020). Therefore, we carefully chose the wetland transect of interest to reduce heterogeneity (e.g. uniformity in vegetation and elevation gradient).

We collected transect elevation points and water level data from an on-site tidal gauge (Figure 1-1), which we used together to calculate exceedance probabilities of inundation using the function fdc() in the R software package hydoTSM (Figure 1-2; Zambrano-Bigiarini, 2020). We obtained specific probabilities of inundation at each wetland position through regressing measured water levels with calculated exceedance probabilities. Then we selected upper, middle, and lower wetland positions that encompassed a range of tidal inundation given the available width of the wetland platform.

1.2.3. Site Characterization

We gathered ancillary field data at each delineated wetland position (upper, middle, and lower), including the assessment of vegetation, measurement of *in-situ* surface water conditions, and collection of shallow wetland pore water and surface water samples. To assess vegetation, we measured three 1 m^2 vegetation plots along the elevation contour of each of the three wetland positions for plant species, plant count, plant height, canopy width, and percent cover through four subdivided quadrants (0.25 m^2 area per quadrant). Prior to surface water collection, we measured *in-situ* surface water conditions— dissolved oxygen (DO), temperature, pH, salinity, and electrical conductivity— using a handheld water quality meter. We used the recorded *in-situ* surface water temperature to set the laboratory temperature where soil core incubation took place. Next, we collected shallow wetland pore water (10 cm below ground) samples using a mini-piezometer with a syringe. We also collected triplicate

surface water samples to measure the *in-situ* conditions of replacement surface water to be used during soil core incubation. Following figure 1-3, we partitioned each bulk water sample into three bottles for three separate analyses.

1.2.4. Surface Water Collection and Soil Core Extraction

During the incoming tide, we collected surface water in carboys using a peristaltic pump and kept the carboys cool through storage in a nearby shaded area until transport. To eliminate biogeochemical processing in the surface water, it is recommended to filter collected water using an inline filter cartridge attached to a peristaltic pump or to filter at the laboratory using a series of vacuum filter towers (PES 0.7 μm or finer). The total volume of water we collected was equal to the volume of all incubation chambers plus the sample volume to be extracted from cores during incubation (200 liters).

As the tide receded and the wetland surface became exposed, we extracted triplicate soil cores at each of the upper, middle, and lower wetland positions (n = 9). To eliminate plant uptake of nitrogen by assimilation in the cores, we pushed aside vegetation to expose the bare soil surface at each wetland position. Next, we placed the core tube bottoms to the bare soil surface. Using a rubber mallet, we gently hammered core tubes into the unvegetated soil surface and carefully extracted soil cores 13 cm in length and 10.2 cm in diameter.

We extracted the soil cores by digging around the core tube (two to three times the core tube diameter). To ensure the soil did not slump out of the core tube, we

secured the core bottom with both hands to extract. Finally, we sliced off excess soil at the core base using a serrated knife. On a hard surface (e.g. transport cooler), we secured a rubber stopper to the core base. We topped extracted cores with several centimeters of collected surface water (stored in carboys) and a loosely fitted lid using plastic sheeting and rubber bands. We stored extracted soil cores upright in an iced cooler for transportation to the laboratory.

1.2.5. Core Equilibration and Incubation

We mimicked *in-situ* conditions by submerging soil cores in a water bath filled with collected surface water and by setting the laboratory to the measured surface water temperature at the time of soil core extraction. We placed triplicate soil cores (labeled #1-3 for each wetland position) from a single wetland position plus one blank or soil-free core (labeled #4) together in a designated incubation chamber (Figure 1-4). As a result, three incubation chambers represented each wetland position: upper, middle, and lower. We used the blank core to correct for any water-column derived nitrogen activity taking place over the course of soil incubation, which we modified to have the same headspace volume as the soil cores (409 mL). Separation of cores by chamber avoided position-related contamination and allowed for inter-site comparison between wetland positions. We filled incubation chambers with replacement surface water, removed temporary soil core plastic lids, and gently submerged associated soil cores and a single blank core into each incubation chamber. We secured cores into place using a cord of string and waterproofed donut magnets (coated in plasti-dip), where we

placed magnets on the inside and outside of the chamber walls to secure. Next, we added bubblers to each incubation chamber for an overnight equilibration of uncapped cores and surface water. Equilibration maintained field temperatures of surface water and ensured the collected surface water did not go anaerobic prior to the experiment. Equilibration and incubation took place under dark conditions, with the use of red light only when monitoring and sampling to avoid microbial photosynthesis and uptake (i.e. assimilation) of nitrogen.

We incubated cores the following day for 12 hours and sampled them every 3 hours for dissolved gases (N_2, Ar, O_2) and nutrients (NO_3^-, nitrite: NO_2^-, NH_4^+). A 12-hour incubation maintained ≥ 50% O_2 saturation, which occurs *in situ* (Owens & Cornwell, 2016). We pumped bubbled surface water into individual carboys via peristaltic pump and secured carboys on a shelf above each incubation chamber (≥ 30 cm) to allow for gravity-fed surface water replacement to cores. The volume of replacement surface water flowing into cores during sampling is equivalent to the volume of sample extracted at each sampling interval. We noted sample volumes extracted from each core, for later application of a dilution equation. For example, we extracted 13 mL of water for gas analysis (8 mL vial + ~5 mL overflow) and 15 mL for nutrient analysis, for a total of approximately 30 mL removed from each core per sampling round. We fitted soil and blank cores (Figure 1-5) with a magnetic spinning lid (Figure 1-6) that mixed the core headspace and included inflow and outflow ports. We carefully fitted core lids onto each core until a bubble-free environment was achieved. Capping soil cores forced soil-trapped air out of the soil and into the

headspace, resulting in a repetitive capping and recapping of cores until we made all cores bubble-free.

Once we capped all cores in the first incubation chamber, we placed a central magnetic stirring unit over the center of the incubation chamber (Figure 1-7). We allowed the central magnetic stirring unit to run for 5 minutes to ensure proper magnetic tee rotation among all cores in the chamber, and then we began core sampling (round 1, t = 0). After we successfully "started" the first incubation chamber, we moved to the next and final chambers and repeated core capping, placement of central magnetic stirring unit, and core sampling. We sampled soil cores and blank cores in order (chamber #1-3, cores #1-4), and we followed this sequence every 3 hours for 12 hours for a total of 5 sampling rounds. During the first and last sampling rounds, we collected an additional 15 mL sample from each core for *in-situ* measurement of DO, temperature, pH, salinity, and electrical conductivity using a handheld water quality meter. For all sampling rounds, we collected an 8 mL dissolved gas sample and a 15 mL nutrient sample from each core. Following the collection of each dissolved gas sample, we immediately preserved the sample by pipetting 166 μL of 50% zinc chloride into the sample vial to prevent further biogeochemical processing. Next, we tightly closed the dissolved gas sample vial and checked for air bubbles by turning the vial up-side-down. Then, we placed the bubble-free vial into a deionized water bath in the temperature controlled laboratory until MIMS analysis. Following dissolved gas collection, we collected 15 mL of water from each core for nutrient analysis. We

filtered the nutrient samples using a syringe and disk filter (PES 0.45 μm), and stored the samples in a freezer until analysis.

Once we completed the 12-hour incubation, we extracted intact soil cores from the core tubes. We froze, freeze dried, and sieved (2 mm) the top 5 cm of each soil core for quantification of soil nutrients (NO_3^-, NH_4^+, carbon to nitrogen ratio; C:N) by the University of California, Davis Analytical Lab. Soil nutrient results help to untangle the biogeochemical processes that took place in the wetland soil during incubation.

1.2.6. N_2, O_2, and Nutrient Concentration Calculations

We analyzed dissolved gas samples on a MIMS within 24 hours of soil core incubation. We converted concentrations of N_2 and Ar using constants (Table 1-1) and equations 1 and 2 as follows from Hamme and Emerson (2004):

(1) $$\mathbf{T_s} = \frac{\mathbf{\ln(298.15 - t)}}{\mathbf{273.15 + t}}$$

$\mathbf{T_s}$: Scaled temperature of replacement surface water in ºC

t: Average temperature of replacement surface water in ºC

(2) $$\mathbf{\ln C = A_0 + A_1 T_s + A_2 T_s^2 + A_3 T_s^3 + S(B_0 + B_1 T_s + B_2 T_s^2)}$$

C: Concentration of gas (N_2 or Ar) at equilibrium with the atmosphere at 1-atm in μmol/kg

$\mathbf{T_s}$: Scaled temperature of replacement surface water in ºC

S: Average salinity of replacement surface water in PSU

O_2 is an important result and can be measured with the MIMS if a copper reduction column is installed on the MIMS (Eyre et al., 2002). As this modification was not available on our MIMS, we monitored DO concentrations at the start and end of the incubation with a handheld water quality meter. We converted raw N_2 in torr from the MIMS to cm^3 STP/g by linear regression of reference MIMS signals to known concentrations in air-equilibrated deionized water standards. Next, we converted N_2 to N_2 excess (resulting from biotic nitrogen removal) using equations 3 and 4:

(3) $$\mathbf{N_2} = \frac{\mathbf{N_2}}{\mathbf{Ar}} \times \mathbf{Ar_{equilibrium}}$$

$\mathbf{N_2}$: Di-nitrogen gas in µmol/kg

$\frac{\mathbf{N_2}}{\mathbf{Ar}}$: Ratio of di-nitrogen gas to argon gas measured on the MIMS

$\mathbf{Ar_{equilibrium}}$: Concentration of argon gas equilibrated with the atmosphere at 1-atm in µmol/kg or **C** in equation 2

(4) $$\mathbf{N_2\ Excess} = \mathbf{N_2} - \mathbf{N_{2\ equilibrium}}$$

$\mathbf{N_2\ Excess:}$ Di-nitrogen gas excess in µmol/kg

$\mathbf{N_2}$: Di-nitrogen gas in µmol/kg

$\mathbf{N_{2\ equilibrium}}$: Concentration of di-nitrogen gas equilibrated with the atmosphere at 1-atm in µmol/kg or **C** in equation 2

We corrected N_2 excess and nutrients (NO_3^-, NO_2^-, and NH_4^+) for dilution due to sample extraction during the experiment using equation 5 from Heffner (2013), with the total volume of sample extracted during each sampling round:

(5) $\mathbf{T_0} = (\mathbf{C_0} \times \mathbf{V_{over}})$

$\mathbf{T_1} = (\mathbf{C_1} \times \mathbf{V_{over}}) + (\mathbf{C_0} \times \mathbf{V_{removed}})$

$\mathbf{T_2} = (\mathbf{C_2} \times \mathbf{V_{over}}) + (\mathbf{C_0} \times \mathbf{V_{over}}) + (\mathbf{C_1} \times \mathbf{V_{removed}})$

$\mathbf{T_{\#}}$: Dilution-adjusted concentration of N_2 excess, O_2*, or nutrients in µmol

$\mathbf{C_{\#}}$: Concentration of N_2 excess, O_2*, or nutrients in µmol/L (equivalent to µmol/kg)

$\mathbf{V_{over}}$: Volume of overlying water in core headspace in L

$\mathbf{V_{removed}}$: Volume of water removed at sampling interval in L

*O_2 measurements following guidelines by Eyre et al. (2002)

Using gas and nutrient concentrations resulting from the blank cores, we calculated concentrations and fluxes for N_2 excess and nutrients (NO_3^-, NO_2^-, and NH_4^+) for each soil core (equation 6-7; Owens & Cornwell, 2016) as follows:

(6) $\mathbf{C_{final}} = \mathbf{C_{soil}} - \mathbf{C_{blank}}$

$\mathbf{C_{final}}$: Final concentration of N_2 excess, O_2*, or nutrients in µmol/L

$\mathbf{C_{soil}}$: Concentration of N_2 excess, O_2*, or nutrients in µmol/L in soil core

$\mathbf{C_{blank}}$: Concentration of N_2 excess, O_2*, or nutrients in µmol/L in blank core

*O_2 measurements following guidelines by Eyre et al. (2002)

(7) $\mathbf{F} = (\mathbf{\Delta C}/\mathbf{\Delta t_{soil}} - \mathbf{\Delta C}/\mathbf{\Delta t_{blank}}) \times \frac{\mathbf{V}}{\mathbf{A}}$

F: Flux of N_2 excess, O_2*, or nutrients in µmol/m^2/hr

C: Concentration of N_2 excess, O_2*, or nutrients in µmol/L

t: Incubation time in hours

V: Volume of overlying water in core headspace in L

A: Area of core soil-water interface in m^2

*O_2 measurements following guidelines by Eyre et al. (2002)

Finally, we created a time series for N_2 excess, NO_3^-, NO_2^-, and NH_4^+ accumulations and fluxes during the course of incubation.

1.3. Discussion

While nitrogen flux method development has become more popular in fully-inundated environments (Devol, 1991; Middelburg et al., 1995; Nielsen & Glud, 1996; Nowicki et al., 1999; O'Brien et al., 2012; Smith et al., 2009) there remains limited *in-situ* field methods for systems with diverse tidal regimes. Marchant et al. (2017) found that nitrogen removal was stimulated by fluctuations in the environment between aerobic and anaerobic conditions, which may occur in intertidal wetlands. As nitrogen-

transforming processes within ecosystems along the terrestrial-aquatic continuum are explored, methods for measuring nitrogen removal grow in complexity and associated difficulty (Groffman et al., 2006). Nonetheless, the ongoing process of method exploration allows for the discovery of measurement limitations, applicability (refined by ecosystem type, e.g. the intertidal wetland), and enhances future method design and development.

This study was motivated by the need for nitrogen removal method development in intertidal wetlands, which experience a series of tidal inundation and exposure over a 24-hour timescale. Benthic primary production was not considered in this study, but deserves consideration in dark-environment laboratory incubations as to avoid underestimation of nitrogen removal via denitrification in light-environments (e.g. *in situ*; An & Joye, 2001). Quantifying nitrogen removal during periods of inundation across a diverse array of intertidal wetlands— saltwater and freshwater marsh, mangrove, hardwood forested— will allow for more accurate estimations of global nitrogen removal. Continued point measurements of nitrogen removal across space (e.g. elevation gradients) and through time (e.g. diel, seasonal, annual) in intertidal wetlands, is necessary to their assessment as sinks to anthropogenic nitrogen pollution.

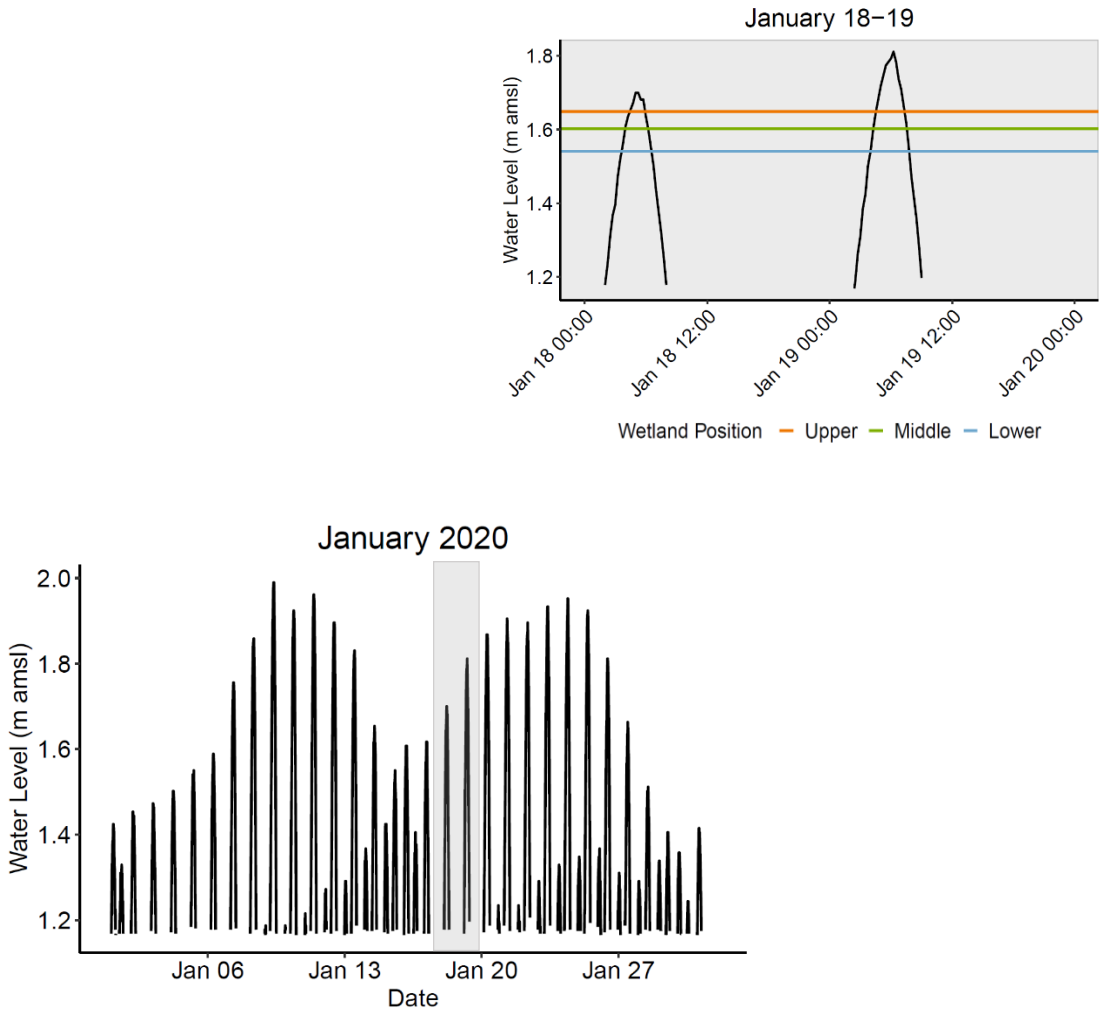

Figure 1-1 An example of tidal gauge data. A month of water level data (bottom), and two days of water level data (top) in meters above mean sea level (amsl) from Cowell Ranch in the Elkhorn Slough, CA, USA. Note that the tidal gauge only captures the high tides (high-high and high-lows), due to its position in the landscape 1.16 m amsl.

