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Bio-enhanced silicate weathering

Coupled with sequestration of CO2

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Civilingenjörsprogrammet i molekylär bioteknik

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Abstract

Weathering of silicate minerals has long been a known source of natural CO2 sequestration, that could be increased in the presence of microorganisms.
Bio-enhanced weathering of silicate minerals could increase the sequestration of CO2 from the atmosphere.

The aim of this project was to evaluate the potential for a new Neutral emission technology (NET), using four different organisms, Aspergillus Niger, Knufia Petricola, Bacillus Subtilis and Cupriavidus Metallidurans and their potential to increase olivine weathering (dunite). Straw, manure and digestate was used as carbon sources. In total 9 biotic - and 9 abioitc reactors were made, containing a mixture of dunite and one of the three carbon sources. In total 250 mL of water was added to each reactor per week, for 6 weeks, and collected at the end of the week for analysis. Geochemical analyses of the leachate were performed, including pH, conductivity, alkalinity, total organic carbon (TOC), total inorganic carbon (TIC), cations, anions and three organic acids: citrate, acetate, and oxalate. Scanning emission microscope (SEM) was used to monitor potential differences pre- and post-treatment.

Straw reactors produced the most growth, both on the carbon source and the dunite grains. Likely due to the increased labile organic carbon concentrations. The total inorganic carbon and alkalinity demonstrated that inoculation of the reactors promoted weathering for all carbon sources, most significantly for the straw reactors.
This observation was evidenced by etch pits in the SEM images and higher TIC, alkalinity, and magnesium values. Microbially enhanced silicate weathering has demonstrated it could be used for the development of NETs for the sequestration of atmospheric carbon.

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Det finns ett behov av att minska våra utsläpp av växthusgaser om vi vill nå Parisavtalets mål att hålla jordens medeltemperatur under 2 ℃. Då det är väldigt svårt att helt reducera utsläppen till noll behövs det tekniker och sätt att binda in och lagra koldioxid under längre tid.

Som tur är finns det naturliga processer som reglerar kolet kretslopp och därmed mängden koldioxid som finns i atmosfären. En av dessa processer är vittring av bergarter. Många kanske associerar vittring med något negativt, så som slitage av statyer eller byggnader, men vittring behöver inte alltid vara negativt. Vittring förekommer i lite olika former men i det här sammanhanget är vi mest intresserade av något som kallas för kemisk vittring. I kemisk vittring bryts mineraler i bergarterna ner via interaktioner med olika kemiska föreningar, vilket leder till att metallföreningar frigörs från mineralen. Dessa metaller, som kan förekomma som laddade joner, kan bilda karbonatföreningar så som kalciumkarbonat som är stabila, icke-reaktiva föreningar. Karbonat, eller bikarbonat bildas från koldioxid i luften vilket innebär att bildandet av föreningar som kalciumkarbonat fungerar som ett naturligt sätt att binda in koldioxid. Denna process är som sagt relativt långsam, men det finns sätt att påskynda den. Tidigare studier har visat att vittring kan påskyndas av biologisk aktivitet hos mikroorganismer som svampar och bakterier. En tänkt anledning till detta är att mikroorganismer producerar enzymer, organiska syror och andra molekyler som antingen påskyndar frigöringen av metalljoner eller bildandet av karbonatföreningar. Om processen påskyndas skulle teoretiskt sätt mer koldioxid bindas in, vilket vore positivt.

I detta arbete testade vi detta koncept genom att fylla små reaktorer med en lättvittrad mineral (olivin) och fyra olika mikroorganismer; *Aspergillus Niger*, *Knufia Petricola*, *Bacillus Subtilis* och *Cupriavidus Metallidurans*. Till detta användes tre olika kolkällor, halm, gödsel och ”digestate” som fördelades jämt mellan alla reaktorer. Kolkällorna är viktiga att ha i reaktorerna eftersom de fungerar som en energikälla åt mikroorganismerna. Hälften av reaktorerna innehöll en blandning av de ovan nämnda organismerna och den andra halvan fungerade som bakterie – och svamplösa kontroller, för att se om det finns en skillnad mellan systemen. Totalt tilldelades alla reaktorerna 250 mL vatten över fem veckodagar. I slutet av veckan samlades lakvattnet upp, alltså vattnet som runnit igenom reaktorerna, för att sedan mätas på pH, alkalinitet, ledningsförmåga, mängd organiska syror samt hur mycket organisk/inorganiskt kol som har bildats. I slutet av experimentet visade det sig att reaktorerna som innehöll halm som kolkälla hade bättre tillväxt av mikroorganismer samt högre värden av vittring. Då karbonatföreningar bildas under vittringsprocessen kunde den uppmätta mängden av inorganiskt kol fungera som en uppskattning av vittring. En anledning till denna skillnad skulle kunna vara att halm har en större mängd labilt kol, dvs en större mängd kol som mikroorganismerna kan använda för att utvinna energi och därmed växa.

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Abbreviations

EDX	Energy dispersive X-ray
LMWOAs	Low-molecular weight organic acids
IC	Ion-Chromatography
NET	Negative emission technology
OM	Organic matter
SEM	Scanning electron microscope
TOC	Total organic carbon
TIC	Total inorganic carbon

1 Introduction

The current climate goal, set out by the Paris Agreement, is to maintain increasing global temperatures within 2 ℃ (preferably within 1.5 ℃) of pre-industrial levels (Courvoisier et al. 2018). There are many proposed strategies which seek to mitigate the rising CO_2 levels, such as the development and implementation of negative emission technologies (NETs) (Courvoisier et al. 2018). There are many kinds of NETs, this project focuses on carbon capture through biomineralization by bio-mediated silicate weathering.

Silicate weathering is a natural process that driven by both abiotic and biotic factors (Sokolova 2011). It has been suggested to have an important role in regulating global CO_2 levels throughout Earth's history (Isson et al. 2020). In the weathering process, CO_2 is converted into carbonate (CO_3^{2-}) through an interaction with the silicate material (e.g. olivine). The dissolution of olivine in the presence of CO_2 can be simply described as seen in (1) (Isson et al. 2020):

$$(1) \qquad MSiO_3 + 2CO_2 + 3H_2O \rightarrow M^{2+} + 2HCO_3^- + H_4SiO_2$$

Here M represents a cation with a double positive charge such as Ca^{2+}, Mg^{2+} or Fe^{2+} (Isson *et al.* 2020).This process can occur under both biotic and abiotic conditions. The carbonate can precipitate in the soil itself or leach into surrounding waters where it can precipitate as $CaCO_{3\,(s)}$ (Vicca *et al.* 2022). This second process is slower but represents longer-term storage of the sequestered carbon (Vicca *et al.* 2022). For every 2 moles of bicarbonate produced during olivine dissolution in the presence of CO_2 (equation 1), 1 mole of CO_2 is sequestered upon precipitation as CaCO3, as shown in reaction (2) (Vicca et al. 2022).

$$(2) \qquad Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O$$

Even though it releases one mole of CO_2 it still retains one mole within the calcium carbonate molecule, which is more stable than in the bicarbonate form. Other silicate minerals, such as basalt and basanite, sequester both moles of HCO_3^-, however their dissolution rates are orders of magnitude lower.

While this process has helped to regulate the CO_2 levels throughout earth's history, it is still not efficient enough to keep pace with current rate of CO_2 emissions, evidenced by the rising temperatures (Donnini *et al.* 2016, Trenberth 2018). Luckily, it is known that microorganisms like bacteria and fungi can affect minerals and soils in general, but have also been seen to affect and enhance the weathering of silicates (Shashank et al. 2016). They achieve this by releasing molecules like chelators (small molecules that can bind tightly to metal atoms) or

organic acids that can either directly interact with certain nutrients within the silicate mineral or locally affect the pH to enhance the natural chemical reaction. Hence, they are effectively mining for nutrients to enable survival in nutrient poor environments through the release of molecules like low-molecular weight organic acids (LMWOAs) and chelators. Fungi are especially prone at creating acidic environments around the mineral to increases the weathering rate (Shashank et al. 2016).

