EPFL – SIE

Environmental microbiology laboratory

Role of the hydraulic regime on the microbial degradation of organic matter

Master project

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Abstract

Six cycles (replicates) of an experiment were designed in a lysimeter, with full saturation of a soil for a few days (5 days, increased to 12 days starting in cycle 3). These cycles were separated into two triplicates (one without carbon amendment, and one with carbon amendment) to investigate the effects of carbon availability, as well as the periodicity (or memory effect) on the microbial processes. Redox potential and moisture content were recorded continuously. Water samples were taken from the bottom of the lysimeter at the end of each cycle, and analyzed for ferrous iron and sulfide content (spectrophotometry), as well as Nitrate, Sulfate (ion chromatography), DOC. DNA samples were also taken, but they will analyzed in the future. The experiment was slightly modified after initial results were analyzed and when issues were encountered, as one of the goals of the experiment was also to refine future protocols. The carbon amendment, composed of equal concentrations of cellobiose and amylopectin, was shown to boost the reduction of iron. More surprisingly, it was also shown to form an orange precipitate on the surface of the lysimeter, under the pooling water. That precipitate is most likely iron (III), indicating the presence of oxygen, and oxygen mixing from the air into the water. Some evidence of air entrapment inside the saturated lysimeter was also found and will need to be addressed more specifically in future experiments. Moisture content “drifting” of the probes was also recorded, and will also need to be investigated further, as no clear explanation was found.

Keywords

Microbial degradation; soil moisture; redox potential; lysimeter

Résumé

Une expérience composée de six cycles (réplicas) a été préparée en utilisant un lysimètre, rempli de sol et complètement saturé d'eau pendant quelques jours (initialement 5 jours, augmenté à 12 jours à partir du cycle 3). Ces cycles ont été séparés en deux séries de triplicatas, l'une avec de l'eau de pluie simple, l'autre avec de l'eau amendée avec du carbone dissous, afin d'investiguer les effets du carbone, ainsi que les effets de périodicité et de mémoire du sol, sur les différents procédés microbiens. L'humidité du sol et le potentiel redox ont été mesurés en continu durant les six cycles. Des échantillons d'eau ont été prélevés à la fin de chaque cycle, et analysés pour les concentrations de fer ferreux et de sulfide (par spectrophotométrie), pour le nitrate et le sulfate (par chromatographie d'ions), et pour le COD. Des échantillons d'ADN ont également été prélevés par filtration, mais ils seront analysés ultérieurement. Le protocole d'expérience a été légèrement adapté à la suite de l'analyse des résultats provisoires, ainsi que lorsque différents problèmes ont été identifiés, étant donné que l'un des objectifs de ce projet était également de peaufiner les protocoles futurs pour les analyses sur lysimètres. L'amendement de carbone, composé de concentrations équivalentes de cellobiose et d'amylopectine, a permis d'accélérer la réduction du fer ferrique en fer ferreux. Plus étonnamment, cet ajout de carbone dissout a également conduit à la formation d'un précipité orangé à la surface du sol du lysimètre, sous 1 à 2 cm d'eau stagnante. Ce précipité est très probablement du fer ferrique reprécipité, ce qui indiquerait la présence d'oxygène, qui diffuserait donc depuis l'interface air / eau au-dessus. Cela indiquerait que la surface du lysimètre est donc contaminée par de l'oxygène atmosphérique. Des indications d'air capturé dans le sol saturé a également été discuté dans ce projet à la suite des résultats obtenus pour les potentiels redox au milieu du lysimètre, et il faudra confirmer ces soupçons pour les prochaines expériences. Des indices de déviation des mesures d'humidité ont enfin été rencontrés, mais aucune explication satisfaisante n'a pu être apportée concernant ce point, et il faudra réussir à le résoudre pour les prochaines expériences.

Mots-clés

Dégradation microbienne ; humidité du sol ; potentiel redox ; lysimètre

Acknowledgments

I would like to thank in no particular order Simiao Wang for all his help and availability during the project, Prof. Bernier-Latmani and Prof. Rinaldo for their insights on specific processes, Jantina van der Meer for her help in sampling and preserving samples, Paolo Benettin and Mitra Asadollahi for their help and advice on the lysimeter, the data management, and any problem on running the experiment, Manon Frutschi and Colin Violet for their help on the filtration of DNA samples and any lab question, Marie Corre on helping me with the autoclave, Karine Vernez and Sylvain Coudret for handling the IC and DOC analyses promptly. And finally, the EML students and scientists for their help around the lab and making me feel welcome for those 4 months.

This project would not have been possible without their help.

1. Introduction and goals

1.1. Motivation

The soil is a very complex system, influenced by a multitude of external drivers. One of the most important ones is the climate, mostly through precipitation, which influences the moisture content and the soil redox state. In the future, it is predicted that the hydrological cycle will change, resulting in longer drying periods and more intense precipitations (Huntington, 2006; Hoegh-Guldberg et al., 2018). One of the questions is whether this change to the hydrological cycle will change the soil saturation, the soil redox state, the microbial community distribution, its metabolism, to a new equilibrium.

Climate also controls the carbon inputs to the soil. According to Zak et al. (1994), carbon inputs to soil do not allow for net annual increase to the biomass, barely meeting the maintenance energy requirements of soil microorganisms. It is thought that soil respiration, the primary path by which CO_2 returns to the atmosphere, is likely to increase due to changes in the Earth's condition (Schlesinger & Andrews, 2000).

But the question remains as to how soil respiration will change when considering changes to the hydrological regime, as a switch from anaerobic to aerobic conditions leads to an increase in mineralization rate (Keiluweit et al., 2017), it can be expected that in our case, periods of intense rain leading to fully saturated conditions, can cause a decrease in respiration rates. In the future, we might either see a decrease of the carbon cycling due to periods of fully saturated soil, or on the other hand, an increase of mineralization rates due to deeper soil layers, anaerobically protected in the past, now exposed to oxygen during prolonged droughts. On that topic, Keiluweit et al. (2017) mention that even in well-aerated soils, anaerobic microsites impose drastic metabolic constraints on mineralization rates, protecting soil carbon against microbial decomposition. DeAngelis et al. (2010) have also seen microsites with sufficiently low redox for methanogenesis to occur, during oxic periods.

It also is of interest to know how quickly the redox potential decreases when changing from anaerobic to aerobic conditions, or from aerobic to anaerobic conditions, and therefore how quickly the microbial community can adapt to a changing environment. Reddy and Patrick (1974) have already investigated to some extent how alternate cycles influence the redox potential and the organic matter decomposition, and their work creates a great starting point for our experiment. In addition, Evans and Wallenstein (2012) have shown that precipitation history can influence the rates of the biogeochemical processes, or in other words that microbial communities have a memory, and that if environmental conditions are similar to ones seen in the past, the community will be able to readapt more quickly. Similarly, DeAngelis et al. (2010) have shown that rapidly fluctuating redox promotes microbial communities specifically adapted to these conditions.

1.2. Redox potential

Redox potential will be one of the most used parameters during this experiment. It is therefore important to give some background and notes on how it is relevant, and what kind of information it gives us.

The redox potential is measured in volts, in reference to H_2, a standard substance (Madigan et al. 2010). It represents the tendency of the redox couple to be an electron donor or an electron acceptor. Madigan et al. (2010), give a list of of redox couples and their corresponding potential. The ones of interest for our experiment are NO_3/NO_2 (0.42 V), Fe_3/Fe_2 (0.2 V) and SO_4/H_2S (-0.22 V), and of course oxygen, which is the strongest electron acceptor of any significance in nature (Madigan et al., 2010). The driving principle is that, if both an electron donor and an electron acceptor are present, the reaction that would yield the highest energy would happen. Therefore, first oxygen will be used, then nitrate, then iron, and finally sulfate, in our system.

The redox potential measured by the probes during the experiment will give us information on the potential reactions that are happening at that depth. But they are measurements at specific points and depths. Therefore, it is likely that we might measure sulfite even though the redox measurements are not as low as needed 0.22 V, due to anaerobic microsites (Keiluweit et al., 2017) or simply spatial heterogeneity.

1.3. Objectives

The two main objectives to this project are (1) to investigate the relationship between redox potential and soil moisture at different depths inside the lysimeter and (2) to investigate the influence of organic carbon on the microbial activity and the redox potential throughout the experiment, and its fate. To do so, a lysimeter will be used to saturate a soil with water for a set amount of time, and then sample and analyze that water. The experimental setup and the protocols are detailed in the methodology, chapter 2.1.

It is expected that soil moisture will remain relatively constant with depth for this experiment, given that the lysimeter will be fully saturated and fully drained successively. Redox potential, on the other hand, is expected to somewhat vary with depth, with more reductive conditions the deeper in the lysimeter, as the upper layers of the lysimeter are closer to the water surface, and potential oxygen mixing.

The goal of the organic carbon amendment is to investigate if the microbial activity in the lysimeter is limited by carbon availability, and how additional carbon would influence the redox potential decrease. The target is to reach -200 mV (from 500 mV). In addition to recording the redox potential, the chemical analyses and the DNA extraction performed on the samples will give us important insights on the different microbial processes taking place with carbon amendment, in contrast to without it.

In addition, this project is also an opportunity to test and refine the different protocols, sampling procedures and analyses for the subsequent works on the Synergia project. Exploring the potential issues with sampling, running the experiments, the quantities of samples needed to have accurate results for the DNA analysis, for example.

1.4. DOC amendment background

In the earlier tests on the lysimeter, the redox potential changes were quite slow when switching from aerobic to anaerobic conditions. It was supposed that the lack of organic carbon in soil (approximately 1.2 %), was the reason for this slow process. Schellenberger et al. (2011) described a similar behavior, with an anaerobic slurry stable at approximately 350 mV, whereas after addition of cellobiose, the redox potential quickly dropped to negative values.

In order to boost the redox potential decrease, it would be easiest to add readily bioavailable organic carbon, quickly degradable by the microorganisms, but in future experiments, organic rich soil will be mixed to the current soil, and Schutter and Dick (2001) have shown that the carbon substrate available plays a major role on microbial communities. On the other hand, van Hees et al. (2004) have discussed that low molecular weight compounds may still be significant in relation to total soil CO_2, despite their low concentrations. Therefore, it was decided that the amendment should contain a mix of low molecular weight carbon, readily bioavailable, and high molecular weight carbon, more persistent, and more representative of a carbon-rich soil, in equal concentrations.

Cellobiose was chosen as the low molecular weight compound, given its solubility in water, and that it is a byproduct of microbial hydrolysis of cellulose (Lynd et al., 2002; Schellenberger et al., 2011). Cellulose being the most important skeletal component in plants (Klemm et al., 2005), it was deemed a representative compound to find in soils. Glucose could have been used instead. For the high molecular weight compound, cellulose was not an adequate choice, due to its insolubility in water. Therefore, amylopectin was chosen.

The concentration of DOC needed to be large enough for effects to be seen, but small enough to be realistic and relevant for the future experiments, where organic rich soil, or leaves, would be the source of carbon. Schellenberger et al. (2011) used an addition of 7.2 mmol of DOC, 100% cellobiose, in their experiment. Phan et al. (2019) added cellobiose to reach a total DOC of 8.33 mmol, including the DOC present in sediments. Therefore, given that the soil used by Phan et al. (2019) had more carbon than the one used in our experiment, it was decided to add a total of 6 mmol of DOC per cycle.

In case the carbon addition was not sufficient, it was at first thought that a third set of experiments could be run, with a different carbon concentration or carbon type. However, it was finally decided to run cycles for longer, based on the results of the initial cycles.

2. Materials and methods

This chapter describes the general methodology designed before starting the experiment, and the few adjustments made throughout the cycles based on the different results.

The different instruments used to monitor the experiment, the specific steps carried out for the collection and preservation of the water samples, and the subsequent analyses performed, are also detailed in this chapter.

2.1. Methodology

The general methodology was to conduct three cycles with each set of conditions, to have a better understanding of the behavior of the system and to have more confidence in the results. A one-time experiment might be subject to random effects (for example due to the heterogeneity of the soil), and the repetition of the process three times allows for comparison between the results and increased confidence in the conclusions.

A few parameters are recorded during this experiment. The moisture content and redox potential are recorded over the entire duration of the experiment (6 cycles, 10 weeks). After each anaerobic cycle, the water is sampled into four bottles, to get some spatial layering, even though preferential flow paths will cause the layering to not be fully accurate. Those water samples are tested for ions (nitrate, nitrite, sulfate, ammonium), DOC, ferrous iron, sulfide and DNA.

The first set of conditions was to use rainwater without any dissolved organic carbon, to have a baseline of how the system reacts. These cycles were run for a total of one week each. During that week, 1 day was used for lysimeter filling and reaching anaerobic conditions and steady state inside the lysimeter, 4 days of kept anaerobic conditions, and then 2 days for complete drainage. These cycling patterns might structure the microbial communities, as shown by Pett-Ridge and Firestone (2005), even though they had more frequent fluctuations than in our experiment.

The lysimeter was already filled with 9 layers of 5 cm each of soil, added 1 month earlier. The tenth layer was added just before the beginning of the first cycle. (2’740 g of dry soil). It contains the top redox probe. Some additional soil was replenished at the beginning of cycle 3, due to settling of soil uncovering the probe. 539 g were added.

The filling of the lysimeter was done using autoclaved bottles of 2 L of water (see chapter 2.3 for details), pumped through an irrigation system and connected to a shower head, effectively raining water down on top of the soil.

The lysimeter was consistently filled until a pool of 2 cm of water sat above the soil, ensuring anaerobic conditions throughout the entire soil column. This accounted to an average 3.5 L of water being used. The two notable exceptions were cycle 1 and 3 (the two cycles with addition of dry soil), which had more water consumption. To reach the expected pooling at the top.

During the drainage period, a suction of 30 kPa (during the first cycle) or 60 kPa (all the subsequent cycles) was continuously applied by a pump built-in and controlled by the dgt logger.

Cycle 3 was kept under anaerobic conditions for an additional week, to see the effects on the redox potential, as well as the chemical concentrations. Following these results, it was decided that the second set of experiments (cycles 4 to 6) would all be kept anaerobic for 12 days instead of the previous 5 as well.

In addition to that time length change, the second set of experiments also used rainwater amended with dissolved organic carbon (see chapter 2.3).

All bottles used for sample collection were autoclaved before use.

2.2. Lysimeter setup

The lysimeter used for this experiment can be seen in Figure 1. It is supplied by UGT. It has a diameter of 30 cm and is filled with 10 layers of 5 cm of soil each. Each layer was added successively and saturated before adding th e next one. The soil used was taken in Lausanne forest, dried and sieved before being added to the lysimeter.

At the bottom, a tube (1 in Figure 1) is connected to the lysimeter and is used for draining it. This tube is connected to a pump through the bottle cap (2 in Figure 1). That cap is connected to the bottles used to collect the pore water.

On top of the lysimeter, a cap (3 in Figure 1) is added during the saturation period to prevent evaporation.

An irrigation system (4 in Figure 1), coupled to a shower head is used to fill the lysimeter with water. Both the irrigation system and the shower head are cleaned after the irrigation process by circulating 2 L of deionized water through them, and then some ethanol, to prevent any clogging or contamination.

Several redox probes (5 in Figure 1) are set up at varying depths (4 cm, 12 cm, 20 cm, 28 cm, 35 cm, 43 cm).In addition, a calibration probe (6 in Figure 1) is also inserted into the soil. Those are redox electrodes from Ecotech with a platinum rod, and a range from -1 to 1 Volt.