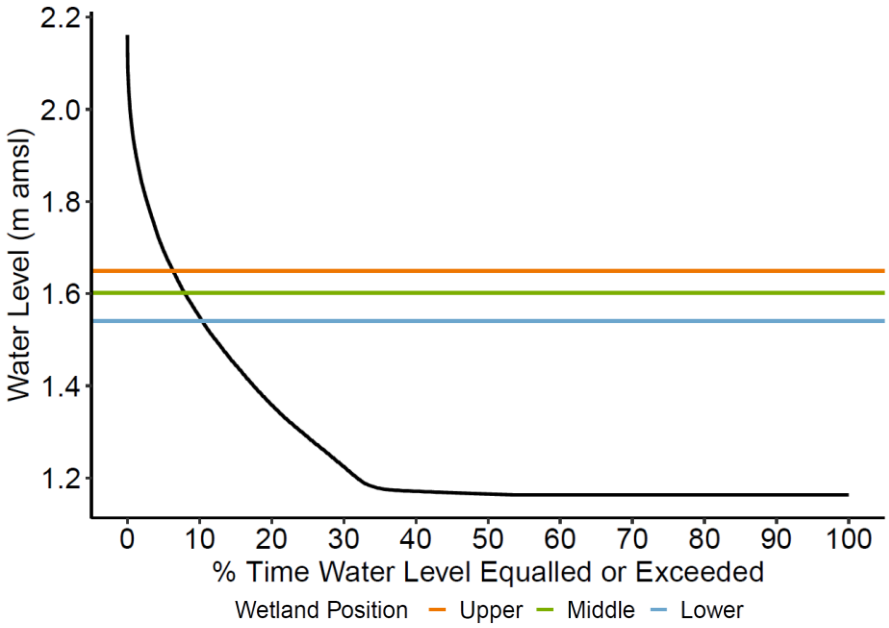

Figure 1-2 A formatted exceedance probability curve output. Water level data from February 2019 to June 2020 used to make an exceedance probability curve for Cowell Ranch in the Elkhorn Slough, CA, USA.

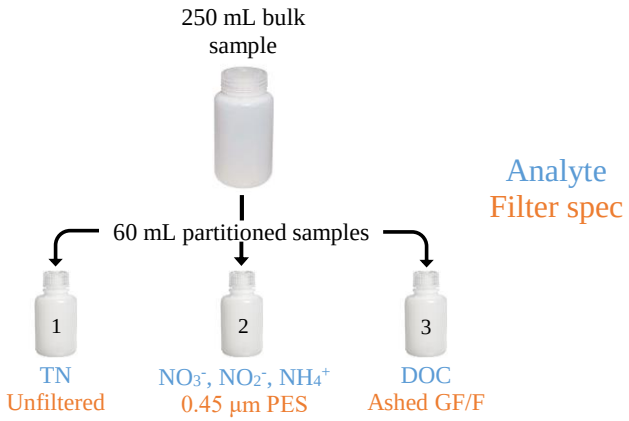

Figure 1-3 Solute sampling scheme from bulk sample to analytes of interest. The bulk sample is partitioned into 3 separate bottles for 3 separate analyses with specified filter specifications: (1) TN: total nitrogen, (2) NO_3^-: nitrate, NO_2^-: nitrite, NH_4^+: ammonium, and (3) DOC: dissolved organic carbon.

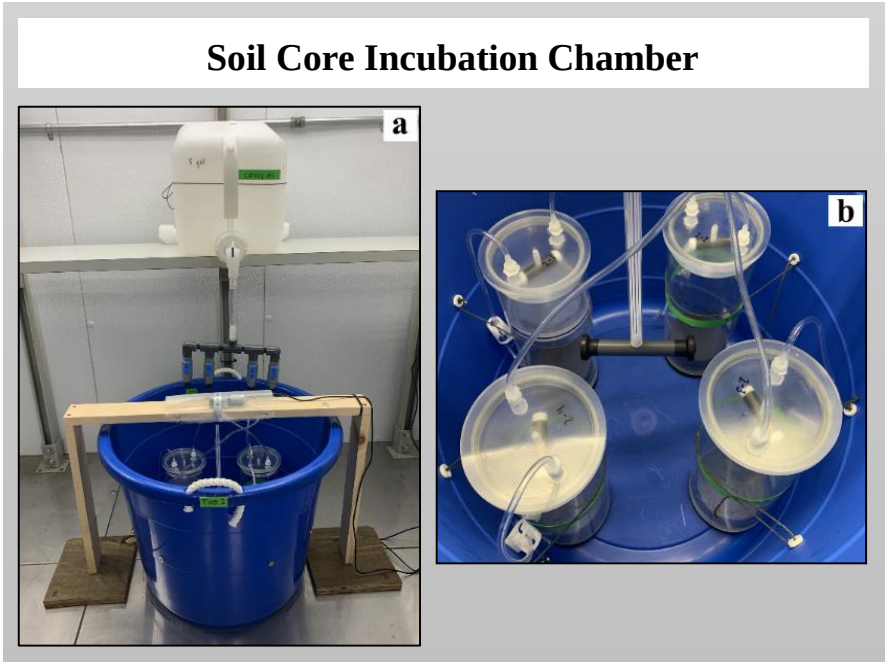

Figure 1-4 Incubation chamber setup with cores in place. (**A**) A single incubation chamber with replacement surface water feeding into cores from an elevated carboy, through the 4-way valve unit, and with the central magnetic stirring unit in place. Note the plastic water-resistant cover over the motor. (**B**) Capped cores arranged around the incubation chamber and secured with magnetic lines. All magnets— central magnetic stirring unit tee, core magnetic tees, and donut magnets used to secure cores— are plasti-dipped (x2 or more coats) to prevent rusting in brackish incubation water.

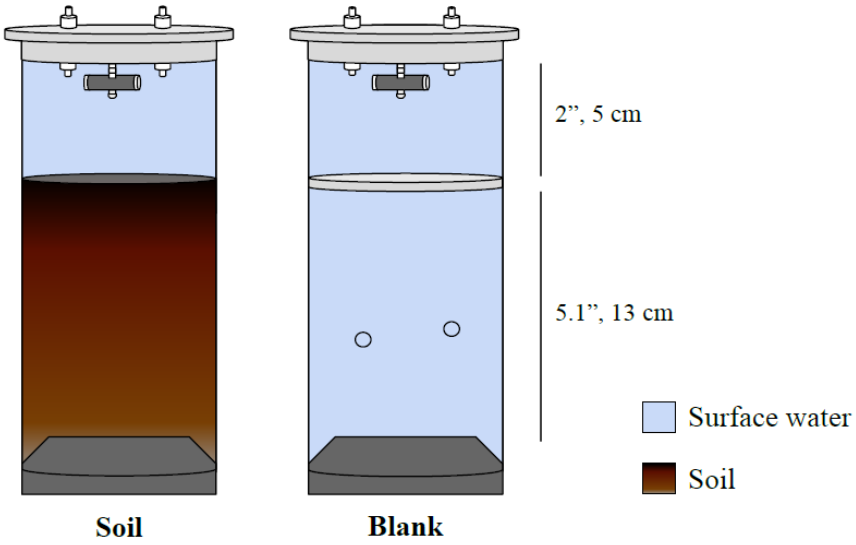

Figure 1-5 Soil and blank incubation cores fitted with magnetic spinning lids. A 5 cm headspace is consistent among all cores, represented in the figure as the surface water above the soil-water interface in the soil core and the surface water above the divider in the blank core. Holes are drilled below the blank core divider to allow water into the bottom section of the core. Note that 5 cm is lost due to the inserted lid and black stopper, which leaves a soil core total length of 13 cm.

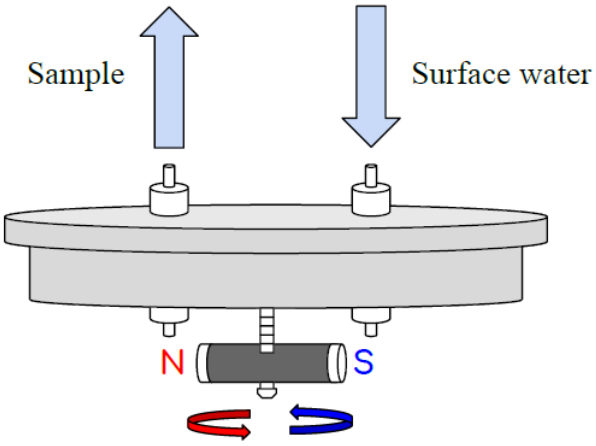

Figure 1-6 Magnetic spinning lid designed to fit cores. The light-blue directional arrows indicate flow of water in and out of ports during soil incubation— as a sample volume is pulled, the same volume is replaced with surface water. Meanwhile, the magnetic spinner mixes the headspace as it is controlled by the central magnetic stirring unit.

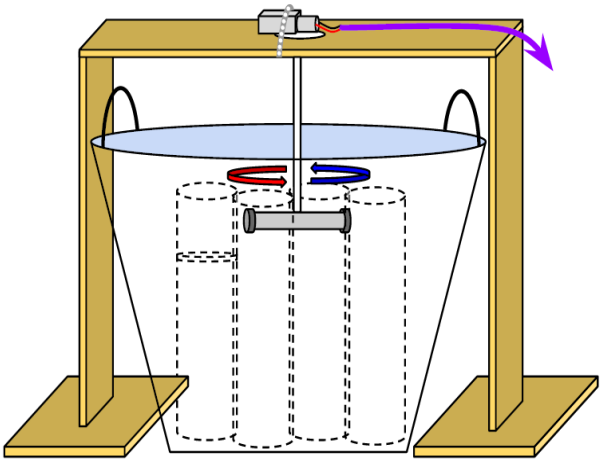

Figure 1-7 Incubation chamber and central magnetic stirring unit. The large magnetic tee is controlled by a DC motor. The motor is wired to a control box (not shown, purple arrow indicating wire to motor control box). When powered on, the large magnetic tee rotates and creates an incubation chamber-wide magnetic field— causing all magnetic stirrers to rotate within individual cores.

Table 1-1 Nitrogen and argon constants used in solubility equations 1 and 2 from Hamme & Emerson (2004).

	N_2 (µmol/kg)	Ar (µmol/kg)
A_0	6.42931	2.7915
A_1	2.92704	3.17609
A_2	4.32531	4.13116
A_3	4.69149	4.90379
B_0	-0.00744129	-0.00696233
B_1	-0.00802566	-0.0076667
B_2	-0.0146775	-0.0116888

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CHAPTER 2: **Quantifying Nitrogen Removal Across a Salt Marsh During Winter**

Abstract

Anthropogenic activities like fossil fuel combustion and fertilizer application have altered the global nitrogen cycle. In the Mediterranean climate of coastal California, winter precipitation has the potential to flush nitrogen pollution from the terrestrial uplands to the marine bottomlands. Located at the interface of estuaries, salt marshes are capable of biogeochemical retention and removal of this incoming nitrogen. However, it is unclear what the nitrogen removal potential of salt marsh ecosystems are during this time when vegetation lies dormant and is not contributing to nitrogen uptake via assimilation. We sought to determine how nitrogen removal rates varied across a common pickleweed (*Salicornia pacifica*) dominated salt marsh during winter (January 2020) in the Elkhorn Slough, CA, USA. During a 12-hour laboratory experiment, we calculated total nitrogen removal from denitrification and anammox by measuring the accumulation of di-nitrogen gas (N_2) in soil cores extracted across a salt marsh. We delineated wetland positions by degree of tidal inundation, which varied between 6 and 10% (upper: 6.3%, middle: 7.9%, lower: 10.3%). Between 499 to 980 μmol/m^2/hr of nitrogen was removed during incubation, indicating the potential for the intertidal wetland to act as a nitrogen sink. Nitrogen removal was highest in the lower wetland position and decreased with increasing elevation. Results showed a deficit of combined nitrate and nitrite (NO_X) at the upper wetland position and a surplus of NO_X and/or ammonium (NH_4^+) at the middle and lower wetland positions by the experiment's end, assuming available NO_X and NH_4^+ in the overlying water contribute to nitrogen removal. We hypothesize that the upper wetland position underwent

nitrogen removal via coupled nitrification-denitrification, while the wetland positions experiencing more frequent inundation (middle and lower wetland positions) underwent retention through dissimilatory nitrate reduction to ammonium (DNRA). Pools of bioavailable NH_4^+ and vegetation density likely played primary roles in determining the magnitude of microbe-mediated nitrogen cycling, even during winter senescence of *S. pacifica*. Increased vegetation density corresponded with the greatest N_2 flux at the middle and lower wetland positions. Microbial communities may be resistant to seasonal transitions, as the dense rhizosphere of *S. pacifica* and abundant organic carbon offered by the salt marsh may lead to established nitrogen transforming communities. Additional nitrogen removal measurements during the growing season are necessary to further our understanding of vegetation-related influences on nitrogen removal in California salt marshes.

2.1. Introduction

Industrial fixation of synthetic fertilizer is the leading source of nitrogen inputs into the biosphere, altering the global nitrogen cycle (Fowler et al., 2013; Galloway et al., 2004; Gruber & Galloway, 2008; Vitousek et al., 1997). Accounting for and managing this new source of nitrogen has proven difficult (Singh & Bakshi, 2013), especially with the discovery of legacy pools or areas of nitrogen in the landscape that are variable in spatial extent, concentration of nitrogen, and duration of release (Van Meter, Basu, Veenstra, & Burras, 2016). Eutrophication from nitrogen pollution occurs in low-lying environments (e.g. lakes, rivers, estuaries, and oceans) at the terrestrial-

aquatic continuum and results in harmful algal blooms (Anderson et al., 2002; Hallegraeff, 2014; Heisler et al., 2008), hypoxia (Conley, Carstensen, & Duarte, 2009; Gray, Wu, & Or, 2002; Rabalais et al., 2010), and dead zones (Altieri & Diaz, 2019; Diaz & Rosenberg, 2008; Joyce, 2000). Effects of climate change, such as increased air and sea temperatures, ocean acidification, land-cover change, and sea-level rise, worsen symptoms of eutrophication (Altieri & Gedan, 2015; Dokulil & Teubner, 2016; Glibert, 2020; Moss et al., 2010; Rabalais et al., 2010; Rabalais, Turner, Diaz, & Justic, 2009; Sinha, Michalak, & Balaji, 2017). Estuaries act as filters to pollution by modifying, transforming, and retaining organic and inorganic material from riverine and marine inputs (Kennedy, 1984). The estuarine landscape is mosaicked by wetlands. Although wetland ecosystems are referred to as the "kidneys of the landscape" for their role in water quality and quantity regulation (Mitsch and Gosselink, 1986), global wetland area remains threatened by hydrologic alteration (i.e. drainage; Leibowitz et al., 2018), agricultural conversion (Mitsch and Gosselink, 2005), and sea level rise (Spidalieri, 2020).

Nitrogen removal processes are carried out under anaerobic or oxygen (O_2)-limited conditions (Maier, 2015) that occur in saturated wetland soils. Under anaerobic conditions, soil microorganisms chemically reduce nitrogen species— nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+)— to inert di-nitrogen gas (N_2) via denitrification and anammox removal pathways (Figure 2-1). While the relative contributions of denitrification or anammox removal to total nitrogen removal in estuarine environments are actively being investigated (see Table 1, Devol, 2015), quantifying

nitrogen removal as the sum of both processes allows insight into total removal by ecosystem type.

Tidal exchange has been cited as the most significant nitrogen transport mechanism in salt marshes (Valiela & Teal, 1979). While the influence of tidal inundation on salt marsh productivity is well known (Steever, Warren, & Niering, 1976), less is known on how nitrogen removal varies across the salt marsh platform as a function of tidal inundation duration. Here, we seek to quantify nitrogen removal across a salt marsh delineated by degree of tidal inundation. To do this, we measure N_2 accumulation as the product of total nitrogen removal through pairing soil core incubation with membrane inlet mass spectrometry (MIMS; Owens & Cornwell, 2016).

2.2. Methods

2.2.1. Study Site

The study site is located within the Elkhorn Slough National Estuarine Research Reserve (ESNERR) in central coastal California (Figure 2-2). Elkhorn Slough feeds into the Monterey Bay National Marine Sanctuary and is a Wetland of International Importance (Ramsar, 2018). Conservation designations to parts of the watershed and neighboring bay highlight the need for increased protection, as Elkhorn Slough faces substantial pressure from upstream pollutants. Cropland makes up 23% of the total watershed area (Caffrey, Brown, Tyler, & Silberstein, 2002), resulting in failing grades annually for surface water quality parameters indicative of widespread fertilizer use (see interactive Water Quality Report Card: http://water.elkhornslough.org/). The state

of California lacks an integrated nitrogen management policy, despite being home to a globally competitive agricultural sector; consequently, California carries many nitrogen-related externalities for the rest of the United States and other regions worldwide (Tomich, Brodt, Dahlgren, & Scow, 2016).

Cowell Ranch is an emergent wetland (System: estuarine, subsystem: intertidal; Cowardin, 1979) positioned in the upper reaches of the Elkhorn Slough watershed, roughly 9 km from the estuary mouth along the main channel (Figure 2-2). Despite this significant distance from the estuary mouth, surface water at Cowell Ranch routinely experienced marine ranges in salinity during 2019 (Table 2-1). The wetland is dominated by the native perennial herb, *Salicornia pacifica*, and is underlain by extensive and partially decomposed organic soil. Elkhorn Slough experiences an ebb-dominant mixed semidiurnal tidal regime (Nidzieko, 2010), where daily high tides inundate Cowell Ranch (see elevations in Table 2-2). Across the 25 m transect at Cowell Ranch, the upper and lower wetland positions differ by 11 cm in vertical elevation.

2.2.2. Field Methods

2.2.2.1. Wetland Position Delineation

We delineated Cowell Ranch into upper, middle, and lower wetland positions through pairing surface water level data and a topographic survey (Table 2-2). We retrieved ~15 months of water level data recorded at 5 minute intervals from an on-site tidal gauge (Solinst LTC M5 3001). We conducted a topographic survey using a total

station (Sokkia SCT6) across the 25 m wetland transect, from the interface of the terrestrial upland to the start of the unvegetated mudflat. We surveyed elevation every 0.5 m to capture small-scale changes in microtopography, as wetland microtopography was found to be a key driver in local-scale hydrology and subsequent vegetation evolution (Liu, Du, Xu, Kardol, & Hu, 2020). We coupled water level and elevation data to calculate percent inundation using the flow duration curve function in the hydroTSM R software package (Zambrano-Bigiarini, 2020). We selected three experiment locations to capture a range in tidal inundation from 1.5 to 2.4 hours of average daily tidal inundation during the period of record (Table 2-2). The Cowell Ranch marsh delineation follows closely with the ESNERR marsh delineation, where *S. pacifica* occurs at "mid" and "low" positions between mean high higher water and mean high water or between 1.68 m and 1.46 m (compare to elevations in Table 2-2; Woolfolk & Labadie, 2012).

2.2.2.2. Soil Characterization

We characterized soil profiles down to 1.3 m depths at the upper and lower wetland positions of Cowell Ranch. We attached an open-faced auger bucket 5.7 cm in diameter to a cross handle and two 1.2 m long extensions (AMS). We delineated soil horizons (master horizons, length, texture, and color), and noted the presence of *S. pacifica* rooting depths and soil redoximorphic features. Prior to the experiment, we installed *in-situ* redox sondes 10 cm below the soil surface at all wetland positions (12

cm diameter; Paleo Terra). With this, we measured redox data at 1-minute intervals for the 24-hour period before, during, and after soil core extraction.