Carbonic anhydrase (CA) and urease are two enzymes that are thought to be important for the enhanced weathering process (Vicca et al. 2022). CA is a very efficient enzyme that is highly present in higher domains of life such as animals and plants, but is also found among microorganisms like bacteria and fungi (Xiao *et al.* 2015). It helps to catalyze the reaction between CO_2 formation into HCO_3^- (Xiao *et al.* 2015). The presence of CA in the environment may increase the rate of bicarbonate formation, and hence more CO_2 could be captured and stored (Xiao et al. 2015)**.** Urease catalyses the hydrolysis of urea to ammonia (Koçak 2020). This causes an increase in pH (due to the release of ammonium) which leads to carbonate precipitation (Vicca et al. 2022).
Low molecular weight organic acids (LMWOAs) can also have a significant effect on the weathering rate (Sokolova 2020)**.** They tend to generally be more potent in dissolving minerals than some mineral acids found at the same pH (Sokolova 2020).

Microbial communities need a source of organic carbon (OC) for survival (Egli *et al.* 1993). However, the type of organic carbon differs between different carbon sources. Some carbon sources might have long polymeric chains like cellulose, others might have shorter and more easily processed carbon molecules like sugars, meaning that the availability of the OC differs between carbon sources. The cost, longevity and accessibility are also important factors when considering the type of carbon source to be used. In this project three different carbon sources were chosen, straw, manure and digestate. All three are producible at a larger level (industrial) with low manufacturing costs, hence making them suitable for testing.

As discussed above, mineral selection can significantly impact sequestration rates. There are many important factors to consider here, such as cost of production and its environmental impact, the weathering rate, general and local abundance, and its accessibility. Olivines, such as the dunite used in this project, are often suggested for this type of application due to its well-known weathering mechanics and the generally fast weathering rate (Köhler et al. 2010)**.**

2 Background

Below follows a more in-depth description of the different important components of the project, such as the organisms used, minerals, enzymes and organic acids analysed.

2.1 Fungi and bacteria used

2.1.1 Aspergillus Niger

Aspergillus Niger is a well-established filamentous fungi that is widely used for production of different food-grade products, such as citric acid, various enzymes and secondary metabolites (Cairns *et al.* 2018). It has a long history within applied biotechnology with a readily available sequenced genome and plenty of resources on culturing and trouble shooting. It has been shown that *A. Niger* can increase the weathering rate of biotite, a silicate mineral, by forming fungal-mineral aggregates and releasing LWMOAs which interact with the mineral and causes ions such as K^+, Mg^2 and Fe^{3+} to be released (Wang W *et al.* 2016).

2.1.2 K. Petriocola

Knufia Petricola A95 is a part of the diverse group of melanised microcolonial fungi (MCF), that is known for its ability to withstand very stressful environments (Noack-Schönmann *et al.* 2014). The group has various secondary metabolites to help them increase their stress tolerance such as microsporines, melanins and carotenoids (Noack-Schönmann *et al.* 2014). Production of extracellular polymeric substances (EPS) and the formation of biofilms also helps it to live in harsh climates by giving protection to UV-light, helping to retain water and acting as a matrix to interact with other organisms (Breitenbach *et al.* 2018). This group of fungi is also known for colonising bare surfaces such as rock by forming biofilms (Breitenbach *et al.* 2018). The attachment of the biofilm plays the most significant role in olivine weathering rather than the actual composition of the biofilm itself (Gerrits *et al.* 2021).

2.1.3 Bacillus Subtilis

Bacillus Subtilis is often described as a soil-dwelling bacteria, but it can be found in many different places, both aquatic and terrestrial (Earl *et al.* 2008). Its ability to form endospores makes it more resilient to lack of nutrients in the environment (Earl *et al.* 2008). It has been reported to affecting the weathering of granite through the formation of biofilms, etch pits on the mineral surface (Song W *et al.* 2007). It has also been reported to produces chelates, which enhance dissolution through the formation of complexes with dissolved metals which are subsequently transported away, creating a localised solution-solid phase disequilibrium and increase the resilience of the biofilm(Kretschmer & Lieleg 2020) (Welch *et al.* 2002).

2.1.4 Cupriavidus Metallidurans

Cupriavidus Metallidurans CH34 is a unique bacteria that has gained some attention for its ability to resist heavy-metal stress (von Rozycki & Nies 2008). It owes this to a set of genes

found on the pMOL28 and pMOL30 plasmids and to some parts of its chromosomal genes (von Rozycki & Nies 2008). These genes code for an efflux system that actively pumps toxic metal species to the outside, where polysaccharides on the surface of the membrane can act as nucleation sites to bind the metal complexes. (Diels *et al.* 2009). The transcription of these genes is triggered by environmental stimulus, which means that they are only active under the right physiological conditions (von Rozycki & Nies 2008). It can also produce siderophores which can help the bacteria to gain a source of iron in scarce environments (Diels *et al.* 2009). *Cupriavidus Metallidurans* can form biofilms, and is a chemolithotroph – meaning it can utilise inorganic molecules within minerals for energy, enabling it to survive and thrive in environments with a high scarcity of carbon (Diels *et al.* 2009).

2.2 Silicate and carbon source(s)

2.2.1 2.2.1 Silicate minerals used

Mineral weathering is dependent both on the chemical, physical, and biological environment of the material but also directly dependent on the material itself, i.e., its composition, and size. Silicate minerals that contain high amounts of magnesium or calcium are often used in carbon sequestration studies (Krevor & Lackner 2009). Stable magnesium and calcium carbonates can be formed from the relatively easily weathered silicate minerals, increasing the solid inorganic carbon (SIC) pool (Lal *et al.* 2015). Dunite was selected as the source of olivine for this project.

2.2.2 Organic carbon sources

Three different types of carbon sources were chosen for this project, straw, manure and digestate to act as energy-sources for the bacteria and fungi. Each carbon source differed in the degree of decomposition, resulting in a range of easily available carbon. There might also be a difference in nitrogen levels, ability to store moisture, aerobic and anaerobic microenvironments and more. Importantly, each is the by-product and require little additional carbon to produce, thus are potential candidates for large-scale carbon sequestration.

The availability of carbon and nitrogen will likely affect the growth of the inoculated bacteria and fungi (Gao *et al.* 2007, Meidute *et al.* 2008). Furthermore, it is known that microorganisms can help increase the micronutrients in nutrient poor environment, meaning that potential nutrient lacking from the carbon sources could be mined from the added mineral (Calvaruso *et al.* 2006, Zhu *et al.* 2014). Bacteria and fungi play an important role in regulating the supply of nutrients to the surrounding environment, particularly in highly weathered soils (Hoffland *et al.* 2004, Uroz *et al.* 2011). For example, the temperate forests growing in acidic soils where a larger pool of nutrients bound within difficult to weather

minerals are made available to the surrounding environment by fungi and bacteria (Hoffland *et al.* 2004, Uroz *et al.* 2011).

2.3 Organic matter (OM)

2.3.1 Enzymes

Enzymes are catalysts that are efficient at speeding up a reactions including mineral weathering and degradation of organic carbon. Enzymes are applicable to the process of chemical weathering, and consists of an array of different steps depending on the reaction pathway (Sokolova 2011, Xiao *et al.* 2015). The central reaction to fixating CO_2 in the weathering process is through precipitation of metal ions in the soil by forming carbonates, see (1).