Moisture probes are placed at four different depths (10 cm, 20 cm, 30 cm, 42 cm), but are not visible from this angle. Those are SMT-100 probes from UGT. They have an accuracy of 3%.

The redox data is recorded by an envilog from Ecotech (redox probes supplier) every three minutes. The moisture data is recorded by a datalogger from UGT (lysimeter supplier), every minute.

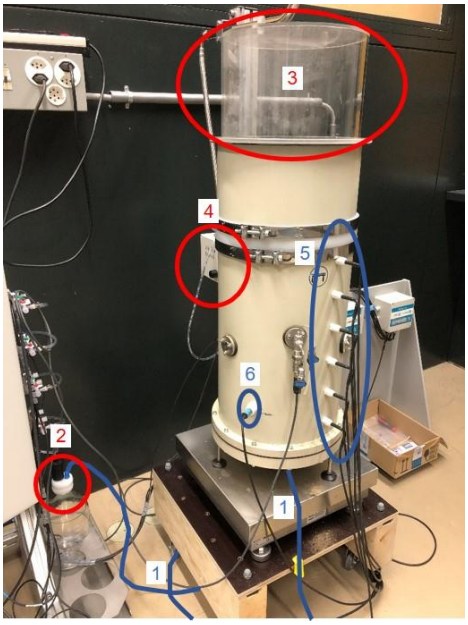

Figure 1: Lysimeter setup: the legend is detailed in chapter 2.2. 1. Collection tube; 2. Cap for water collection; 3. Anti-evaporation cap; 4. Irrigation pump; 5. Redox probes; 6. Redox reference probe

2.3. Composition of the rainwater fed to the lysimeter

The water added to the lysimeter was milli-Q water, amended with multiple salts (NH_4HCO_3, NH_4Cl, $KHCO_3$, $CaCl_2$, $MgSO_4$, Na_2SO_4, $NaHCO_3$, $NaNO_3$, $NaNO_2$). The exact recipe with the concentrations of those salts is presented in Appendix 1.

This recipe was the basis for all of the water added to the lysimeter, but two different types of water have been used over the course of the experiment. The first type was simply the basic recipe. In the second type, some organic carbon (equal carbon concentrations of cellobiose, a low molecular weight and easily degradable compound, and amylopectin, a high molecular weight less readily bioavailable compound) was also added to the solution. The solution was amended with 3 mM of DOC from cellobiose and 3 mM of DOC from amylopectin (resulting in an addition of 80 mg/L of amylopectin and 85 mg/L of cellobiose).

For consistency and practicality, two 100 times concentrated solutions (one containing all the salts except $CaCl_2$, the other only containing $CaCl_2$) were made at the beginning and autoclaved. Each was 1 liter in volume. Each 2 L porewater bottle was then made by mixing 20 ml of each solution, and filling to 2 L using milli-Q water.

2.4. Preparation of solutions used for preservation and analysis

Multiple different solutions and reagents were needed for the preservation and subsequent analysis of the water samples, as can be seen in the protocol (Appendix 2). The calibration standards were made each week just before the analysis, and are detailed in the appropriate sections (chapters 2.6.3 and 2.6.4).

2.4.1. Sulfide

For the sulfide preservation of samples, a 2 molar Zinc acetate solution (43.92 g of Zinc acetate in 100 ml milli-Q water), as well as a 1 molar NaOH solution (4.02 g NaOH in 100 ml milli-Q water) were made.

For the sulfide reagent, the concentrations from Cline (1969) were used. The reagent was made for sulfide concentrations ranging 3-40 µM. 1.54 g of ferric chloride ($FeCl_3$-$6H_2O$) and 1.01 g of diamine oxalate were diluted to 175 ml using milli-Q water. 75 ml of HCl 25% was added to reach a 250 ml solution of 50% (v/v) HCl.

A 1 molar HCl solution was made adding 13 ml of HCl 25% to 87 ml of milli-Q water. This solution is used just before the analysis of samples, to neutralize the NaOH.

The solutions were then kept at 4°C.

2.4.2. Ferrous iron

The 1 molar HCl solution described in chapter 2.4.1 is used for the preservation of the ferrous iron samples.

Following Stookey (1970), an acid reagent was made mixing 511.6 mg of ferrozine with 9.91 g of hydroxylamine hydrochloride in 30 ml of milli-Q water. 50 ml of HCl 25% was added and the final solution was diluted to 100 ml with milli-Q water. A buffer solution was also made, mixing 30g of ammonium acetate in 15 ml of milli-Q water, adding 26.25 ml of ammonia 25% and diluting to 75 ml with milli-Q water.

The solutions were then kept at 4°C.

2.5. Collection and preservation of water samples

During the drainage period, all of the water was collected into four bottles. The first two were 1 L bottles, the third one was a 2 L bottle, and the last one was a 1 L bottle.

Drainage started at 10h00 the first day, bottle 1 was collected from 10h00 to 13h00, bottle 2 from 13h00 to 17h00, bottle 3 overnight until 10h00 the second day, and bottle 4 from 10h00 the second day to 14h00 the third day, usually. Any deviation from this timeframe is specified in the results.

The bottles were put into an anaerobic chamber as soon as they were removed from the drainage setup, and samples were collected from each bottle separately for each analysis.

All the samples except the ones for DNA extraction were also filtered with a 0.2 µm filter, following a protocol from Asta, Maria P. et al. (2019).

Starting with cycle 4, the drainage was slightly altered to modify the sample collection for DNA extraction (see 2.5.5 for further explanations). To that regard, each bottle drained was subdivised into a small (100 to 250 ml) bottle for sample preservation and a large (1 L) bottle for filtering in a laminar flow hood. The small bottle was removed when full to maintain it anaerobic as much as possible and put into an anaerobic chamber for sample preservation. The larger bottle was removed to be consistent with the cycles 1-3 separation of bottles.

2.5.1. DOC analysis

In an anaerobic chamber, 25 ml of sample water were filtered into a 50 ml falcon tube using a 5 ml syringe and a Filtropur S 0.22 µm filter.

They were then stored in a 4°C fridge before being sent for analysis.

2.5.2. Ions analysis

In an anaerobic chamber, 10 ml of sample water were filtered into a 15 ml falcon tube using a 5 ml syringe and a Filtropur S 0.22 µm filter.

They were then stored in a 4°C fridge before being sent for analysis.

2.5.3. Sulfide analysis

In an anaerobic chamber, 10 ml of sample water were filtered into a 15 ml falcon tube using a 5 ml syringe and a Filtropur S 0.22 µm filter. 20 µl of the 2 M Zinc acetate solution and 100 µl of the 1 M NaOH solution were added for preservation of the sample, as described by the National Environmental Methods Index, method 4500-S2-G.

They were then stored in a 4°C fridge for up to 6 days.

2.5.4. Ferrous iron analysis

In an anaerobic chamber, 10 ml of sample water were filtered into a 15 ml falcon tube using a 5 ml syringe and a Filtropur S 0.22 µm filter. 100 µl of the 1 M HCl solution was added for preservation of the sample, as described in the sample preservation and sampling guide for liquid matrices, from eurofins (consulted in September 2020).

They were then stored in a 4°C fridge for up to 6 days.

2.5.5. DNA extraction

The protocol for collection of samples for DNA extraction changed two times during the experiment. The three different sample collections will therefore be described.

The initial method (used for cycles 1 to 3) consisted of taking 40 ml of unfiltered sample water into a 50 ml falcon tube, using a 5 ml syringe. The sample was then centrifuged at 10'000 g for 10 min at 4°C. The supernatant was dumped, and the pellet was stored at -80°C.

To complement that initial method, starting with cycle 2, 50 ml of water was filtered through a Filtropur S 0.22 µm filter. The filter was then stored at -80°C in a sterile bag.

From cycles 4 to 6, the DNA extraction was changed. Water samples used for DNA extraction were collected separately from anaerobic samples, and then filtered through a 0.22 µm filter, in a laminar flow hood. Approximately 300 ml of water was filtered for each sample (the exact volumes of water filtered were recorded for each filter). The filter was then stored at -80°C in a sterile bag.

2.6. Analysis of water samples

2.6.1. DOC analysis

The DOC concentration was measured by the GR-CEL laboratory at EPFL, using a DOC/TOC analyzer (Elementar Vario TOC Cube). It measured DOC (NPOC) using wet oxidation and nondispersive infrared sensor methods. The detection limit is 6 µg/L carbon. The measurement accuracy is 1.5% of maximum carbon.

Two measurements were made for each sample, yielding an average concentration and a confirmation of the precision of the device.

2.6.2. Ions analysis

The different ions were measured by the GR-CEL laboratory at EPFL by ion chromatography, using a Thermo Scientific Intergrion HPIC. It measured anions and cations, using a conductivity detector and a single quadrupole mass spectrometer ISQ EC MS. The eluent generation is automated.

Five different known concentrations of each ion were used for calibration. One measurement was made for each sample.

Figure 2:IC setup at GR-CEL

2.6.3. Sulfide analysis

Calibration standards of known concentrations (0, 5, 10, 20, 30, 40 µM) were made using a sodium sulfide solution. The sodium sulfide solution was made by mixing 24 mg of NaS_2 in 100 ml of water (reaching a 1mM solution), and then diluting 50 µL, respectively 100, 200, 300, 400 µL of that solution to 10 ml with milli-Q water.

Following Cline (1969), all samples and calibration standards were mixed with a 1-1 ratio between sample and reagent, caped and mixed gently. After 20 min, their absorbance was measured at 670 nm by using an UV-2501PC spectrophotometer from Shimadzu.

A calibration curve was built using the 5 calibration standards and by forcing the 0 µM concentration of the regression curve as the blank measurement. From that calibration curve, the concentrations of the samples were deducted from the absorbance value.

2.6.4. Ferrous iron analysis

Calibration standards of known concentrations (0, 2, 5, 10, 20, 50 µM) were made using a ferrous sulfate solution. The ferrous sulfate solution was made by mixing 27.8 mg of $FeSO_4$-$7H_2O$ in 100 ml of milli-Q water (reaching a 1 mM solution), and then diluting 20 µL, respectively 50, 100, 200, 500 µL of that solution to 10 ml with milli-Q water. An additional 100 µM standard was made when the spectrophotometer absorbance values of the samples were higher than the 50 µM calibration standard.

Following Stookey (1970), all samples and calibration standards were diluted to 20 ml in a 50 ml Erlenmeyer flask. 0.4 ml of the acid reagent solution was added, and the solution was then heated on a hot plate and held at boiling point for 10 min. 0.4 ml of buffer solution was then added. The absorbance was measured at 562 nm by using a spectrophotometer.

A calibration curve was built using the 5 calibration standards and by forcing the 0 µM concentration of the regression curve as the blank measurement. From that calibration curve, the concentrations of the samples were deducted from the absorbance value.

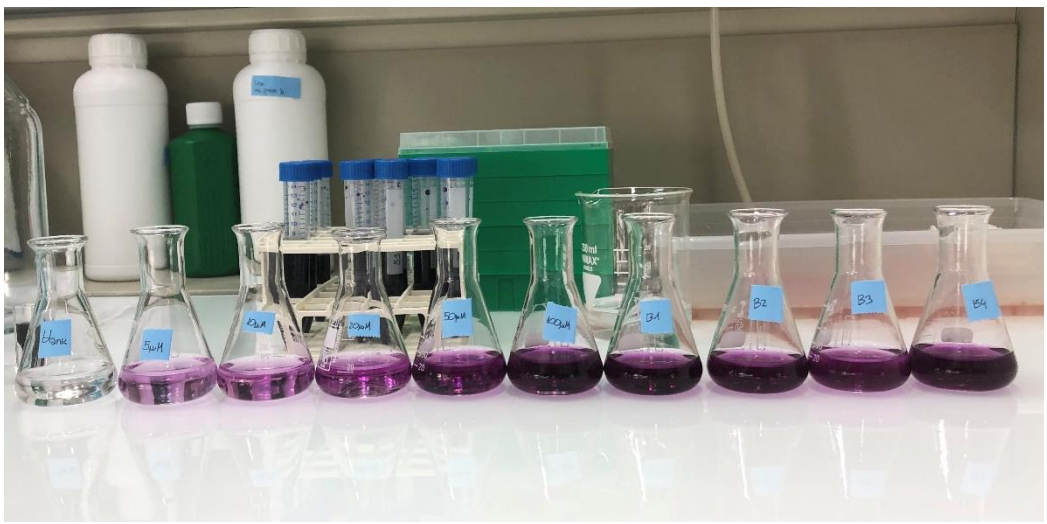

Figure 3: Ferrous iron samples and calibration standards after addition of buffer solution for color development.

2.6.5. DNA extraction

The DNA extraction and sequencing will be conducted in the future and are not included in this report.

3. Results

The results will be presented and quickly described in this chapter. They are separated based on carbon amendment. What we can learn from those results and the new limitations that have risen will be discussed in detail in chapter 4.

Raw results (either received from the lab for DOC and IC, or measured by spectrophotometry for Sulfide and Ferrous iron) are presented in the appendices (DOC in Appendix 3, IC in Appendix 4, Sulfide in Appendix 5 and Ferrous iron in Appendix 6). Moisture and redox potential data used for the plots are available on demand in csv files.

3.1. Lysimeter general data

General data, not influenced by the different cycles, will be presented in this first chapter.

3.1.1. Lysimeter temperature

The temperature is recorded by the moisture probes, at depths of 8, 20, 30 and 42 cm. The behavior at the 4 depths is very similar. Therefore, only one depth is plotted for clarity.

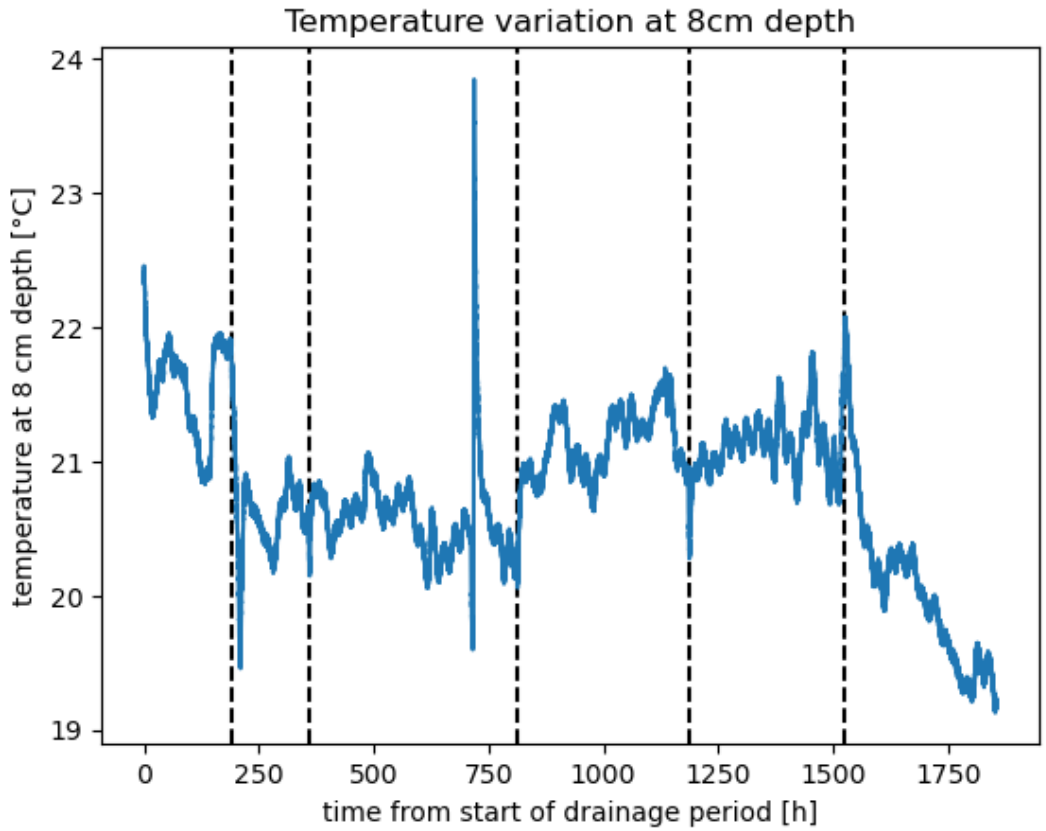

Figure 4: Temperature (°C) variation at 8 cm depth during the 6 cycles. Vertical black lines represent the start of cycles 2 to 6.

