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Using Aquatic Mesocosms to Assess the Effects of Soil and Vegetation for Informing Environmental Research

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Abstract: An aquatic mesocosm facility consisting of thirty 15,000 L tanks was constructed in Vegreville, Alberta to support environmental research. In 2017, an experiment was conducted as an inaugural run for the facility; this study continued through the winter of 2017/18 (over-wintering is a unique capability of the facility) and concluded in the fall of 2018. Here, we report key methods used to evaluate the effects of two independent variables: (1) a soil layer covering the floor of the mesocosms, and (2) vegetation installed in the mesocosms. Although a range of response variables were measured during this study, we limit our analysis here to the physicochemical (e.g., pH, turbidity, conductivity, and dissolved oxygen) and biological/ecological response variables (e.g., macrophytes, phytoplankton/metaphyton, and macroinvertebrates) that differed due to these two variables. The presence of a soil layer covering the floor of the mesocosm was associated with increased turbidity on some days and depths in 2017. Specific conductivity was higher in the presence of soil and its associated adventitious vegetation. During this initial study, we gained a better understanding of the characteristics and mechanics of the mesocosms, which informs design and implementation of future experiments.

Keywords: aquatic mesocosms; water chemistry; brine rejection; aquatic macrophyte; soil

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1. Introduction

Mesocosms—defined here as having a volume of 1 to 1000 m^3 [1]—are simplified physical models of 'real world' ecosystems containing a variety of structural and functional components [2], and represent a balance between the control and replicability of bench-scale experimentation and the realism of field studies. Odum defined a mesocosm as a "bounded and partially enclosed outdoor experiment to bridge the gap between the laboratory and the real world in environmental science" [3]. Aquatic mesocosms have been used for environmental risk assessment for decades [4]. A recent review examining opportunities and limitations of mesocosm-scale research related to mine pit lakes [5] suggested that mesocosms are appropriately-scaled for confirmation of results from bench-scale experiments, as they enable some degree of quasi-realistic response to exposure to industrial materials [6].

To support applied research related to industry, including de-risking potential reclamation and remediation technologies and approaches, an outdoor aquatic mesocosm facility was constructed at InnoTech Alberta's site in Vegreville, Alberta in 2016. This facility was explicitly designed to safely house experiments incorporating potentially noxious industrial materials.

Every mesocosm facility tends to exhibit distinct ecological characteristics/behaviors through time. For example, an indoor mesocosm facility was equipped with a rotating paddle inside the mesocosms to prevent excessive periphyton/plankton growth on the tank walls [7,8]. Characteristics such as these may influence the extent to which a mesocosm

is able to develop and support a semi-realistic ecosystem, which impacts the degree to which data collected from these mesocosms can be applied to larger scale systems. For instance, an aquatic experimental system of 1–5 m^3 could be considered as a mesocosm based on spatial scale [8]. Although some response variables may exhibit relatively little sensitivity to factors such as mesocosm volume (e.g., algal abundance [9]), small volumes associated with some mesocosm facilities limit the evaluation of impacts to other response variables (e.g., fish communities [10]). While past studies can provide some guidance, there is no effective means of predicting exactly how a given response variable will be influenced by the structural and functional characteristics of a mesocosm; these effects must be determined empirically. To this end, the first experiment using the mesocosms (initiated in 2017) was designed, in part, to gain an understanding of (1) operational aspects of experimentation in this facility, (2) whether the mesocosms could support simplified ecosystems, and (3) how the mesocosms would react to perturbation [11].

During this initial experiment, the 30-mesocosm array was split into groups defined by treatments and internal structures, and a variety of response variables were measured. These included water quality (e.g., specific conductivity, turbidity, etc.), plant growth and tissue chemistry (e.g., height and bio-uptake), toxicity (e.g., 96 h rainbow trout), periphyton (e.g., biomass), zooplankton (e.g., diversity), macroinvertebrates (e.g., relative abundance), and phytoplankton/metaphyton (e.g., relative abundance) [12]. While several aquatic mesocosm studies have been implemented at our facility from 2017 to 2023, here we limit our discussion to a subset of the project data collected in 2017 and 2018. Our focus here is on those initial data that differed significantly between mesocosms treatment groups with different internal structures and those relevant to monitoring aquatic systems which may be impacted by industrial operations. For example, *Ceratophyllum demersum* (hornwort) is considered a sensitive indicator species in wetlands associated with oil sand mining [13], so growth and development data for this species are included here, while other macrophyte data are excluded. More complete descriptions of the project can be found elsewhere [6,7].

The overall aim of this article is to convey what we have learned about the response of our mesocosms to specific factors—including installed vegetation and/or a soil layer that covers the entire floor—to inform the design and implementation of future mesocosm studies. We also provide insights into some of the challenges associated with conducting mesocosm experiments that include an overwintering period, which can be an important factor in high latitudes.

2. Materials and Methods

2.1. Mesocosm Facility

The aquatic mesocosm facility is located in Vegreville, Alberta, approximately 100 km east of Edmonton (see Supplementary Materials Video S1). The facility is composed of 30 in-ground mesocosms, each consisting of two nested polyethylene tanks. An inner tank (3.63 m diameter, 1.81 m deep, 15.9 m^3 maximum volume, and 13.6 m^3 operating volume) contains the water, sediment, and biota necessary to maintain the model ecosystem. An outer tank (4.47 m diameter, 1.72 m deep, and 25.0 m^3 maximum volume) serves as a secondary containment vessel, preventing the escape of test materials into the environment should the inner tank leak. A water jacket between the inner and outer tanks allows thermal conduction between the mesocosm and the surrounding earth, and provides hydrostatic support for the inner tank. The level of the water jacket is maintained 10 to 20 cm lower than that of the inner tank, thereby preventing floatation of the inner tank and providing an easy way to detect leaks from the inner tank; such a leak would result in the equalization of water levels between the inner and outer tanks. The pair of nested tanks is surrounded by a tunnel liner (4.8 m diameter and 0.8 m high) buried approximately 0.4 m below ground level (Figure 1). This galvanized steel ring protects the relatively flexible tanks from lateral compressive forces exerted by passing vehicles.

Figure 1. Mesocosm protected by steel tunnel liner and overflow tank housed within a culvert, visible during construction in 2016.

To mitigate the risk of tank overflow associated with heavy precipitation or melting snow, the inner and outer tanks are connected by hoses to two smaller in-ground tanks installed immediately to the east of each mesocosm, with one tank nested inside the other to provide containment. These tanks are referred to, collectively, as overflow tanks. Unlike the mesocosms, the overflow tanks are not in direct contact with the surrounding earth. Rather, they are contained within a galvanized steel culvert (Figure 1).

High groundwater, when present, exerts buoyant forces on both the mesocosms and the overflow tanks. As long as these tanks are full of water, their mass more than compensates for their buoyancy. However, if the tanks are empty (e.g., during tank cleaning) their buoyancy is sufficient to cause floatation. In the case of the mesocosms, floatation would result in the subsidence of the surrounding earth, necessitating excavation and reinstallation of the tanks, an expensive and time-consuming process. Because the overflow tanks are isolated from the surrounding earth by a steel culvert, their floatation results only in ineffective overflow protection (i.e., overflow tanks floating above the level of the mesocosm cannot receive excess water via gravity drainage). To mitigate these contingencies, a semi-autonomous solar-powered dewatering system was added to the facility (Figure 2). This system draws groundwater from the area around each mesocosm and pumps it to a drainage ditch running along the western side of the facility. Given sufficient time, this system can lower groundwater levels across the facility, thereby protecting the infrastructure.

A simple network of gravel roads allows vehicles up to the size of loaded hydrovac trucks to access the mesocosms. The geometry of the road network restricts larger vehicles (e.g., tractor-trailer trucks) to access the main road (Figure 3). Posts erected near the mesocosms allow the installation of monitoring equipment, mesocosm identification tags, wildlife deterrents, and boundary lines. Wooden walkways facilitate mesocosm access and provide flat, vegetation-free areas to organize gear.