The re-precipitation is dependent on the availability of carbonate in soil around the solubilised free metal ions (Xiao *et al.* 2015). As discussed above, this reaction releases one mole of CO_2 from the two moles of CO_2 sequestered as HCO_3^-. Carbonate formation is accelerated by Carbonic Anhydrase (CA) - one of the most efficient and abundant enzymes found in nature (Vicca *et al.* 2022). It has previously been shown that an increase in CA-gene expression has had a negative correlation with CO_2 concentration and the amount of soluble Ca^{2+} in the context of wollastonite dissolution (Xiao *et al.* 2015).

Urease has been used in microbially increased carbon precipitation (MICP) applications due to its ability to produce CO_3^{2-} which can subsequently form precipitates (e.g. $CaCO_3$) (Konstantinou *et al.* 2021).

$$(4)\ CO(NH_2)_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-}$$

Urease catalyses the reaction of ammonium and bicarbonate from urea, which is an irreversible reaction (Koçak 2020). The increase in available bicarbonate can help to increase the formation of carbonate species in soil or other materials as seen below (Konstantinou *et al.* 2021)

$$(5)\ Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3$$

Urease activity is also related to an increase in local pH since the carbonate will interact with water to form bicarbonate and hydroxide ions that increase the local pH, which benefits carbonate species that usually have lower solubility at higher pH (Li M *et al.* 2013).

2.3.2 Organic acids

LMWOAs are important for the dissolution of minerals in soils and are mainly produced by plants and microorganisms (Sokolova 2020). LMWOAs can favour increased dissolution both by changing the local pH, but also from a direct interaction with the mineral (Sokolova 2020). The local pH around the mineral affects cation dissolutions (through acidification) and the complex formation (chelation) affects its solubility within the soil (Adeleke *et al.* 2017). The complexation capacity, meaning the ability of a LMWOA to form complexes with other species, can affect weathering efficiency of different minerals (Sokolova 2011). The availability of organic ligands for complexation of metal species within the minerals has been reported to outweigh the effect of pH (Sokolova 2020). This means that a soil that has a lower pH but a different composition of organic ligands, does not necessarily have a higher weathering rate (Adeleke *et al.* 2017, Sokolova 2020). It follows that the relative importance of acidification and complexation will depend on the nature of the system, e.g. carbon and mineral source.

Tricarboxylic acids are more potent than dicarboxylic acids with monocarboxylic acid being the least potential for mineral mobilisation (Adeleke *et al.* 2017). A common tricarboxylic acid that are often associated with increased weathering is citric acid. *Aspergillus Niger* has the ability to produce a large amount of citric acid given the right carbon sources (Behera 2020). Two other aliphatic LMWOAs that have a potential to affect weathering are oxalate and acetate (Sokolova 2020).

Ion chromatography will be used to measure the direct concentration of citrate, acetate, and oxalate. Other types of organic acids will not be identified.

3 Materials and methods

All culturing, and handling of microorganisms and leachate was performed under sterile conditions in a laminar flow fume hood. Most species were technical rules for biological agents (TRBA) classification 1, except *Aspergillus Niger* (TRBA 2). Appropriate personal protection, such as a face mask and gloves, were used to further ensure personal safety.

3.1 Culturing

All microorganisms discussed in the background were grown in liquid media. All Bacteria and *Aspergillus Niger* were bought from dsmz (Germany) and *Knufia Petricola* from Westerdijk Institute (Neatherlands). Bacterial cultures were grown in a nutrient broth, at 35 °C on a shaker plate. *Knufia Petricola* (grown in malt extract broth) and *Aspergillus Niger* (grown in potato dextrose broth) were left on shakers at room temperature (21 °C). A 500 mL solution of meat extract broth was prepared by mixing 1,5 g of meat extract, 2,5 g of bacto

peptone and 2,5 g of NaCl into 500 ml of MilliQ water. Likewise, 500 mg of casein hydrolysate, 10 g of malt extract and 10 g of D-(+)-glucose was mixed in 500 mL of MilliQ water for the malt extract broth. The potato extract broth was prepared by boiling 200 g of scrubbed and sliced potatoes in 500 mL of MilliQ water for 1 hour. The potato mixture was sieved using a fine mesh, 10 g of D-(+)-glucose was added and the solution was made to 500 mL with distilled water. All the above media was then autoclaved at 121 °C to ensure sterile conditions and left to cool down back to room temperature before further use.

Culturing was done in autoclaved 250 mL culturing flasks (E-flask). Roughly 100 mL of the appropriate media was transferred to the proper culturing flask in a laminar-flow hood. A pasture pipette was used to inoculate *B. subtilis* and *C. Metallidurans* from previous liquid cultures into two (separate) new ones each. Around 1/100 of the total volume was taken from the existing liquid cultures and transferred into the newly prepared growth media. A similar process was done for the two fungi, using media mentioned above. Here two 100 mL replicates were made as well, but instead of transferring liquid from previous cultures, some of the fungi were directly transferred by using a pasture pipette or inoculation loop into the new media. The newly made cultures were then left on the appropriate shaker plates until further use.

3.2 Assembly of reactors

Magenta boxes® were used to prepare 18 mini-reactors. Each Magenta box was rinsed with water and washed using a dishwasher and left to dry. Two magenta boxes were then stacked on top of each other. The top box had a small, predrilled, hole at the bottom, roughly 0,5 cm in diameter to allow water to flow through; the bottom box was completely intact. A piece of filter mesh as cut out to cover the bottom of the upper box, covering the hole. The mesh allowed added liquid to drain through the OM-mineral composite that would sit on top of the mesh. Each of the 18 reactors were then filled with 200 g of dunite (Sibelco) and 5 g of one of the three carbon sources were used to fill six of the reactors, such that there was 6 reactors replicates of each combination. All of the three carbon sources were dried in an oven at 40 °C over night before use. The fully assembled reactors with the dunite and OM were then autoclaved three times to ensure make sure that the conditions were as sterile as possible before inoculation. The small gap between the lower and upper magenta box was sealed with parafilm to reduce the risk of contamination from the external environment.

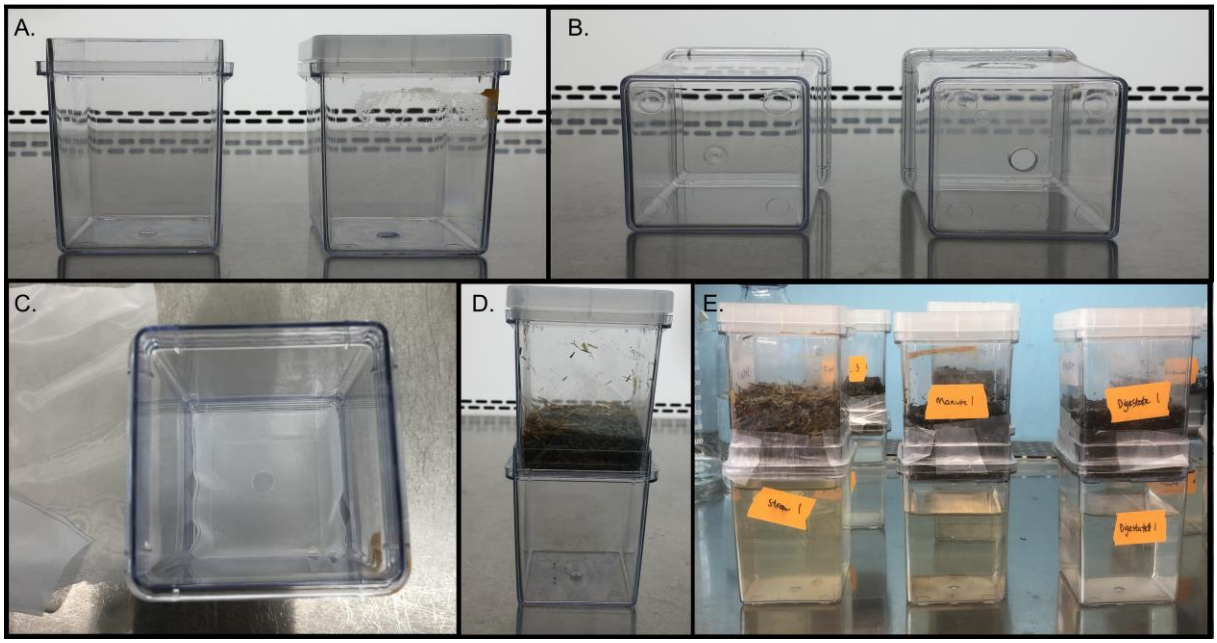

Figure 1: Reactor assembly. A, B: Two magenta boxes, left box collected the leachate (lower compartment) and the right held the mineral/carbon source media (upper compartment) C: Mesh filter overlaying hole in top compartment. D. Fully assembled reactor with dunite and either straw, digestate or manure inside. E. Labelled reactors, with specific carbon source, and parafilm wrapped the opening. Water is added from the top of the reactor, drains through the OM-mineral composite and collects at the bottom box.