The peak of 24 °C at 750 hours is also a water input, with the first try of the 4th cycle, which was aborted due to a leak over the first weekend.

Temperature variations will be further discussed in chapter 4.

3.1.2. Lysimeter weight

The lysimeter weight can give us information on potential water loss (due to evaporation, a leak, change in temperature), as seen in Figure 5 and Figure 6, as well as on the similarity of the drainage between cycles, as shown in Figure 7.

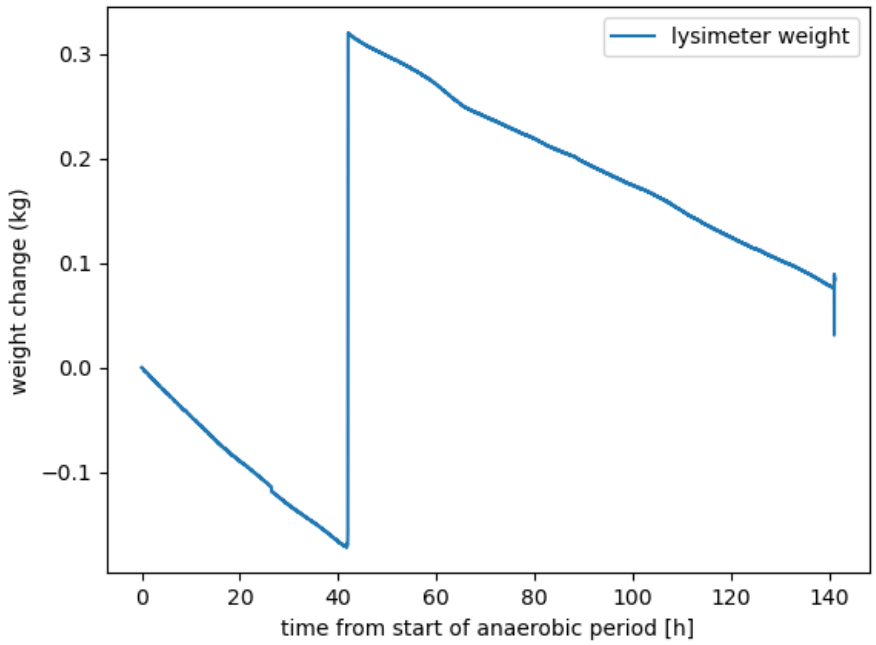

Figure 5: Lysimeter weight change during cycle 1 (anaerobic period without drainage).

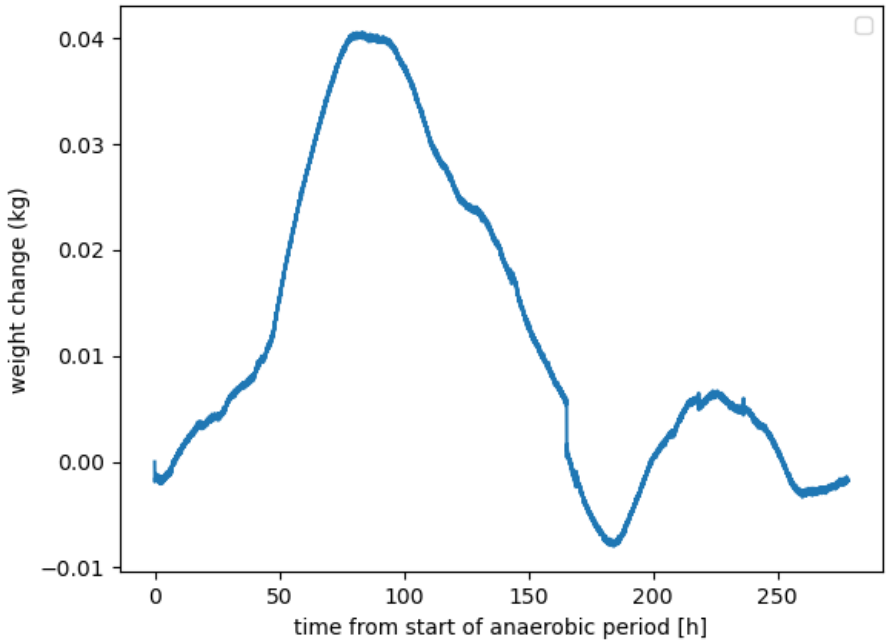

Figure 6: Lysimeter weight change during cycle 4 (anaerobic period without drainage)

Figure 5 shows the losses due to evaporation. The increase at 40 hours is due to the addition of 500 ml of water just before the weekend (fear of not having 100% saturated conditions during the weekend otherwise).

After cycle 1, a cap (see Figure 1) was installed at the top of the lysimeter to prevent evaporation losses. It is clear looking at Figure 6 that there are no evaporation losses anymore. 400 ml were evaporated during cycle 1, without accounting for potential evaporation losses during drainage (given that ponding water disappeared after a few hours of drainage, this should not cause much evaporation losses, compared to the 140 hours of the first cycle).

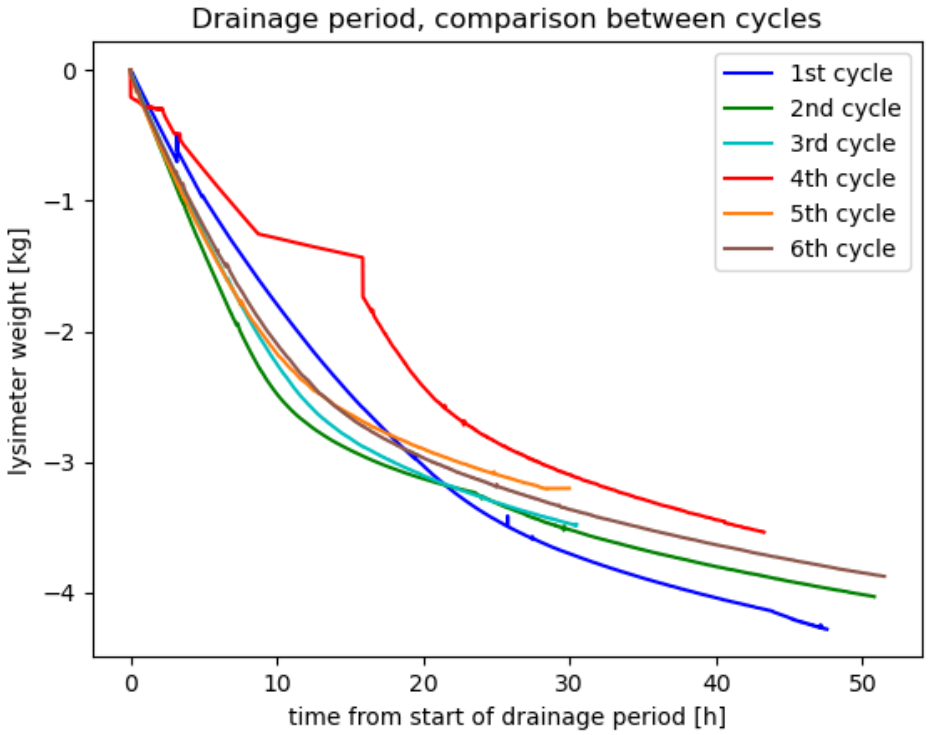

Figure 7: Lysimeter weight change during the drainage, for every cycle.

The slope of cycle 1 is more flat, because the suction applied was 30 kPa for that cycle, instead of 60 kPa for the others, but the linear decrease lasts for longer because more water was present in the lysimeter, therefore it was easier to drain water for longer.

Cycle 4 had issues for the first day (leak leading to 0 suction applied), explaining the slow drainage during the first 15 hours.

Drainage was stopped early in cycles 3 and 5 (after one and a half day instead of 52 hours), due to the impossibility of collecting the last sample after 50 hours.

3.2. Cycles 1 to 3, no carbon amendment

3.2.1. Redox potential

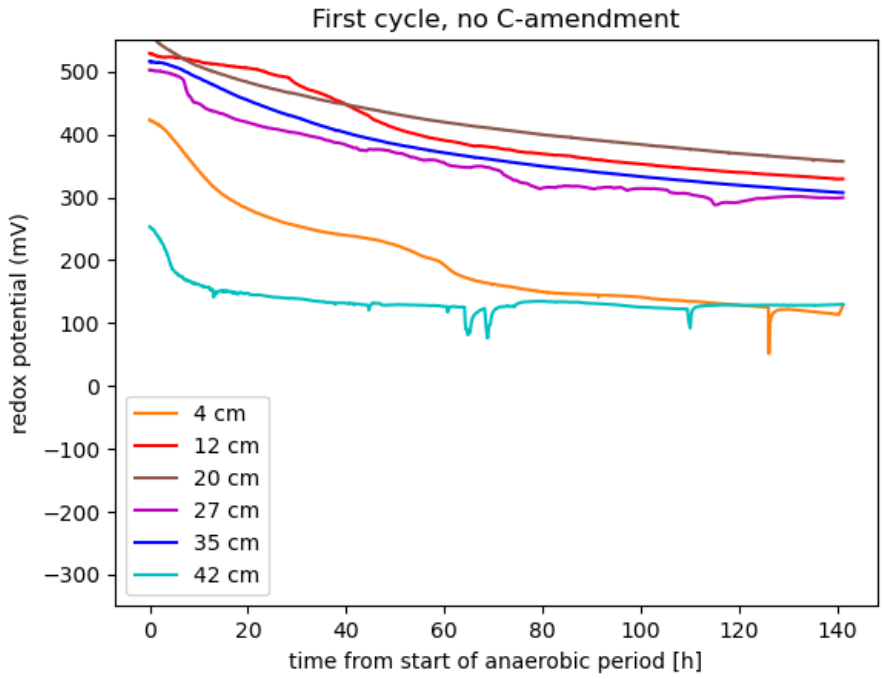

Figure 8: Redox potential [mV] evolution during the anaerobic (fully saturated) period of cycle 1

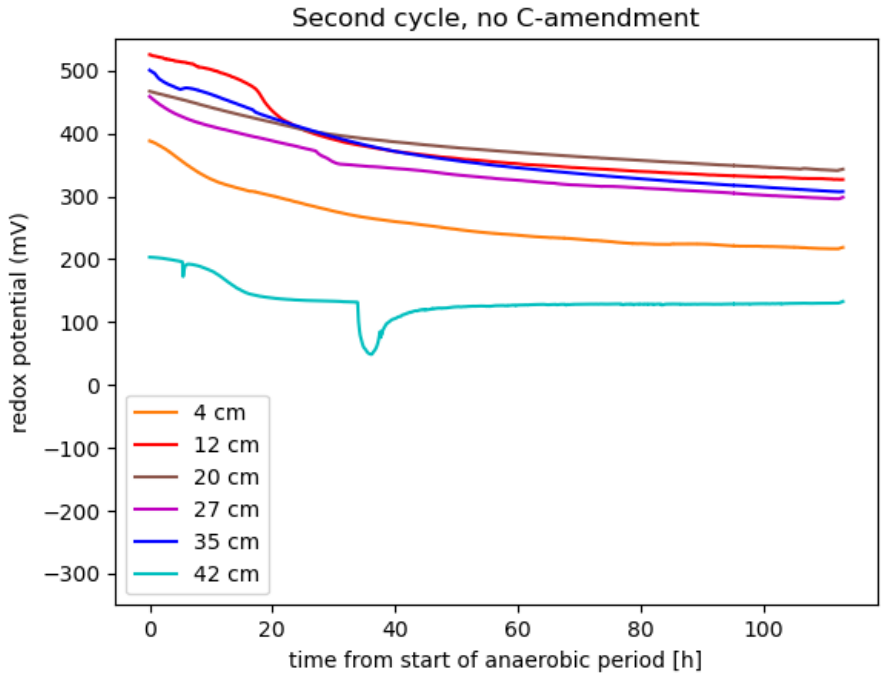

Figure 9: Redox potential [mV] evolution during the anaerobic (fully saturated) period of cycle 2

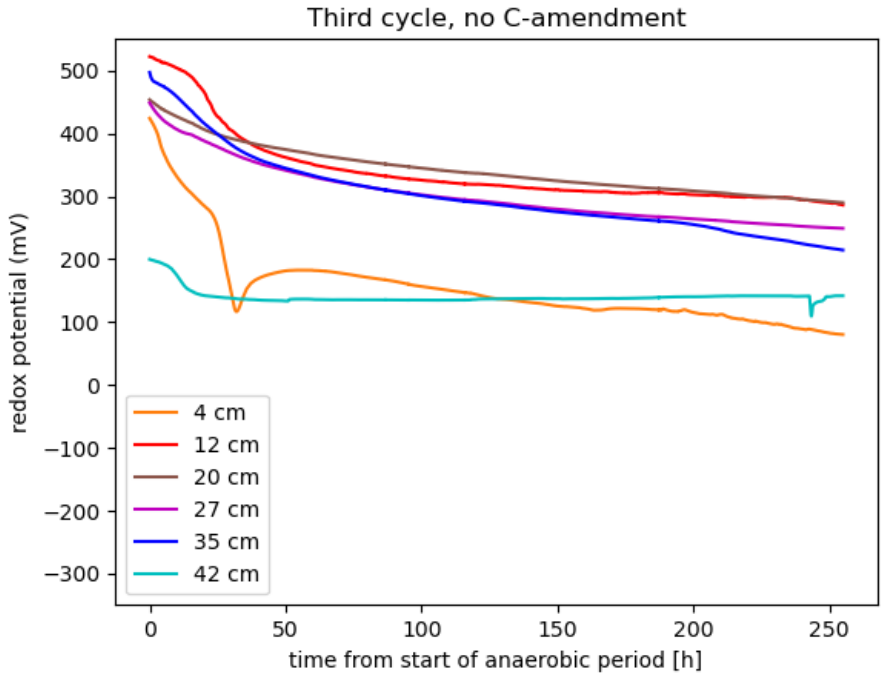

Figure 10: Redox potential [mV] evolution during the anaerobic (fully saturated) period of cycle 3

Cycle 1 is 6 days long, cycle 2 is 5 days long and cycle 3 is 11 days long.

The redox potential tends to decrease over time during a cycle. The initial redox potential is around 500 mV but varies between cycles (400 mV to 550 mV range, except for the 42 cm probe which does not ever rise above 300 mV).