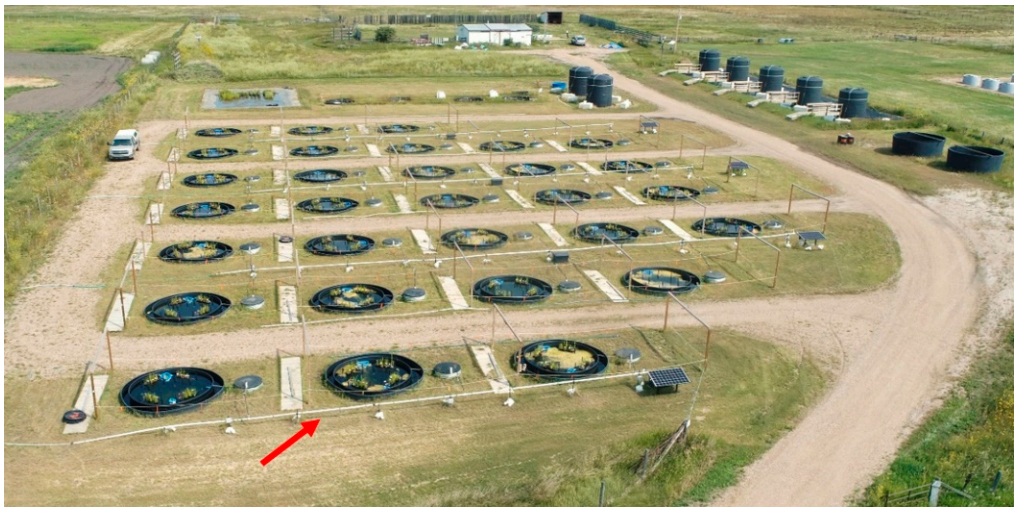

Figure 2. Aquatic mesocosm facility in 2018. Note the presence of wooden walkways and posts near each mesocosm. The dewatering system can be seen as slim white pipes running from one side of the facility to the other (indicated by red arrow).

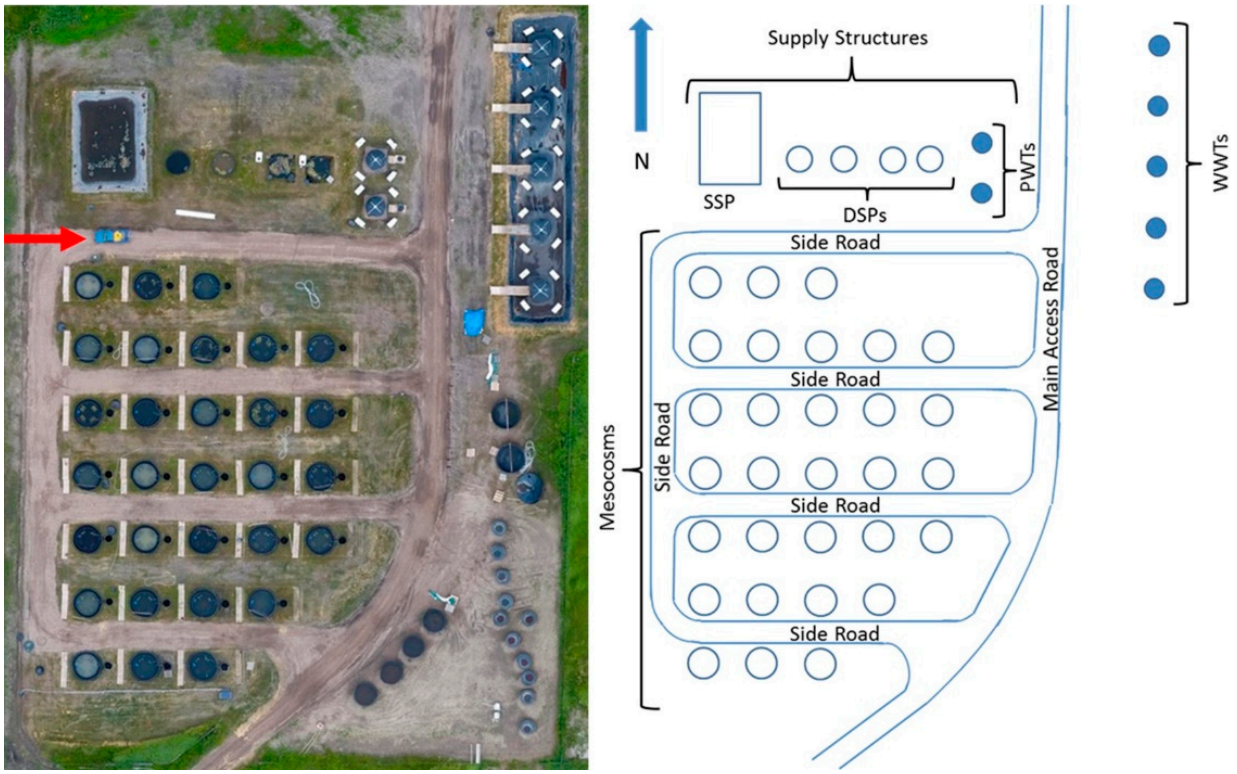

Figure 3. Aquatic mesocosm facility in 2017. Left—air photo of mesocosm facility; right—schematic diagram of the same facility. Legend: SSP = shallow supply pond, DSPs = deep supply ponds, PWTs = potable water tanks, WWTs = wastewater tanks. Note the photo was taken before the dewatering system was installed. Four holding tanks for surface water were set parallel to the road in the southeast corner of the facility. A pickup truck (indicated by red arrow) parked in the facility provides an idea of scale. A series of ancillary tanks that are used to house water for short periods of time during delivery and handling are located east of the main access road and south of the wastewater tanks.

In hot, dry weather, the mesocosms can lose substantial volume to evaporation, and potable water is used to offset these losses. Distilled or deionized water would

be chemically superior to treated potable water but these products are not readily available in the necessary volumes or with the required frequency at our site. Potable water is stored in two large vertical tanks (50 m^3 total volume) to the north of the mesocosms (Figure 3). These potable water tanks are filled by tanker truck and drained into the mesocosms using a network of polyvinyl chloride (PVC) hoses.

Wastewater storage tanks to the northeast of the mesocosms (125 m^3 total volume) are used to hold test materials or liquid wastes. These tanks are housed within a geomembrane-lined containment berm to protect the surrounding environment from leaks or spills. Other tanks used to hold river water or other materials are stored in the eastern and southeastern portions of the facility.

To the north of the mesocosms are shallow and deep supply ponds used for propagating aquatic plants for use in experiments at the site.

2.2. Mesocosm Design

The inaugural 2017–2018 study included a number of different experimental groups, but here we focus on three that differed across two experimental factors: a soil layer and plants installed in pots. Three experimental groups were defined based on these factors: plants and soil (designated as +Plant +Soil), plants and no soil (+Plant −Soil), and neither plants nor soil (−Plant −Soil). The mesocosms in the +Plant +Soil group contained 1.6 m^3 (equivalent to a 20 cm layer across the entire floor of the mesocosm) of unconditioned topsoil and installed plants. The mesocosms in the +Plant −Soil group lacked a layer of topsoil but were otherwise identical to the +Plant +Soil group. The mesocosms in the −Plant −Soil group contained no soil or installed plants. Unconditioned topsoil, which was used in place of sediment, is defined as material that had been housed underwater for less than three months and is not expected to exhibit sediment-like properties [14]. The use of natural wetland sediments was not feasible due to logistical and regulatory limitations.