3.3 Inoculation of reactors

The inoculate was prepared by using an electric mixer to homogenize all the four different organisms, and break up the fungi colonies for pipetting. Fungal cultures were filtered to remove excess liquid media. The bacterial and fungal cultures were then mixed using a tabletop kitchen grade mixer and poured into a 40 mL falcon tube. A pasture pipette was then used to equally distribute the inoculate across 9 of the reactors; 3 straw, 3 manure and 3 digestate. An equal amount of reactors were kept inoculum-free to act as abiotic controls. The remaining inoculate was saved for PLFA analysis to determine microbial biomass of the starting inoculum.

3.4 Watering, collection of leachates and filtering

Each of the reactors were watered once every day during the (weekdays) with 50 mL of distilled autoclaved water using a volumetric flask. This step of opening the reactors and re-sealing them was done in a laminar flow hood to reduce the risk of contamination from the surrounding air. The water was poured so it was equally distributed across the surface of the mineral-OM composite.

The leachate was collected weekly on the last day of watering (i.e. every Friday) once all the liquid had drained through. As the experiment progressed, it was clear that the OM-mineral composite became more compacted, and in some cases, biofilms developed over the drainage hole, resulting in a slower flow-through in some reactors. In these cases standing water mass was allowed to run through over the weekend and the leachate was collected the following Monday morning. Watering was omitted if a reactor still had a noticeable standing water mass and resumed once the previous weeks water had run through completely. Hence, the total volume of added water differed between reactors and weeks, however accurate volumes were recorded for subsequent flux calculations.

The leachate collected was stored in 500 mL Schott bottles and left at 4 °C over the weekend. Around 80 – 100 mL (or everything if the total volume was below 80 mL) of leachate was then filtered (0,2 µm) and subsequently collected in 100 mL Schott bottles. This was done using a sterile 50 mL sterile syringe (insert type). The same syringe was used for all the samples but was rinsed with milli-Q water between each sample. Filters were changed between sample types, meaning that the same filter could be used for replicates of the same type (e.g. Biotic Straw), but had to be changed when replicates of different types were to be filtered (e.g. changing from biotic straw to biotic manure). The filtered samples were then stored at 4 °C until further use. Subsamples, for ion chromatography, were frozen at -20 °C.

3.5 Alkalinity, pH, and conductivity measurements

Filtered leachate samples were measured for alkalinity, pH and conductivity measurements. The stored samples (4 °C) were first allowed to rise to room-temperature, either by resting outside the fridge on the bench-top or by being put in a 20 °C water bath for ~ 30 minutes. Around 40 mL of leachate was transferred to a separate 100 mL glass Schott bottle to be used for pH measurements, the amount used was recorded gravimetrically.

A pH-probe was calibrated using a 7,0 and a 4,0-calibration solution. Alkalinity was measured via end-point titration, meaning the initial pH was recorded and enough volume of 0,02 mol L^{-1} HCl was added using an auto-titrator, while stirring, to have the solution reach a pH of 4. The volume of added HCl was then used to back-calculate the amount of $CaCO_3$ in the sample.

A stirring bar was used to mix HCL into the solution to let the solution equilibrate before taking the pH value. The stirring bar was washed between each different sample using milliQ water.

Conductivity measurements were performed using a conductivity probe, which was first calibrated using KCl calibration solution to 1413 µs cm^{-1}. No mixing was done for these measurements.

3.6 TOC analysis

The same filtered leachate samples that were used to for conductivity measurements were used for TOC analysis, using a high sensitivity TOC analyser (Sievers M9 ® system). However, due to the high initial carbon content, most samples required dilution (to be below 40 mg L^{-1} calibration maximum). Samples from the first week were diluted by a factor 20 (1:20), second and third week by a factor 10, fourth week and biotic straw from week five and six by factor 2. The remaining samples were left undiluted. All dilutions were made using milliQ water gravimetrically. The leachate samples were either transferred directly to or diluted in 40 mL cleaned TOC bottles.

3.7 Analysis using Ion-chromatography

Ion-chromatography was used to measure the concentration of organic acids in the leachate samples. Standards (0.01 – 20 mg L^{-1}) were prepared gravimetrically, from citrate, oxalate and acetate stock solutions (1 g L^{-1}). Cation and anion standards were also prepared from Anion-multi-element standard I (Certipur ®), Anion-multi-element standard II (Certipur ®) and Multi Cation standard 1 for IC (TraceCERT ®).

Metrohm ion chromatography system 883 basic IC Plus and a 919 Autosampler Plus was used for measurements for all IC measurements. Using the Metrosep A Supp 5 (250x4.0 mm) separation column, for both citrate and anion measurements, in combination the Dionex IonPac NG1 (4x35mm) and Metrosep A supp 4/5 guard columns. Citrate and anion measurements also used the same flow rate (0,7 ml min^{-1}) and loop volume (20 µL), though different eluents were used. 4.5 mmol L^{-1} NaOH and 14.5 mmol L^{-1} Na_2CO_3 for citrate and 3.2 mmol L^{-1} Na_2CO_3 and 1.0 mM $NaHCO_3$ for anions. During the cation measurements a Metrosep C6 (250x2.0mm) separation column was used together with a Metrosep C6 guard column. Eluent used for cation was composed of 4.0 mmol L^{-1} nitric acid and 1.0 mmol L^{-1} dipicolinic acid, using a loop volume of 400 µL and a flow rate at 0,9 ml min^{-1} .

All chromatograms produced were processed and visualized using Magic IC Net 3.3. Further analysis of the data was done using MATLAB R2020b. The area under the peaks were integrated and converted to concentrations based on calibration curves. Quality control samples were used to ensure quality of calibration curves.

3.8 Scanning Electron Microscopy (SEM)

SEM was used to gather visual data of the carbon sources and the minerals before and after treatment. Any potential biofilms or other points of interested were also analysed using SEM. Before analysis biotic samples were fixed using a modified drying and fixing protocol for SEM, to maintain the structural integrity of the samples and prevent the collapse of the microbial cells.

Before the solution could be added to the samples excess water was filtered off under vacuum. This was done by placing a 0,8 µm membrane filter on the top of a stericup and vacuum flask. Fixation of the cells were made by gently removing the filters and placing a smaller section of the filter in a beaker containing roughly 4 mL of glutaraldehyde solution together with 4 mL of phosphate buffer (1 M) and 32 mL of deionised water, and left for 4 hours with parafilm placed on top. The filters were then washed by subsequently soaking them in 0,1 M phosphate solution and left to rest for 10 minutes. This step was repeated twice, followed by drying with ethanol (50 % v/v), 70%, 80%, 90%, 95% and 100% v/v for 10 min each. The 100 % v/v ethanol wash was repeated three times. After the final ethanol drying, the filters were placed in HMDS for 5 minutes and then left to air dry until the following day.