3.2.2. Moisture content

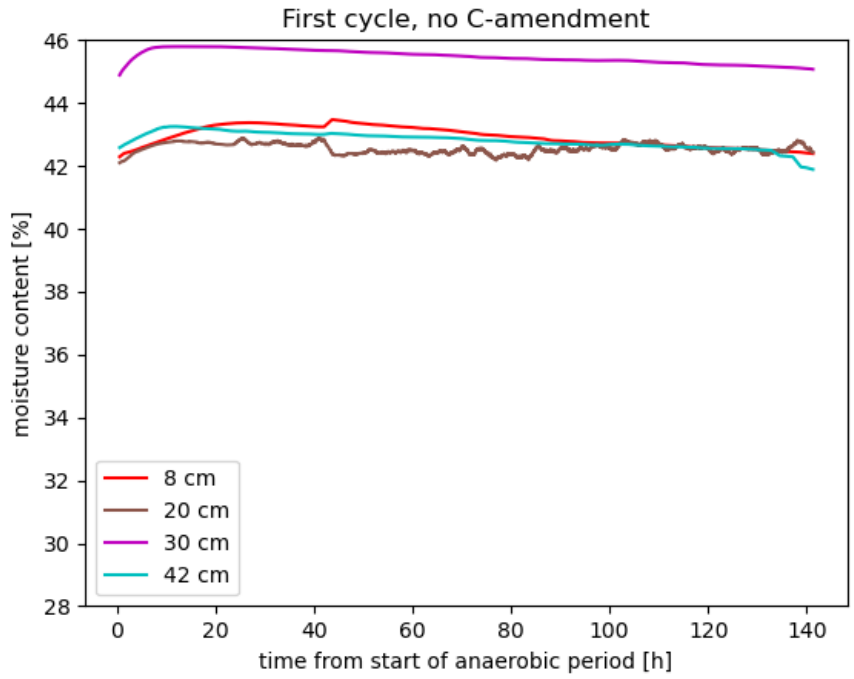

Figure 11: Moisture content variation during the anaerobic (fully saturated) period of cycle 1

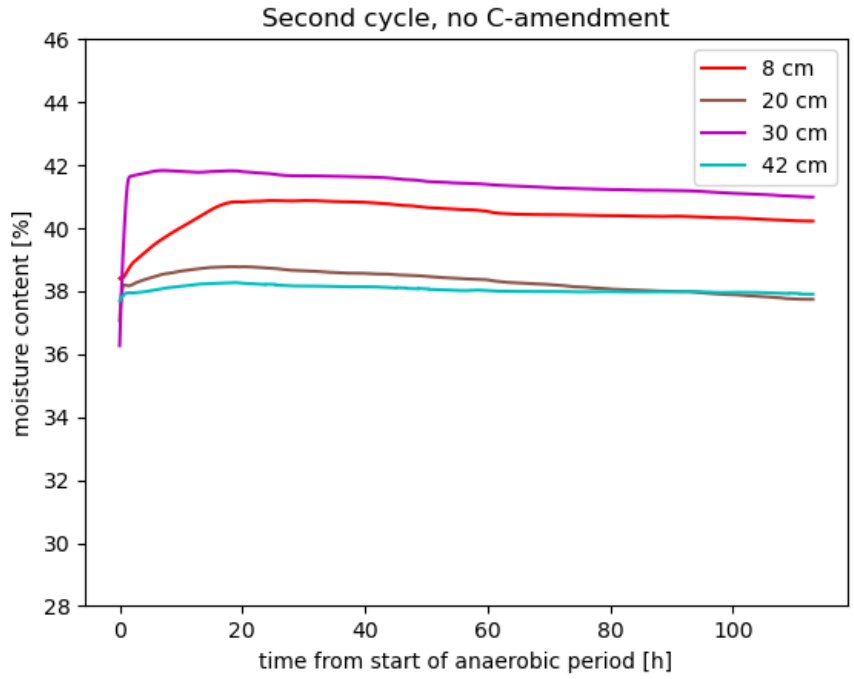

Figure 12: Moisture content variation during the anaerobic (fully saturated) period of cycle 2

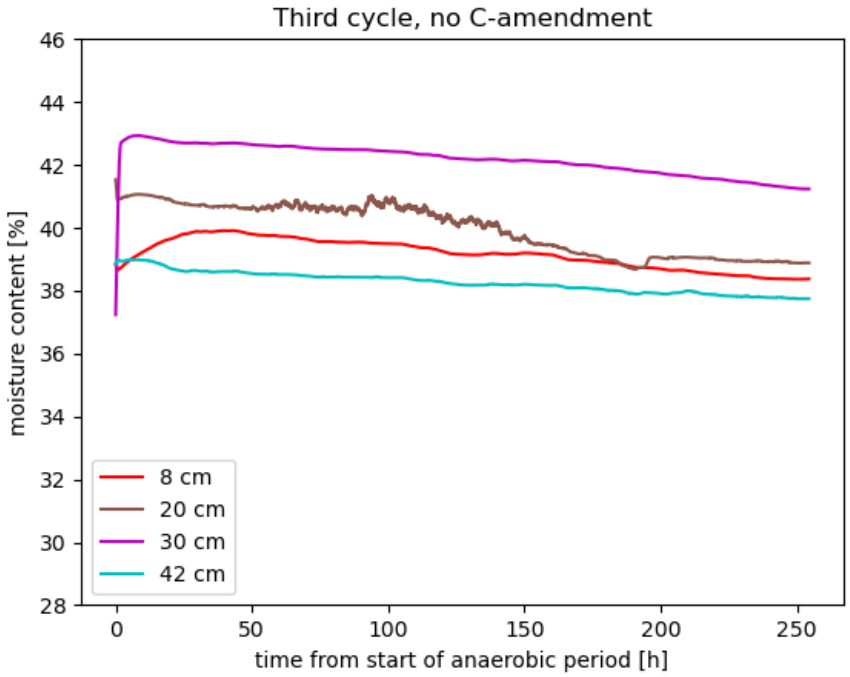

Figure 13: Moisture content variation during the anaerobic (fully saturated) period of cycle 3

Cycle 1 is 6 days long, cycle 2 is 5 days long and cycle 3 is 11 days long.

From cycle to cycle, the moisture content at a given depth varies. Moreover, the moisture content tends to decrease during a cycle, even though there is no water loss, nor any other external disturbance. However, it stays within the accuracy threshold of the probe (3%). This behavior will be discussed in chapter 4.

The data was filtered (100 steps, moving window filter), which explains the noise of the 20 cm probe (data points were jumping between two values).

3.2.3. Chemical analyses

The results of all the chemical analyses, either sent to the CEL lab or measured by spectrophotometry, are presented in this chapter.

Table 1: Chemical analyses for cycles 1 to 3. C1 represents cycle 1, B1 represents the first sampling bottle of that cycle. The volume represents how much water was collected for sampling. The sampling volumes are described in chapter 2.5.

Sample	Volume [ml]	Ferrous iron [µM]	Sulfide [µM]	Sulfate [mM]	Nitrate [mM]	Nitrite [mM]	Ammonium [mM]	DOC [mM]
C1-B1	1'000	4	1	-	-	-	-	-
C1-B2	2'000	8	1	0.09	1.93	-	-	1.71
C1-B3	700	4	3	0.10	1.86	-	-	1.78
C1-B4	700	4	1	0.09	2.08	-	-	0.58
C2-B1	1'000	7	2	0.12	1.49	-	-	0.54
C2-B2	1'000	6	4	0.12	1.18	-	-	0.98
C2-B3	1'550	3	2	0.11	1.30	-	-	0.47
C2-B4	550	1	-3	0.10	1.58	-	-	0.40
C3-B1	850	12	5	0.18	0.08	0.00	0.44	1.74
C3-B2	800	9	2	0.18	0.02	0.00	0.41	1.71
C3-B3	1'650	8	4	0.16	0.00	0.00	0.43	1.82
C3-B4	200	3	1	0.17	0.00	0.00	0.40	1.39

Samples for ions and DOC were not sent for analysis for the first bottle of the first cycle.

During the first cycle, bottle 2 was sampled overnight, instead of the regular procedure detailed in chapter 2.5, as the suction applied by the pump was lower (30 kPa) and the drainage speed was consequently lower as well.

Ferrous iron and sulfide were measured by spectrophotometry, as explained in chapter 2.6.3 and 2.6.4. These measurements are not 100% accurate, as it is based on absorbance values and a calibration curve. The negative sulfide value recorded in cycle 2 is due to that uncertainty. See Appendix 5 for the measured absorbance and the calibration curve.

Nitrite and ammonium were not measured during the first two cycles. They were measured starting in cycle 3, based on analysis of the results of the first two cycles, to try to identify the nitrate concentration behavior. This will be discussed further in chapter 4.

0.00 values for Nitrate and Nitrite mean below the detection value (0.008 mM for Nitrate, 0.002 mM for Nitrite).

Each DOC sample sent to the lab was measured twice, the measurements presented here are the mean of those two measurements. The detailed measurements are available in Appendix 3.

Soil was added at the beginning of cycles 1 and 3, as explained in chapter 2.1. It could be linked to the DOC measurements. This will be discussed further in chapter 4.

3.3. Cycles 4 to 6, carbon amendment

3.3.1. Redox potential

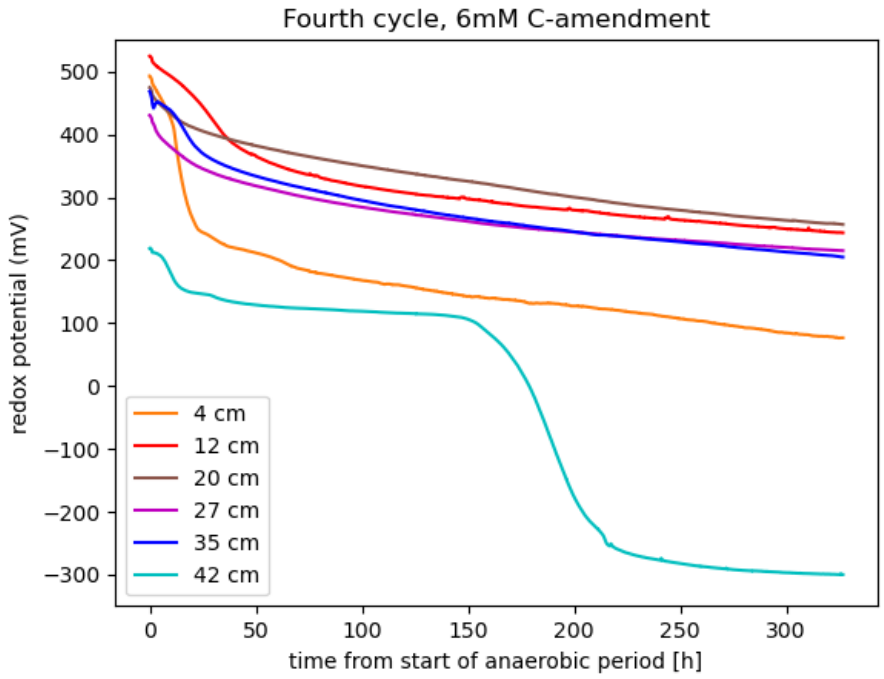

Figure 14: Redox potential [mV] evolution during the anaerobic (fully saturated) period of cycle 4

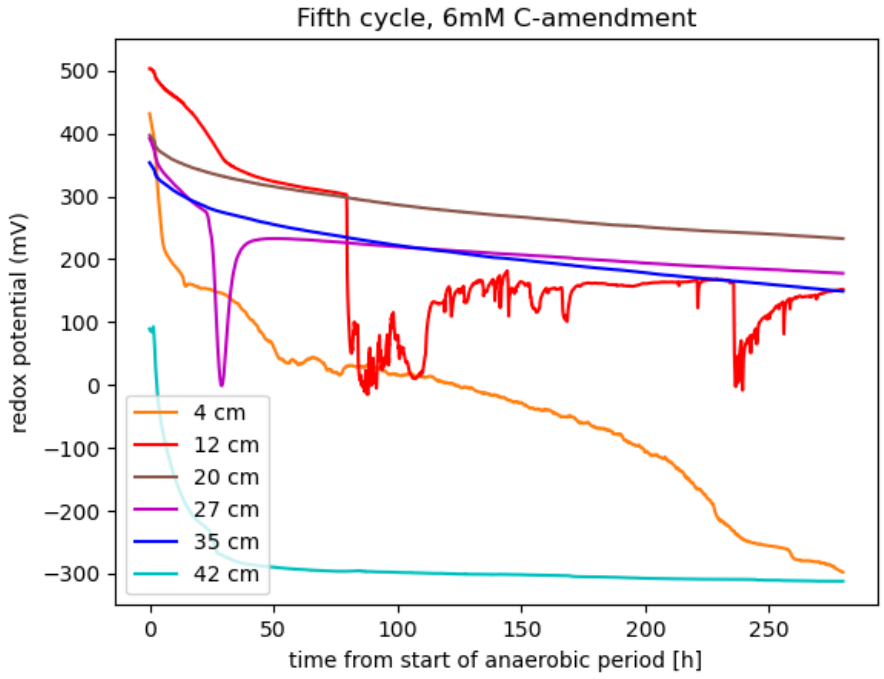

Figure 15: Redox potential [mV] evolution during the anaerobic (fully saturated) period of cycle 5

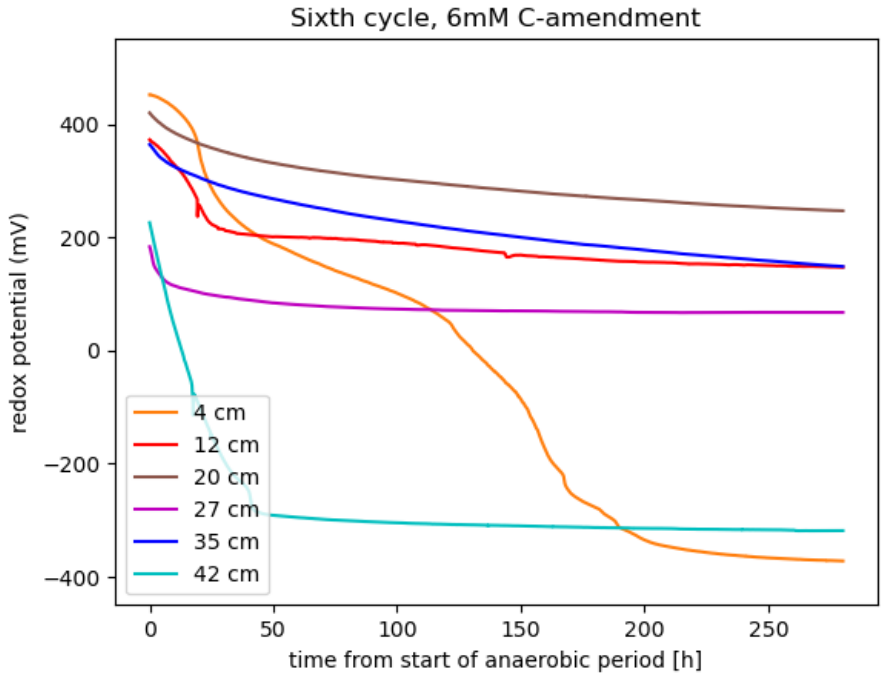

Figure 16: Redox potential [mV] evolution during the anaerobic (fully saturated) period of cycle 6

Cycle 4 is two days longer than cycles 5 and 6.

The y axis is extended to -450 mV in cycle 6 to display the 4 cm value fully.