2.2.2.3. Vegetation Surveying

On the day of soil core extraction, we conducted vegetation surveys at each wetland position prior to extracting soil cores. We took three 1 m^2 surveys along the elevation gradient of each wetland position (Table 2-2). From this, we calculated percent cover, canopy width, and canopy height of individual *S. pacifica* plants. We also noted observed senescence of *S. pacifica*.

2.2.2.4. *In-Situ* Pore Water and Surface Water Collection

Following vegetation surveying, we collected wetland pore water samples at 10 cm depths below the ground surface with a mini-piezometer to analyze for NO_3^-, NO_2^-, and NH_4^+ concentrations at each wetland position. Using an Orion Star A321 handheld water quality meter, we measured surface water for dissolved oxygen (DO), pH, electrical conductivity, salinity and temperature. We collected 200 liters of surface water using a peristaltic pump for the incubation experiment. To keep collected surface water cool, we stored carboys in a shaded area until transport. The volume of surface water collected was equivalent to the volume of each incubation chamber plus the volume of sample extracted (dissolved gas + nutrient) from each core during incubation. For this experiment, we did not filter the collected surface water used during incubation.

2.2.2.5. Soil Core Extraction

Using 10.2 cm diameter clear core tube housing, we extracted triplicate soil cores to a depth of 13 cm below the surface at each wetland position. Once extracted, we topped the soil cores with 5 cm of surface water. This water helped to maintain soil saturation and reduce introduction of N_2 rich air, which is necessary for accurate MIMS analysis. We sealed and placed cores upright in a cooler with ice for transport to the laboratory.

2.2.3. Laboratory-Based Soil Core Incubation

2.2.3.1. Equilibration

We submerged triplicate soil cores plus one blank (soil-free) core for each wetland position into individual water-filled chambers. We kept wetland positions separated by incubation chambers, which permitted comparison between wetland positions. A 12-hour equilibration of uncapped cores took place overnight under oxygenated (via bubbler) and dark conditions. We set the temperature controlled laboratory to the lowest possible temperature (13 ºC), somewhat higher than the measured surface water temperature in the field (7.4 ºC).

2.2.3.2. Sampling Preparation

Prior to the experiment, we modified the blank cores to have a headspace volume equal to that of the soil cores (409 mL), which underwent the same treatment as the soil cores. Following equilibration, we pumped the oxygenated surface water

from the incubation chambers into individual carboys to prepare for the 12-hour incubation. We elevated each carboy ≥ 30 cm above the incubation chambers so that the carboys supplied gravity-fed surface water to cores during sample extraction (Owens & Cornwell, 2016). We capped the cores, taking care to eliminate bubbles in the water-filled core headspaces. A bubble-free environment is necessary for accurate MIMS dissolved gas analysis, as atmospheric gas would reduce resolution of dissolved gases of interest. Therefore, we assumed dissolved N_2 accumulation in the overlying water (i.e. core headspace) was the result of biogeochemical processing of nitrogen in the underlying soil.

2.2.3.3. Core Sampling

We incubated and sampled the headspaces above the soil cores and associated blanks for 12 hours. Every 3 hours, we collected 8 mL dissolved gas (N_2 and argon; Ar) samples and 15 mL nutrient (NO_3^-, NO_2^-, NH_4^+) samples for a total of 5 sampling rounds.

2.2.4. Laboratory Analysis and Calculations

2.2.4.1. Soil Analysis

Immediately following incubation, we extracted the soil cores from the core tubes. We collected the top 5 cm of each soil core by slicing off a disk using a serrated knife. We froze the soil disks completely and then freeze dried them, which took approximately 48 hours. Once dried, we ran the samples though a 2 mm sieve to

homogenize the soil. We sent the soil samples (60 g; n = 9) to the University of California, Davis Analytical Lab to be analyzed for carbon and nitrogen (C:N) using flash combustion paired with a thermal conductivity detector (AOAC, 1997) and nutrients (NO_3^- and NH_4^+) using nitrogen extraction paired with flow injection (Hofer, 2003; Keeney & Nelson, 1982; Knepel, 2003). The lab also completed hydrochloric (HCl) acid additions on all samples as an inference to total inorganic C.

2.2.4.2. Water Analysis

Prior to analysis, we filtered (PES 0.45 μm) and froze the water samples collected in the field and during core sampling. We measured nutrient (NO_3^-, NO_2^-, NH_4^+) concentrations through flow injection on a Lachat QuikChem nutrient analyzer (≤ 5% error). We ran nutrient check standards that encompassed the observed range in concentrations of NO_3^-, NO_2^-, and NH_4^+ every 10 samples. Using data collected from the nearby ESNERR Kirby Park sampling station (7 km from the estuary mouth along the main channel; 36°50'26"N, 121°44'40"W), we averaged surface water NO_3^- concentrations for the months of January 2008-2020 (ESNERR, 2020).

2.2.4.3. Excess N_2 and Nutrient Calculations

The product of microbe-induced nitrogen removal, N_2, is used to infer total nitrogen removal, independent of process. Optimized by Kana et al. (1994), the MIMS analytical method allows for rapid and precise analysis of environmental water samples for dissolved N_2 and Ar. Ar is measured as the reference gas, assuming Ar remains

constant throughout the experiment and is unaffected by changes in temperature and salinity (Eyre, Rysgaard, Dalsgaard, & Christensen, 2002). Thus, the N_2:Ar ratio output from the MIMS elucidates the change in N_2 through time.

To calculate the concentrations of N_2 and Ar at equilibrium with the atmosphere, we applied solubility constants and fitting equations from Hamme & Emerson (2004) using temperatures and salinities of replacement surface water during incubation. We converted raw N_2 in torr from the MIMS to cm^3 STP/g using reference MIMS signals against known concentrations in air-equilibrated deionized water standards. N_2 is converted to N_2 excess (resulting from biotic nitrogen removal) using equations 1 and 2:

(1) $\mathbf{N_2} = \frac{\mathbf{N_2}}{\mathbf{Ar}} \times \mathbf{Ar_{equilibrium}}$

$\mathbf{N_2}$: Di-nitrogen gas in µmol/kg

$\frac{\mathbf{N_2}}{\mathbf{Ar}}$: Ratio of di-nitrogen gas to argon gas measured on the MIMS

$\mathbf{Ar_{equilibrium}}$ $*$: Concentration of argon gas equilibrated with the atmosphere at 1-atm in µmol/kg

$*$variable **C** in equation 2 from Hamme & Emerson (2004)

(2) $\mathbf{N_2\ Excess} = \mathbf{N_2} - \mathbf{N_{2\ equilibrium}}$

$\mathbf{N_2\ Excess:}$ Di-nitrogen gas excess in µmol/kg

$\mathbf{N_2}$: Di-nitrogen gas in µmol/kg

$\mathbf{N_{2\ equilibrium}}$ ∗: Concentration of di-nitrogen gas equilibrated with the atmosphere at 1-atm in µmol/kg

∗variable **C** in equation 2 from Hamme & Emerson (2004)

We applied a dilution equation to N_2 excess and nutrient (NO_3^-, NO_2^-, NH_4^+) concentrations using equation 3 from Heffner (2013) with the volumes of sample extracted during each sampling round:

(3) $\mathbf{T_0} = (\mathbf{C_0} \times \mathbf{V_{over}})$

$\mathbf{T_1} = (\mathbf{C_1} \times \mathbf{V_{over}}) + (\mathbf{C_0} \times \mathbf{V_{removed}})$

$\mathbf{T_2} = (\mathbf{C_2} \times \mathbf{V_{over}}) + (\mathbf{C_0} \times \mathbf{V_{over}}) + (\mathbf{C_1} \times \mathbf{V_{removed}})$

$\mathbf{T_{\#}}$: Dilution-adjusted concentration of N_2 excess or nutrients in µmol

$\mathbf{C_{\#}}$: Concentration of N_2 excess or nutrients in µmol/L (equivalent to µmol/kg)

$\mathbf{V_{over}}$: Volume of overlying water in core headspace in L

$\mathbf{V_{removed}}$: Volume of water removed at sampling interval in L

To remove any possible biogeochemical activity that took place in the core headspace water, we compared N_2 excess and nutrient (NO_3^-, NO_2^-, NH_4^+) concentrations and fluxes between soil cores and blank cores. Fluxes represent the differences in concentrations, normalized by the core surface area and time. We did this using equations 4 and 5 from Owens & Cornwell (2016) as follows:

(4) $\mathbf{C_{final} = C_{soil} - C_{blank}}$

$\mathbf{C_{final}}$: Final concentration of N_2 excess or nutrients in µmol/L

$\mathbf{C_{soil}}$: Concentration of N_2 excess or nutrients in µmol/L in soil core

$\mathbf{C_{blank}}$: Concentration of N_2 excess or nutrients in µmol/L in blank core

(5) $\mathbf{F} = (\mathbf{\Delta C}/\mathbf{\Delta t_{soil}} - \mathbf{\Delta C}/\mathbf{\Delta t_{blank}}) \times \frac{\mathbf{V}}{\mathbf{A}}$

F: Flux of N_2 excess or nutrients in µmol/m^2/hr

C: Concentration of N_2 excess or nutrients in µmol/L

t: Incubation time in hours

V: Volume of overlying water in core headspace in L

A: Area of core soil-water interface in m^2

By the end of the experiment (t = 12), we conducted a stoichiometric mass balance on N_2 and nutrient species available for uptake (i.e. NO_3^-, NO_2^-, NH_4^+). For example, 2 moles of NO_3^- are necessary to produce 1 mole of N_2 during denitrification; 1 mole of NH_4^+ and 1 mole of NO_2^- are required to produce a single mole of N_2 via anammox (Figure 2-1).

2.3. Results

2.3.1. Vegetation Survey

S. pacifica was the only species observed (Table 2-3), with the highest number of individuals at the middle wetland position. The number of plants were significantly

different (Kruskal-Wallis test: $p = 0.03$, $H = 7.26$, $df = 2$) between middle and lower wetland positions (Dunn test: $p = 0.02$); the median of the middle wetland position was equal to 27 plants, while the median for the lower wetland position was 7 plants. Percent cover was significantly different (ANOVA test: $p = 0.005$, $df = 2$, $F = 14.79$, means in Table 2-3) between the upper and middle wetland positions ($p = 0.04$) and between the middle and lower wetland positions ($p < 0.001$) under the post-hoc Tukey HSD test. Variances in percent cover between wetland positions were not different (Levene's test: $p > 0.05$), which confirmed homogeneity of variance for the null hypothesis. Canopy width was significantly different (Kruskal-Wallis test: $p = 0.04$, $H = 6.49$, $df = 2$) between middle and lower wetland positions (Dunn test: $p = 0.03$). We found no significant difference (Kruskal-Wallis test: $p > 0.05$) in plant height across wetland positions.

2.3.2. Wetland Soil

Soil characterization revealed an active rhizosphere to 18 cm below the soil surface at the upper and lower wetland positions (Table 2-4). We classified the upper wetland position as an typic agrixeroll and the lower wetland position as a typic haplofibrist, with both positions following a xeric moisture regime (Soil Survey Staff, 2015). The upper wetland position consisted of a peaty, dark bluish gray (Gley 2 4/10B) Oi horizon from 0-8 cm, a silty-peat, dark bluish-gray (Gley 2 4/5B) Ag horizon from 9-19 cm, and a sandy-clay, blueish-gray (Gley 2 6/10B) Bgs horizon from 20-110 cm (Munsell, 2009). The Bgs horizon contained orange-stained iron (Fe) concretions

throughout the gleyed horizon. The lower wetland position contained a single mucky-peat Oi horizon from 0-130 cm that transitioned between shades of bluish gray (Gley 2 6/5B to Gley 2 5/10B) at 56 cm (Munsell, 2009). During the time of auguring in winter (March 2019), we observed no surface water presence as the tide was on the rising limb of the diel high-low tide. However, we did observe groundwater at a depth of 5 cm below the surface at the upper wetland position and 19 cm below the surface at the lower wetland position.

We calculated the median redox potential 10 cm below the surface on the day we extracted soil cores from Cowell Ranch (Table 2-4). Medians measured -389.2 mV at the upper wetland position, -92.2 mV at the middle wetland position, and -389.3 mV at the lower wetland position. Redox potential was significantly different (Kruskal-Wallis test: $p < 0.001$, $H = 2879.3$, $df = 2$) between upper and middle wetland positions (Dunn test: $p = 0$) and between middle and lower wetland positions (Dunn test: $p = 0$).

Following incubation, we analyzed the top 5 cm of wetland soils for a suite of variables that have been shown to influence the biotic removal of nitrogen (Table 2-4). There was a significant difference in C:N (Kruskal-Wallis test: $p = 0.03$, $H = 7.2$, $df = 2$) between the upper and lower wetland positions (Dunn test: $p = 0.02$) with medians 14.3 and 11.5, respectively. No significant difference existed among NH_4^+ ($p = 0.06$) and NO_3^- ($p = 0.07$) in wetland soils when the Kruskal-Wallis test was applied. Yet, NH_4^+ concentrations increased by 366.5 μmol/kg dry soil from upper to middle wetland positions, but decreased by 390.3 μmol/kg dry soil from middle to lower wetland positions. NO_3^- concentrations increased by 62.4 μmol/kg dry soil from upper to middle

wetland positions, but decreased by 65.5 μmol/kg dry soil from middle to lower wetland positions. We found a significant difference between pH (Kruskal-Wallis test: $p = 0.03$, $H = 7.2$, $df = 2$) at the upper and lower wetland positions (Dunn test: $p = 0.02$). Upon HCl addition to soil samples, no reaction was witnessed; the volume of carbon-dioxide released by acid addition is a measure for total inorganic C (Nimmo, 2005), where a lack of reaction indicates no appreciable levels of inorganic C exist.

2.3.3. *In-Situ* Pore Water and Surface Water

Concentrations of NO_3^-, NO_2^-, and NH_4^+ in the sampled pore water are presented in Table 2-5. NH_4^+ concentrations in pore water were highest at the lower wetland position (85.3 μmol/L) and lowest at the upper wetland position (2.1 μmol/L). NO_3^- in the lower wetland position pore water was lower (0.3 μmol/L) than middle (3.4 μmol/L) and upper (2.3 μmol/L) wetland positions.

Average surface water NO_3^- concentrations and parameters measured using a handheld water quality meter are presented in Table 2-6. In winter, the surface water pH was near-neutral (pH of 7) and salinity was brackish (between 0.5 and 30 ppt).

2.3.4. Accumulation of N_2, NO_X, and NH_4^+ in Wetland Soils

N_2 accumulated in the core headspaces at all wetland positions (Figure 2-3), which could indicate microbially induced nitrogen removal from the wetland soil cores. Accumulation is marked by a positive slope in N_2 over time, which represents N_2 production in the soil that diffuses into the overlying water of the core headspace. NO_X

represents combined NO_3^- and NO_2^-, with NO_3^- being the dominant nitrogen species in NO_X during the experiment. NO_X concentrations decreased over the experiment at all wetland positions. The negative slope is interpreted as the consumption of NO_X from the overlying water by microbial communities in the soil. NH_4^+ concentrations increased at the middle and lower wetland positions and stayed constant in the headspace of the upper wetland position.

2.3.5. Total Flux of N_2, NO_X, and NH_4^+ in Wetland Soils

We calculated total fluxes of N_2, NO_X, and NH_4^+ in each incubated soil core (Figure 2-4). The N_2 flux was not significantly different among wetland positions (ANOVA: $p > 0.05$), with a removal rate of 499 μmol/m^2/hr at the upper wetland position, and 971 μmol/m^2/hr and 980 μmol/m^2/hr at the middle and lower wetland positions, respectively. The Levene's test confirmed the variance in N_2 across wetland positions was not significantly different ($p > 0.05$). NO_X fluxes were different between the upper and middle wetland position (Kruskal-Wallis test: $p = 0.05$, $H = 5.96$, $df = 2$). The median flux of NO_X at the upper wetland position was -580 μmol/m^2/hr and the median flux was -880 μmol/m^2/hr at the lower wetland position. NH_4^+ fluxes showed no difference across all cores ($p = 0.07$). Yet, NH_4^+ flux was 1,130 μmol/m^2/hr higher (34-fold) between upper and middle wetland positions and 910 μmol/m^2/hr higher (28-fold) between upper and lower wetland positions.

2.4. Discussion

2.4.1. Soil Core Incubation Elucidates Nitrogen Removal Across the Salt Marsh

Accumulation of N_2 took place over the 12-hour incubation in all soil core headspaces, which represented all wetland positions. N_2 accumulation in the water-filled headspace is suggestive of microbial removal via denitrification and/or anammox from the soil cores (Erler, Eyre, & Davison, 2008). The accumulation of N_2 across sampling intervals occurred contemporaneously with loss of NO_X (Figure 2-3). NO_3^- is the first of eight inorganic nitrogen species utilized along the reduction pathway, which is composed of 14 redox reactions carried out by microorganisms (see Figure 1 in Kuypers, Marchant, & Kartal, 2018). At the upper wetland position, a deficit of 10 μmol/L of NO_X and/or NH_4^+ resulted at the end of the experiment, assuming available NO_X and NH_4^+ contributed towards N_2 accumulation in that order. The middle wetland position had 16 μmol/L and 28 μmol/L extra of NO_X and NH_4^+, respectively, while the lower wetland position had an excess of 9 μmol/L NH_4^+ by the experiment's end. When NH_4^+ was available, we suggest NH_4^+ was consumed in place of missing NO_X, anaerobically via anammox or aerobically via nitrification, which is a less efficient two-step process (Maier, 2015).

2.4.2. Pools of Bioavailable NH_4^+ Discovered Across the Salt Marsh

Based on an observed positive N_2 accumulation, a minimal decease in NO_X accumulation, and near-zero NH_4^+ accumulation (Figure 2-3), we suggest the upper wetland position underwent nitrogen removal through coupled nitrification-

denitrification. The upper wetland position remains free of overlying tidal surface water 94% of the time (Table 2-2). We hypothesize that a nitrifying community may be supported there, as nitrification is predominantly carried out by aeorbic chemoautotrophs (Maier, 2015) and can be followed by instantaneous denitrification, which is supported by plant roots (Reddy, Patrick, & Lindau, 1989). Wankel et al. (2011) suggested that higher rates of nitrification in wetlands of the Elkhorn Slough may contribute to a reduced capacity for nitrogen uptake, which may have occurred at the upper wetland position. Total nitrogen flux was 95% greater at the middle wetland position and 96% greater at the lower wetland position when compared to the upper wetland position (Figure 2-4). When taking into account the average inundation per day at Cowell Ranch (Table 2-2), the wetland position inundated most often (i.e. lower wetland position) had the highest potential to remove nitrogen (Figure 2-4).