2.3. Experimental Setup

2.3.1. Mesocosm Commissioning and Establishment

The commissioning and establishment phases represent the preparation of the mesocosms for an experiment. Commissioning encompasses installation of internal structures and materials. During commissioning, each mesocosm was inoculated with 2 L of sediment collected from a nearby pond to facilitate development of a representative invertebrate community. Commissioning is followed by a relatively short two-week establishment period, which allowed for the formation of simplified food webs, and homogenization of water chemistry and nektonic communities across all mesocosms [15]. This two-week period began when water was homogenized (circulated) between mesocosms. Surface water, obtained from the Athabasca River in the Fort McMurry area of Alberta (Canada), was used to fill the mesocosms during commissioning in the spring of 2017. Surface water in the mesocosms was homogenized by circulating water across rows and columns of mesocosms, twice, within one month in 2017. Homogenization minimized chemical and biotic differences between mesocosms. After homogenization, a river water sample was collected for baseline material characterization prior to the installation of soil into the mesocosms. The results showed a nitrate (NO^{-3}) concentration of 2.82 mg/L and a phosphorus (P, dissolved) concentration of 56 µg/L.

Polyethylene shelving units (91 cm length × 61 cm width × 135 cm height) were installed in each mesocosm to support emergent vegetation contained within plastic pots (23 cm diameter × 22 cm height, high density polyethylene (HDPE) and polypropylene). Mesh socks—cylindrical nets (25 cm diameter × 135 cm height) composed of UV-resistant polyester suspended from a foam ring float—were used to contain submerged, free-floating plants.

All mesocosms contained 2 pots each of *Carex aquatilis* (water sedge), *Typha latifolia* (common cattail), and *Potamogeton zosteriformis* (flatstem pondweed), and one mesh sock containing approximately 25 g of *Ceratophyllum demersum* (hornwort or coontail) in 2017.

These installed plant species were selected because they occurred naturally in Alberta's boreal region, were commercially available, and exhibited a range of sensitivities to industrial byproducts. For example, the relative frequency of occurrence of *C. aquatilis* has been reported to be negatively related to salinity [16]. *Typha* spp. has been suggested as a standard test species for ecological risk assessments [17]. *P. zosteriformis* is known to exhibit alkali tolerance [18] and *Potamogeton* spp. indicates trophic conditions [19]. *C. demersum* is considered an indicator species sensitive to industrial processes and has been documented to be absent in industrial wetlands [13].

Installed plants were initially placed in all mesocosms, and then subsequently removed from the −Plant −Soil mesocosms following the establishment phase. By installing vegetation in +Plant −Soil while removing it from −Plant −Soil, the role which plants played in the development and behavior of the mesocosms could be examined. Note that vegetation intentionally installed in pots in the mesocosms (installed plants) is distinguished here from adventitious vegetation, which grew spontaneously within the mesocosms. All installed vegetation was purchased from Bearberry Creek Greenhouse Nursery and Water Gardens (Sundre, AB, Canada).

Unconditioned soil was obtained from REDA Enterprises (Bonnyville, AB, Canada). During initial setup of the mesocosm experiment, unconditioned soil was added to those mesocosms designated for the +Plant +Soil. To install soil, half of the water in the mesocosm was evacuated to a clean utility tank (an above-ground ancillary tank used for the temporary storage of material), unconditioned soil was placed in the mesocosm, then the water from the utility tank was returned to achieve the desired final depth. A layer of floating organic material was observed following the addition of the soil; this material was removed from each mesocosm using pool skimmers over the next several days.

Shelves, mesh socks, and installed plants were removed or destructively sampled in the fall of 2017, then re-installed during the week of 30 April 2018 for the second year of the study. The mesocosms remained full until the fall of 2018, when the experiment was concluded.

2.3.2. Decommissioning

Mesocosm decommissioning refers to the process whereby the last, often destructive, samples are collected, and the tanks are prepared for overwintering or refurbished for a new study. In the fall of 2018, the mesocosms described herein were decommissioned by pumping water to the onsite drainage ditch. Where soil was present on the mesocosm floor, an excavator truck was contracted to remove the soil layer. Finally, all mesocosms were pressure-washed with hot soapy water, which was then removed via vacuum.

2.4. Water Chemistry Analyses

Field water quality data were collected using a multiparameter EXO2 data sonde (YSI, Ohio) at approximately 17 cm below the surface and 125 cm below the surface, slightly above the soil layer (where present). Autonomous dissolved oxygen (DO) loggers (HOBO U26, Onset Computer Corporation, Cape Cod, MA, USA) and conductivity loggers (Aqua Troll 100, In-Situ Incorporated, Fort Collins, CO, USA) were installed in 3 mesocosms from each group, and attached to the southwestern shelving unit at a depth of 15 to 20 cm. The purpose of the DO loggers was to monitor the effect of mesocosm configuration on diel oscillations of dissolved oxygen. These oscillations were expected to become more extreme with increasing sediment and associated establishment of adventitious submerged vegetation. While a range of water chemistry parameters were collected using the data sonde and loggers, only pH, specific conductivity (µs/cm), dissolved oxygen (mg/L), and turbidity (FNU) are analyzed and reported here. Details of the collection, analysis, and results of additional physical and chemical parameters can be found elsewhere [11].

2.5. Biological Measurement

2.5.1. Emergent Plants

While 3 emergent plant species were installed in pots and several parameters were collected (e.g., leaf length and biomass) for each species, only survival data for *T. latifolia* are reported here to inform future experiments after this initial project. More information on other species and related parameters can be found elsewhere [12].

2.5.2. Submerged Plants

In order to collect repeated plant growth measurements, fresh plant weight was recorded biweekly by collecting *Ceratophyllum demersum* from its mesh sock with a sieve and transferring it to a Ziploc® bag. Any excess water was gently shaken from the plant material, and any visible non-plant material (e.g., snails) was removed prior to weighing. The weight of the bag and its contents were determined using a spring scale (Pesola® Medioline, Dynamic Aqua-Supply Ltd., Surrey, BC, Canada). The plant material was then returned to its sock, the weight of the bag plus any residual water was recorded, and the net weight of the plant material was calculated. To avoid weighing errors associated with the effects of wind, the bag and its contents were suspended inside a bucket during the weighing procedure.

2.5.3. Phytoplankton/Metaphyton

Although not quantified in 2017, incidental observations suggested that mesocosms could support substantial populations of phytoplankton. Here, the term phytoplankton/metaphyton is used to describe all visible photosynthetic free-floating organisms. In 2018, visual estimation of phytoplankton/metaphyton cover was recorded as an index of relative abundance near the surface (0–5 cm depth). Visual estimates can be biased, so several techniques were employed to increase objectivity, such as digital imaging [20–23].

2.5.4. Aquatic Macroinvertebrates

Aquatic macroinvertebrate communities were assayed using round 14-plate Hester-Dendy samplers [24]. One sampler was deployed at a depth of 60 cm in each mesocosm for 6 weeks [25], after which it was collected via rapid withdrawal of the sampler from the mesocosm followed by immediate transfer into a wide mouth sample bottle containing 90% denatured ethanol. To collect invertebrates, the sampler was disassembled in a plastic tray and the plates brushed into the ethanol-filled sample bottle, which had contained the intact sampler. The contents of the sample bottle and tray were filtered through two separate 200 µm Nitex mesh patches. The mesh patches were then transferred to a 500 mL HDPE sample storage jar containing 10% buffered formalin. Samples were sent for taxonomic analysis to the family level. Species were assigned to ecological functional groups based mainly on the Southwest Association of Freshwater Invertebrate Taxonomists (SAFIT) database (https://www.safit.org/TVFFG.php, accessed on 10 March 2023) (Personal communication, Scott Finlayson at Cordillera Consulting, 21 January 2022).

2.6. Data Analyses

Mean value ± standard error is presented for each response variable and values of each group (e.g., +Soil +Plant) are jittered in each figure to avoid complete overlap. Effects of soil or installed vegetation were identified by comparing +Plant +Soil to +Plant −Soil, and +Plant −Soil to −Plant −Soil. Scatter plots were constructed with lines and error bars defined as ± standard error. Statistical analyses were conducted using R Version 3.4.3 [26]. Response variables were compared using a repeated-measures analysis of variance to determine if statistically significant differences were observed between groups and over time. Statistical significance was declared when $p \leq 0.01$.