Between cycles 5 and 6, the 12 cm probe was taken out, cleaned with deionized water, and put back in at the same depth, due to the unusual variations seen in Figure 15. The probes present at 27 cm and 42 cm were also taken out, cleaned, and switched at the same time, to get a confirmation on the values recorded.

The redox potential tends to decrease over time during a cycle. The initial redox potential is around 400 mV, but varies between cycles (350 mV to 500 mV range, except for the 42 cm probe which does not ever rise above 200 mV, and the 27 cm probe at the beginning of cycle 6, which is simply the 42 cm probe just switched to its new depth).

3.3.2. Moisture content

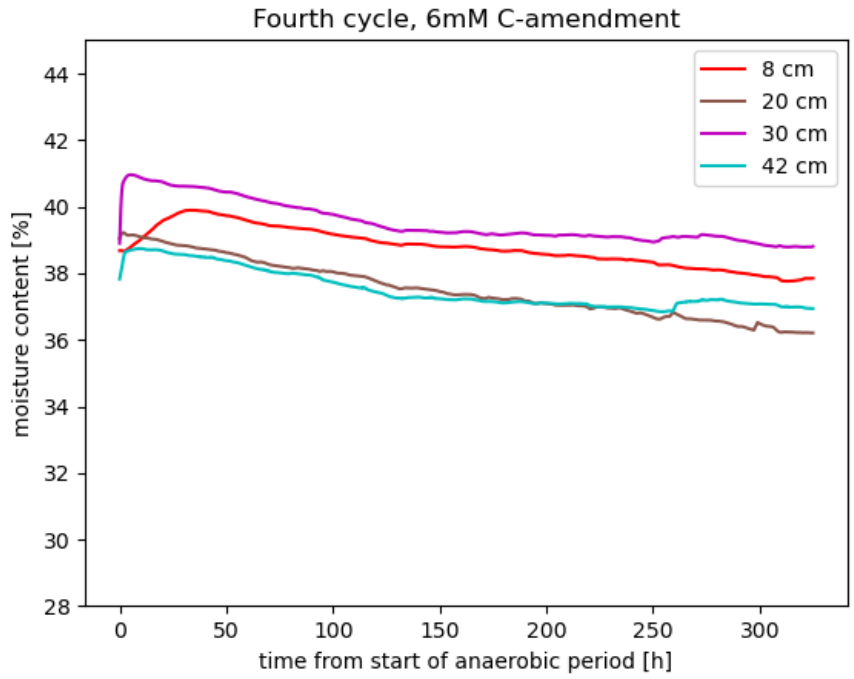

Figure 17: Moisture content variation during the anaerobic (fully saturated) period of cycle 4

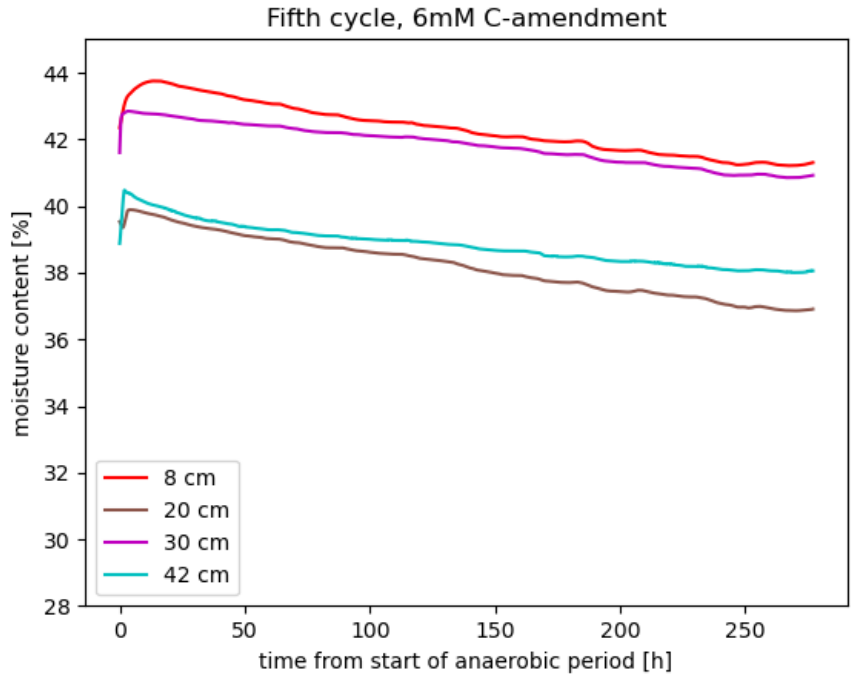

Figure 18: Moisture content variation during the anaerobic (fully saturated) period of cycle 5

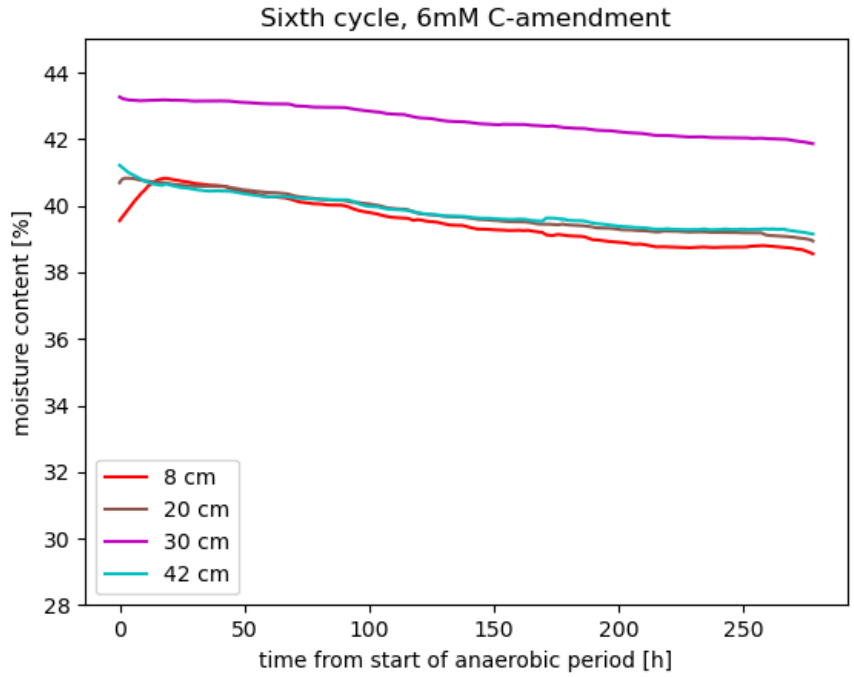

Figure 19: Moisture content variation during the anaerobic (fully saturated) period of cycle 6

Cycle 4 is two days longer than cycles 5 and 6. The y axis is fixed for the 3 graphs to allow for an easier comparison.

From cycle to cycle, the moisture content at a given depth varies. Moreover, the moisture content tends to decrease during a cycle, even though there is no water loss, nor any other external disturbance. However, it stays within the accuracy threshold of the probe (3%). This behavior will be discussed in chapter 4.

3.3.3. Chemical analyses

The results of all the chemical analyses, either sent to the CEL lab or measured by spectrophotometry, are presented in this chapter.

Table 2: Chemical analyses for cycles 4 to 6. C4 represents cycle 4, B1 represents the first sampling bottle of that cycle. The volume represents how much water was collected for sampling. The sampling volumes are described in chapter 2.5.

Sample	Volume [ml]	Ferrous iron [µM]	Sulfide [µM]	Sulfate [mM]	Nitrate [mM]	Nitrite [mM]	Ammonium [mM]	DOC [mM]
C4-B1	500	27	0	0.20	0	0	0.41	3.66
C4-B2	1'400	56	19	0.20	0	0	0.39	2.32
C4-B3	850	47	0	0.18	0	0	0.37	2.04
C4-B4	850	19	0	0.20	0	0	0.40	1.75
C5-B1	850	87	6	0.19	0	0	0.41	3.45
C5-B2	800	79	14	0.18	0	0	0.38	3.35
C5-B3	1'400	81	4	0.18	0	0	0.38	2.94
C5-B4	400	116	2	0.17	0	0	0.39	3.96
C6-B1	800	99	19	0.19	0	0	0.39	6.97
C6-B2	600	96	15	0.19	0	0	0.37	4.10
C6-B3	1'800	97	15	0.18	0	0	0.36	3.82
C6-B4	700	101	12	0.20	0	0	0.39	4.05

The drainage of cycle 4 has some problems during the first day (air leak, no suction applied), which delayed the bottle collection. Only bottle 1 was collected the first day, bottle 2 was collected overnight. Bottle 3 was collected the second day and bottle 4 was collected as usual.

Additional Sulfate, Nitrate, Nitrite and Ammonium measurements are available for cycle 5 in Appendix 4. This is because each sample was duplicated, and each duplicate was amended with 1 ml of concentrated Nitrate solution, resulting in a final concentration of 0.26 mmol. Each duplicate has lower concentrations of ammonium and sulfate, due to the dilution (11 ml sample instead of the regular 10 ml).

Those amendments gave confidence in the precision of the IC results received from the lab.

Nitrate and Nitrite measurements were below the detection limit (0.008 mM for Nitrate, 0.002 mM for Nitrite).

Each DOC sample sent to the lab was measured twice, the measurements presented here are the mean of those two measurements. The detailed measurements are available in Appendix 3.

4. Discussion

4.1. External factors influencing the experiment

As Conant et al. (2011) have shown in their review, temperature plays a role on organic matter decomposition through multiple processes. Therefore, it would be wise to try and keep the temperature of the soil column as constant as possible over the duration of the experiment in the future, because a difference of 2.5 °C over the last cycle, as can be seen in Figure 4Figure 4: Temperature (°C) variation at 8 cm depth during the 6 cycles. Vertical black lines represent the start of cycles 2 to 6., over 2 weeks, probably influences the microbial activity in a way that cannot be quantified. Similarly, the water used for filling the lysimeter should be autoclaved well in advance, so that it has time to cool down to the room temperature. We can see higher temperature fluctuations at the beginning of the cycles on Figure 4, especially at the beginning of cycles 4, 6 and the aborted cycle because the water was autoclaved the day of the feeding, and the water was still at a higher temperature, a few degrees above room temperature. The opposite can be seen at the beginning of cycle 2. The water was kept in another lab, with lower room temperature, which caused the lysimeter temperature to decrease rapidly.

Another crucial factor to account for at the beginning of our experiment was evaporation, measured by weight loss, as seen on Figure 5 and Figure 6. The issue regarding evaporation was solved after cycle 1, with the addition of an evaporation cap above the lysimeter, but it might have influenced cycle 1 results.

Finally, during the anaerobic cycles, the water at the top of the lysimeter is in contact with oxygen. Therefore, some oxygen could be replenished to the system through the top of the lysimeter. This oxygen flux might not be very large, as there is no turbulent flow, but it might be of interest to quantify it and see how deep in the water column that oxygen can travel. Once that is discovered, the lysimeter could be filled with more ponding water, to ensure the dissolved oxygen stays above the soil column and does not diffuse downwards.

Figure 20:Surface of the lysimeter at the end of cycle 6. Orange patches are probably iron (III) precipitates

This last point is of particular interest given that an orange precipitate started forming at the top of the soil column during cycle 5, as can be seen in Figure 20. This orange precipitate is most likely an iron precipitate.

Iron (III) precipitates in contact with oxygen to form this orange color. This would indicate that there is oxygen and ferric iron, the oxidized form of iron, present during the anaerobic phase, which is expected to be a reducing environment, given the redox potentials measured at the surface in Figure 15 and Figure 16. Or it could be the anaerobic precipitation of iron (III) with nitrate as the electron acceptor (Benz et al., 1997; Straub et al., 2000).

However, the precipitation of iron (III) at the surface started during cycle 4, with the carbon amendment. It is clear then, that nitrate could not act as the electron acceptor, as there was no nitrate in the system anymore at the time of the iron precipitation. The precipitate formation could be explained by one of two things that changed after cycle 3. One would be that Nitrate, present during cycles 1 and 2, as can be seen in Table 1, was inhibiting the iron cycle, since Nitrate reduction yields more energy than Iron reduction (higher reduction potential). That could explain why the iron precipitates did not form until all the nitrate was gone, and iron started to get reduced. Without iron reduction, there was no iron (II) available to be oxidized back to iron (III). In Table 1 we can also see that some Iron (II) started to be measured in the sample at the end of cycle 3. The other possible explanation would be that the carbon amendment increased the microbial activity, by adding the carbon source needed for growth (a starting assumption was that the system was carbon-limited, as discussed in chapter 1.3). To decide between these two possible explanations, the assumption regarding carbon limitation of the system now needs to be verified with the data collected, in chapter 4.2.

4.2. Carbon limitation

In order to evaluate whether the lysimeter system was carbon-limited, the results of the 6 cycles need to be separated into three categories. (1) without carbon amendment, but with addition of dry soil that contains some (~1.3%) organic carbon, cycles 1 (2’740g of dry soil) and 3 (539 g of dry soil), (2) without carbon amendment, cycle 2, and (3) with carbon amendment, cycles 4, 5 and 6. This distinction is important to evaluate the chemical results of Table 1 and Table 2, because it can be seen that cycles 1 and 3 yielded higher DOC concentrations that cycle 2, indicating that dry soil addition also added organic carbon to the system, and that that organic carbon was not consumed completely.

It can clearly be seen that ferrous iron concentrations rose with organic carbon amendment. But that could simply be due to the depletion of nitrate and iron being the next electron acceptor available in the redox chain. However, what can also be seen, is the nitrate consumption rate similarity between cycle 2 and cycle 3, with one of those cycles having some organic carbon available (from soil addition), and the other having no carbon addition. Moreover, that soil addition seems to only affect the top of the lysimeter, based on Figure 8, Figure 9 and Figure 10. But the nitrate concentration is quite constant between samples of a cycle, as is the DOC concentration. This would therefore indicate that sampling of water cannot even remotely be associated to lysimeter depths, and that all samples should be considered a mixture of the entire lysimeter depth range, due to preferential flow paths. And that the carbon present in the soil column was sufficient for the nitrate consumption. On the other hand, between cycle 3 and cycles 4 to 6, the only experimental difference is the amendment of carbon. That addition clearly started the iron reduction process, with ferrous iron concentrations barely reaching 10 µM in cycle 3 (Table 1), and going above 100 µM in cycles 5 and 6 (Table 2).

Overall, the results indicate that carbon was probably the limiting nutrient for the microbial activity to take place.

4.3. Nitrate reduction

We can now investigate in depth the nitrate reduction process that seems to take place during cycles 1 to 3 (see Table 1). Nitrate can be reduced using two main pathways. (1) Denitrification is a process of the nitrogen cycle, the reduction of nitrate to nitrogen gas, usually happening under anaerobic conditions (Madigan et al., 2010), even though it can happen aerobically under certain conditions, as reviewed by Ji et al. (2015). (2) The reduction of Nitrate to Ammonia, which can happen through multiple pathways, such as dissimilative reduction of nitrate to ammonium and ammonification, the assimilation of nitrate in organic nitrogen compounds, later decomposed (Madigan et al., 2010). This process happens under anaerobic conditions.

According to literature (for example Madigan et al., 2010; Kraft et al., 2014; Tiedje J., 1988), nitrate-reducing bacteria exploit the pathway (2) when nitrate is limiting, due to more electrons being consumed.

In our case, the conditions are thought to be carbon-limiting, at least during part of the experiment. By not measuring ammonium concentrations during cycles 1 and 2, it is not possible to give a final answer as to which pathway was used in this case, but the final ammonium concentrations being lower than the initial nitrate concentrations (0.4 mM ammonium, 2 mM nitrate, see Table 1) indicate that 1.6 mM of nitrate at least was either incorporated into biomass or went through the denitrification pathway.