In other salt marsh studies, coupled nitrification-denitrification was promoted under the presence of ferric iron (Fe^{3+}), which was found to alleviate the inhibition of nitrification caused by sulfide (Dollhopf et al., 2004). The upper wetland position exhibited greater maturity in mineral soil horizon development when compared to the lower wetland position, suggesting aerobic conditions allowed for Fe^{3+} and ferrous (Fe^{2+}) redoximorphic concretions (Schaetzl & Anderson, 2005). The middle wetland position experienced a redox potential of -98.0 mV, the upper and lower wetland positions were much lower at -389.5 mV and -389.4 mV, respectively (Table 2-4). Highly-reduced soils (from -100 mV to -300 mV) are characteristic of peat-dominated wetlands rich in organic matter (Tokarz & Urban, 2015). Furthermore, measured redox

potential has been observed to be lower than theoretical redox potential (Brady & Weil, 2010).

The middle and lower wetland positions accumulated NH_4^+ in the headspace (Figure 2-3) by three likely processes: fixation, mineralization, or dissimilatory nitrate reduction to ammonium (DNRA). Nitrogen fixation occurs under aerobic and anaerobic conditions, with a variety of molecular functions responding to nitrogen, redox, and O_2 levels (Dixon & Kahn, 2004). Furthermore, the nitrogen-fixing community is likely dominant in surface soils of *S. pacifica* (Moseman, 2007).

When the energetic needs of microbes are met by local organic C sources, mineralization rates increase and NH_4^+ dominates the soil nitrogen pool (see Figure 2, case C in Schimel & Bennett, 2003). Significant pools of NH_4^+ were measured in wetland soil across all wetland positions (Table 2-4). NH_4^+ represented a second pool of nitrogen in the middle and lower wetland position pore waters with concentrations measured at 22.9 μmol/L and 85.3 μmol/L, respectively (Table 2-5). Other research has shown that NH_4^+ concentrations in pore water are typically much higher than those measured in coastal surface waters (see Table 2-5; Capone, Bronk, Mulholland, 2008). Weakly-bound NH_4^+ in intertidal wetlands has been shown to be exported to the water column upon tidal flooding, as inundation causes a mixing and transport of interstitial pore water, sea water, and O_2 (Caetano, Falc̃o, Vale, & Bebianno, 1997). The balancing point between NH_4^+ mineralization (nutrients available to biota; inorganic forms) and immobilization (nutrients unavailable to biota; organic forms) lies at a C:N ratio of 20; a C:N ratio below 20 has been shown to indicate net mineralization of NH_4^+, which

results in increased inorganic C and N (Maier, 2015). While all soil samples measured a C:N ratio less than 20 (Table 2-4), the middle and lower wetland position C:N ratios were 14.8 and 13.3, respectively. Cowell Ranch soils almost exclusively contained organic C, as no reaction was observed following the addition of HCl to soil samples. Nutrient availability is often low in organic soils, which results in low bioavailability of inorganic nutrients (Mitsch & Gosselink, Wetlands, 2015). Cowell Ranch soils are classified in the first and second most organic orders— histosol and mollisol— where histosols exclusively occur in wetland ecosystems (Brady & Weil, 2010). Although the highest total C (23.9%) was measured at the upper wetland position, mineralization does not seem to play a role in the accumulation of NH_4^+ (or lack thereof) in upper wetland position soils (Figure 2-3).

DNRA has been shown to be enhanced in estuaries exhibiting low pore water NO_3^- concentrations and high Fe^{2+} availability (Kessler, Roberts, Bissett, & Cook, 2018). While Fe was not measured in this study, lower wetland position pore water experienced the lowest NO_3^- concentrations at 0.3 μmol/L (Table 2-5). Concentrations of pore water NO_3^- at the middle wetland position were not distinctly lower than other wetland positions ($p > 0.05$). Under sulfide presence, salt marsh soils retained nitrogen through DNRA (Murphy, Bulseco, Ackerman, Vineis, & Bowen, 2020). Although we did not measure sulfide, Cowell Ranch emits the characteristic “rotten egg” smell produced by anaerobic decomposition, especially when more frequently inundated soils are disturbed (i.e. middle and lower wetland positions). Sulfide concentrations measured 11 μmol/L from burrow-water (species *Urechis caupo*) in mudflats of the

Elkhorn Slough (Arp, Hansen, & Julian, 1992), and is produced at the highest rate under neutral pH conditions (Mitsch & Gosselink, Wetlands, 2015). Sulfate reduction likely plays a key role in coastal wetlands, but its importance is often overlooked (Pester, Knorr, Friedrich, Wagner, & Loy, 2012). We suggest nitrogen retention through mineralization and DNRA likely dominated the middle and lower wetland positions. However, we cannot rule out fixation as a source contributing to the measured NH_4^+ accumulation, especially at the upper wetland position.

It is possible labile C inputs— from surrounding soil and water pools— into Cowell Ranch are low, allowing for primary removal by denitrification and secondary removal by anammox, especially when C:N ratios are low; if Fe plays a key role in Cowell Ranch biogeochemistry, denitrifcation would likely dominate (see Figure 4 in Burgin & Hamilton, 2007). Pools of NH_4^+ existed across the salt marsh in soil (Table 2-4) and pore water (Table 2-5), which deserve greater exploration at Cowell Ranch. Within the first 3 hours of incubation, which represents the time interval each position remains tidally inundated at the study site (Table 2-2), soils at the middle and lower wetland positions accumulated a similar amount of NH_4^+, at an average of 9 μmol/m^2/hr and 8 μmol/m^2/hr, respectively (Figure 2-3).

2.4.3. Vegetation Presence Correlated to Nitrogen Removal During Winter

S. pacifica covered all wetland positions, with middle and lower wetland positions distinct among the number of plants, canopy width, and percent cover. The lower wetland position had the greatest canopy width (43.2 cm) and percent cover

(87.3%) when compared to the middle wetland position, yet the middle wetland position had the highest plant count at 25 individuals (Table 2-3). This suggests that plant area could compensate for plant count in net nitrogen removal, as N_2 flux was similar between the middle and lower wetland position at 972 μmol/m^2/hr and 980 μmol/m^2/hr, respectively (Figure 2-4). Roots of *S. pacifica* can extend up to 25 cm in length (Mahall & Park, 1976), but primarily occupy the top 5 cm of soil (Mahall & Park, 1976), and were observed at an average depth of 18 cm at Cowell Ranch. Aboveground plants have been found to regulate their belowground rhizospheres, which host microbial communities responsible for nutrient cycling (Henneron, Kardol, Wardle, Camille, & Fontaine, 2020). Chen et al. (2016) found a positive correlation between nutrient removal and root exudates. The greatest number of plants— and presumed rhizosphere area— at the middle wetland position may host a rich microbial community capable of the greatest NO_X uptake observed in this study (Figure 2-4).

Winter rates of nitrogen removed from estuaries around the world are presented in Table 2-7. Few studies quantified nitrogen removal across a salt marsh, with exception to Kaplan et al. (1979), who used tidal inundation as a proxy for delineating salt marsh vegetation. Our results followed a similar trend to those measured by Kaplan et al. (1979), where nitrogen removal rates increased from high elevation to low elevation across the salt marsh. These findings could indicate the importance of tidal inundation on *in-situ* soil development and the successive microbial community, as intertidal inundation has been shown to influence environmental properties in soil (e.g. salinity, O_2 depletion, redox; Delaune, Pezeshki, & Patrick, 1987). Joye et al. (1996)

measured nitrogen removal in a pacific coast estuary 175 km north of the Elkhorn Slough, which was the only study to present similar rates of nitrogen removal when compared to this study. Acetylene inhibition could have influenced rates measures by Joye et al. (1996), as the method has a long-standing history of underestimating nitrogen removal measurements via adverse elemental interactions (e.g. low NO_3^- concentrations, presence of sulfide) and nitrification inhibition (Knowles, 1990). Interestingly, Joye et al. (1996) measured nitrogen removal in an unvegetated subtidal regime, while removal rates from vegetated intertidal marshes were lower (Cabrita & Brotas, 2000; Kaplan et al., 1979) or higher (Sousa et al., 2012) when compared to our study. Nitrogen removal rates may have been underestimated by Cabrita & Brotas (2000) and Kaplan et al. (1979), as their measurements were taken during low tide which promotes aerobic soil conditions.

Elevation differences between the upper and lower wetland position (11 cm; Table 2-2), may have played a difference in nitrogen removal due to tidally-induced *in-situ* soil formation. Two vegetation species measured in genus *Salicornia* (*S. fruticosa and S. perennis*) were shown to contribute the greatest to nitrogen sequestration when compared to other salt marsh plants of genus *Spartina* and *Halimione* (Sousa, Lillebø, Pardal, & Caçador, 2010). However, Sousa et al. (2012) measured the highest rate of nitrogen removal in a *Spartina martima* marsh with combined dark and light results. Benthic primary production was not considered in this study, but deserves consideration in dark-environment laboratory incubations as it may underestimate nitrogen removal via denitrification in light-environments (e.g. *in situ*;

An & Joye, 2001). Therefore, we recommend considering the influence of day versus night on nitrogen removal.

2.4.4. The Potential for Salinity to Influence Microbial Communities

Salinity may play a role in the observed nitrogen fluxes at Cowell Ranch. Excavation of a harbor at the mouth of the Elkhorn Slough has resulted in increased tidal energy and volume (Van Dyke & Wasson, 2005), washing high-salinity marine waters into the upper channel (Table 2-1). Distinct groups of denitrifying bacteria were shown to occupy low and high salinity environments along the channel of a Pakistani estuary; the highest count of denitrifying bacteria occurred in a high-salinity site measuring 23 ppm (value close to that measured in Table 2-1; Fozia et al., 2020). Mosier and Francis (2008) observed that ammonia oxidizers or those microbes responsible for anammox removal were abundant in San Francisco Bay sediments, where archaea had a positive correlation with salinity and bacteria had a negative correlation. Additional measurements of microbial genetics in wetland soil across the salinity-defined wetland transect may provide further insight into which microbes are responsible for nitrogen removal (e.g. denitrifying bacteria, ammonia oxidizers) and retention (e.g. ammonia reducers, nitrifying bacteria, nitrogen fixers).

2.5. Conclusion

We measured nitrogen removal rates in soil cores extracted from three elevations in a *S. pacifica* dominated salt marsh in the Elkhorn Slough, CA, USA. We

paired a soil core incubation method with MIMS using N_2 as a proxy for nitrogen removal during January 2020 when plants were dormant. During the 12-hour laboratory-based incubation experiment, we observed between 499 and 980 μmol/m^2/hr of nitrogen removed, highlighting the salt marsh's potential as a nitrogen sink.

Stoichiometric analysis of N_2 accumulation revealed a higher NO_X loss than anticipated at the upper wetland position while a surplus of NO_X and/or NH_4^+ occurred at the middle and lower wetland positions. We suggest the upper wetland position underwent coupled nitrification-denitrification. The upper wetland position likely permits aerobic nitrification, as it showed evidence of Fe accumulation in soils. Mineralization and DNRA processes may have driven NH_4^+ accumulation at the middle and lower wetland positions. Soils from these wetland positions also measured low in C:N, which may promote sulfide production. We suggest that pools of bioavailable NH_4^+ and vegetation presence (including plant genus: *Salicornia*) played a role in nitrogen removal across the intertidal wetland. Plant area may have compensated for plant count in net nitrogen removal, as N_2 flux was similar between the middle (972 μmol/m^2/hr) and lower wetland position (980 μmol/m^2/hr). Belowground rhizosphere area and associated microbial communities may have influenced nitrogen-cycling, even during winter senescence of *S. pacifica*.

Future experiments are needed across the growing season to fully capture the role of *S. pacifica* in nitrogen removal and retention across Cowell Ranch, especially as pools of bioavailable NH_4^+ in pore water and wetland soil were observed.

Investigation of Fe (Fe^{2+}, Fe^{3+}), gaseous sulfur (H_2S), and microbe genetics could prove complementary to the characterization of the coastal California salt marsh as a nitrogen sink or source on seasonal and annual timescales.

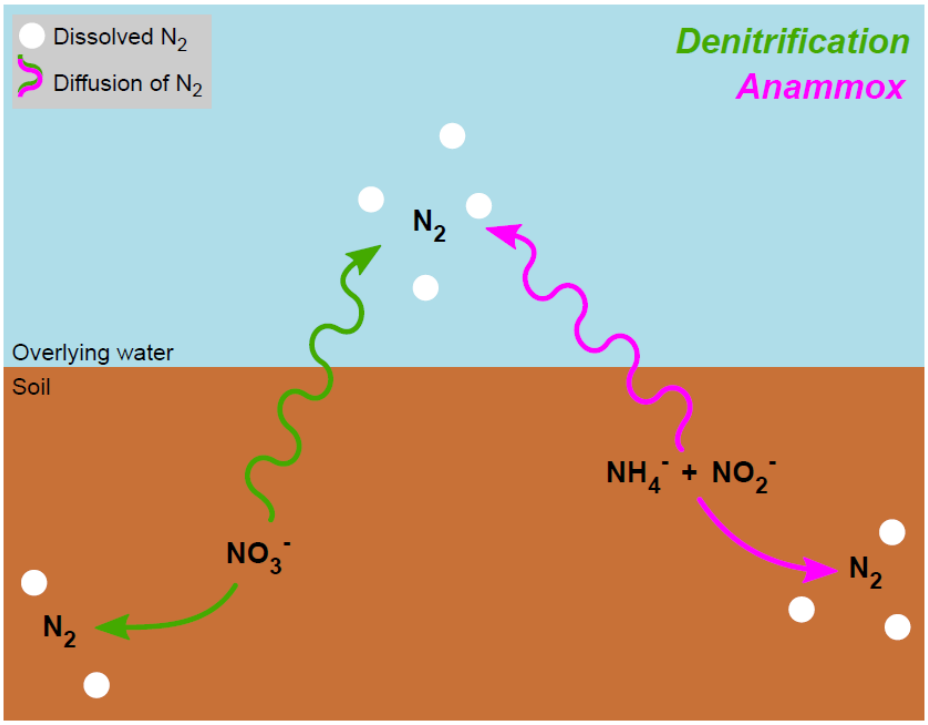

Figure 2-1 Nitrogen removal processes of interest. Denitrification and anammox occur in anaerobic wetland soil, resulting in the release of N_2. Dissolved N_2 diffuses from the soil to the overlying water; N_2 is measured in water samples extracted from the headspaces of soil cores, and analyzed on a MIMS.

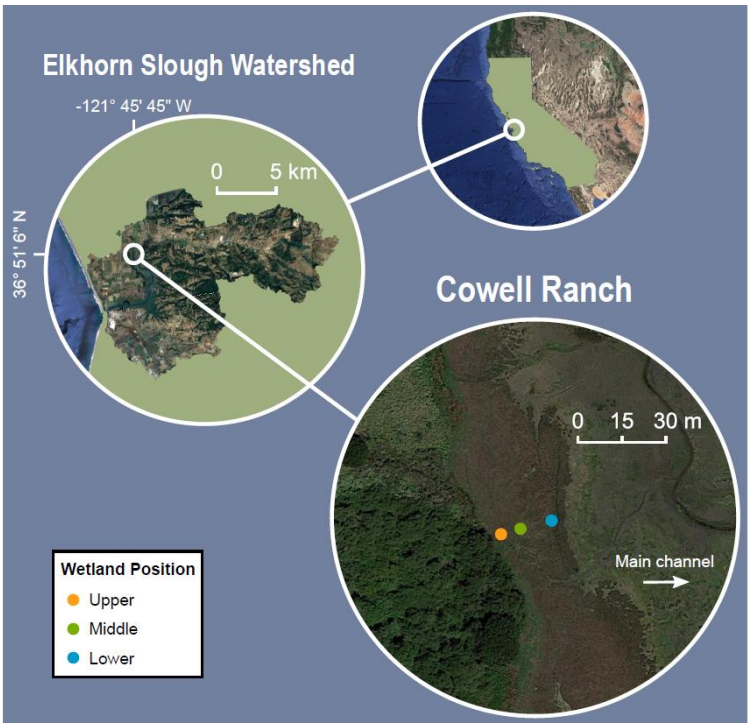

Figure 2-2 The Cowell Ranch experimental transect in Elkhorn Slough, CA, USA. Elkhorn Slough is located adjacent to Monterey Bay, in central coastal California, USA. Sampling positions across the Cowell Ranch salt marsh transect are delineated according to degree of intertidal inundation. Note the Cowell Ranch imagery was captured at low tide, with the mudflat area (to the right of the wetland positions) exposed to the atmosphere.

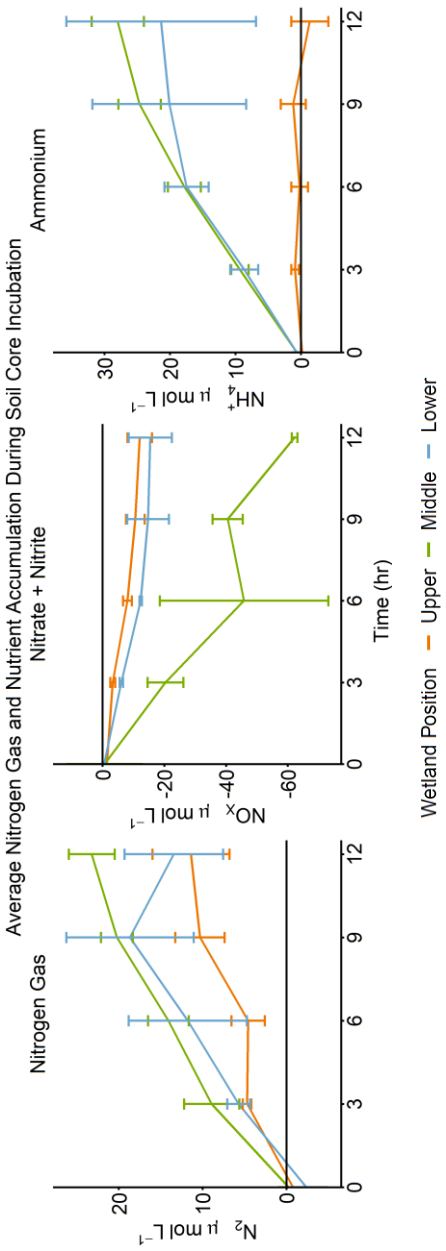

Figure 2-3 N_2, NO_X, and NH_4^+ accumulation during the 12-hour incubation. Measured changes in N_2, NO_X, and NH_4^+ in headspace waters above the soil cores during the 12-hour incubation experiment. Error bars represent the standard deviation of the averaged soil cores (n = 3) for each position.