3. Results

3.1. Water Quality

3.1.1. pH

The presence of a soil layer was associated with significant changes in pH in both years (Figure 4). During the first 5 weeks of the study, pH was significantly lower in +Plant +Soil group than the +Plant −Soil group ($p < 0.0001$) at the surface and bottom. However, this relationship was reversed in the last 5 weeks of 2017, where the pH was significantly higher in the +Plant +Soil group than the +Plant −Soil group ($p < 0.01$) at both depths.

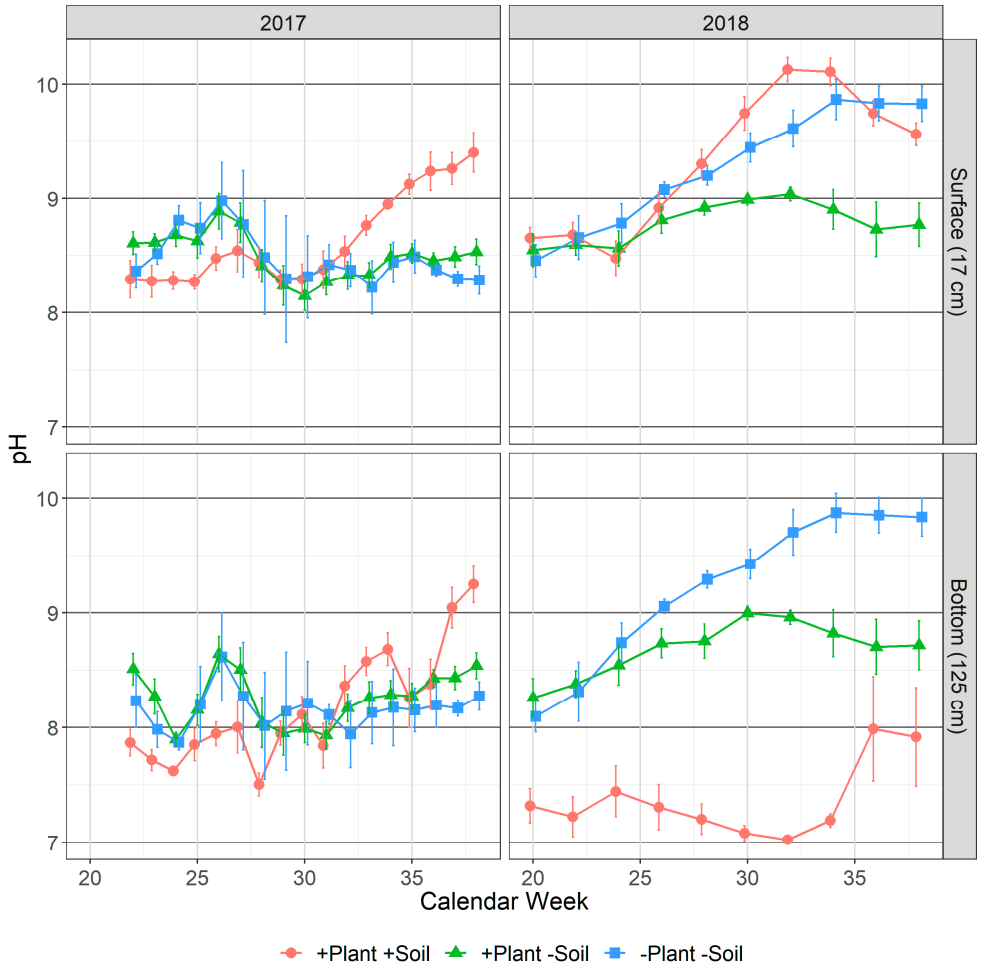

Figure 4. Mean pH readings from data sonde across depth and time in different groups (+Plant +Soil, n = 6; +Plant −Soil, n = 6; −Plant −Soil, n = 3). Error bars represent standard error.

In 2018, mesocosms with soil (+Plant +Soil) had a significantly lower pH at the floor and significantly higher pH at the surface than their +Plant −Soil counterparts ($p < 0.0001$). Installed vegetation did not significantly affect pH in 2017 ($p > 0.01$) but resulted in significantly lower pH over much of 2018 when the +Plant −Soil and −Plant −Soil groups were compared ($p < 0.0001$).

3.1.2. Turbidity

+Plant +Soil mesocosms were more turbid than +Plant −Soil mesocosms, but this difference was only significant at the bottom of the mesocosms and sporadically throughout the study (Figure 5). The turbidity of +Plant −Soil and −Plant −Soil mesocosms only differed at the bottom on week 22 of 2017, but never differed significantly after that at any depth ($p > 0.01$).

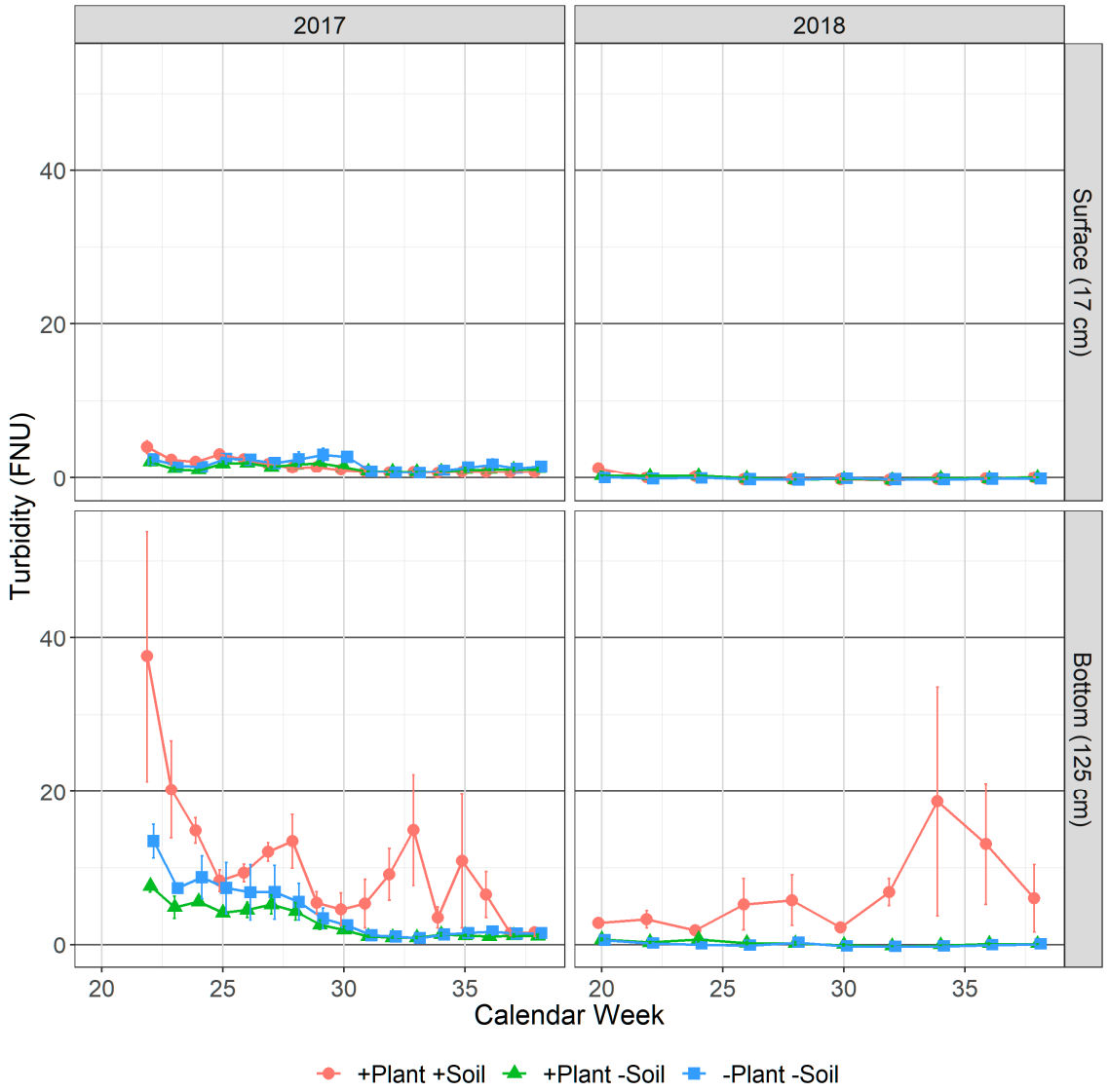

Figure 5. Mean turbidity readings from data sonde across depth and time in different groups (+Plant +Soil, n = 6; +Plant −Soil, n = 6; −Plant −Soil, n = 3). Error bars represent standard error.