Nitrate was produced through nitrification, an aerobic process, during the long aerobic period before our experiment started. It could be interesting, in the future, to measure ammonium and nitrite during the first cycles in case there is some nitrate build up again, to get some confirmation on the nitrate reduction processes taking place.

This could be especially important given that nitrate seemed to be inhibiting other, less energy favorable reduction processes, that we were measuring.

4.4. Sulfur cycle

The sulfur chemistry is measured in our experiment with sulfide and sulfate.

We have some sulfate input to our system, 90 µM (Appendix 1). The expected reaction would be to have sulfate reduction to sulfide, with a decrease in sulfate content, and a corresponding increase in sulfide concentration, in the samples collected. Sulfate respiration only happens under anaerobic conditions (Madigan et al. 2010), and sulfate is not a good electron acceptor (see chapter 1.2), so that reaction should be the last one to happen, and indicate a very low redox potential and a highly reductive environment.

The sulfide could be measured, but it could also react quickly with iron (II) to form a black precipitate, mackinawite (Morse et al., 1987), that would stay in the lysimeter column.

From the results shown in Table 1 and Table 2, we see that, starting in cycle 3, the Sulfate concentration increased, above the sulfate inputs. Clearly indicating that some sulfate was removed from the system. Some sulfur is present in the soil used to fill the lysimeter (~400 mg/kg soil), so the source of Sulfate is known. But it is interesting to realize that the Sulfate concentration increase coincided with the longer cycles, and not with the carbon amendment.

We also measure some sulfide, especially in cycle 6, indicating that sulfate reduction takes place, first only in some microsites (cycles 4 and 5, low concentration measured in one sample), but then more uniformly over the soil column (sulfide concentrations in the 4 samples). The redox potential values measured during cycles 4 to 6 (Figure 14, Figure 15 and Figure 16) would however indicate that at the lowest depth, the sulfate reduction should be happening during the 3 cycles with carbon amendment. And that no sulfide reduction should happen in cycle 6 for the middle of the lysimeter.

The explanation for the sulfate reduction to sulfide in the 4 samples of cycle 6 is that (1) samples do not represent depth, due to preferential flow paths in the soil column. It is not possible to link the samples to a depth. And (2) that since we measure the redox potential at one specific point, microsites with lower redox conditions are expected, as discussed before.

It is more difficult to explain why we do not measure sulfide in the first sample of cycles 4 and 5. Even with preferential flow paths in the soil column, it is quite unlikely that water containing sulfide in the 10 lowest cm of the lysimeter, is only sampled after two liters were already collected. Another explanation could be the precipitation with iron (II)., which would explain the lower iron (II) concentration in cycle 4. But cycle 5 remains an enigma.

4.5. Redox potential

The redox potential, as explained briefly in chapter 1.2, gives us a lot of information on the potential microbial activity taking place at the different depths of the lysimeter for each experiment.

There are three main results that we can investigate, with this experimental setup and methodology. (1) is to look at depth differences during a cycle and to try to explain why certain depths have more reductive conditions than others. (2) is to look at differences between cycles with the same initial conditions. Such differences would mostly be between cycles 1 and 2 (Figure 8 and Figure 9), and between cycles 4, 5 and 6 (Figure 14, Figure 15 and Figure 16), as they have the same lengths of saturation period, and the same carbon amendment conditions. Finally, (3) is to investigate the influence of the carbon amendment on the redox potential. This has been discussed to some extent already in chapter 4.2, but will be explained more now, based on redox potential results.

The result (1) was unexpected and needs to be investigated thoroughly. No proper final explanation has been found to fully explain this behavior, but here are some ideas. At 42 cm depth, and at 4 cm depth, the redox potential decreases, whereas at the other depths, it stays quite constant. A first assumption, based on the results from cycles 1 to 3, was that the bottom of the lysimeter, as expected, constantly had the most reductive conditions, and that the soil addition at the top of the lysimeter, by also adding organic carbon, was the driver of microbial activity at the top of the lysimeter. But based on that explanation, it would have been expected that the carbon amendment would boost the microbial activity at all depths, which clearly is not the case. The organic carbon clearly reaches all depths, as we can measure a large amount of organic carbon in the sampling water as well. Which means it is not completely getting consumed. We especially see an increase in DOC concentrations during cycles 5 and 6 (Table 2).

For the redox potential to remain that high, and for carbon to not be consumed, a possible explanation could be that oxygen remains trapped inside the lysimeter during the saturation process. Bubbles of trapped air have been seen rising to the surface during the filling of the lysimeter, but it was always assumed that, after a few hours, all the air was gone from the pores, and that the soil was completely saturated. But some trapped air could explain why the moisture measurements vary between cycles. Those moisture readings will be discussed in chapter 4.6. Faybishenko (1995) has shown that the volume of entrapped air depends on the method used for the initial saturation, and that in our case of ponded infiltration, entrapped air ranged from 5 % to 10% for his experiment, with the presence of mobile air. Similarly, Wang et al. (1998) have shown that there is more entrapped air left in a soil column when the air can only escape through the soil surface, as is the case in our experiment.

Therefore, there could be consequent amounts of entrapped air inside the lysimeter, inhibiting anaerobic microbial processes, and resulting in the redox potentials measured in this experiment. The redox probe closest to the surface would not be subject to air entrapment, as air could escape more easily. Other ways to saturate the soil could potentially be used to reduce the air entrapment effect. Faybishenko (1995) mentions two methods that yielded almost complete water saturation. One is replacement of the pore air by CO_2, which is not possible in our case due to risks of completely disturbing the microbial processes, but another is the vacuum elimination of air from the pore space. But since our experiment aims to replicate precipitation patterns, it might be of more interest to look for more appropriate ways to irrigate and saturate the soil in the first place. With lower (more realistic) intensities, over a longer period of time. This could already reduce sensibly the amount of entrapped air present in the

soil column. Another option could be to fill the lysimeter while the drainage pipe is left open, to let air and water freely move downwards for a time.

Now looking at (2), in Figure 8, Figure 9 and Figure 10, we probably see the effect of the soil addition at the beginning of cycle 1, and 3, leading to a greater decrease in the redox potential at the surface of the lysimeter, since the 4 cm probe was put in the middle of the layer added before cycle 1. At the other depths, there is no visible change in the redox potential.

In Figure 14, Figure 15 and Figure 16, there are two main processes that can be highlighted. One is that, similarly to cycles 1 to 3, the redox potential decreases at the surface, but also at the bottom of the lysimeter. The other is the effect of memory and successive cycles. We see that the redox potential decreases more quickly in the later cycles. This is expected, since biomass is already present in the later cycles. Specifically for the 27 cm probe in cycle 6 (Figure 16), the redox value measured is constantly lower than what was measured in cycles 4 and 5, surprisingly compared to the other probes in the middle of the lysimeter. In this case, it could be due to the probe slightly disturbing the soil when switched and measuring slightly different redox conditions. But a more likely reason is that the probe is uncalibrated, and measures redox potentials below 300 mV constantly, as it was placed at the bottom of the lysimeter for 6 months and has never risen above 300 mV in that time. That is confirmed by the probe that was at 27 cm for the first 5 cycles and placed at the bottom of the lysimeter for the 6^{th} cycle. It measured the same very reductive (-300 mV) conditions during the saturation cycle, but then rose to +400 mV quickly during drainage. The potentially faulty probe should be looked at and recalibrated before the next set of experiments.

Finally, considering (3), the influence of the carbon amendment is clear on the 4 cm and 42 cm probes in Figure 14, Figure 15 and Figure 16. In Figure 14, we see that at 42 cm, the redox potential suddenly drops to -300 mV after 200 hours, indicating very reductive conditions and potential sulfate reduction, even though sulfide was not measured in the sample, and sulfate concentration did not vary much compared to cycle 3. We also see that in cycles 5 and 6, that redox potential was reached after only 50 hours, again highlighting memory effects. The same effect to some extent was seen at 4 cm depth, even though the decrease in redox potential is slower, probably indicating that there is a very small amount of air trapped at that depth already.

Surprisingly, the effects of the carbon amendment on the other depths is very difficult to identify from those graphs, contrary to what we would expect. The presence of trapped oxygen, which is the main supposition at this point, would explain that behavior as well. This further confirms that oxygen entrapment is a likely issue in this experiment.

4.6. Moisture content

There are two main findings regarding the moisture content with this set of experiments. One is that the moisture content between each cycle varies significantly, and the other is that the moisture content decreases almost linearly, at all depths, during a cycle.

The difference in moisture content between cycles is probably the result of a few factors, such as air entrapment, as discussed in chapter 4.5, but also soil structure differences due to drying / rewetting stress or small differences in ponding water height between cycles (which influences water pressure). Air entrapment would directly decrease the moisture content, so based on that and if we assume that everything else is staying homogeneous, which is unlikely, we could get as much as 5% air entrapment over the entire column, assuming there is no air entrapment when the moisture content is at its highest value, in cycle 1. On that note, cycle 1 is by far the cycle with the highest moisture content, due to two factors. One is that the lysimeter experienced a very long dry period before the start of this experiment, making water infiltration much easier. And the second factor is that the top 5 cm of the lysimeter was new soil freshly added, completely dry. The combination of these two factors made water infiltration very easy and caused the very high moisture content, by limiting the air entrapment.

The linear decrease in soil moisture is more problematic, and difficult to explain. It could simply be a drift in the sensors, but that would not explain why the sensors reset after each cycle. If it was a drift, we would expect the error to propagate between cycles.

This is most likely not due to air bubbles trapped in the system either, as we would then expect a quick rise in moisture content whenever the bubble bursts and is able to escape to the surface of the lysimeter, rather than a linear decrease.

This issue needs to be solved for future experiments.

5. Conclusion

This experiment has improved our understanding of multiple processes related to the microbiology inside a saturated soil for further experiments conducted with lysimeters. It has shown that carbon amendment causes an increase in microbial activity, which leads to a drop in redox potential, the reduction of iron and some sulfide formation. It has also shown that not all the added DOC was consumed during the duration of the cycle (12 days), especially in the later cycles (5 and 6).

Moreover, evidence of other processes than iron reduction and sulfate reduction, such as nitrate reduction and iron precipitation, have been recorded. Nitrate reduction, especially, was seen without the amendment of carbon, and even during shorter cycles (5 days long).

This experiment has also shown that processes, and the redox potential measured, have a memory effect, meaning that conditions reached in the past are easier to be restarted in a future cycle.

But some questions remain open, especially regarding the relation between the moisture content and the redox potential, as multiple issues need to be figured out. These issues range from the method used to fill the lysimeter, which seems to cause a large volume of air entrapment, to the precision of the moisture probe (3% right now), to the drift seen in all moisture content measurements, that has not been explained with enough confidence yet.

Outside of the two objectives listed at the beginning, some other relevant data was also collected. We have seen that more sulfate was collected in the samples at the end of the longer cycles (3-6), than in the first 2 cycles. We have also seen that those sulfate concentrations were higher than the inputs in the rainwater solution used to fill the lysimeter. This is evidence that sulfate is removed from the solid, soil-phase.

We have also seen that nitrate build up can be an important process to take into account, especially if before the start of an experiment, the soil is left to dry for a long period of time. The effects of evaporation during the experiment have also now been minimized with the addition of a cap, which works well, and we have seen that temperature might influence the experiment and will need to be controlled more effectively.

For the future, it will be especially interesting to analyze the DNA results to clearly link the chemical results to microbial processes and see the evolution of the microbial community over the cycles. It will also be of interest to have the possibility to sample water from different depths, to better relate samples to specific redox potential measurements. It might also be interesting to try to fill the lysimeter with an open bottom (drainage valve open), to let entrapped air escape by the open bottom as well. While that might not fully solve the issue of air entrapment, it might help reduce it to values more realistic of the real world, where water (and entrapped air bubbles) are usually free to move downwards.

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Appendices

- Appendix 1: Composition of the rainwater used for the feeding of the lysimeter
- Appendix 2: Porewater sample collection and analysis protocol
- Appendix 3: DOC results from the CEL laboratory
- Appendix 4: IC results from the CEL laboratory
- Appendix 5: Sulfide spectrophotometer results
- Appendix 6: Ferrous iron spectrophotometer results

Appendix 1: Composition of the rainwater used for the feeding of the lysimeter

Appendix 1

Composition of the rainwater used for the feeding of the lysimeter

Substrate	Concentration		Mass of substrate (in mg) in 1L 100 times concentrated solution
	µM	mg/L	
NH_4HCO_3	328.72	25.97	2596.89
NH_4Cl	71.47	3.82	382.36
$KHCO_3$	91.2	9.12	912.03
$CaCl_2$	55.96	6.21	621.16
$MgSO_4$	60.98	7.32	731.78
Na_2SO_4	26.69	3.79	378.93
$NaHCO_3$	30.06	2.52	252.49
$NaNO_3$	22.23	1.89	188.95
$NaNO_2$	17.87	1.23	123.28

Appendix 2: Porewater sample collection and analysis protocol

Porewater Sample Collection and Analysis Protocol

1. General Preparation

(1) Put collected water sample in an anaerobic chamber

(2) Filter water sample with 0.2μm filter

(reference: Asta, Maria P., et al. "Microbially Mediated Release of As from Mekong Delta Peat Sediments." Environmental science & technology 53.17 (2019): 10208-10217.)

2. DOC analysis

(1) Collect 20-25 mL filtered water sample into a 50mL falcon tube

(2) Taking it out of anaerobic chamber

(3) Frozen it in -20°C freezer before taking it to CEL for TOC analysis

3. Sulfate and Nitrate analysis

(1) Collect 5-10 mL filtered water sample in a 20mL falcon tube

(2) Store it in -20°C freezer

(3) Send it to CEL for further analysis

4. Sulfide analysis

(1) Preservation:

Prepare 2mol/L zinc acetate solution and 0.1 mol/L NaOH solution; Add zinc acetate solution and NaOH solution in bottle first, and then add filtered sample into bottle. For 100mL sample, 0.2mL zinc acetate is needed if sulfide concentration is less than 64mg/L. NaOH should be added to adjust the pH value to be at least 9. Headspace should be minimized to avoid volatilization. Store it in fridge.

Record the volume of sample, zinc acetate solution and NaOH solution used to calculate dilution times of sulfide sample.

(reference: Standard Methods: 4500-S2- G: Sulfide by Ion-Selective Electrode, National Environmental Methods Index, EPA, https://www.nemi.gov/methods/method_summary/7419/)

(2) Reagent preparation:

TABLE 1. *Suggested reagent concentrations and dilution factors to be used in the determination of sulfide-sulfur in the stated concentration ranges*

Sulfide concn (μmoles/liter)	Diamine concn (g/500 ml)	Ferric concn (g/500 ml)	Dil. factor (ml : ml)	Path length (cm)
1–3	0.5	0.75	1 : 1	10
3–40	2.0	3.0	1 : 1	1
40–250	8.0	12.0	2 : 25	1
250–1,000	20.0	30.0	1 : 50	1

Based on the estimation sulfide concentration range, dissolve the

amounts of *N, N*-dimethyl-*p*-phenylenediamine sulfate and ferric chloride ($FeCl_3$•6H2O) in 500ml of cool 50% (v/v) reagent grade hydrochloric acid. **Keep it in dark bottles under refrigeration.**
(reference: Cline, J. D. (1969). Spectrophotometric determination of hydrogen sulfide in natural waters 1. Limnology and Oceanography, 14(3), 454-458.)