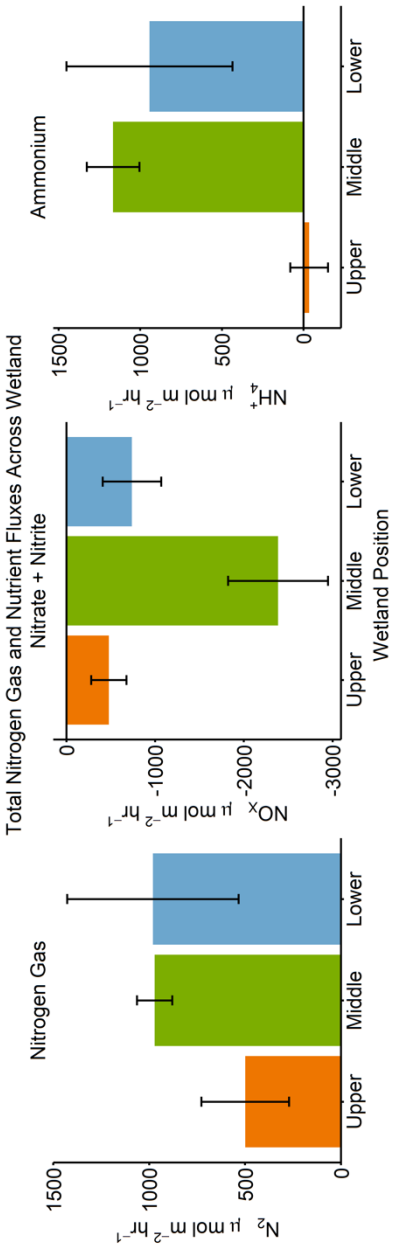

Figure 2-4 N_2, NO_X, and NH_4^+ flux averages following the 12-hour incubation. Fluxes of N_2, NO_X, and NH_4^+ at each wetland position. Error bars represent the standard deviation of the averaged soil cores (n = 3) for each position.

Table 2-1 Surface water salinities at Cowell Ranch.

	2019 Surface Water Salinities (ppt)
Summer	34.9 ± 1.1
Fall	37.4
Winter	23.7

± indicates the standard deviation of the mean (n = 3)

Table 2-2 Upper, middle, and lower wetland position elevations, inundation percentages, and average time inundated at Cowell Ranch.

Position	Elevation* (m)	Inundation (%)	Average Time Inundated (hrs/day)
Upper	1.65	6.3	1.5
Middle	1.60	7.9	1.9
Lower	1.54	10.3	2.4

* indicates elevation in meters using NAVD88 (2011)

Table 2-3 Vegetation survey results across Cowell Ranch.

	Salicornia pacifica Survey		
	Upper	Middle	Lower
Plant number	13 ± 2	25 ± 6	6 ± 2
Percent cover (%)	79.0 ± 1.3	66.3 ± 5.4	87.3 ± 6.1
Canopy width (cm)	33.7 ± 5.2	28.6 ± 4.0	43.2 ± 6.1
Plant height (cm)	41.4 ± 2.7	41.8 ± 0.7	39.0 ± 2.9

± indicates the standard deviation of the mean (n = 3)

Table 2-4 Wetland soil characterization across Cowell Ranch.

	Wetland Soil		
	Upper	Middle	Lower
Active root depth (cm)	18	-	18
Great group	Agrixeroll	-	Haplofibrist
Order	Mollisol	-	Histosol
Redox potential (mV)	-389.5 ± 0.7	-98.0 ± 36.5	-389.4 ± 1.4
C:N*	16.7 ± 0.5	14.8 ± 0.1	13.3 ± 0.4
C (%)*	23.9 ± 0.9	19.0 ± 0.5	13.9 ± 1.2
N (%)*	1.7 ± 0.1	1.5 ± 0.0	1.2 ± 0.1
NO_3^- (μmol/kg dry soil)*	143.7 ± 8.4	206.1 ± 35.7	140.6 ± 19.0
NH_4^+ (μmol/kg dry soil)*	1651.6 ± 73.3	2018.1 ± 215	1627.8 ± 65.4
pH˜	7.5 ± 0.1	7.7 ± 0.0	7.8 ± 0.0

± indicates the standard deviation of the means

* indicates results from the top 5 cm of soil cores post-incubation

˜ indicates measurements taken from headspace water at the start and end of the experiment

Table 2-5 Concentrations of *in-situ* wetland pore water across Cowell Ranch.

	In-Situ Pore Water (μmol/L)		
	Upper	Middle	Lower
NO_3^-	2.3 (5%)	3.4 (5%)	0.3 (5%)
NO_2^-	0.3 (5%)	0.5 (5%)	0.3 (5%)
NH_4^+	2.1 (ND)	22.9 (ND)	85.3 (ND)

% indicates analytical error
"ND" indicates no difference between samples and standards

Table 2-6 Surface water measurements taken during soil core extraction at Cowell Ranch.

	Surface Water
NO_3^- (µmol/L)	8.5 ± 8.5
Temperature (ºC)	7.4 ± 0.3
DO (mg/L)	8.2
pH	7.8
Salinity (ppt)	23.7
Conductivity (mS/cm)	39.9

± indicates the standard deviation of the means

Table 2-7 Nitrogen removal rates from estuaries around the world during winter.

Study	Location	Tidal Regime	Vegetation Present?	Method	Nitrogen Removal Rate ($\mu mol/m^2/hr$)
Present study*	Elkhorn Slough, CA, USA	Intertidal	Yes; *Salicornia pacifica*	Laboratory; N_2:Ar (MIMS)	499-980
Cornwell & Owens (2012)*	San Francisco Bay, CA, USA	Subtidal	No	Laboratory; N_2:Ar (MIMS)	20-70
Sousa, Lillebø, Risgaard-Petersen, Pardal, & Caçador (2012)	Tagus Estuary, PT, EU	Inertidal	1) Yes; *Spartina maritima* 2) No	Laboratory; Isotope pairing technique	1) 1,700 2) 1,250
Ferguson & Eyre (2007)*	Deception Bay, QL, AUS	Subtidal	No	Laboratory; N_2:Ar (MIMS)	52-55
Cabrita & Brotas (2000)*	Tagus Estuary, PT, EU	Intertidal	No	Laboratory; Isotope pairing technique	30-90
Ogilvie, Nedwell, Harrison, Robinson, & Sage (1997)	River Colne Estuary, UK, EU	Subtidal	No	Laboratory; Isotope pairing technique	1.5-82.5
Joye, Smith, Hollibaugh, & Paerl (1996)	Tomales Bay, CA, USA	Subtidal	No	Laboratory; Acetylece block technique	42-1,458
Kaplan, Valiela, & Teal (1979)	Great Sippewisset Marsh, MA, USA	Intertidal	*1)* Yes; *Spartina alterniflora* (tall, short), *Spartina patens, Distichlis spicata* 2) No	*In-situ* (Vegetated), Laboratory (Unvegetated); N_2:Ar (MIMS)	1) 9-64 2) 32

* indicates dark results

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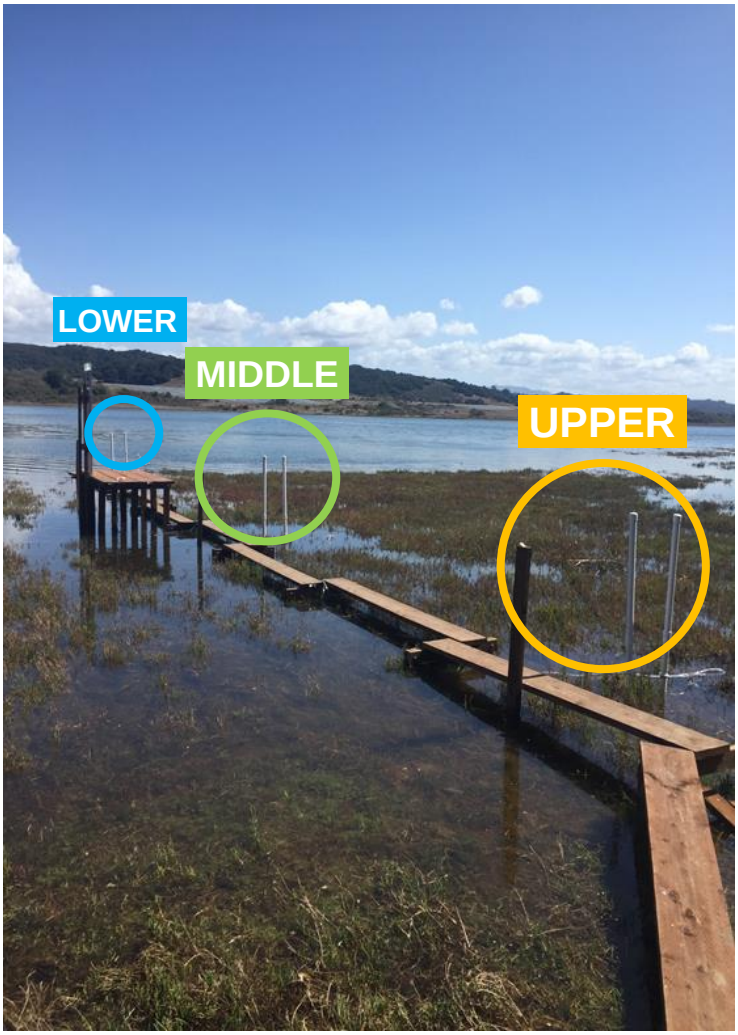


Figure 2-S1 Wetland positions upper, middle, and lower across the salt marsh. The upper wetland position experiences an average of 1.5 hours of tidal surface water inundation per day, while the middle and lower wetland positions experience 1.9 hours and 2.4 hours of inundation per day, respectively.

Figure 2-S2 Drone orthomosiac of Cowell Ranch following the winter (January 2020) soil core incubation experiment. Note the exposed sinuous tidal creek at low tide, marked by bright-green mats of sea lettuce (*Ulva .spp.*). Photo taken by John Haskins and processed by Fuller Gerbl of ESNERR.

Figure 2-S3 The contrast of *S. pacifica* between winter and summer at Cowell Ranch. The winter photograph (left) was taken on the day of soil core extraction (January 18, 2020), while the summer photograph (right) was taken during routine pore water sampling (June 11, 2019). *S. pacifica* is a native perennial herb, which undergoes above-ground senescence of plant tissue during winter months.

Figure 2-S4 Fe concretions among a reduced soil at the upper wetland position of Cowell Ranch. Fe concretions were identified between 20 and 110 cm depth below ground, indicative of wetting and drying induced by surface water presence and absence (e.g. tides, precipitation).

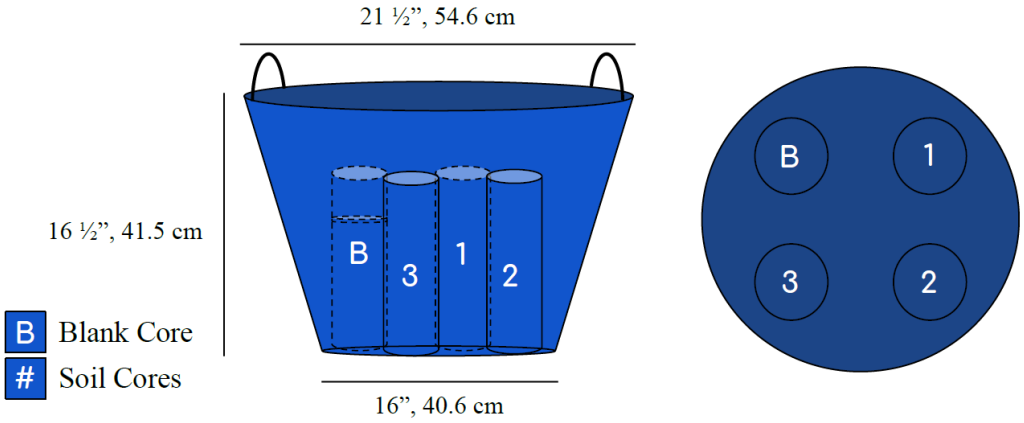
Appendix

3.1. Design Specifications

Soil core incubation design specifications are presented as exact measurements unique to the presented research project. Specifications are subject (and encouraged) to change, allowing for flexibility in desired research questions and goals. Inspired by Owens and Cornwell (2016): "The Benthic Exchange of O_2, N_2 and Dissolved Nutrients Using Small Core Incubations", the outlined design is cost-effective, relies on minimal machined parts, and allows for do-it-yourself construction.

3.1.1. Incubation Chambers

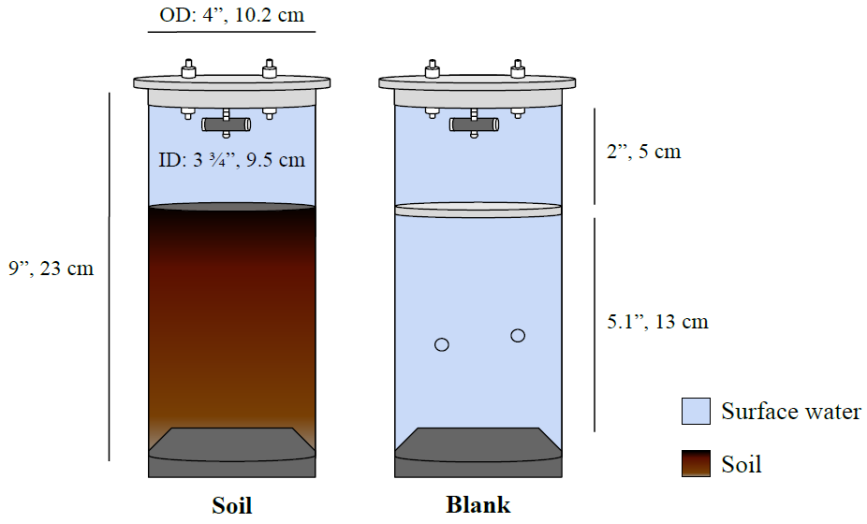
Incubation chambers serve as the micro-environment for soil core incubation at each wetland position. During soil core equilibration, the incubation chamber is filled with collected surface water. A total of three incubations chambers represent selected wetland positions along a tidal inundation gradient (i.e. chamber 1: upper, chamber 2: middle, chamber 3: lower). Each incubation chamber houses triplicate soil cores and a single blank core, with a total of four cores per incubation chamber. To secure cores in place, an adjustable string line is fitted with donut-shaped magnets.



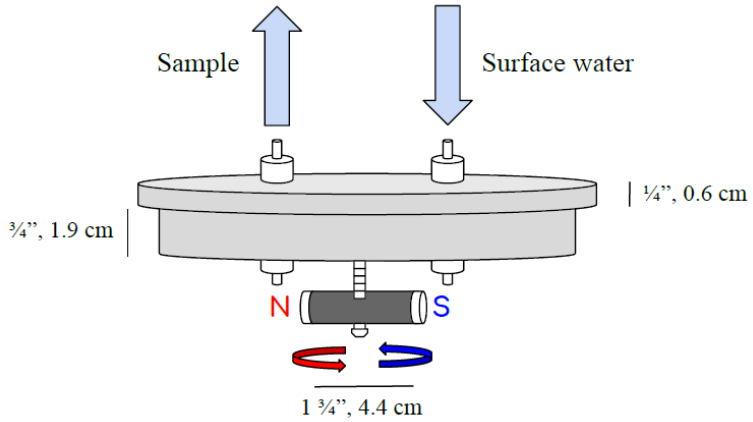
A1 A single incubation chamber with cores inside. The incubation chambers house cores from different wetland positions along a tidal inundation gradient. Each incubation chamber represents a single wetland position and contains three soil cores and one blank core with a fixed headspace.

3.1.2. Soil and Blank Cores Fitted with Magnetic Spinning Lid

An enclosed clear core with the addition of a lid with a magnetic spinner are designed for three functions (1) serve as an apparatus for holding soil, (2) enable sample water extraction and surface water replenishment in core headspace during incubation, and (3) obtain an evenly mixed headspace during incubation. An additional blank core with a fixed headspace is used in conjunction with the soil cores to obtain soil-water interface rates of nitrogen removal. Nine soil cores and three blank cores are assembled, for a total of 12 cores, and 12 lids fitted with magnetic spinners and hardware. Assembling additional cores and at least one lid is advised, for the case of core cracking or lid leaks (e.g. hardware threads incorrectly tapped). Although acrylic was used in this study, impact-resistant polycarbonate is recommended to reduce incidents of cracking.



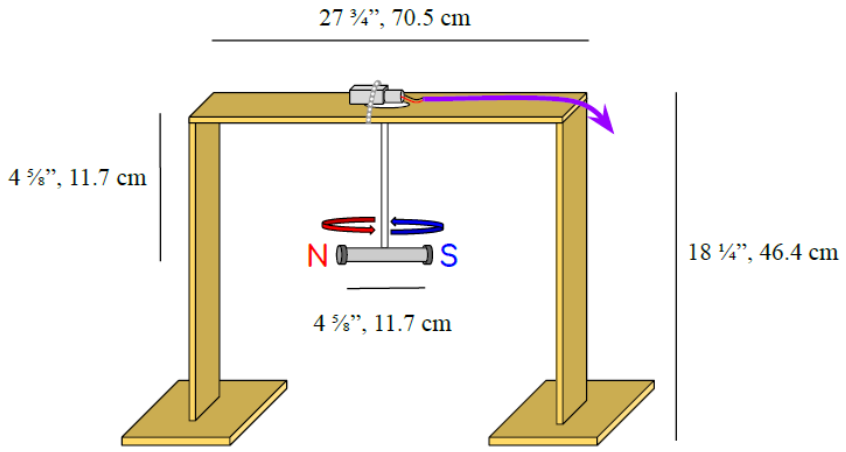
A2 Soil and blank incubation cores fitted with magnetic spinning lids. A 5 cm headspace is consistent among all cores, represented in the figure as the surface water above the soil-water interface in the soil core and the surface water above the divider in the blank core. Holes are drilled below the blank core divider to allow water into the bottom section of the core. Note that 5 cm is lost due to the inserted lid and black stopper, which leaves a soil core total length of 13 cm.