3.1.3. Specific Conductivity

The presence of soil was associated with increased specific conductivity (Figure 6). At both the bottom and surface, specific conductivity in +Plant +Soil mesocosms was higher than in +Plan −Soil mesocosms throughout 2017. In 2018, a similar relationship was observed consistently at the bottom of the mesocosms, and sporadically at the surface. However, the specific conductivity of +Plant −Soil and −Plant −Soil mesocosms never differed significantly at either depth in either year ($p > 0.01$), although this difference approached significance at the end of 2018 as the two groups diverged.

In 2017, +Plant +Soil specific conductivity was significantly higher at the mesocosm bottom compared to the surface for the first 7 weeks of the study ($p < 0.0001$). Experimental groups that did not include a soil layer (+Plant −Soil and −Plant −Soil) did not exhibit significant depth-related differences in specific conductivity in that year ($p > 0.1$ in all cases). Specific conductivity was substantially lower at the surface than the floor in the +Plant +Soil group throughout 2018, but only for the first two weeks of the 2018 season in the other two groups. Interestingly, the specific conductivity increased near the surface and decreased near the floor at the start of the 2018 study season for all three groups.

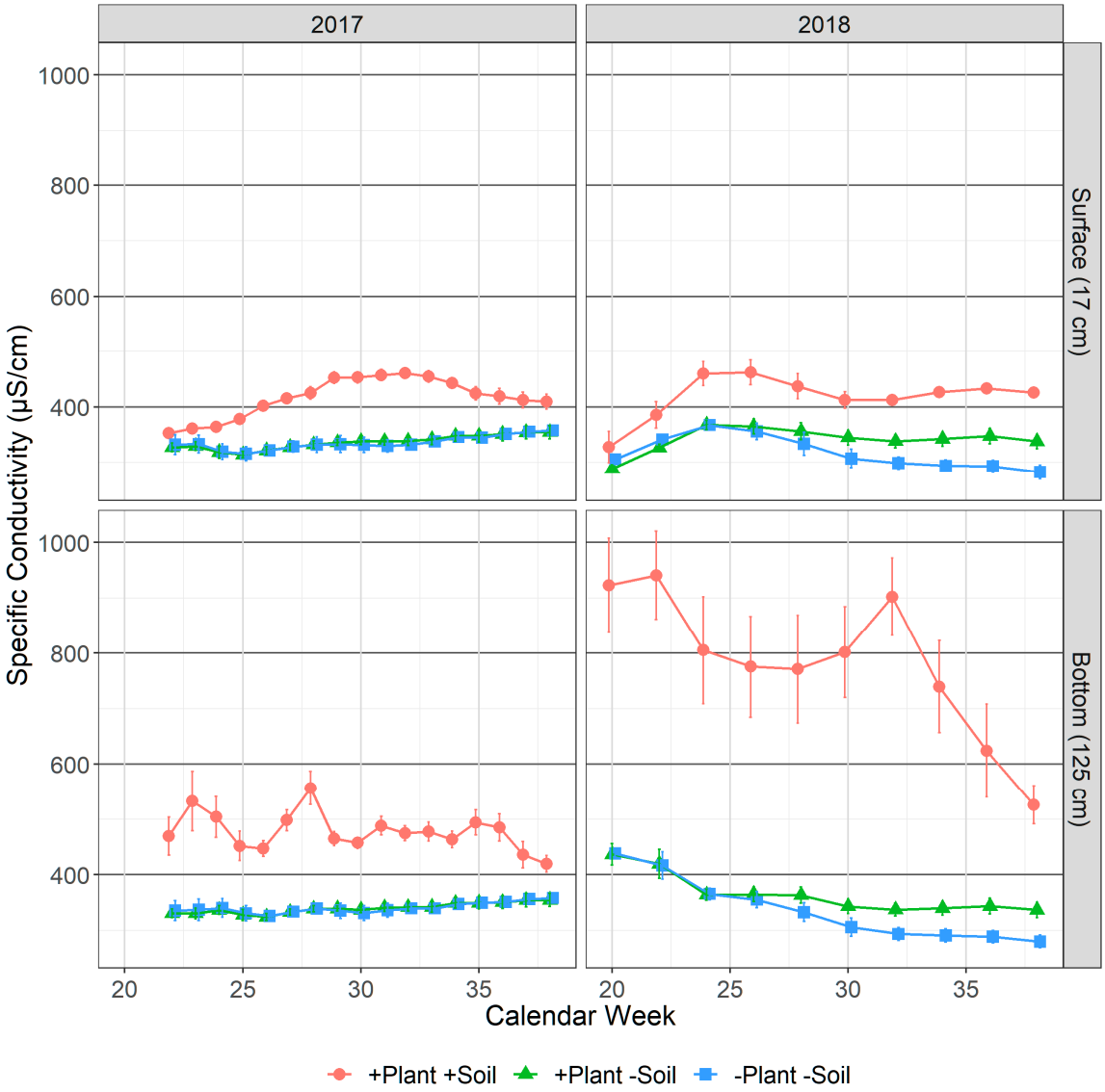

Figure 6. Mean specific conductivity readings from data sonde across depth and time in different groups (+Plant +Soil, n = 6; +Plant −Soil, n = 6; −Plant −Soil, n = 3). Error bars represent standard error.

3.1.4. Dissolved Oxygen

In 2017, the +Plant +Soil mesocosms exhibited steadily increasing dissolved oxygen levels. No similar trend was observed in +Plant −Soil or −Plant −Soil groups (Figure 7). While +Plant +Soil and +Plant −Soil produced similar diel swings (changes over a 24 h period) in dissolved oxygen throughout the study, −Plant −Soil appears to have produced larger swings and within-group variance between calendar weeks 27 and 30 of 2017 (data not shown). By the end of 2017, higher dissolved oxygen concentrations were observed in +Plant +Soil mesocosms, relative to other groups. In 2018, diel concentrations and range of dissolved oxygen also varied between the three groups; in +Plant +Soil and −Plant −Soil mesocosms, dissolved oxygen levels increased overall, while DO levels plateaued after Week 27 in +Plant +Soil. However, +Plant −Soil mesocosms exhibited mid to late season decreases, followed by an increase in the early 2018 season values.