(3) Calibration Standards:
Based on the range of estimated sulfide concentration, prepare sodium sulfide solutions of 5 different known concentrations within the range as well as a milliQ water sample.

(4) Measurement:
Based on the dilution factor listed in table above, mix samples (both unknown and calibration standards) with reagents in a bottle/falcon tube. Cap it to reduce volatilization and mix them gently.
After 20 minutes, put them in appropriate cuvette, and measure their absorbance at 670nm by using the spectrophotometer.
A calibration curve can be built with the 5 calibration standards and the unknown sulfide concentration can be determined based on its absorbance and the calibration curve.
(Terminology: Path length: In chemistry, the path length is defined as the distance that light (UV/VIS) travels through a sample in an analytical cell.)

5. Ferrous ion analysis

(1) Preservation:
After filtration, add 1mol/L HCl into sample to adjust pH<2, store it in a dark, brown glass bottle with minimized headspace to avoid the effect of oxygen. Store it in fridge.
Record the volume of sample, HCl solution used to calculate dilution times of ferrous sample.
(reference: SAMPLE PRESERVATION AND SAMPLING GUIDE Liquid Matrices, eurofins, https://cdnmedia.eurofins.com/apac/media/168333/sample-preservation-guide-liquid-matrices.pdf)

(2) Reagent Preparation:
Acid reagent: Dissolve 5.14 g of ferrozine and 100 g of hydroxylamine hydrochloride in a small amount of water. Cautiously add 500 ml of concentrated hydrochloric acid. Cool to 20 °C and dilute to 1 liter with demineralized water.
Buffer solution: Dissolve 400 g of ammonium acetate in water. Add 350 ml of concentrated ammonium hydroxide and dilute to 1 liter with demineralized water.

(3) Calibration Standards:

Based on the range of estimated ferrous concentration, prepare ferrous sulfate solutions of 5 different known concentrations within the range as well as a milliQ water sample

(4) Measurement:

Place 50.00 ml of freshly drawn sample water in a 125 mL Erlenmeyer flask. Add 1.00 mL of acid reagent solution. Heat the solution on a hot plate and hold at the boiling point for ten minutes. Cool to 20 °C and transfer the contents quanti- tatively to a 50 mL volumetric flask. Add 1.00 mL of buffer solution and dilute to the mark with demineralized water. Mix thoroughly and allow 1 minute for full color development.

Measure the absorbance at 562 nm against a calibration curve prepared from standard solutions treated in the same manner.

A calibration curve can be built with the 5 calibration standards and the unknown sulfide concentration can be determined based on its absorbance and the calibration curve.

(reference: Stookey, L. L. (1970). Ferrozine---a new spectrophotometric reagent for iron. Analytical chemistry, 42(7), 779-781.)

6. DNA extraction

(1) Weight Balance:

Measure the weight of each falcon tube used as well as the weight of filtered water sample added into them. **Make sure the total weight of tube and sample are the same for centrifuge.**

(2) Centrifuge:

Place falcon tube on centrifuge plate, centrifuge them at 1000g for 30 minutes at 4 degrees.

(3) Storage:

Dump the supernatant and store the pellets in -80°C freezer.

7. Naming of samples

L1-C1-Analysis Target-Sampling Time-Sampling person

L1 refers to Lysimeter 1

C1 refers to the first cycle

Appendix 3: DOC results from the CEL laboratory

Retti Ivan

Appendix 3
DOC results from the CEL laboratory

Without C-amendment			
Sample	DOC [mg/L]	DOC [mmol/L]	Average [mmol/L]
C1-B1	-	-	-
C1-B1	-	-	
C1-B2	21.30	1.77	1.71
C1-B2	19.80	1.65	
C1-B3	21.95	1.83	1.78
C1-B3	20.78	1.73	
C1-B4	6.81	0.57	0.58
C1-B4	7.12	0.59	
C2-B1	6.48	0.54	0.54
C2-B1	6.44	0.54	
C2-B2	11.90	0.99	0.98
C2-B2	11.59	0.97	
C2-B3	5.73	0.48	0.46
C2-B3	5.40	0.45	
C2-B4	4.75	0.40	0.39
C2-B4	4.67	0.39	
C3-B1	20.89	1.74	1.73
C3-B1	20.73	1.73	
C3-B2	20.50	1.71	1.71
C3-B2	20.55	1.71	
C3-B3	21.72	1.81	1.82
C3-B3	21.99	1.83	
C3-B4	16.43	1.37	1.38
C3-B4	16.75	1.40	

With C-amendment			
Sample	DOC [mg/L]	DOC [mmol/L]	Average [mmol/L]
C4-B1	44.26	3.69	3.66
C4-B1	43.57	3.63	
C4-B2	27.74	2.31	2.32
C4-B2	27.92	2.33	
C4-B3	24.50	2.04	2.04
C4-B3	24.57	2.05	
C4-B4	21.06	1.76	1.75
C4-B4	20.98	1.75	
C5-B1	41.28	3.44	3.45
C5-B1	41.57	3.46	
C5-B2	39.92	3.33	3.35
C5-B2	40.49	3.37	
C5-B3	35.20	2.93	2.94
C5-B3	35.32	2.94	
C5-B4	47.34	3.95	3.96
C5-B4	47.72	3.98	
C6-B1	82.67	6.89	6.97
C6-B1	84.61	7.05	
C6-B2	49.12	4.09	4.10
C6-B2	49.36	4.11	
C6-B3	46.24	3.85	3.82
C6-B3	45.54	3.80	
C6-B4	49.36	4.11	4.05
C6-B4	47.84	3.99	

C1 represents the cycle 1, B1 the sample 1. For each sample, the lab measured the concentration twice.

Appendix 4: IC results from the CEL laboratory

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Appendix 4
IC results from the CEL laboratory

Sample		Sulfate [mg/L]	Sulfate [mmol/L]	Nitrate [mg/L]	Nitrate [mmol/L]	Nitrite [mg/L]	Nitrite [mmol/L]	NH_4^+ [mg/L]	NH_4^+ [mmol/L]
Without C amendment	C1-B1	-	-	-	-	-	-	-	-
	C1-B2	9.1	0.09	119.70	1.93	-	-	-	-
	C1-B3	9.6	0.10	115.20	1.86	-	-	-	-
	C1-B4	9.1	0.09	128.80	2.08	-	-	-	-
	C2-B1	11.5	0.12	92.40	1.49	-	-	-	-
	C2-B2	11.7	0.12	73.18	1.18	-	-	-	-
	C2-B3	10.8	0.11	80.51	1.30	-	-	-	-
	C2-B4	9.8	0.10	98.25	1.58	-	-	-	-
	C3-B1	17.5	0.18	4.97	0.08	<0.1	<0.002	7.96	0.44
	C3-B2	17.3	0.18	0.92	0.01	<0.1	<0.002	7.37	0.41
	C3-B3	15.6	0.16	<0.5	<0.01	<0.1	<0.002	7.67	0.43
	C3-B4	16.4	0.17	<0.5	<0.01	<0.1	<0.002	7.15	0.40
With C amendment	C4-B1	19.2	0.20	<0.5	<0.01	<0.1	<0.002	7.290	0.41
	C4-B2	19.2	0.20	<0.5	<0.01	<0.1	<0.002	7.03	0.39
	C4-B3	17.4	0.18	<0.5	<0.01	<0.1	<0.002	6.70	0.37
	C4-B4	18.8	0.20	<0.5	<0.01	<0.1	<0.002	7.19	0.40
	C5-B1	17.8	0.19	<0.5	<0.01	<0.1	<0.002	7.38	0.41
	C5-B2	17.0	0.18	<0.5	<0.01	<0.1	<0.002	6.75	0.38
	C5-B3	17.4	0.18	<0.5	<0.01	<0.1	<0.002	6.85	0.38
	C5-B4	16.5	0.17	<0.5	<0.01	<0.1	<0.002	7.09	0.39
	C6-B1	18.4	0.19	<0.5	<0.01	<0.1	<0.002	7.07	0.39
	C6-B2	18.5	0.19	<0.5	<0.01	<0.1	<0.002	6.70	0.37
	C6-B3	17.3	0.18	<0.5	<0.01	<0.1	<0.002	6.48	0.36
	C6-B4	19.0	0.20	<0.5	<0.01	<0.1	<0.002	6.96	0.39

C1 represents the cycle 1, B1 the sample 1.

Appendix 5: Sulfide spectrophotometer results

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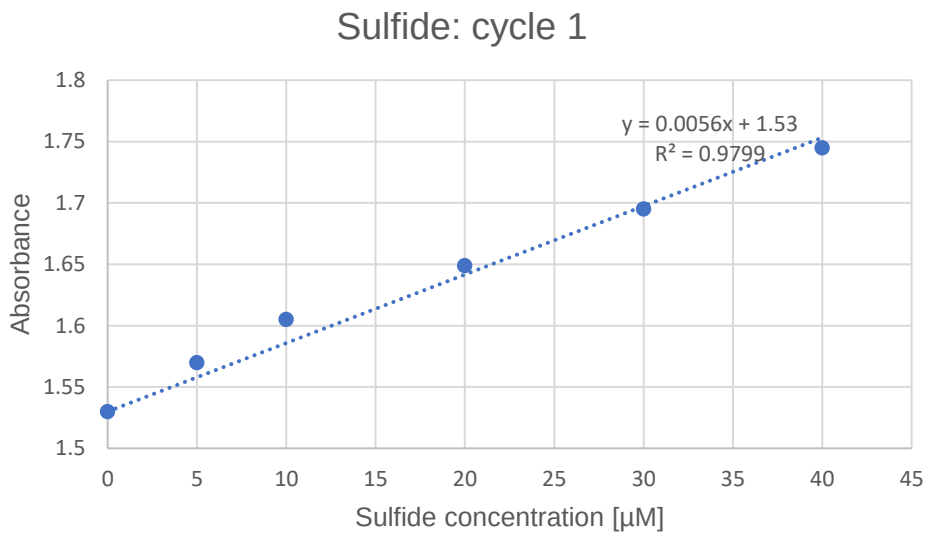
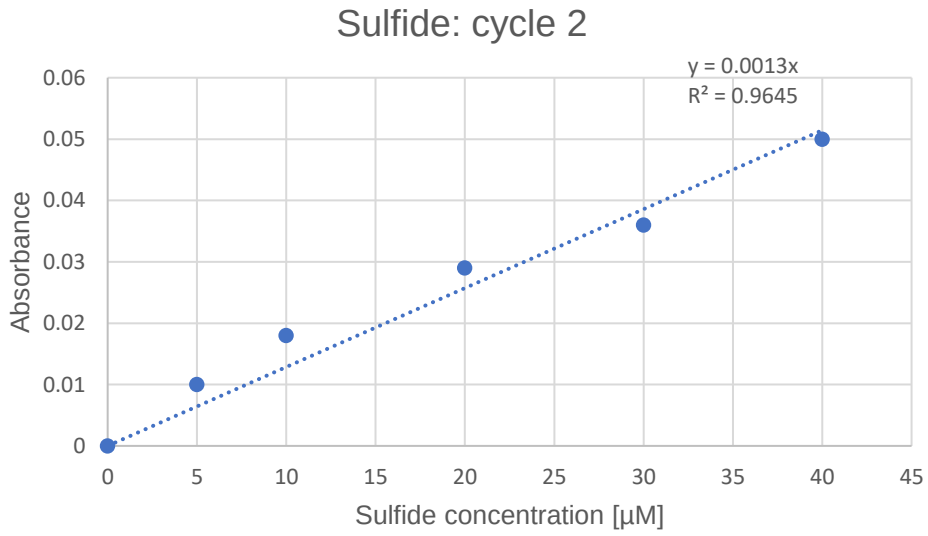
Appendix 5
Sulfide spectrophotometer results

Cycle 1		
Known concentration [µM]	Absorbance measured	Resulting concentration [µM]
0	1.53	
5	1.57	
10	1.605	
20	1.649	
30	1.695	
40	1.745	
B1	1.538	**1**
B2	1.535	**1**
B3	1.547	**3**
B4	1.535	**1**

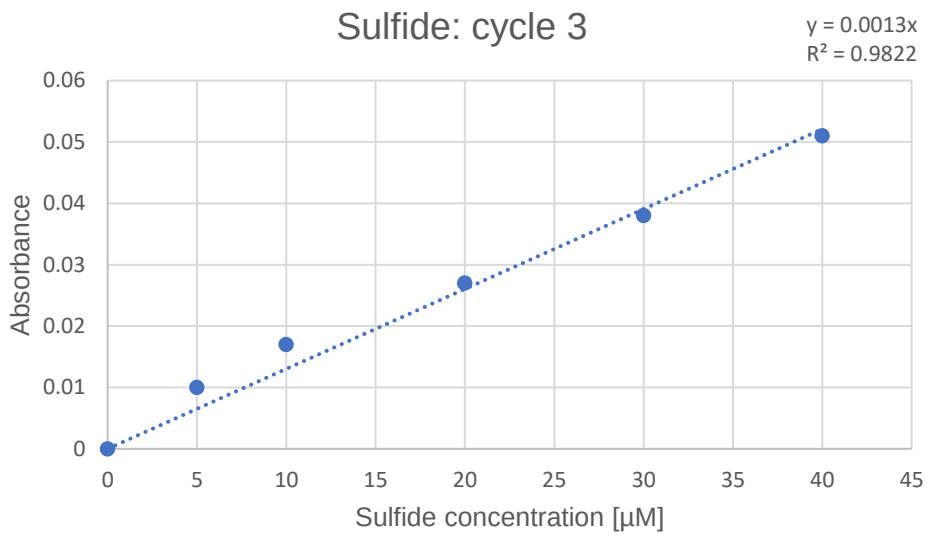


Cycle 2		
Known concentration [µM]	Absorbance measured	Resulting concentration [µM]
0	0	
5	0.01	
10	0.018	
20	0.029	
30	0.036	
40	0.05	
B1	0.003	**2**
B2	0.005	**4**
B3	0.002	**2**
B4	-0.004	**-3**

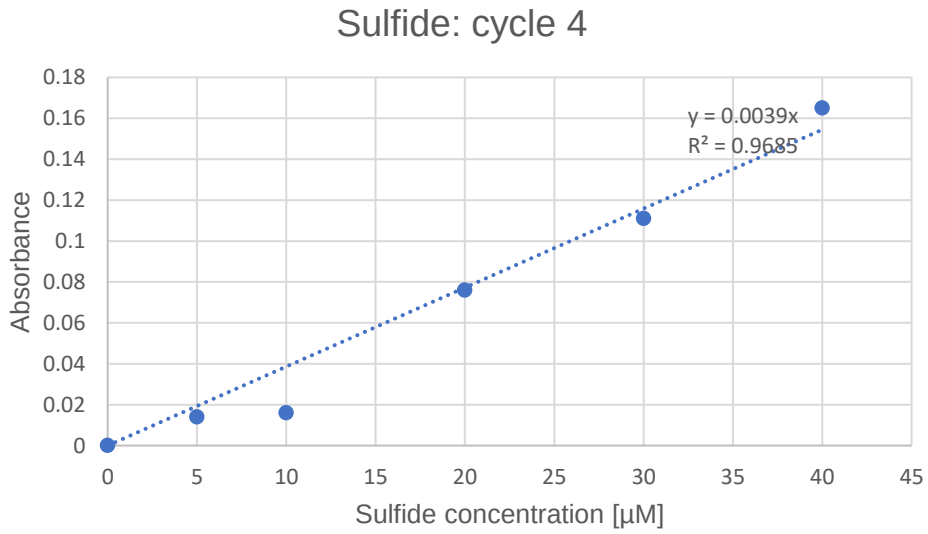


Appendix 5
Sulfide spectrophotometer results

Cycle 3		
Known concentration [µM]	**Absorbance measured**	**Resulting concentration [µM]**
0	0	
5	0.01	
10	0.017	
20	0.027	
30	0.038	
40	0.051	
B1	0.006	**5**
B2	0.003	**2**
B3	0.005	**4**
B4	0.001	**1**