All samples were coated with palladium/gold prior to the SEM analysis. The SEM analysis were performed using a Zeiss Supra 35 VP (Carl Zeiss SMT, Oberkochen, Germany) field emission SEM. All elemental analysis were made using a EDAX Apex 4 (Ametekh, Mahwah, USA) EDS-detector for X-ray microanalysis.

4 Results

All values shown in the graphs of the result section are mean values taken from the three different biological replicates for each organic matter. For example, the pH value of biotic straw is a mean value of the measured values from all three replicates of the biotic straw reactors. Note that error bars are missing, this is because a lot of value are displayed on the same x-value (since the values are displayed as a time series) which would have made the graphs very messy to read if they had been included. P-values from a performed t-test can be found in the appendix for all the values displayed below (Table S.2). Most p-values were below 0,05 except for acetate concentrations for biotic digestate, biotic manure, abiotic digestate and phosphate concentrations for abiotic straw. However, this didn't interfere with the main conclusion (section 6) from these result that are discussed in section 5.

4.1 Geochemical and TOC analysis

The pH measurements of day 4 was the highest for all categories except for manure biotic and abiotic that had their highest point after 11 days (Figure 2. B). A general trend found was the difference in pH between the biotic reactors and their control (abiotic). The biotic reactors (regardless of carbon source) all had a higher measured pH than their corresponding control. The change of pH was also mirrored by their respective control expect for straw which displayed a different trend than its control. Most reactors displayed a drop in pH between day 4 and day 32, except for manure control and straw biotic that both saw a minor increase in pH

between day 11 and 18 (Figure 2. B). A pH increase was also seen between day 32 and day 39 (except manure biotic that kept decreasing), with a higher increase found in the biotic reactors compared to their control. The largest pH increase was observed for biotic straw that almost rose above its initial pH value (~ 7.8) between day 32 and day 39.

A similar trend to pH can be seen in alkalinity and conductivity (Figure 2. A, C). The highest point for both parameters in all reactors was after 4 days. The alkalinity produced by the biotic reactors is greater than the corresponding control throughout the experiment (Figure 2.A). Alkalinity decreased for reactors from day 4 up until day 32 and continued through day 39, except for biotic straw, biotic digestate and digestate control where there was a small increase from day 32 to day 39 (Figure 2. A). In contrast to alkalinity and pH, the control reactors produced the largest conductivity (Figure 2.) Biotic digestate and biotic straw did also produce an increase between day 32 and 39 as was observed in the alkalinity and pH measurements (Figure 2. A, B).

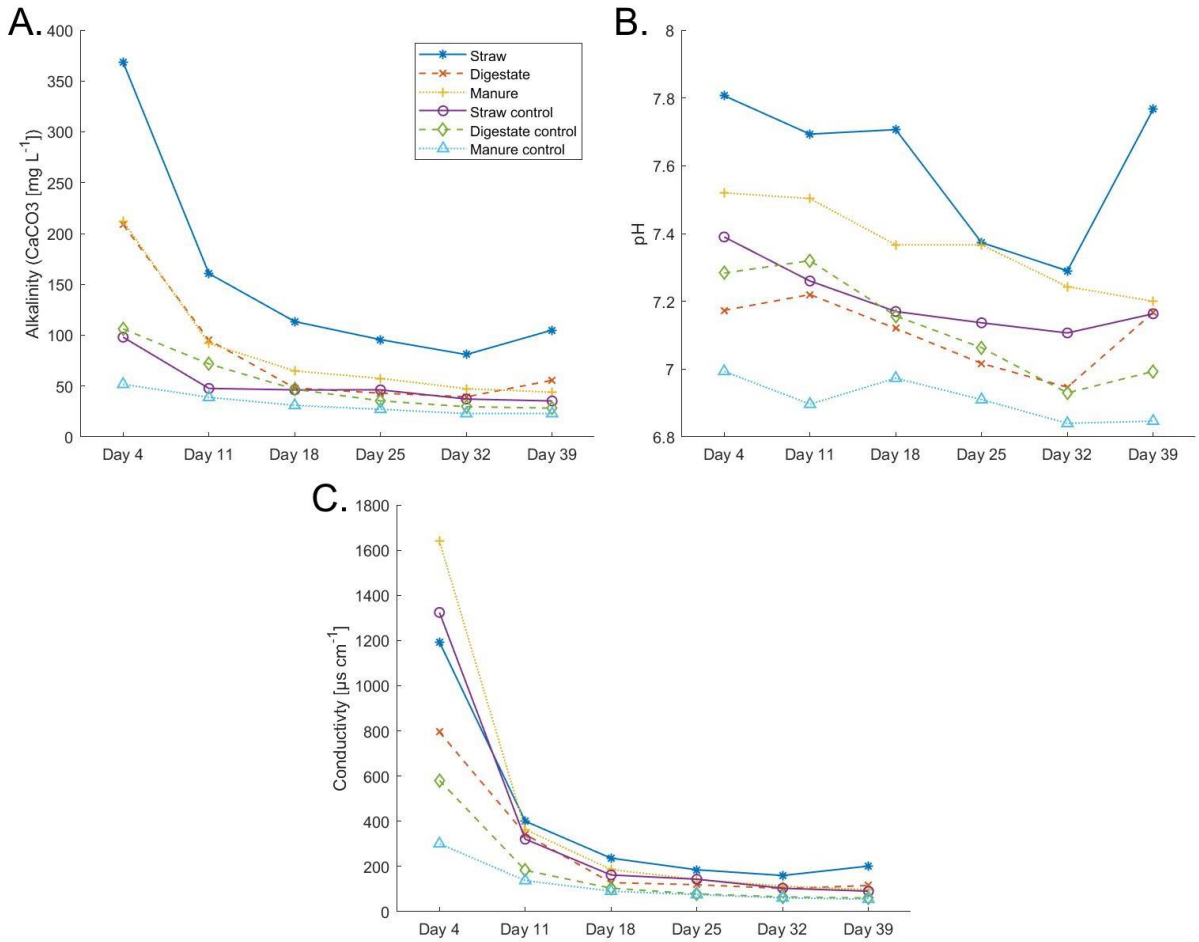

Figure 2 A: Alkalinity. B: pH. C: Conductivity. A general decrease in all three graphs is seen throughout the time series. Alkalinity is generally higher for biotic treatments as compared to abiotic treatments, were biotic straw has the highest recorded value (A). The pH is higher for biotic treatments as compared to abiotic treatment; biotic straw has

the highest value (B). Highest conductivity was measured in biotic manure, biotic and abiotic straw were more closely linked here than previous measurements (C). Overall higher levels in biotic treatments were once again seen (C).

The organic carbon (OC) concentration in the leachate had a less obvious trend, compared to alkalinity, pH and conductivity (Figure 3.). The controls produced more organic carbon than their corresponding biotic reactors, although the difference between manure and it's control was slight (Figure 3. A). A decrease in OC was seen throughout experiment, with a minor increase in both biotic straw and straw control between day 32 and day 39. The inorganic carbon (IOC) content was significantly higher for the biotic reactors after 4 days than the controls (Figure 3. B). All the controls had an IOC of roughly 10 mg C L^{-1} while the biotic reactors were more spread out. The biggest difference between control and biotic reactor observed were that between straw; with roughly 5,5 times higher IOC levels seen in biotic straw (Figure 3. B). Overall trend for both OC and IOC was a decrease in concentrations throughout the time series, with a few exceptions. A slight increase in both OC and IOC can be seen for biotic straw and straw control, which has been a trend for alkalinity, pH and conductivity as well (Figure 2, Figure 3). Between day 11 and day 18 for straw control, there was an increase of ~ 6 mg C L^{-1}. This increase seems to not have affected the alkalinity, pH or conductivity as there was no noticeable change between those days (11 and 18) (Figure 2, Figure 3. B)