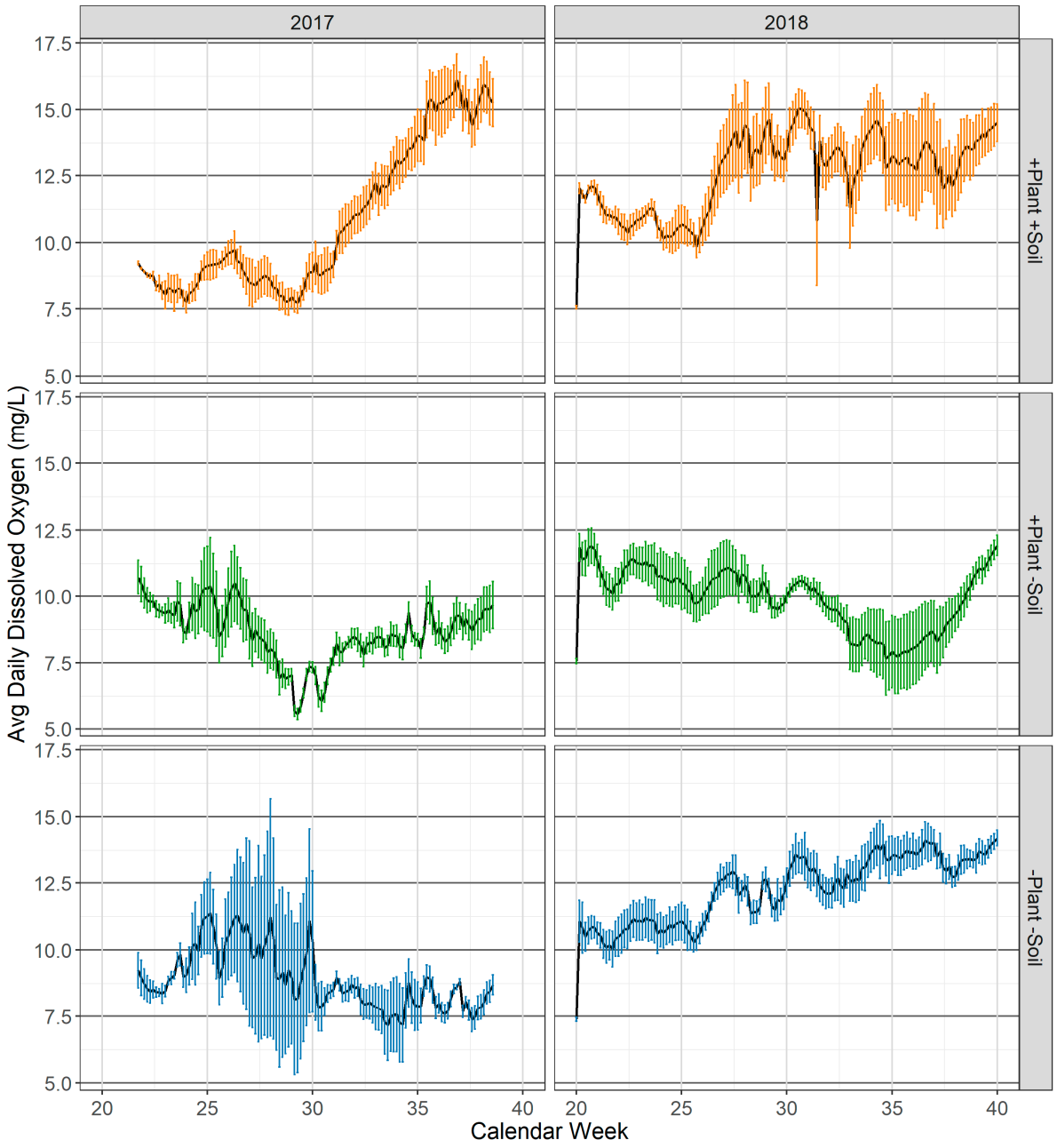

Figure 7. Mean dissolved oxygen readings from data loggers in different groups (n = 3 for all treatment types). Error bars represent standard error.

3.2. Plant Growth

3.2.1. *Typha latifolia*

All *T. latifolia* individuals grew over time except for three plants from +Plant +Soil, and one plant from +Plant −Soil, all four of these plants died in the first three weeks of 2017. In 2018, four plants died in +Plant +Soil and six plants died in +Plant −Soil at the beginning of the year.

3.2.2. *Ceratophyllum demersum*

Initially, *C. demersum* biomass decreased in both +Plant +Soil and +Plant −Soil in 2017 (Figure 8). However, changes in *C. demersum* biomass in +Plant +Soil and +Plant −Soil were difficult to evaluate in the second half of 2017 due to substantial amounts of adherent metaphyton. By the end of the 2017 season, *C. demersum* from three of the +Plant

+Soil mesocosms and three of the +Plant −Soil mesocosms was sufficiently burdened with metaphyton that *C. demersum* biomass measurement could potentially been confounded. We believe the increased within-group variability, observed in Figure 8, was associated with the presence of this metaphyton. In those mesocosms where *C. demersum* survived to the end of the 2017 season, it appeared to be healthy and of substantial biomass. In contrast to 2017, there was a decrease in *C. demersum* biomass across both +Plant +Soil and +Plant −Soil mesocosms in 2018.

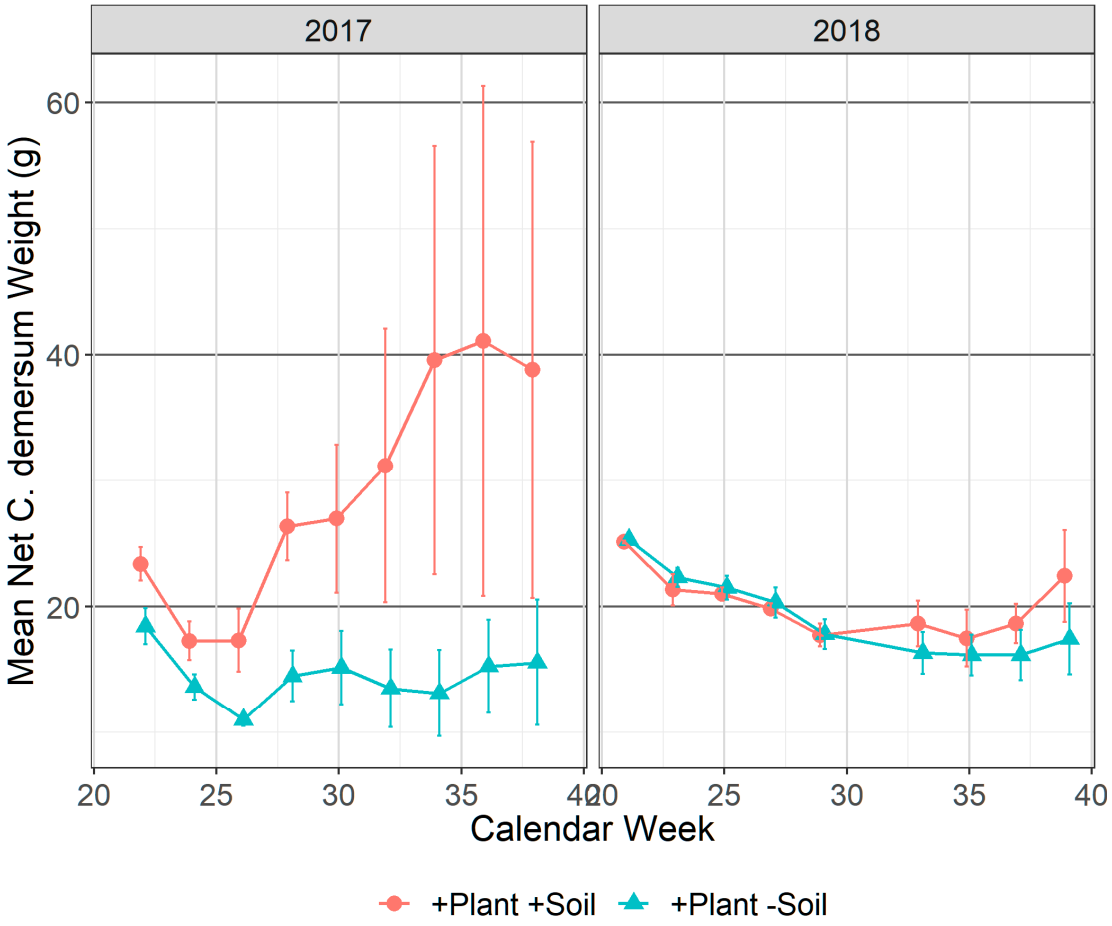

Figure 8. Mean net weight of *C. demersum* over time with (+Plant +Soil, n = 6) or without soil (+Plant −Soil, n = 6) during 2017 and 2018. Error bars represent standard error. Each mesocosm received 25 ± 0.5 g of viable *C. demersum* at the beginning of each year.