Cycle 4		
Known concentration [µM]	**Absorbance measured**	**Resulting concentration [µM]**
0	0	
5	0.014	
10	0.016	
20	0.076	
30	0.111	
40	0.165	
B1	0	**0**
B2	0.076	**19**
B3	0	**0**
B4	0	**0**



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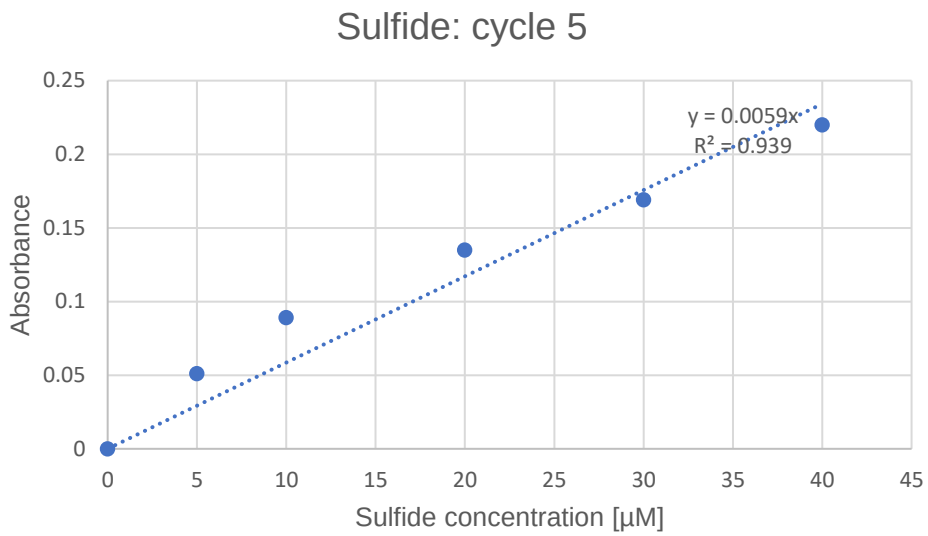
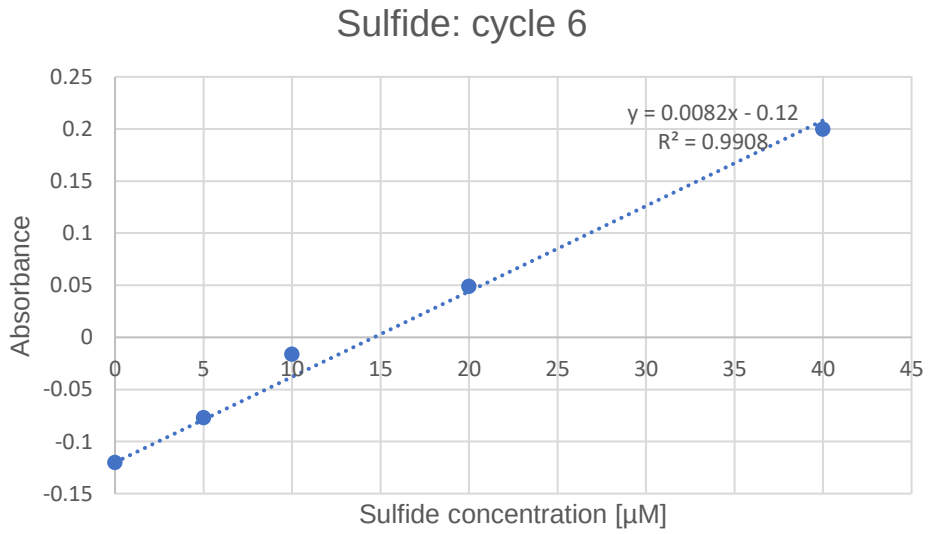
Appendix 5
Sulfide spectrophotometer results

Cycle 5		
Known concentration [µM]	**Absorbance measured**	**Resulting concentration [µM]**
0	0	
5	0.051	
10	0.089	
20	0.135	
30	0.169	
40	0.22	
B1	0.037	**6**
B2	0.08	**14**
B3	0.023	**4**
B4	0.013	**2**



Cycle 6		
Known concentration [µM]	**Absorbance measured**	**Resulting concentration [µM]**
0	-0.12	
5	-0.077	
10	-0.016	
20	0.049	
40	0.2	
B1	0.033	**19**
B2	0	**15**
B3	0	**15**
B4	-0.02	**12**



Appendix 6: Ferrous iron spectrophotometer results

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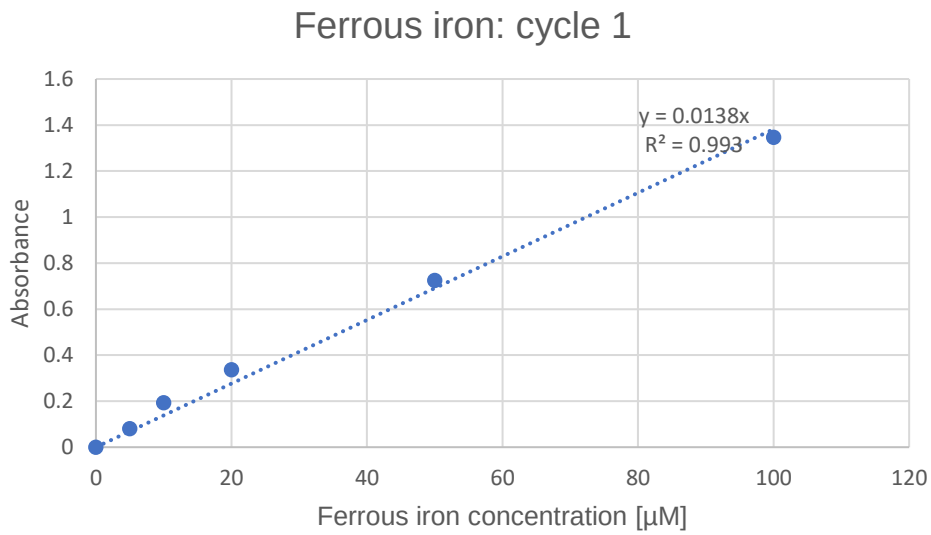
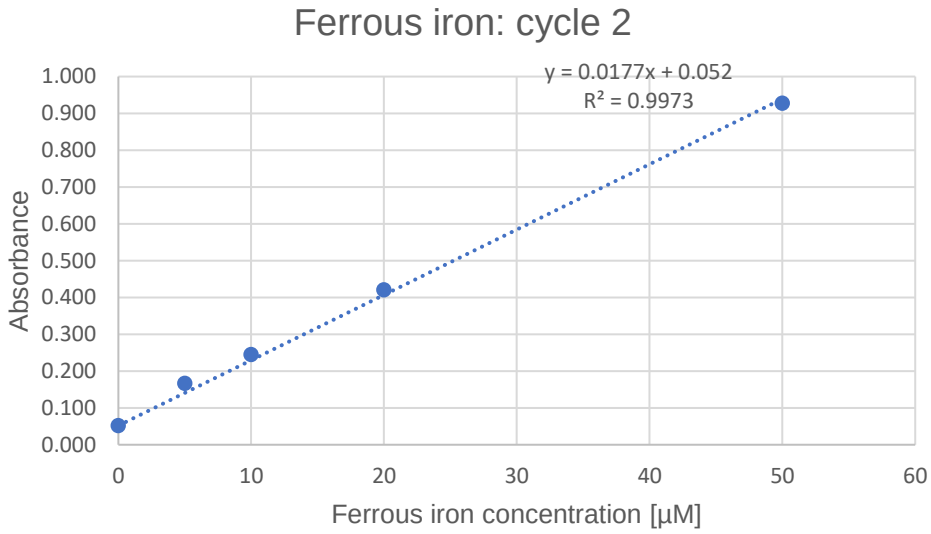
Appendix 6
Ferrous iron spectrophotometer results

Cycle 1		
Known concentration [µM]	**Absorbance measured**	**Resulting concentration [µM]**
0	0	
5	0.081	
10	0.193	
20	0.336	
50	0.725	
100	1.347	
B1	0.054	**4**
B2	0.112	**8**
B3	0.055	**4**
B4	0.058	**4**

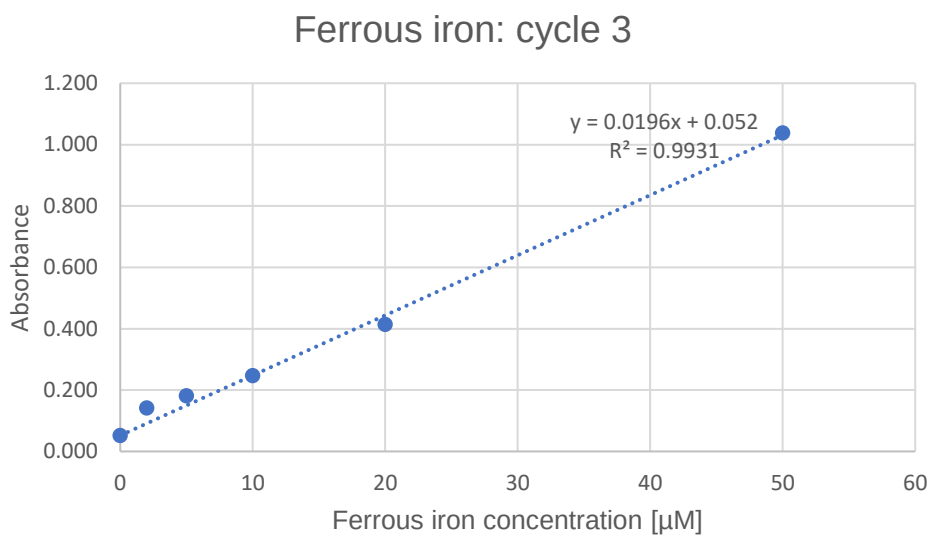


Cycle 2		
Known concentration [µM]	**Absorbance measured**	**Resulting concentration [µM]**
0	0.052	
5	0.167	
10	0.245	
20	0.421	
50	0.928	
B1	0.173	**7**
B2	0.165	**6**
B3	0.11	**3**
B4	0.07	**1**

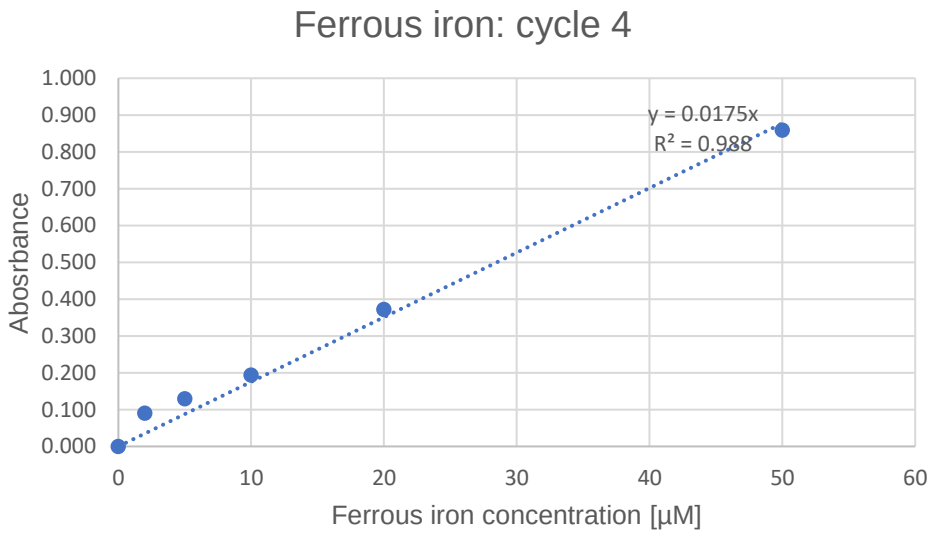


Appendix 6
Ferrous iron spectrophotometer results

Cycle 3		
Known concentration [µM]	**Absorbance measured**	**Resulting concentration [µM]**
0	0.052	
2	0.142	
5	0.181	
10	0.247	
20	0.414	
50	1.038	
B1	0.284	**12**
B2	0.234	**9**
B3	0.208	**8**
B4	0.101	**3**



Cycle 4		
Known concentration [µM]	**Absorbance measured**	**Resulting concentration [µM]**
0	0	
5	0.09	
10	0.129	
20	0.194	
30	0.372	
40	0.859	
B1	0.466	**27**
B2	0.985	**56**
B3	0.817	**47**
B4	0.341	**19**



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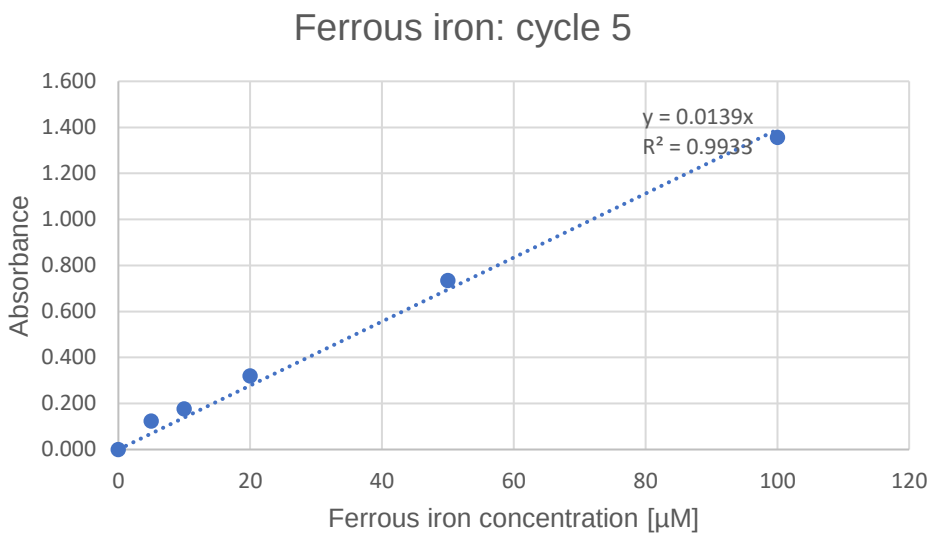
Appendix 6
Ferrous iron spectrophotometer results

Cycle 5		
Known concentration [µM]	**Absorbance measured**	**Resulting concentration [µM]**
0	0	
5	0.176	
10	0.343	
20	0.82	
50	0.734	
100	1.356	
B1	1.213	**87**
B2	1.105	**79**
B3	1.122	**81**
B4	1.61	**116**



Cycle 6		
Known concentration [µM]	**Absorbance measured**	**Resulting concentration [µM]**
0	0	
10	0.176	
20	0.343	
50	0.82	
100	1.348	
B1	1.407	**99**
B2	1.363	**96**
B3	1.377	**97**
B4	1.434	**101**