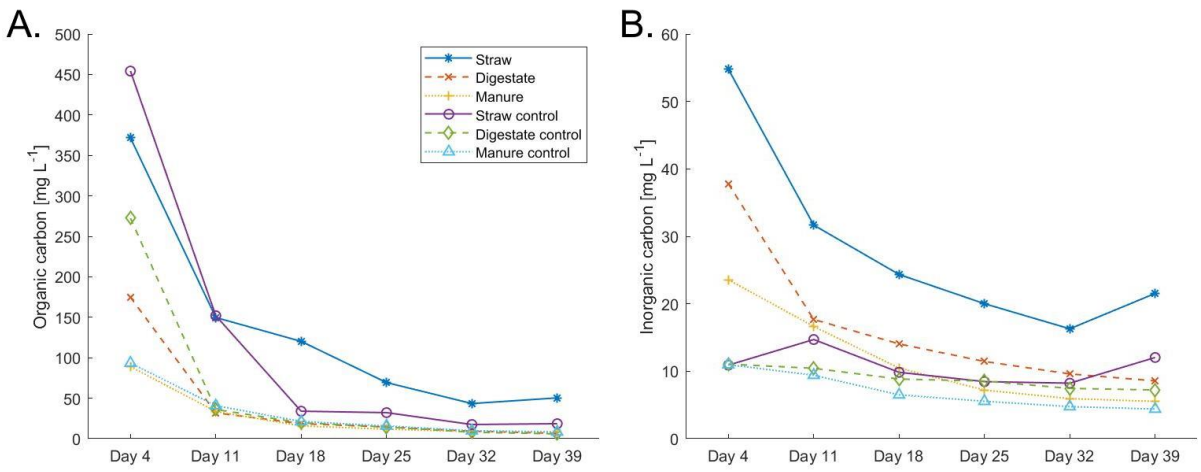

Figure 3 A: Organic carbon. B: Inorganic Carbon. Organic carbon content is higher for abiotic treatments as compared to biotic treatments (A). The biotic treatments for the different carbon sources follow a similar change in organic carbon to their abiotic control for the respective carbon source throughout the time series (A). Inorganic carbon is generally higher in the biotic treatments, largest difference seen between biotic and abiotic straw (B).

There was a clear linear correlation in alkalinity against TOC and IC (Figure 4. A,B). The alkalinity-TOC relationship is clearly defined between treatments, which seems to be split into two different groups, the biotic and abiotic treatments. In the abiotic systems alkalinity increased slowly, even as TOC increased rapidly. In comparison, the alkalinity increased at

roughly the same rate as the TOC for the biotic treatments (Figure 4. A). In contrast, TIC seems to have a greater control on alkalinity, as abiotic and biotic treatments follow a similar linear trend (Figure 4. B). Importantly, clustering of the abiotic treatments at low TIC together with the generally higher TIC and alkalinity levels for the biotic treatments suggests that the biotically treated reactors are experiencing higher weathering rates (Figure 4. B). The TIC reflects the products from weathering better than the TOC data. This demonstrates the importance of separating the two components in this sort of analysis (Figure 4. C).

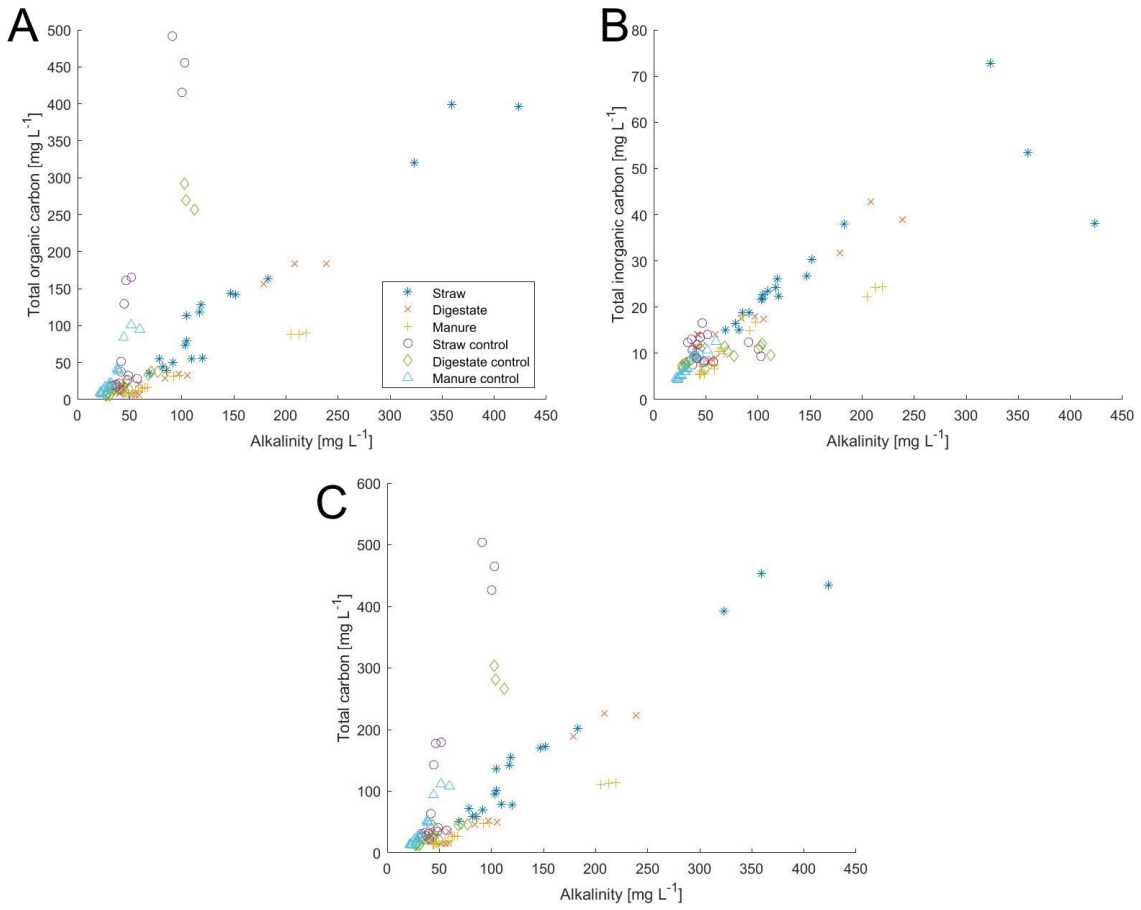

Figure 4. A: Alkalinity against total organic carbon. B: Alkalinity against total inorganic carbon. C: Alkalinity against total carbon. The biotic and abiotic treatments follow different trends, data points from the biotic treatments had a stronger correlation between total organic carbon and alkalinity as compared to data points from abiotic treatments (A). Both the biotic and abiotic treatments have a high correlation between the two parameters; biotic treatments are generally clustered at higher concentrations compared to abiotic treatments (B). The total carbon content is misleading since the high correlation observed in B disappears, hence appearing like there is no correlation between alkalinity and inorganic carbon (C).

4.2 IC data

For all reactors and treatments, citrate was generally below detection limit. Abiotic straw is the only system that gave multiple points, seen from day 4 to day 25 (Figure 5). The citrate

concentration declined over time until the concentrations were below the detection limit. There is a single data point for digestate at day 25 (< 0,1) (Figure 5).