3.2.3. Adventitious Vegetation

Adventitious vegetation (also known as plant colonization) was commonplace. Noteworthy was the large amount of submerged vegetation found in the +Plant +Soil mesocosms. Based on morphology, these plants were tentatively identified as *Callitriche hermaphroditica* (northern water starwort), *Potamogeton pusillus* (slender pondweed), and *Chara* spp., though the last species is a green alga and not a vascular plant. The same species were found in +Plant −Soil and −Plant −Soil mesocosms, though in much lower density.

3.3. Phytoplankton/Metaphyton Coverage

Phytoplankton/metaphyton surface area coverage exhibited a bell-shaped relationship over time in 2018. While there were no significant differences between the three groups at any of the weeks ($p > 0.01$), phytoplankton/metaphyton coverage was significantly lower in both +Plant −Soil and −Plant −Soil than +Plant +Soil across the entire period ($p < 0.01$) (Figure 9).

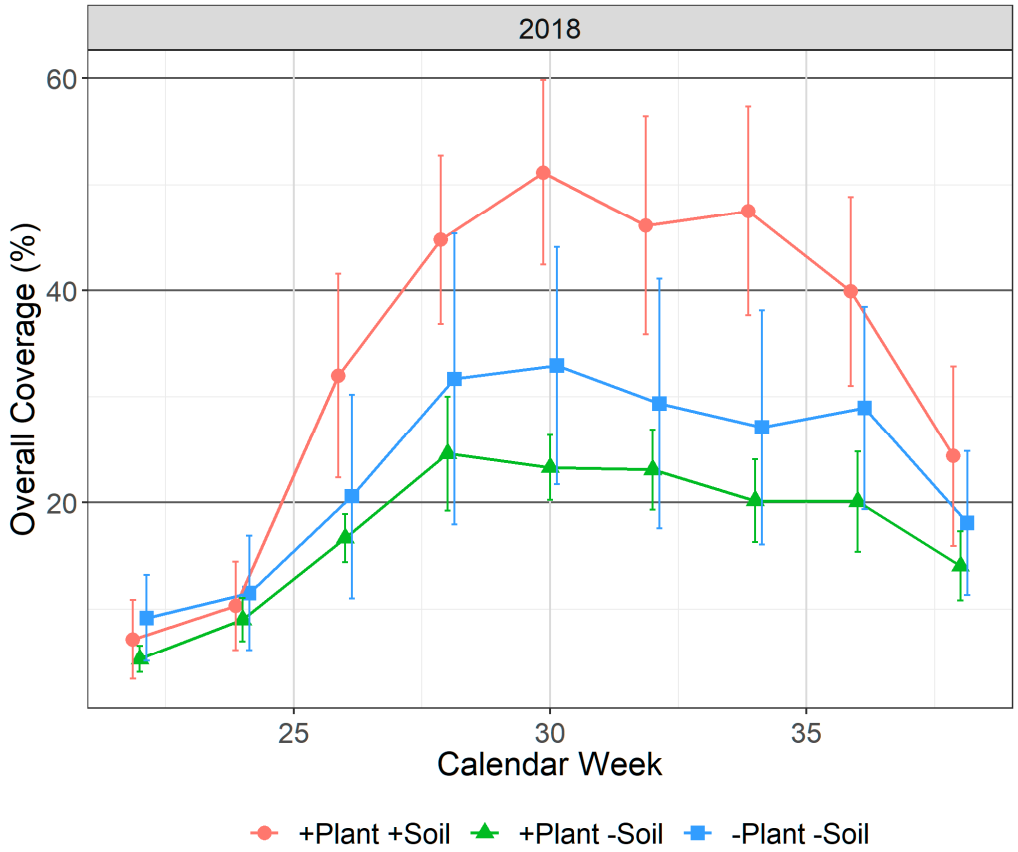

Figure 9. Mean estimated cover of phytoplankton/metaphyton over time with (+Plant +Soil, n = 6) or without soil (+Plant −Soil, n = 6; −Plant −Soil, n = 3) during 2018. Error bars represent standard error.

3.4. Macroinvertebrate Functional Groups

Abundance of macroinvertebrates was lower in 2018 in comparison to 2017 in all the groups. Macroinvertebrate ecological functional groups shifted in most experimental groups in 2018 compared to 2017. Most notably, the collector-filterer group seemed to disappear from all treatments in the second year of the study, and a substantial increase in the proportion of scrapers abundance was observed in 2018 (Table 1).

Table 1. Abundance (mean ± standard deviation) and functional groups composition of macroinvertebrate (mean) as sampled at the end of July 2017 and 2018 (+Plant +Soil, n = 6; +Plant −Soil, n = 6; −Plant −Soil, n = 3).

Functional Groups (%)	2017			2018		
	+Plant +Soil	**+Plant −Soil**	**−Plant −Soil**	**+Plant +Soil**	**+Plant −Soil**	**−Plant −Soil**
Predators	7.6%	22.7%	3.5%	21.6%	25.3%	10.6%
Shredder-Herbivores	0.3%	0.6%	0.5%	1.1%	0.0%	0.0%
Collector-Gatherers	60.2%	58.7%	75.5%	33.3%	49.3%	26.6%
Scrapers	3.6%	6.7%	1.0%	41.2%	24.3%	62.1%
Macrophyte-Herbivore	0.0%	0.0%	0.0%	1.4%	0.0%	0.0%
Collector-Filterer	27.6%	10.5%	18.9%	0.3%	0.0%	0.4%
Omnivore	0.0%	0.1%	0.0%	0.3%	0.0%	0.0%
Unclassified	0.7%	0.8%	0.5%	0.7%	1.1%	0.4%
Abundance [1]	351 ± 133	291 ± 239	347 ± 56	58 ± 23	63 ± 55	51 ± 42

[1] Abundance: total number of individual macroinvertebrates captured per Hester-Dendy sampler per mesocosm after a 6-week deployment.

4. Discussion

4.1. The Effect of Overwintering (Brine Rejection)

The aquatic mesocosm study was conducted over two years in ambient outdoor conditions, starting with establishment in the spring of 2017 and decommissioning in the fall of 2018, with the mesocosms freezing over the winter. The in-ground installation of the mesocosms, combined with the water jacket, allows the surrounding earth to partially insulate the mesocosms. As a result, the bottom ~0.5 m of water remained liquid all winter (data not shown), allowing biota to persist. To the best of the authors' knowledge, this capability is unique to our mesocosm facility in Canada.

During the freezing process, elements or compounds tend to be excluded from the ice in a process known as brine rejection. As the surface water freezes, a large proportion of its excluded salt is forced into the underlying water column [27]. In the spring, that surface ice, now greatly reduced in salt content, melts and produces a water layer with comparatively low conductivity. The greater the discrepancy in conductivity between the surface and bottom water layers, the more the mixing of those two layers is inhibited [28]. The influence of brine rejection was observed through changes in conductivity in surface and bottom water layers in the mesocosms in early 2018, although this effect was more obvious in data sets other than those presented here [12]. In the event a study is subject to an overwintering period, a density/salinity gradient can be expected, and sampling techniques and/or timing may need to be adjusted accordingly depending on the research objectives. For example, taking an integrated depth (or composite) sample in subsequent mesocosm experiments may be considered to reflect the water quality of the entire water column and minimize the artifacts in trend analysis [29].

4.2. Effect of Time

Only the +Plant +Soil group exhibited an increase in pH during 2017. However, an overall increase in pH across all three experimental groups was observed over 2018, particularly at the bottom of the mesocosms. The +Plant +Soil mesocosms supported an abundance of submerged adventitious plant growth in 2017 and 2018. The increase in pH might have occurred due to biological factors, such as CO_2 depletion from adventitious plants and algae during photosynthesis [30,31].