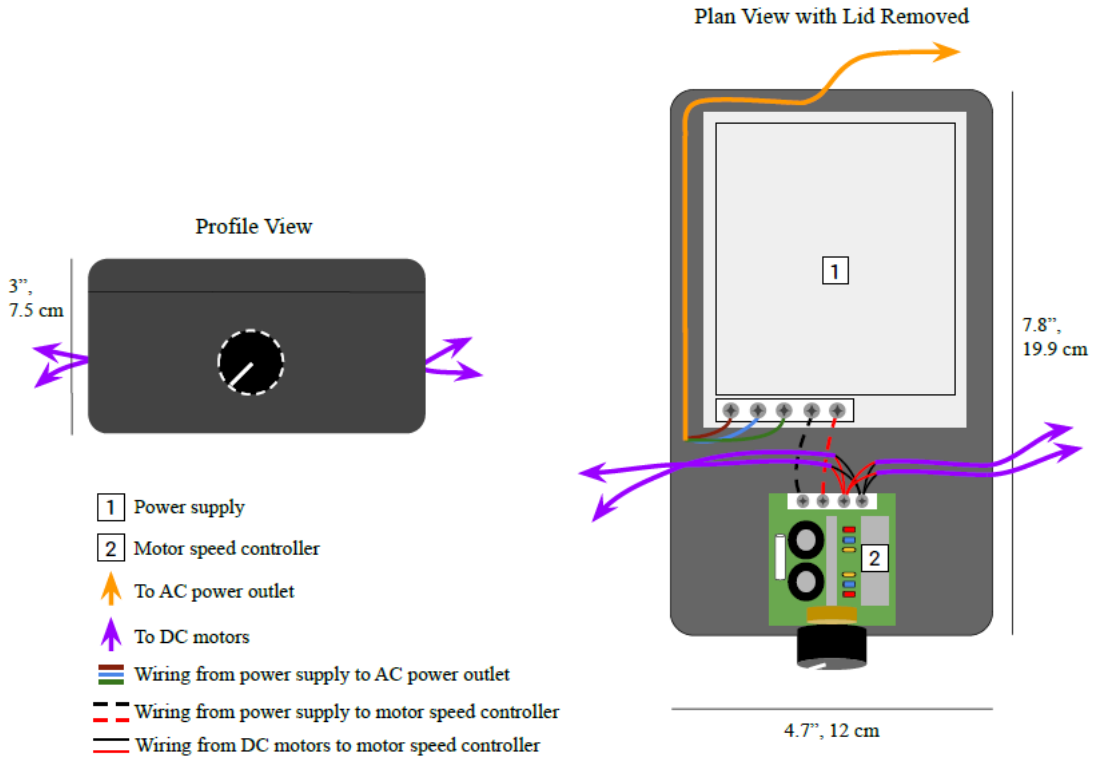
A3 Magnetic spinning lid. The light-blue directional arrows indicate flow of water in and out of ports during soil incubation— as a sample volume is pulled, the same volume is replaced with surface water. Meanwhile, the magnetic spinner mixes the headspace as it is controlled by the central magnetic stirring unit.

3.1.3. Central Magnetic Stirring Unit with Motor Control Box

The role of the central magnetic stirring unit is to create a magnetic field in each incubation chamber, capable of rotating all core lid magnetic spinners in synchrony. A small motor is mounted atop a wooden frame, powering the rotation of a rod with a magnetic tee attached. An alternating current (AC) power outlet is converted to unidirectional flow (DC) via power supply controller, which enables the DC-motor to turn. A motor control box is fitted with an adjustable knob controlling motor speed of all associated motors. Each incubation chamber is fitted with a single central magnetic stirring unit (n = 3).



A4 Central magnetic stirring unit. The large magnetic tee is controlled by a DC motor. The motor is wired to a control box (not shown, purple arrow indicating wire to motor control box). When powered on, the large magnetic tee rotates and creates an incubation chamber-wide magnetic field— causing all magnetic stirrers to rotate within individual cores.

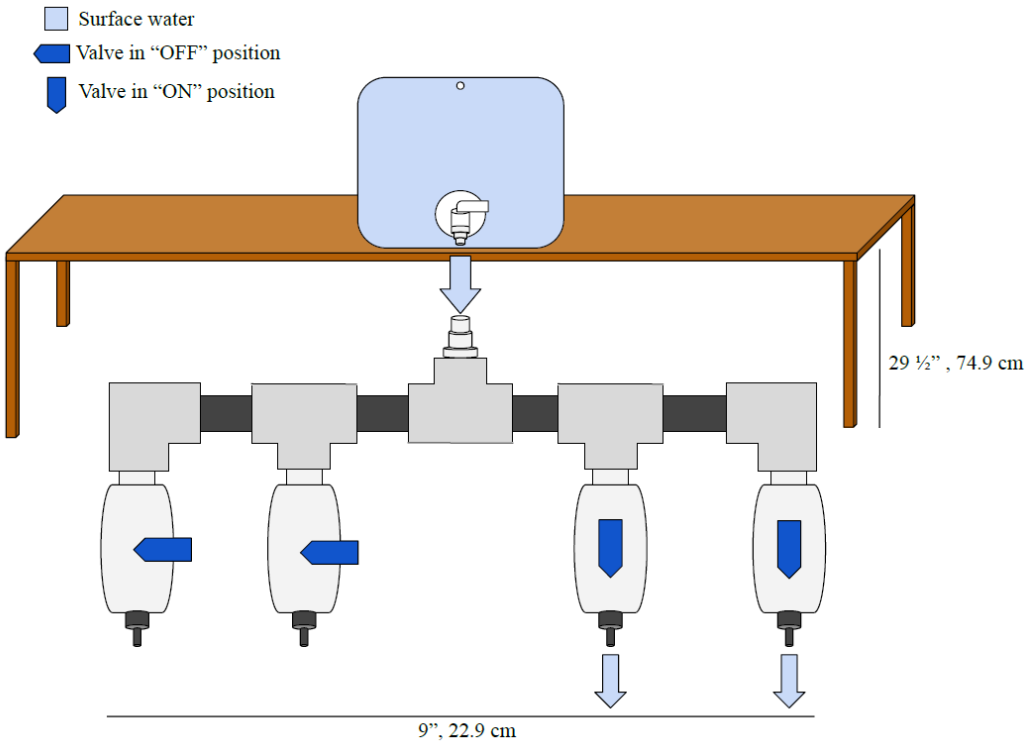


A5 Motor control box. The motor control box houses the power supply (120 V AC to 5V DC) and motor speed controller with an adjustable knob. Note the black and red wiring from the power supply and motor speed controller motor represents negative and positive charges, respectively.

3.1.4. 4-Way Valve Unit

The 4-way valve unit diverts gravity-fed replacement surface water into each core (n = 3 soil, n = 1 blank) belonging to a single incubation chamber. The valves allow for on and off manipulation of incoming surface water during water sample extraction from core headspaces. The carboy must be elevated to ensure gravity flow of surface water to the 4-way valve unit, ~30-40 cm above the tops of the cores is recommended (Owens and Cornwell 2016). The unit is fitted with clear, flexible tubing from the carboy to the 4-way valve unit and into each core. Surface water from the

carboy will be carefully flushed through the connected 4-way valve unit to remove air bubbles, which would cause errors in measurements of dissolved gases from the MIMS.

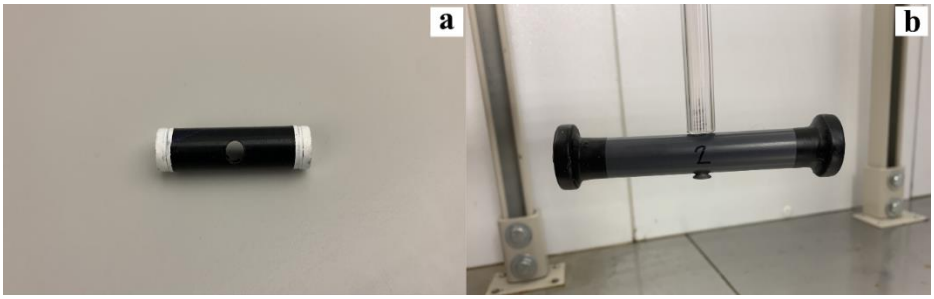


A6 4-way valve unit. Surface water is gravity fed from replacement surface water carboy, through the 4-way valve unit, and finally to the soil and blank cores. Note the light-blue directional arrows indicate flow of water; the 4-way valve unit is enlarged to show detail.

3.2. Experimental Set-Up Photographs

Photographs of the experimental set-up connect the Design Specifications (section 3.1), Soil Core Incubation Standard Operation Procedure (section 3.3), and Parts and Construction (section 3.4) into one cohesive methodology.

A7 Incubation chamber set-up. A single incubation chamber set-up, fit with (1) 4-way valve unit, (2) soil and blank cores fitted with magnetic spinning lids, and (3) central magnetic stirring unit connected to motor box. Note the motor box is not pictured.



A8 Magnetic tees used to create chamber-wide magnetic field. Close-up of (**A**) tee from magnetic stirring lid, and (**B**) tee from central magnetic stirring unit. To avoid rusting, magnet ends were plasti-dipped (x2 coats) and dried promptly, following experiment.

A9 The assembled and labeled 4-way valve unit. Close-up of assembled 4-way valve unit with blue 4-way valves and tubing leading from 4-way valve unit to clearly labeled cores.

3.3. Soil Core Incubation Standard Operating Procedure

The presented method is designed for measuring the rate of nitrogen removal through time in field-collected soil cores using a laboratory-based soil core incubation technique. This technique is coupled with the analysis of dissolved gas ratio N_2:Ar, and is analyzed on a MIMS. Although the collected surface water was not filtered in the experiment presented in Chapter 2, filtering the incubation water is strongly recommended to eliminate undesired biogeochemical activity in the water column.

3.3.1. Considerations

Considerations aid in experiment design, planning, and implementation. Therefore, these considerations should not be ignored.

- Determine degree of soil core replication needed, site-wide statistical design, and amount of small scale spatial variability

    - Determine total number of soil cores: At least 3 cores per wetland position (e.g. upper, middle, and lower)
- Final number of cores dictates volume of surface water needed for soil core incubation
    - See Experiment Calculations Spreadsheet to calculate volume of surface water based on experimental design
- Collection of surface water will likely occur at high tide (i.e. when surface water is present at or near sampling positions)
- Collection of soil cores will likely occur following the recession of high tide (i.e. when surface water is not present at or near sampling positions but soil pore spaces are still saturated)
- Determine desired soil parameters to analyze and soil storage method post-soil core incubation (e.g. freeze drying soil sampling inhibits further biogeochemical processing)

3.3.2. Experiment Timeline

The recommended timeline is outlined below for a successful soil core incubation experiment including an overview of each day's task. A rest day is recommended between day 2 and day 3.

Day 1: Field Collection and Equilibration

- Collect site-specific data by (1) collecting pore water at all sampling positions plus surface water for measurement of TN/TP, NO_X, NH_4^+, PO_4^{3-}, and DOC, (2) conducting vegetation surveys at all sampling positions, and (3) measuring *in-situ* surface water parameters (DO, pH, electrical conductivity, salinity and temperature) using a handheld water quality meter
- Collect soil core incubation materials including (1) soil cores and (2) surface or replacement surface water
- Filter replacement surface water
- Set temperature controlled room to surface water temperature
- Equilibrate soil cores by (1) positioning soil cores in designated incubation chambers, (2) filling incubation chambers with collected replacement surface water, and (3) bubbling each incubation chamber overnight (12 hours minimum)

Day 2: Soil Core Incubation and Soil Sample Extraction

- Prepare and conduct 12-hour soil core incubation experiment
- Collect water samples from soil core and blank core headspaces at 3 hour intervals and store collected samples (gas and nutrient) accordingly
- Extract soil samples from soil cores and store accordingly

Day 3: Gas Analysis on a Membrane Inlet Mass Spectrometer

- Run samples for gases of interest (N_2, Ar, O_2^*) on MIMS

  *Copper reduction column must be installed on the MIMS (see Eyre et al., 2002)

Day 4: Experiment Clean-Up

- Disassemble soil incubation set-up and clean all parts thoroughly with deionized water and mild detergent

Days Following: Soil and Water Sample Analysis

- Partition soil samples for desired analyses, complete analyses
- Run water samples for desired nutrients

3.3.3. Day 1: Field Collection and Equilibration

Thorough field collection offers site-specific knowledge and is the premise for soil incubation during day 2. Soil cores should be collected with precision and care. Due to the large volume of surface water carried from the field, it is advised to adjust crew size based on carboy load (2 carboys per person; 7 gallon carboys of saltwater weighs ~60 lbs). Crew sizes can be adjusted based on material hauling distance.

Preparation

1. Calculate the total volume of surface water to be collected using the Experiment Calculations Spreadsheet
2. Gather supplies for each objective on day 1 from the Supply List (section 3.3.8)

Pore Water Collection

1. With nitrile gloves on, collect triplicate pore water samples (10 cm) at each wetland position (upper, middle, and lower) and triplicate surface water for TN/TP, NO_X, NH_4^+, PO_4^{3-}, and DOC
2. Record time of sample collection on raw sample bottle
3. Filter and transfer triplicate raw samples from wetland positions and surface water into labeled samples bottles, accordingly
    a. See Filtering Protocol
4. Store samples in the freezer until analysis

Vegetation Survey

1. Starting at wetland position of interest
    a. Face upland
    b. Place 1 m^2 vegetation quadrat over area to be sampled
    c. Flag the four corners of the quadrat
    d. Take photographs of
        i. The entire quadrat

ii. The entire quadrat with a cardboard cutout in quadrant I

iii. Close-ups of each quadrant (I-IV)

e. Starting at quadrant I

i. If ≤ 4 individual plants are present measure every individual

ii. If > 4 individual plants are present measure only 1 individual

f. For each individual measure and record on Prepared Sampling Spreadsheet

i. Species (if species cannot be identified, take a photo and a sample)

ii. Diameter (in plan view) across the widest part of the plant(s)

iii. Circumference of the canopy at the widest portion of the plant(s)

iv. Circumference below the canopy (basal circumference) of the plant(s)

v. Height from base to tallest stem

vi. Percent cover of plant(s) canopy using the cardboard cutouts (larger cutout: 6.25% of total area, smaller cutout: 1.56% of total area)

Note: choose an area that is representative of the entire site (i.e. one that is not completely bare/disturbed)

2. Continue to step 1a, shifting to the left of the first quadrat to complete quadrat #2

3. Continue to step 1a, shifting to the right of the first quadrat to complete quadrat #3

Surface Water Collection

1. Measure *in-situ* surface water conditions based on the considerations above
    a. Record time of *in-situ* surface water condition measurements on Prepared Sampling Spreadsheet
    b. Using *in-situ* meter, measure and record: DO, pH, electrical conductivity, salinity and temperature with all sondes
        i. Calculate the average temperature and record on the Prepared Sampling Spreadsheet
    c. Contact a lab member to set temperature controlled room to the averaged *in-situ* water temperature
2. Collect replacement surface water based on the considerations above
    a. On Prepared Sampling Spreadsheet, record start time of surface water collection

        Note: when collecting surface water, avoid tidal creeks and stagnant pools.
    b. Assemble peristaltic pump with inline filter cartridge (PES 0.7 μm or finer)
        i. Pump bottom water (unstratified) into carboys

Note: if inline filter cartridge is not available, filter (PES 0.7 μm or finer) with a series of vacuum filter towers in the laboratory

c. Record end time of surface water collection

Soil Core Collection

1. Record start time for soil core collection on Prepared Sampling Spreadsheet
2. Collect cores based on the considerations above (start: upper wetland position)
    a. Record core sample location using a GPS unit and flag
    b. Core insertion
        i. Place core tube over the soil surface
        ii. If vegetation is present: (1) find a bare surface or move vegetation aside to expose a bare surface and (2) trim vegetation to soil surface if absolutely necessary (collect in ziploc bag)
        iii. Using a rubber mallet, gently mallet core tube straight down into soil using scrap wood to prevent core fracturing upon insertion
            1. Insert core tube until top of taped line— representing proper headspace volume with lid and stopper inserted— is flush with soil surface
            2. Place plastic sheet with double rubber bands on soil core top, acting as the lid for transport
    c. Core extraction

i. Using garden trowel, carefully extract soil core by digging around (~10 cm) inserted core

ii. Reach into the hole, and pull out core ensuring the bottom remains intact by digging out core deeper than actual length (i.e. digging below core bottom several centimeters)

iii. Using a serrated knife, cut soil flush with bottom of core tube

iv. Place black stopper on soil core bottom, inserting against a hard surface (cooler lid) until secure

Note: this does not compact the soil, as the soil slides up the core walls

v. Wipe off core exterior with towel until completely clean and dry

vi. Using duct tape, tape across black stopper in an X-fashion and tape around the core-stopper

vii. Store soil core upright in cooler, chilling with ice packs

d. Using a peristaltic pump and one carboy of previously collected replacement surface water

i. Fill the empty core headspaces with several centimeters of surface water to avoid soil pore spaces from drying out

e. Repeat step 2a-2d until all cores at all positions are extracted and covered with surface water

f. Backfill holes with washed sand at earliest convenience

Soil and Surface Water Equilibration

1. Upon arrival to the laboratory, check temperature has been set correctly in temperature controlled room
2. Slowly, pour all collected surface water from carboys into each incubation chamber, alternating between chambers and filling chambers equally with collected surface water
3. Push stoppers into cores one more time using a hard surface, keeping black stoppers on core bottom

   Note: this step is to double-check stopper seal and is the time to check for leaks at stopper!
4. Remove plastic sheeting and rubber bands from soil core tops
5. Slowly, one core at a time, lower core into chamber water
6. Complete for desired number of soil cores plus one blank soil core in each incubation chamber
7. Secure cores with magnetic lines
8. Remove magnetic spinners from all spinning lids
9. Place designated core lids plus plastic screw (from magnetic spinner) in bottom of designated incubation chambers
10. Drop designated stone bubbler in the center of each chamber
11. Turn on aquarium bubbler and record equilibration start time on Prepared Sampling Spreadsheet
12. Check that all incubation chambers are bubbling

13. Fill gas sample bin with deionized (DI) water

    Note: this is where dissolved gas samples will remain until MIMS analysis to stay at incubation experiment temperature

14. Turn off lights in temperature controlled room
15. Bubble chambers overnight for a minimum of 12 hours

3.3.4. Day 2: Core Incubation and Soil Sample Extraction

Core incubation allows the user to obtain N_2 accumulation through time, an indicator of microbe induced nitrogen removal via denitrification and anammox. A 12-hour incubation is conducted after all cores are capped, where dissolved gas, nutrient, and *in-situ* measurements are collected through time. Soil samples are extracted post-incubation, allowing for further analysis of factors contributing to observed N_2 cycling in the soil cores.