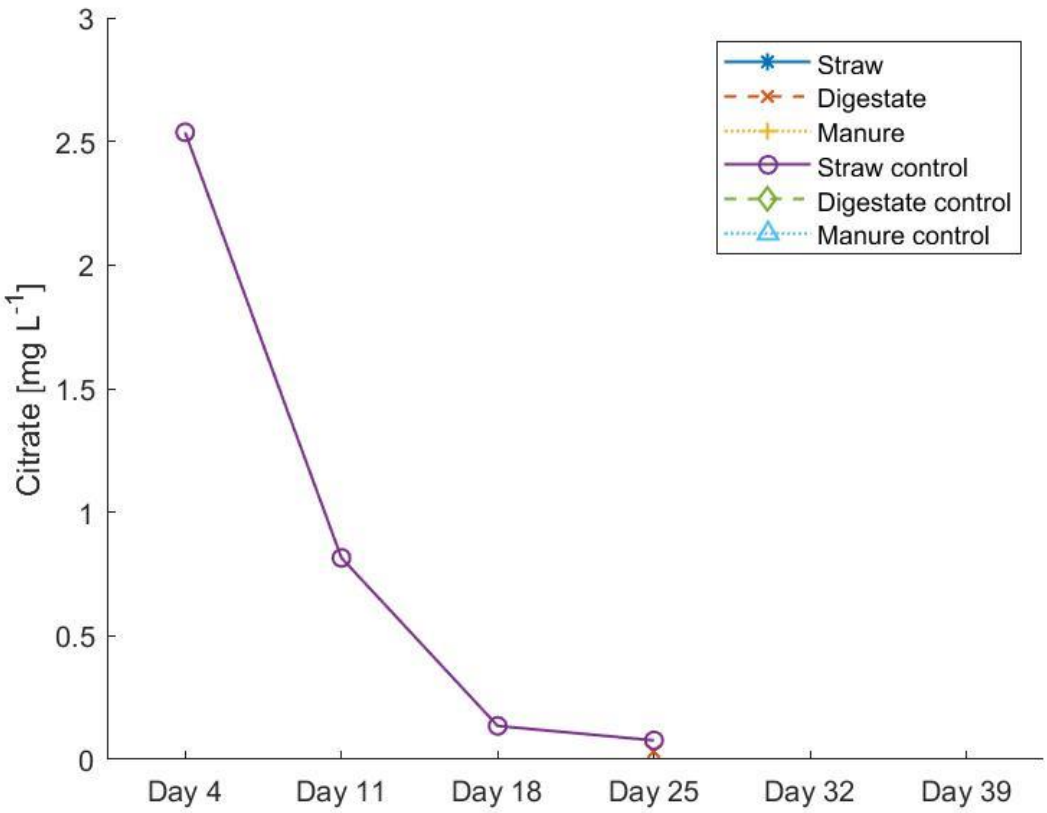

Figure 5. Change in citrate concentrations over 39 days. Abiotic straw was the only category that had any citrate concentrations above the detection limit, except for one data point observed for biotic digestate. The rest of the carbon sources, despite treatment, had citrate levels below the detection limit.

Numerous cations were measured that were deemed relevant for mineral weathering which included potassium, magnesium, and calcium (Figure 6). Looking at potassium levels in the different system shows that straw had the highest initial potassium content out of all carbon sources, both biotic and abiotic straw were roughly equal (Figure 6. A). This was also the case for biotic and abiotic manure, although these had roughly half the potassium concentrations that was seen in straw (Figure 6. A). Manure had a noticeable difference between the biotic and abiotic system for the initial measurement, but later cojoined at the next measurement (day 11) (Figure 6. A). Overall, it seems like potassium levels sank over time, all treatments and carbon sources reached very similar concentrations by day 25. Most of the concentrations stayed at the same level during day 32 and 39 except for potassium that was a bit higher on both day 32 and 39 (Figure 6. A).

Measured magnesium was initially highest in the biotic and abiotic digestate, which both had very similar concentrations (Figure 6. B). However, both dropped in concentration quite rapidly and was below biotic straw at day 18. Overall, magnesium levels dropped over time, with a noticeable rise during day 39 for both biotic and abiotic straw (Figure 6. B).

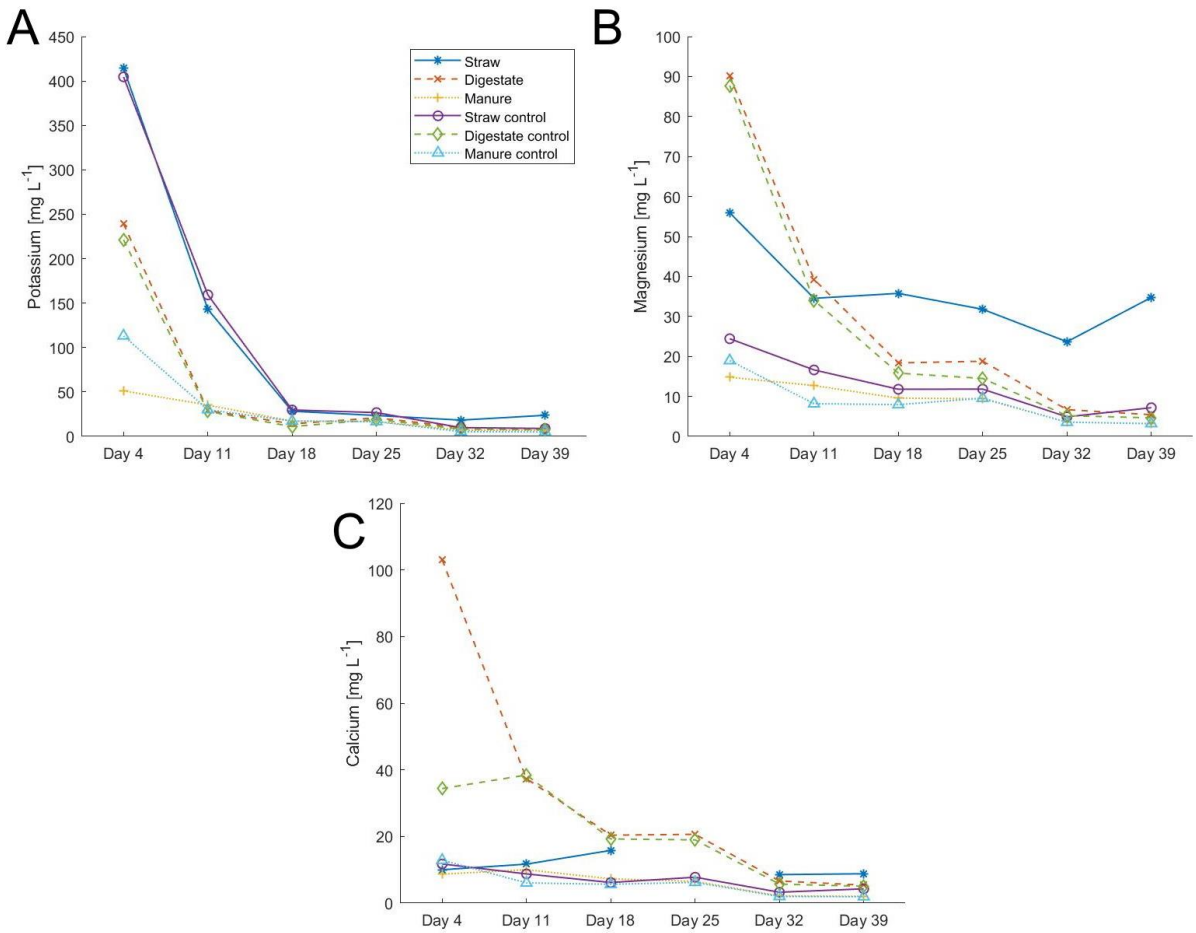

Figure 6. Change in concentration for cations, including A: Potassium, B: Magnesium, C: Calcium, over 39 days. Concentrations displayed are mean values for each carbon source, straw, digestate and manure, for both biotic and abiotic treatments. Cation concentrations below detection limit are not displayed in the graph, hence some data points appear empty. Biotic and abiotic are closely linked between all three carbon sources in A, except for manure. Magnesium levels appear to be higher in biotic straw compared to abiotic straw in B. Biotic digestate is almost 3 times higher for biotic digestate compared to abiotic digestate in C, but only for day 4. Other calcium concentrations are similar between biotic and abiotic treatment.