The artificial nature of the mesocosm environment may help explain the higher turbidity levels in the +Plant +Soil mesocosms at the bottom in 2017, since some fraction of the soil added to the tanks remained suspended in the water column following installation. The higher turbidity levels at the bottom resolved over time in +Plant +Soil mesocosms due to sedimentation of the suspended soil. The observed increase in turbidity at depth in 2018 in the +Plant +Soil mesocosms may have been due to the propagation of phytoplankton, or suspension of sediments by the data sonde touching the soil-covered floor during sampling.

Dissolved oxygen increased dramatically in +Plant +Soil but less so in +Plant −Soil and −Plant −Soil mesocosms in 2017, likely as a result of photosynthesis by the abundant submerged vegetation in the +Plant +Soil group. In 2018, the mid to late season decrease in dissolved oxygen in the +Plant −Soil group could plausibly be explained by several factors, including increasing algal abundance and the decomposition of detritus. Overall, the non-uniformity of patterns in DO levels over time between groups could be a result of photosynthesis and respiration, related to the abundance of plants and phytoplankton [32]. Future examination of phytoplankton/periphyton abundance may be beneficial to further determine the influence of these groups on community metabolism. Dissolved oxygen data from the loggers demonstrated that the water column was never anoxic, which was an initial concern during the project design phase, and a major driver for installation of these sensors in the mesocosms.

T. latifolia, an emergent plant, and *C. demersum*, a submerged unrooted macrophyte, demonstrated dramatically different growth trends over the two years of the study. While *T. latifolia* grew consistently in 2017 and 2018 [12], *C. demersum* tended to lose biomass over each year, which may be related to the distribution and biomass of phytoplankton

from possible shading [33]. Other potential explanations for the decline in *C. demersum* biomass could include changes in water chemistry or low nutrient availability in the mesocosm water.

Due to relatively low winter survival, all *T. latifolia* plants were replanted with fresh stock in the spring of the 2018 study. *T. latifolia* mortality was attributed to water level: plants were left in water deeper than the main shoot [34]. In subsequent experiments, floating rafts were used in place of the polyethylene shelving units to support emergent vegetation, as the rafts maintained their height above the water's surface, irrespective of the changes in water depth.

All mesocosms were found to support populations of macroinvertebrates, including members of higher trophic levels (e.g., predators), through the entire 16-month duration of the project. More detailed macroinvertebrate analyses can be found elsewhere [12]. The decrease in macroinvertebrate abundance in 2018 compared to 2017 may have been due to changes in external factors (e.g., weather patterns) over the course of the study between years or aging of the mesocosm systems [35]. The proportional change in macrophyte-herbivore and scrapers between 2017 and 2018 suggests that the structure of the mesocosm community evolved over time. In the study described here, macroinvertebrates samples were collected via Hester–Dendy samplers, which mostly collected epifaunal (surface-dwelling) invertebrates [36]. In subsequent experiments, activity traps, which primarily collect free-swimming macroinvertebrates, were also used to profile different portions of the macroinvertebrate community (i.e., benthic vs. nektonic).

4.3. Installation of Soil

While suspended material did appear to be higher in +Plant +Soil mesocosms immediately after soil addition, settling occurred quickly enough and homogenization was effective enough that turbidity was only significantly different at the tank bottom in 2017 between +Plant +Soil+ and +Plant −Soil or −Plant −Soil ($p < 0.01$). By restricting the floor area covered by soil (e.g., by housing relatively small amounts of soil in a number of submerged buckets), associated artefacts can be minimized in future experiments.

4.4. Effect of Soil

The presence of a soil layer tended to increase turbidity at depth and conductivity throughout the mesocosm. The large amount of submerged vegetation in +Plant +Soil mesocosms dramatically increased dissolved oxygen and pH as a result of photosynthetic activity [37]. However, in the absence of adventitious plants, installed vegetation—submerged—had no detectable effect.

Phytoplankton/metaphyton (filamentous) coverage was most expansive in mesocosms where soil was present. This was probably due to nutrients (e.g., sulfur [38]) released from the soil [12], and indicates that soil can impact the structure of aquatic ecosystems that develop in mesocosms. As described in Section 2.5.3, the term phytoplankton/metaphyton in the current study is used to describe what visually appears to be algal material but is free-floating in the water column. Taxonomic analysis can be considered for future experiments to obtain a more detailed understanding of algal community response to different experimental treatments. In addition, laboratory measurement of chlorophyll a can be used as a surrogate for total phytoplankton biomass [39].

4.5. Effects of Vegetation

No substantial differences were found between +Plant −Soil and −Plant −Soil for most response variables, such as specific conductivity and turbidity, which suggests that a great deal more plant material needs to be included in the mesocosms before the effects of installed vegetation can be observed. Future investigators may wish to focus on total biomass more than taxonomic diversity if effects attributable to plants are a focus of their work.

Despite the absence of any substantial soil or sediment, adventitious plants were observed to be growing in the −Plant −Soil group, though to a much lesser degree compared to +Soil groups, rendering the initial designation of the −Plant −Soil group as "plant-free" invalid. Based on morphology, these plants were tentatively identified as *Callitriche hermaphroditica* (northern water-starwort), *Potamogeton pusillus* (slender pondweed), and *Chara* spp., though the last species is a green alga and not a true plant. In future experiments, identification of adventitious vegetation (plants or algae) by experienced aquatic vegetation taxonomists would be required to determine what species colonize the mesocosms.

4.6. Extrapolation of Results from Mesocosms Studies

Mesocosms can provide comparative information on different experimental groups in a replicated and semi-controlled fashion. When examining results, caution should be exercised against the over-extrapolation of mesocosm results to larger scales in terms of deriving regulatory acceptable concentrations from different climatic zones [40]. Furthermore, our quasi-natural outdoor mesocosms aimed to minimize extrapolation uncertainty under conditions as realistic as possible over a long time period in order to bridge the gap between laboratory and field scales of research. It should be noted that our aquatic mesocosms are static ecosystems; the appropriateness of extrapolating the results from work in lentic mesocosm systems such as ours to "flowing" (lotic) natural systems is debatable.

Furthermore, we expect that our mesocosm macroinvertebrate communities were simpler than those of a "real-world" ecosystem, although inoculation of sediment from a local borrow-pit pond likely helped increase the realism of the study. The relative lack of taxonomic diversity within such a simple community may tend to over- or underestimate the impact of an industrial material on natural macroinvertebrate communities. However, aquatic mesocosms are outdoor artificial systems, and interpretation of the data in terms of absolute values must be approached with caution. Comparing trends over time and pattern differences across groups should be focused to assess relative risk and potential impacts of industry materials in the future.

5. Conclusions

The present study describes a mesocosm experiment that was used to understand the operation and response of our mesocosm facility. The results demonstrated that a large amount of soil (2 m^3) incurred a significant change in water chemistry, while water quality measures (e.g., pH) exhibited relatively large drift over time in mesocosms without soil. For future experiments, a relatively small volume of soil should be considered to limit variations between chemical characteristics of the surface and bottom waters and avoid artefactual changes in water chemistry parameters. In addition, soil should be contained in relatively small containers, rather than being spread across the floor of the mesocosm, to minimize the potential effects of adventitious plants colonizing the soil while still maintaining adventitious vegetation as potential biological indicators of water conditions.

The changes in conductivity in surface and bottom water layers was linked to the brine rejection effect. In the future, using an integrated depth sampling method to collect water samples would better represent the entire water column and minimize the brine rejection effect in the spring for any overwintered experiment. The mesocosm experiment described here also evidenced macroinvertebrates of several functional groups, indicating that mesocosm experiments are valuable tools in understanding potential impacts of industrial materials on complex ecological systems. Knowledge gained from this study were used to support subsequent mesocosm-based studies at the InnoTech Alberta's facility in Vegreville, Alberta from 2019 to 2023. The results we reported here provide information for scientists who wish to plan and design mesocosm experiments for environmental risk assessment, especially when performed in mesocosm facilities in areas that experience winters with subzero temperatures.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/environments10070129/s1, Video S1: Video of the aquatic mesocosm facility.

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