Preparation

1. Gather supplies for each objective on day 2 from the Supply List (section 3.3.8)

Remove Equilibration Bubblers

1. Turn on red lights in temperature controlled room, keep overhead lights off for the entirety of the experiment
2. Turn off aquarium bubbler and document equilibration end time
3. Remove aquarium bubbler and associated stones, dry, and set aside

Measure *In-Situ* Incubation Chamber Conditions and Collect Replacement Surface Water Samples

1. Starting at chamber of interest (start: chamber 1), drop water quality sondes into water-filled chamber
2. On Prepared Sampling Spreadsheet, record time and measure + record DO, pH, electrical conductivity, salinity, and temperature with all sondes
    a. Check that water temperatures in chambers match or are close to DO and *in-situ* water temperatures from field collection
    b. Remove sondes and rinse with DI over sink
    c. Dry sonde by shaking as best as possible after DI rinse and dabbing with kimwipes
    d. Repeat until all chambers are complete
3. Assistant: collect triplicate replacement surface water samples from each incubation chamber
    a. Put on nitrile gloves
    b. Collect replacement surface water nutrient samples in 60 mL bottle by dunking 50 mL syringe, with plunger removed and finger over tip, into chambers of interest, and attach plunger to end

        Note: carefully turn syringe on side when installing plunger to prevent water from squirting out
    c. Collect replacement surface water gas samples in 8 mL glass vial by dunking into chamber of interest

d. Record time of nutrient and gas collection on Prepared Sampling Spreadsheet

4. Assistant: filter and add preservative to collected samples
    a. Filter raw replacement surface water nutrient sample
        i. Attach PES filter (0.45 μm) disk to labeled syringe end, screwing on in one turn
        ii. Filter sample into labeled 60 mL bottle
        iii. Discard filter disk
        iv. Set aside filtered nutrient sample
    b. Add preservative to replacement surface water gas samples
        i. With nitrile gloves on, pipet 166 μL of 50% $ZnCl_2$ into 8 mL glass vial without touching pipet tip to sample

            Note: ensure concave meniscus is visible
        ii. Carefully cap 8 mL glass vial, checking for air bubbles by turning sample upside-down
            1. If sample has air bubble, uncap and fill with additional $ZnCl_2$ solution, recap, check for air bubbles
        iii. Submerge gas samples in gas sample bin filled with DI water

Pumping Surface Water into Replacement Surface Water Carboys

1. Assemble peristaltic pump with tubing

2. Place one end of the peri-pump tubing into chamber of interest (start: chamber 1) and the other end into an empty carboy (start: carboy 1)
3. Pump previously calculated volume of bubbled surface water from chamber into labeled 5-gallon carboy
    a. Remove peri-pump tubing from chamber water, running in the air until tubing is dry
        i. Assemble carboy spigot
    b. Attach carboy spigot to carboy

       Note: 4-way valve unit and ½" tubing section will already be attached to spigot
    c. CLOSE all 4-way valves
5. Secure carboy in place on shelf
6. OPEN carboy to flush water into 4-way valve unit
7. Carefully move 4-way valve unit around and flick tubing to dislodge bubbles trapped in the unit

   Note: this step should be done carefully, taking several minutes to dislodge bubbles from 4-way valve unit
8. Repeat steps 2-7 until all carboys are full of replacement surface water, secured to shelving, and air bubbles in 4-way valve unit dislodged

   Note: 4-way valve units are not attached to cores yet

Attach Core Spinning Lids to Cores

1. Continuing at chamber of interest (start: chamber 1), add plastic tubing clamps to surface water reservoir line and fully attach surface water line to designated core lid port

   Note: ensure tubing lines are not tangled

2. Flush air out of 4-way spigot and associated surface water lines

   Important note: the following steps (steps 2-12) are done with core lids, core hardware, and associated surface water lines submerged in chamber water with the goal of forming an air or bubble free connection between replacement surface water carboy and cores.

   a. Slowly OPEN 1st 4-way valve ¼ turn or less, starting at the left (start: core 1 in chamber 1)
   b. Watch air bubbles run down surface water line, moving around 4-way spigot to dislodge any left-behind air bubbles
   c. Once all air bubbles are clear from line, CLOSE 1st 4-way valve
   d. CLOSE tubing clamp on surface water line

3. Starting with spinning lid of interest (start: core 1), replace magnetic spinner with pre-soaked plastic screw

   Note: use a pipe cleaner to dislodge any bubbles from center screw hole before attaching magnetic spinner!

4. Once magnetic spinner is attached to core lid, spin magnetic spinner several times to dislodge any air bubbles

5. Run your fingers around the lid o-ring, removing the o-ring and replacing it to dislodge any air bubbles
6. With one person positioned at each incubation chamber, carefully attach spinning lid using equal pressure to cap core

   Note: the surface water line and sample line will be connected with valves closed
7. Inspect lid attachment, ensuring (1) no bubbles, (2) that the o-ring has not moved, and (3) that the lid fully inserted

   Note: be patient, capping takes time and often needs to be redone 10 times or more to eliminate all air bubbles; you are doing great!! ☺
8. When all cores appear bubble free, uncap all cores once more
9. Continuing at chamber and core of interest (start: chamber 1, core 1), recap core one final time
10. Add tubing clamp to sample line
11. CLOSE tubing clamp on sample line
12. Assistant: record time core is successfully capped on Prepared Sampling Spreadsheet
13. Re-secure core using associated magnetic line and tuck in sample line
14. Repeat steps 9-13 for all cores in the working chamber
15. Carefully place central magnetic stirring unit over chamber

16. Power on central magnetic stirring unit (by connecting chamber of interest to central magnet stirring unit power box) after core positions in chamber are correct, adjusting cores and magnetic lines as needed

    Note: central magnetics should be rotating smoothly
17. Adjust motor to highest RPM possible (range: 0-36 RPM), without disturbing or suspending soil in cores
18. Begin dissolved gas, *in-situ*, and nutrient sampling of the incubation chamber that was successfully capped (section **Dissolved Gas Sampling** and ***In-Situ* and Solute Sampling**)
19. Repeat 9-18 for all incubation chambers in order (i.e. chamber 1, 2, 3)
20. Take photos of each incubation chamber after first sampling round for reference at the experiment end (i.e. no bubbles in cores after first sampling round)

Dissolved Gas Sampling

1. Sample water in core headspace at predetermined sample intervals (e.g. every 3 hours)
    a. From core of interest (start: core 1), carefully untuck submerged sample line from magnetic line and lift out of chamber water
    b. Wipe sample line with a kimwipe until dry, preventing contamination from chamber water
    c. Remove stagnant water from sample line

i. Place sample line in 10 mL graduated cylinder (start: core 1) over large beaker

ii. Slowly OPEN 1st 4-way spigot knob, starting at the left, ½ turn or less, controlling surface water

iii. OPEN tubing clamp on surface water line

iv. Carefully fill 10 mL graduated cylinder to 3 ml

1. Quickly open and close sample line clamp
2. CLOSE tubing clamp on sample line when 3 mL full
3. Assistant: record time and exact amount (~ 3 mL) extracted on Prepared Sampling Spreadsheet

   Note: this time marks the sample timing of future sampling intervals taking place every 3 hours for 12 hours (discard happens quickly relative to step 1d)
4. Dump discard from 10 mL graduated cylinder into water catchment bin

v. CLOSE tubing clamp on surface water line

vi. CLOSE 4-way spigot knob controlling surface water

d. Collect core sample in 8 mL glass vial

i. Place sample line in labeled 8 mL glass vial (start: core 1) over large beaker

ii. Slowly OPEN 1st 4-way spigot knob, starting at the left, ½ turn or less controlling surface water

iii. OPEN tubing clamp on surface water line

iv. Carefully fill 8 mL glass vial:

1. Quickly open and close sample line clamp
2. CLOSE tubing clamp on sample line when 8 mL glass vial is full
3. Transfer sample overflow to 10 mL graduated cylinder and measure volume
4. Assistant: record exact amount extracted on Prepared Sampling Spreadsheet (~8 mL)

v. Hand 8 mL glass vial to gloved assistant

1. Assistant: pipet 166 µL of 50% $ZnCl_2$ into 8 mL glass vial without touching pipet tip to sample

   Note: ensure concave meniscus is still visible
2. Assistant: carefully cap 8 mL glass vial, checking for air bubbles by turning sample upside down
   a. If sample has air bubble, uncap and fill with additional $ZnCl_2$ solution, recap, check for air bubbles
3. Assistant: submerge sample in sample bin filled with DI water

e. Continue to step 1 in section ***In-Situ* and Solute Sampling** if collecting *in-situ* measurements and solutes, if not continue below

f. CLOSE tubing clamp on surface water line

g. CLOSE 4-way spigot knob controlling surface water

h. Submerge sample line in chamber water, and secure in proper magnetic line holding core

i. Once dissolved gas sampling of all chambers is complete

    i. Discard used kimwipes

    ii. Dump water catchment bin in sink

    iii. Re-prepare sample bin and water catchment bins

In-Situ and Solute Sampling

1. Sample Cores

    a. Place sample line into labeled modified falcon tube (i.e. cut to fit sondes from handheld water quality meter in)

    b. Carefully fill to 15 ml

        i. Quickly open and close sample line clamp

        ii. CLOSE tubing clamp on sample line when 15 mL is reached

        iii. Assistant: record time of sample extraction and exact amount extracted on Prepared Sampling Spreadsheet (~15 mL)

    c. Hand labeled modified falcon tube, with sample inside, to gloved assistant

    d. Assistant: take *in-situ* measurements

        i. Place modified falcon tube into position in styrofoam rack

ii. Place DO sonde into modified falcon tube

iii. Click measure and wait for stabilization

iv. Record DO (mg/L) on Prepared Sampling Spreadsheet

v. Set DO sonde aside by hanging on dry rack

vi. Place pH meter into same modified falcon tube

vii. Click measure and wait for stabilization

viii. Record temperature (C) and pH (pH) on Prepared Sampling E

ix. Set pH sonde aside by hanging on dry rack

x. Connect electrical conductivity sonde to meter

xi. Place electrical conductivity into same modified falcon tube

xii. Click measure and wait for stabilization

xiii. Record temperature (C), electrical conductivity (mS/cm), and salinity (psu) on Prepared Sampling Spreadsheet

xiv. Set electrical conductivity sonde aside by hanging on dry rack

xv. Continue until all modified falcon tubes representing all cores are measured

e. Assistant: filter sample

i. Grab labeled syringe, remove plunger, and add syringe filter

ii. Pour 15 mL contents from modified falcon tube into labeled syringe

iii. Carefully attach syringe plunger to full syringe

iv. Attach PES filter disk to labeled syringe end, screwing on in one turn

v. Filter sample into labeled 60 mL bottle

vi. Discard filter disk

vii. Continue until all modified falcon tubes representing all cores are filtered into 60 mL bottles

f. CLOSE tubing clamp on surface water line

g. CLOSE 4-way spigot knob controlling surface water

h. Submerge sample line in chamber water, and secure in proper magnetic line holding core

2. Repeat step 1 until all cores are sampled (until styrofoam rack is full)

3. Once *in-situ* and solute sampling of all chambers is complete

a. Assistant: (1) finish writing label on all 60 mL sample bottles (date, time sampled), and (2) place all 60 mL sample bottles in the freezer

b. Discard used kimwipes

c. Dump water catchment bin in sink

d. Rinse 10 mL graduated cylinder and large beaker with DI and shake dry

e. Rinse all labeled syringes with DI and shake to dry

f. Re-prepare sample bin and water catchment bins

Bubble Occurrence in Core During Incubation

1. If a gas bubble occurs— **even as small as a pin-prink**— in soil or blank cores during:
    a. Round 2 (t = 3) of incubation, the core must be recapped and effectively restarted from t = 0
        i. Record which core contains the air bubble
        ii. Recap the core as done previously
            1. Carefully attach spinning lid using equal pressure

               Note: the surface water line and sample line will be connected
            2. Inspect lid attachment, ensuring (1) no bubbles, (2) that the o-ring has not moved, and (3) that the lid fully inserted
        iii. Begin dissolved gas, *in-situ*, and nutrient sampling of the incubation chamber that was successfully capped (section **Dissolved Gas Sampling** and ***In-Situ* and Solute Sampling**

             Note: this new time marks the sample timing of future sampling intervals taking place every 3 hours for the remaining experiment duration (t = 9 for recapped core, t = 12 for other cores)
    b. Rounds 3-5 (t = 6-12) of incubation, the cores can be retired (i.e. too much time has gone by)

i. Record time of core retiring

ii. Do not proceed to **Dissolved Gas Sampling** or ***In-Situ* and Solute Sampling** for the duration of the experiment

iii. Continue with **Post-Incubation Replacement Surface Water and Soil Sampling**, taking note of the retired core(s)

Post-Incubation Replacement Surface Water and Soil Sampling

1. Turn lights on in temperature controlled room
2. Take photos of each chamber and all cores
3. Assistant: measure *in-situ* conditions of end of experiment replacement surface water from each replacement surface water carboy
    a. Close spigot of carboy of interest and remove 4-way valve unit (start: chamber 1 carboy)
    b. Over sink, spigot water from carboy into rinsed large beaker
    c. Measure and record DO, pH, electrical conductivity, salinity, and temperature with all sondes
    d. Record time of collection on Prepared Sampling Spreadsheet
    e. Remove sondes and rinse with DI over sink
    f. Dry sonde by shaking as best as possible after DI rinse and tapping with kimwipes
4. Assistant: collect triplicate replacement surface water samples from each replacement surface water carboy

a. Collect nutrient sample in 60 mL bottle
b. With carboy still over stink, spigot water into 60 mL bottle
c. Collect gas sample in 8 mL glass vial
d. With carboy still over stink, spigot water into 8 mL glass vial
e. Record time of nutrient and gas sample collection on Prepared Sampling Spreadsheet

5. Filter and add preservative to collected samples
    a. Filter raw replacement surface water nutrient samples
        i. Ensure nitrile gloves are on
        ii. Grab labeled syringe, remove plunger, and add syringe filter
        iii. Pour contents from raw replacement surface water bottle into labeled syringe
        iv. Carefully attach syringe plunger to full syringe

            Note: carefully turn syringe on side when installing plunger to prevent water from squirting out
        v. Attach PES filter disk to labeled syringe end, screwing on in one turn
        vi. Filter sample into labeled 60 mL bottle
        vii. Discard filter disk
    b. Add preservative to replacement surface water gas samples
        i. With nitrile gloves on, pipet 166 µL of 50% $ZnCl_2$ into 8 mL glass vial without touching pipet tip to sample

Note: ensure concave meniscus is still visible

ii. Carefully cap 8 mL glass vial, checking for air bubbles by turning sample upside down and mixing sample with preservative

iii. Submerge gas samples in sample bin filled with DI water

iv. Repeat steps 2-5 until all carboys are sampled

6. Remove soil sample from core

    a. Remove soil core from chamber of interest (start: chamber 1, core 1), dry with a towel, and set aside on counter top

    b. Put on a new pair of nitrile gloves

    c. Dry outside of core with towel

    d. Carefully remove spinning lid using lip of lid on sink/countertop edge

    e. Move core to ethanol cleaned tray

    f. Carefully remove black stopper

    g. Insert plunger disk into bottom of core (i.e. where the black stopper was)

    h. Stand 1” PVC pipe on tray with core

    i. Place core on top of 1” PVC pipe, so contact with plunger disk and PVC is made

    j. While holding the soil core, push down creating force between the countertop and soil core

    k. Stop at 5 cm and cut soil into one 0-5 cm bulk disk

    l. Place bulk disk on tray

7. Collect microbe sample
    a. Using an ethanol cleaned soil scoop, scoop ~5 g or ~1 tablespoon of soil from the surface of the bulk disk
    b. Carefully place the scooped sample in a labeled whirlpak
    c. Flatten sample in whirlpak into a disk
    d. Using the edge of the counter, push all air out of the whirlpak starting from the bottom edge
    e. On the counter, fold the metal twist tie several times until the flattened fold is near the sample
    f. Bend both ends of the whirlpak towards the inside once
    g. Set aside

        Note: see this YouTube video on proper closing of a whirlpak
8. Collect bulk sample
    a. Place the bulk disk sample in a labeled ziploc bag
    b. Set aside

        Note: freeze dry within 24 hours
9. Set sampled soil core aside
    a. Rinse tray, knife, and scoop with DI water, and dry with paper towels
    b. Wipe down tray, knife, and scoop with ethanol on paper towels
    c. Repeat step 6-9 for all incubation chambers (12 soil cores total)
10. When soil samples are complete, store accordingly:
    a. Microbe samples in whirlpaks: -20 C freezer

b. Bulk samples in ziploc bags: -18 C freezer

   Note: after bulk samples are completely frozen, freeze dry as soon as possible

Same-Day Clean Up

1. Rinse with DI and wipe dry all magnet parts including magnetic spinning tees on each core lid, magnet tees on central magnetic stirring units, and magnetic lines

   Note: this step is extremely important and prevents rust of metal parts before the deep cleaning on day 4

3.3.5. Day 3: Gas Analysis on a MIMS and Freeze Drying Bulk Soil Samples

Dissolved gas samples collected during the soil incubation experiment should be analyzed on a MIMS within 48 hours. Since MIMS design and standard operating procedures vary from instrument to instrument, consult with the expert on the MIMS instrument and request a thorough training alongside their standard operating procedure. The following standard operating is for the MIMS from the Lawrence Livermore National Laboratory, located in the Zimmer Watershed Hydrology Laboratory, UCSC.

Preparation

1. Gather supplies for each objective on day 3 from the Supply List (section 3.3.8)

Preparing Insulated Beaker

1. Prepare insulated beaker
    a. In fume hood, add dry ice to fill insulated beaker ¾ full
    b. Add ~200 mL isopropanol to small beaker
    c. Slowly pour a small amount of isopropanol into insulated beaker filled with dry ice

    Note: contents of insulated beaker will smoke, so pour small amounts in intervals until dry ice is floating, stirring with metal rod to prevent dry ice from sticking together

Connecting Desktop to MIMS and Opening RGA Software

1. Switch the left-most blue Ethernet cable from wall to Ethernet hub connected to the MIMS

    Note: any of the 1-4 ports on the Ethernet hub will work
2. Turn off desktop firewalls
    a. Firewall & network protection → Turn off: Domain network, Private network, and Public network → Exit windows
3. Connect desktop to MIMS IP address
    a. Ethernet settings → Change adapter options → Ethernet → Properties → Internet Protocol Version 4 (TCP/IPv4) → Use the Following IP Address → Enter IP Address: 192.168.0.101 → Enter Subnet Mask: 255.255.255.0 → Okay → Okay → Close → Exit windows

Note: yes, the final number is different than the actual IP address

4. Open RGA software
    a. Desktop → MIMS → Right click rgaApp - Shortcut → Run as Administrator → Yes → rgaApp will open

MIMS Start-Up

1. MIMS should be powered on, and system in vacuum
    a. If not powered on, power on the RGA flipping the switch on the back of the box
2. Connect MIMS to computer by switching IP address (192.168.0.102)
3. Open template RGA file template
    a. Desktop → MIMS → RGA Run Template → yyyymmdd_hhmm_MIMS_name
4. Save RGA file template, allowing user to keep defaulted settings
    a. File → Save as… → Rename → Save as .rga file
5. Link RGA by clicking: 
    a. Update RGA list → Select IP address associated with MIMS (192.168.0.102) → Connect → Close
6. Filament may turn on by default:  (B&W: Off, Color: On)
7. Save often by clicking: 
8. To change gases of interest click: 

Note: Keep default speed of 6, default gases include CH_4, N_2, O_2, Ar, CO_2, H_2O

9. Turn off filament:  (B&W: Off, Color: On)
10. Close valve #1 (metal T)
11. Slowly open valve #2 (round black plastic)
    a. Watch pressure rise, ensure pressure does not exceed 500 mTORR
    b. Continue slowly in intervals until fully open
    c. Turn a half turn in the opposite direction once fully open
12. Place prepared insulated beaker over trap, using metal stir rod to break up dry ice
13. Wait for the red light to go out next to mTORR and pressure reads 0
14. Open valve #1 (metal T)
    a. Open ~12 full rotations
15. Turn on filament:  (B&W: Off, Color: On)

Opening Prepared Excel File

1. Open the Prepared MIMS Spreadsheet created by Ate Visser (Desktop → MIMS → Prepared MIMS Spreadsheet → yyyymmdd_hhmm_MIMS_name), allowing user to keep defaulted settings
    a. Click “File”
    b. “Save as…”

c. Rename and save as .xle file

2. Open the “runtimes” tab in the Prepared MIMS Spreadsheet

Running Dissolved Gas Samples

1. Click go: 
2. MIMS is now running in sample “zero” mode, measuring residual air in the machine and on internal walls
    a. Wait for “zero” to stabilize (represented as a plateau on the graph) as filament warms and trap cools, which takes ~1.5 hrs. Toggle arrow keys to read measured values through time.

    Note: oxygen stabilizes slowly, and water never fully stabilizes. Nitrogen and argon stabilize quickly.
4. While sample “zero” is equilibrating (~1.5 hrs), gather and organize samples according to desired run scheme
5. Enter “Sample” and “Sample_ID” in “runtimes” tab of Prepared MIMS Spreadsheet
6. Once sample “zero” is stabilized, enter the end time in the “runtimes” tab
7. Begin sample “AEW” (i.e. the air-equilibrate water standard)
    a. Swap “home AEW” with sample “AEW”
    b. Ensure auto sampler is rested near the bottom of the sample vial
    c. Turn on peristaltic pump by clicking the counter clockwise arrow button

Note: refill “home AEW” and sample “AEW” with air-equilibrated Milli-Q water

8. Once sample “AEW” is stabilized, enter the end time in the “runtimes” tab
9. Begin sample “1”
    a. Swap sample “AEW” with sample “1”
    b. Ensure autosampler is rested near the bottom of the sample vial
10. Once sample “1” is stabilized, enter the end time in the “runtimes” tab
11. Begin sample “2”, repeating steps 9-10 until samples are complete, making sure to disperse sample “AEW” every 5-10 samples

    Note: during sample run (1) watch sample vial to ensure sample doesn’t run dry when waiting on stabilization, (2) tend insulated beaker when dry ice runs low
12. Begin sample “AEW”
    a. Swap sample “#” (last sample) with sample “AEW”
    b. Ensure auto sampler is rested near the bottom of the sample vial
13. Once sample “AEW” is stabilized, enter the end time in the “runtimes” tab
14. Begin sample “zero”
    a. Turn off peristaltic pump by clicking “Stop”
    b. Swap sample “AEW” with “home AEW”
    c. Ensure auto sampler is rested near the bottom of the sample vial
15. Once sample “zero” is stabilized (~1.5 hrs), enter the end time in the “runtimes” tab

16. Save the Prepared MIMS Spreadsheet and RGA file
17. Click stop: 

MIMS Shut-Down

1. Turn off filament by clicking:  (B&W: Off, Color: On)
2. Close valve #1 (metal T)
3. Remove the trap beaker and place an empty beaker under the trap to catch melting dry ice

   Note: pressure will increase after insulted beaker is removed

   a. Allow light next to mTORR to go out, and pressure return to 0
4. Close valve #2 (round black plastic)
5. Open valve #1 (metal T)
6. Dispose of samples (H_2O + 50% $ZnCl_2$) and isopropanol from the insulated beaker in properly labeled waste bins located under the fume hood

Saving .RGA File

1. Save data in .txt format
   a. File → Save ACSII data → Rename with file name previously used and save as .txt file

Opening .RGA File In Prepared MIMS Spreadsheet

1. Copy and paste .txt file to Prepared MIMS Spreadsheet: “ASCII data” tab

a. Highlight first row → Ctrl + Shift + End → Ctrl + C

b. Paste data in “ASCII data” tab (Ctrl + P)

c. Format data by clicking Data → Text to Columns → Delimited → Next → Comma → Next → Finish

2. Edit elevation of MIMS (~100 m) in “AEW concentration” tab
3. Review that all tabs are correct and email to Ate Visser if needed

Disconnecting Desktop from MIMS

1. Switch the blue Ethernet cable from the Ethernet hub back connected to the MIMS into the wall
2. Turn on desktop firewalls
    a. Firewall & network protection → Turn on: Domain network, Private network, and Public network → Exit windows
3. Connect desktop to computer IP address
    a. Ethernet settings → Change adapter options → Ethernet → Properties → Internet Protocol Version 4 (TCP/IPv4) → Obtain an IP address automatically → Okay → Okay → Close → Exit windows

3.3.6. Day 4: Experiment Clean-Up

All parts used in the soil core incubation— incubation chamber setup and cores— should be disassembled, thoroughly rinsed and scrubbed (where possible) with DI water and mild detergent. Close attention should be paid to magnetic parts, drying

immediately upon cleaning a second and final time to prevent rust. Plasti-dip on magnet ends should be replaced accordingly.

3.3.7. Days Following: Soil and Nutrient Sample Analysis

Bulk soil samples should be completely frozen and freeze dried as soon as possible to prevent any undesired biogeochemical processing. Once freeze dried, soils can be partitioned for desired analysis (e.g. particle size, C:N, soil nutrients). If completing microbe analysis, soil sample DNA will be extracted using a DNeasy PowerSoil Kit (QIAGEN) and tested for desired nitrogen-cycling genes via quantitative real-time polymerase chain reactions or qPCR. *In-situ* water samples (NO_X, NH_4^+, PO_4^{3-}, DOC, TN/TP) and soil core incubation nutrient samples (NO_X, NH_4^+, PO_4^{3-}) should be analyzed accordingly.

3.3.8. Supply List

The supply list is coupled with the Soil Core Incubation Standard Operating Procedure (section 3.3). Supplies are subject to change depending on what the user has available and the desired research goals of the project.

Pore Water Collection

- Pore water lunchbox
    - Ashed 47 mm DOC filters
    - 47 mm PES filters

- 47 mm filter holders
- Tweezers
- Sample label tape
- Sharpies
- Nitrile gloves

- Mini-piezometer, tubing, syringe
- Pre-labeled sample bottles
  - X4 raw 100 mL sample bottles (upper, middle, lower, surface water)
  - X12 filtered 60 mL sample bottles (each raw sample filtered into 3 sample bottles)
- Orion A329 handheld meter
  - DO sonde
  - Electrical conductivity sonde
  - pH sonde
- Large cooler
- Small hand-held cooler
- Ice packs
- Flagging
- Field notebook
- Pencils

Vegetation Survey

- Prepared Sampling Spreadsheet printed on waterproof paper ("Vegetation Survey" tab)
- 1 m^2 vegetation plot
- Vegetation plot cardboard cutouts
- Measuring tape
- Survey tape
- Ziploc bags
- Camera
- Field notebook
- Pencils

Surface Water and Soil Core Collection and Handling

- GPS unit
- X2 carboy backpacks
- X2 hip waders
- X9 cores tubes marked with tape (5 cm headspace volume after lid and black stopper have been inserted), and labeled for each site/wetland position
- X9 black stoppers (one for bottom of each core tube)
- X9 plastic core squares (acting as lids)
- X18 rubber bands

- ☐ Prepared Sampling Spreadsheet printed on waterproof paper (“Core Extraction” tab)
- ☐ Orion A329 handheld meter
  - ☐ DO sonde
  - ☐ Electrical conductivity sonde
  - ☐ pH sonde
- ☐ Scissors
- ☐ Scrap plywood square (~25 x 25 cm)
- ☐ Rubber mallet
- ☐ Garden trowel
- ☐ Towels
- ☐ Large cooler
- ☐ Ice packs
- ☐ Carboys
  - ☐ X10 7 gal (26.5 L)
- ☐ Peristaltic pump
- ☐ Peristaltic pump tubing
- ☐ X3 20 AH batteries
- ☐ Sharpies
- ☐ Serrated blade knife
- ☐ Tape measurer
- ☐ Washed sand (50 lb bag/22.6 kg)

- Field notebook
- Pencils

Soil and Surface Water Equilibration

- Prepared Sampling Spreadsheet printed on waterproof paper (“Equilibration” tab)
- X2 red headlamps
- DI water
    - Bottle with tip
- Kimwipes
- X10 full carboys
- X4 incubation chambers
    - Fit with X4 magnetic lines per chamber
- Aquarium bubbler unit with tubing and stones
- Orion A329 handheld meter
    - DO sonde
    - Electrical conductivity sonde
    - pH sonde
- X16 lids with magnetic spinners removed
- X16 nylon screws (for attaching magnetic stirrers to core lids)
- Gas sample bin filled with DI water
- Camera

- Pencils

Core Incubation Preparation

- Prepared Sampling Spreadsheet printed on waterproof paper ("Post-Equilibration" tab)
- X4 red headlamps
- Incubation chambers with cores, lids, and previously bubbled surface water
- X4 empty 5 gallon carboys
- Peristatic pump
- Peristaltic pump tubing
- X2 20 AH batteries
- X4 carboy spigots (4-way valve unit and ½" tubing attached)
- X4 central magnetic stirring units stands with motors
- Orion A329 handheld meter
    - DO sonde
    - Electrical conductivity sonde
    - pH sonde
- Kimwipes
- X16 loose magnetic stirrers
- X16 nylon screws (for attaching magnetic stirrers to core lids)
- X32 plastic tubing clamps
- Pipe cleaner brush

- Pencils

Dissolved Gas Sampling

- Prepared Sampling Spreadsheet printed on waterproof paper ("Incubation Sampling" tab)
- X66 labeled 8 mL glass vials + extra (5 rounds, start and end of experiment surface water)
- "Sample bin"
    - Kimwipes
    - Labeled glass vials with specified round
    - Clean towel
- "Water catchment bin"
    - 10 mL graduated cylinder
    - 500 mL beaker
- Pipet in µL
- Pipet tips for µL pipet
- 50% $ZnCl_2$ solution in labeled 250 mL sample bottle
- Sharpies
- Notebook
- Pencils

In-Situ and Solute Sampling

- X66 labeled 60 mL bottles + extra (5 rounds, start and end of experiment surface water)
    - Prepare area on table by laying out:
        - Nitrile gloves
        - Kimwipes
        - Labeled 60 mL sample bottles with specified round
        - Labeled syringes (1 for each core + 1 for each carboy)
        - Bagged 25 mm PES filter disks
        - DI water
            - X4 bottles with tip
            - X4 1000 mL bottles
    - Modified falcon tubes in styrofoam rack (50 mL falcon tubes cut in half to fit water quality meter sondes)
    - Pencils
    - Sharpie
    - Sample bottle tape

Post-Incubation Soil Sampling

- Prepared Sampling Spreadsheet printed on waterproof paper (“Post-Incubation”, and “Soil sampling” tab)
- Nitrile gloves

- Towels
- Plunger system (disk + 1” PVC)
- X9 labeled microbe sample ziploc bags
- X9 labeled bulk sample ziploc bags
- Plastic tray
- Tape measurer
- Serrated knife
- DI water
    - Bottle with tip
- Ethanol
    - Bottle with tip
- Paper towels
- Sharpies
- Notebook
- Pencils

MIMS Analysis

- Desktop with RGA software downloaded
- Running MIMS with “home AEW” (vial that rests in position when MIMS is in standby)
- Insulated beaker
- Dry ice (nugget or crushed)

- Isopropanol
- X2 250 mL beakers
    - X1 for prepping insulated beaker
    - X1 for catching melt after MIMS run
- Metal stir rod
- PPE for insulated beaker prep and handling
    - Insulated gloves
    - Nitrile gloves
    - Glasses
    - Lab coat
    - Closed toed shoes
- PPE for MIMS run
    - Nitrile gloves
    - Glasses
    - Lab coat
    - Closed toed shoes
- Labeled samples
- Filled AEW (air equilibrated water) container, which serves as the standard ran at the beginning and end of each sample run
    - Fill with DI water
- Sample waste container
- Prepared MIMS Spreadsheet made by Ate Visser

3.4. Parts and Construction

This section serves as a guide to soil core incubation experiment replication, with detailed part and build explanations, including manufacturer where applicable. While acrylic was the material used in both cores and magnetic spinning lids, impact-resistant polycarbonate is advised, as acrylic has the tendency to chip and crack.

A11 Table of Parts.

Part	Manufacturer	Part #	Specifications
Field Collection			
Mini-piezometer*	Amazon	B0002APYRM	OD: ⅛", ID: 3/16". Cut tubing to desired length, melt with lighter, press end with pliers, and cut a point. Drill small holes into end. Add length of ⅛" tubing and syringe.
⅛" ID tubing	Amazon	B0002563MW	OD: 3/16", ID: ⅛". Used with mini-piezometer, aquarium bubbler, lid ports, 4-way valve unit, and in replacement water carboy vent hole.
Syringes	Fisher Sci	1482343	60 mL. Used with mini-piezometer, filtering with filter holder, and during soil core incubation to filter samples with PES syringe filter disks and to take nutrient samples from carboys and incubation chambers.
Filter holder	VWR	28163-089	Used with 47 mm filters to filter in field.
47 mm filters	(1) Waters Tech (2) Fisher Sci	(1) WAT200538 (2) AP4004705	(1) 0.45 μm PES (2) 0.7 μm GF/F

Handheld water quality meter	Thermo Sci	Orion Star A329: STARA3290	Electrical conductivity, DO, and pH sondes included.
Surface water carboys	Amazon	B001QC31G6	7 gal. Used to haul surface water from the field.
Core tubes*	US Plastic	44546	OD: 4”, ID: 3 ¾”. Machined to 23 cm long. X4 per incubation chamber.
Black stoppers	Fisher Sci	14-130W	Size 15, X1 for each core bottom.
Part	Manufacturer	Part #	Specifications
		Equilibration	
Incubation chambers	Amazon	B06ZZ3W8FK	X1 per wetland position.
Aquarium bubbler	Amazon	B07JBPH4HW	Use with ⅛” ID tubing.
Bubbler stones	Amazon	B01MZ01SM6	Pack of 4. X1 dropped in each incubation chamber.
Magnetic lines	(1) Total Element (2) Amazon	(1) ½” x ⅛” donut magnets (5.7 lbs): N/A (2) String: B0058I3SQW	Plasti-dip magnets (X2 coats), preserving donut hole. Tie 2 magnets on a length of string (same magnets used on magnetic spinning lid) with stopper knots at ends. From outside of the incubation chamber, 2 extra magnets will attract magnets on string inside incubation chamber. Pull on string ends to tighten against core.
Part	Manufacturer	Part #	Specifications
		Incubation	
Replacement water carboys	ULINE	S-12768	5 gal. Fill to 1 ½ gallon line for soil core incubation.

Replacement water carboy spigot	ULINE	S-12768F	ID: ⅝”. Spigot opening threaded to allow for attachment of replacement water carboy barbed adapter.
Replacement water carboy barbed adapter	McMaster-Carr		Barb: ½”, NPT: ⅝”. Screwed into threaded replacement water carboy faucet and glued with silicone.
4-way valve unit	(1-4) McMaster-Carr (5-6) US Plastic	(1) Valves: 4796K38 (2) Tee: 4596K321 (3) 90° Corners: 4589K28 (4) Connectors: 4882K11 (5) ½” to ¼” Barb Adapter: 64817 (6) ¼” to ⅛” Barb Adapter: 65897	Assembled using plumbers tape or pipe joint compound, following 4-way valve unit photograph (see A9).
½” ID tubing	Amazon	B07HF4SYWY	Used to attach 4-way valve unit to replacement water carboy barbed adapter.
Core lids*	McMaster-Carr	8528K55	OD: 4 ½”, rod length: 2’. Machined acrylic^{+} rod to make lids, height: 1” (¼” lip, ¾” insert). Insert machined to 3 ¾” OD. Important that lid material is clear in order to see presence of bubbles during incubation.

Lid hardware	(1) US Plastic (2) Grainger	(1) ¼’ NPT to ⅛” Barb Adapter Port: 65897 (2) Screws: 4DFF3	Screws cut and sanded to make lowest profile possible for hanging magnetic spinner on lid. Ports and screw hole threads tapped into lids for hardware insertion. Ports glued with silicone glue.
Blank core divider*	McMaster-Carr	8528K55	Excess material from the acrylic^{+} rod is machined to make blank core dividers for each incubation chamber. OD: 3 ¾”, height: ¼”. Dividers are set to allow 5 cm headspace with core lid in place, and sealed on both sides with silicone glue. Holes are drilled in the acrylic^{+} core below the divider to allow water in.
Magnetic spinner on lid*	(1) Amazon (2) Total Element	(1) 1” OD Plastic rod: B000FN15T2 (2) ½” x ⅛” donut magnets (5.7 lbs): N/A	Rod cut to 1 ¾”, and center hole drilled to spin freely on screw. Donut magnets screwed into ends and dipped in plasti-dip to prevent rusting during incubation (see A8).
Central magnetic stirring unit*	(1-2, 4-5, 7) Amazon (3) Total Element (6) Home Depot	(1) 1” OD Plastic stir-rod: B000FN15T2 (2) Long rod: B01MYNR9TE (3) 1 ½” x ¼“ donut magnets (35.6 lbs): N/A (4) 36 RPM motor: B07DN6VQSM (5) 6 mm to 12 mm Coupler*: ⅝” OD Rod-	Magnetic tee cut to 4”, donuts magnets screwed into ends and dipped into plasti-dip to prevent rusting. Long rod cut to 9 ½” (or height necessary for magnets to communicate when cores are intact) and attached to magnet tee and motor with coupler. Couplers built with lathe and end mill, fitted with set screws. Wooden frame built with existing scrap

		B00CPRF8VW, Set screws- B07DS7FQYY (6) Wooden Stand*: N/A (7) Motor control box: Box- PF201275, Power Supply- B01MSWS3CT, Motor Switch- B00QVONO20, Power Cords- B072BYGKZZ, B07MYXMGHT	wood. Motor control box powering central magnetic stirring unit assembled (see A5).
Dissolved gas sample vials	VWR	470151-614	8 mL
PES syringe filter disks	VWR	28145-489	0.45 μm. For nutrient samples taken during incubation.
Part	Manufacturer	Part #	Specifications
Soil Sample Extraction			
Soil plunger*	(1) US Plastic (2) Home depot	(1) 8528K55 (2) 1 ½" Schedule 40 PVC: 100135041	Excess material from the acrylic^{+} rod is machined to a disk for pushing out soil from soil cores post-incubation. OD: 3 ¾", height: ½". A 2' section of free 1" PVC pipe is used to push the disk, applying pressure using a flat surface to extract soil.

* indicates custom built items

$^{+}$ indicates acrylic material to be substituted for impact-resistant polycarbonate