Initial calcium concentration was roughly the same for most systems except biotic and abiotic digestate that both had higher measured calcium levels, with biotic digestate being roughly a 10-fold higher than the other carbon sources (Figure 6. C). However, calcium concentration dropped dramatically by day 11 for biotic digestate, and was equal with the other treatments. An increase was seen between day 4 and day 11 for abiotic digestate as well for biotic straw (which lasted until day 18). Overall, the calcium concentration decreased throughout the experiment (Figure 6. C).

Acetate levels were very high for biotic and abiotic straw, with the latter being the highest (Figure 7. A, B). However, both had dropped down to a similar level as the other carbon sources by day 18. A noticeable increase for acetate for biotic straw could be seen between day 32 and 39 (Figure 7. B). Overall acetate seems to decrease with time for all systems. For a large number of samples concentration was below the detection limit, which may have been a dilution effect – similarly to citrate (Figure 7. A, B).

Oxalate levels were within a narrower interval than for acetate. The highest initial value was for biotic manure but it's difficult to trace its' development as there are only to data points available (day 4, day 32) (Figure 7. C). At day 25 the biotic straw's oxalate concentration increased from < 1 mg L^{-1} to ~ 5 mg L^{-1} by day 39. All other systems decreased throughout the experiment. A small increase for abiotic straw occurred between day 4 and day 11 but dropped below the first measurement by day 18 (Figure 7. C).

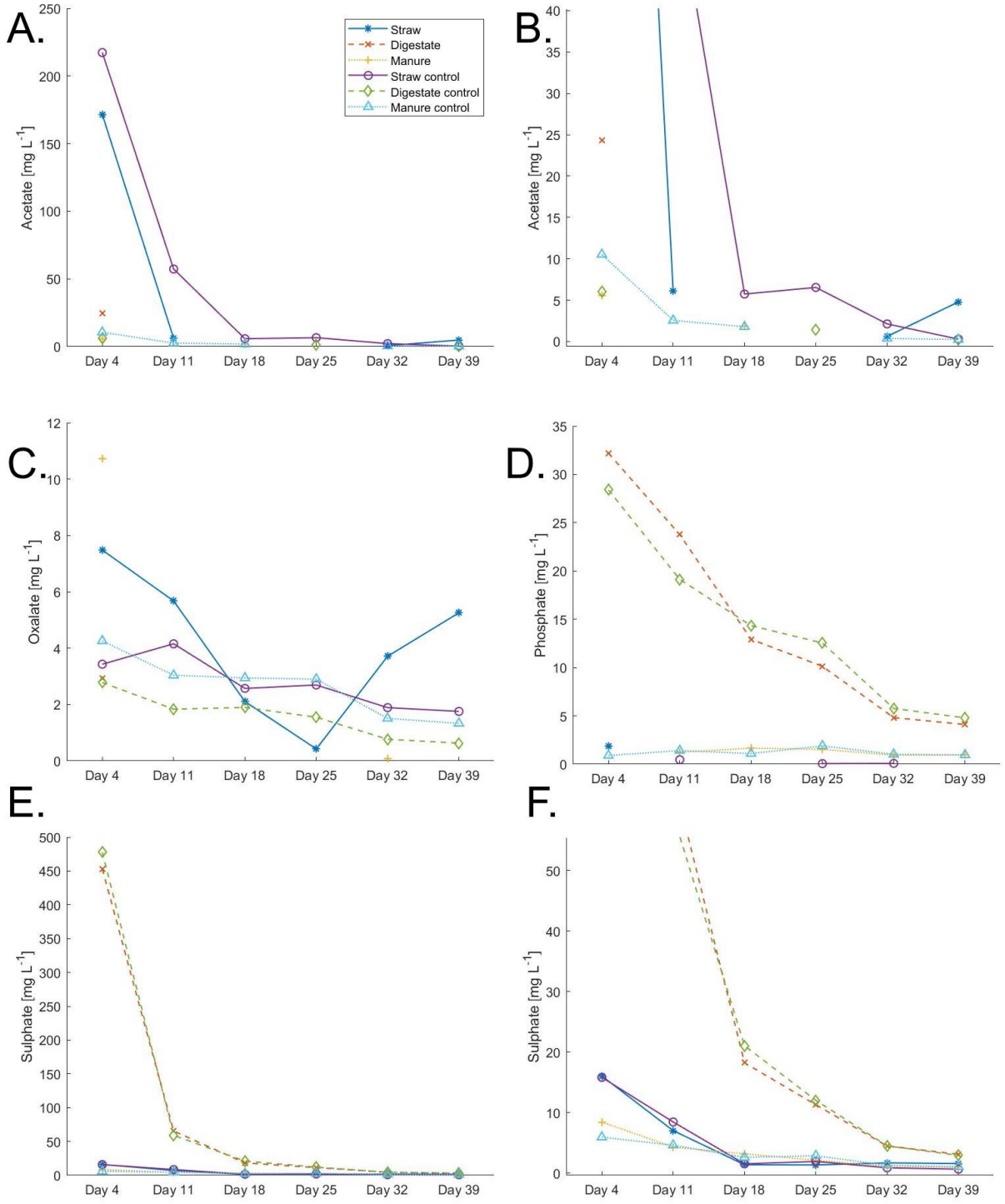

Figure 7 Change in concentrations of anions, including A: Acetate, B Acetate zoomed, C: Oxalate, D: Phosphate, E: Sulphate, F: Sulphate zoomed, over 39 days. Concentrations displayed are mean values for each carbon source, straw, digestate and manure, for both biotic and abiotic treatments. Anion concentrations below detection limit are not displayed in the graph, hence some data points appear empty. Highest acetate concentrations were observed in straw, both for biotic and abiotic treatment, as seen in A,B. Majority of biotic manure and digestate are missing hence no comparison can be made to their abiotic controls for oxalate concentrations (C). Biotic straw dips below abiotic straw during day 25 but rises above the abiotic oxalate concentration for the two remaining days (C). Digestate seems to

have the highest phosphate concentrations as seen in D. Digestate also had the highest sulphate levels, regardless of abiotic or biotic treatment (E,F).

The carbon source appeared to be the greatest control of phosphate and sulphate (Figure 4. D-F). For each carbon source the concentration and trend decreased through time, both for biotic and abiotic treatments. Phosphate levels were very similar for the different systems except for both digestates, which had around 30 times higher concentration than the other systems (Figure 7. D). Both of these decreased to roughly 5 mg L^{-1} at day 39. All the other systems kept a steady concentration at ~ 1 mg L^{-1} throughout the time series.

Initial sulphate concentrations for biotic and abiotic digestate were around 40-50 times higher than for the other carbon sources but dropped substantially by day 18 (Figure 7. E, F). At the last timepoint, both were at a similar concentration level during day 39.

4.3 SEM

Images of the mineral and carbon sources were produced pre and post treatment. Both the grains and carbon sources were checked for visible growth such as biofilm formation, hyphae or single cells attached to either the carbon sources or the dunite grains. Hyphae formation was seen directly on the surface of the biotic straw (Figure 8. A, B). No growth was observed on the straw prior to treatment which indicates that this formation is caused either by *K. Petriocola* or *A. Niger* (Figure 8. C, D). Most of the growth observed in the manure reactors were on the straw that was present in the material (Figure 8. E, F). The structure seen here looks more like a biofilm formation than hyphae, which possibly could be caused by either of the two bacteria *B. Subtills* or *C. Metallidurans*. No such growth was found on the pre-treated digestate (Figure 8. G, H).

The growth on the manure was much harder to find than digestate or straw, potentially due to the nature of the manure itself, it is very uneven with lots of different components or due to the lack of growth more generally. Hence overview of the general growth on the material was near impossible to produce, also due to the limited amount of growth found. Both hyphae formation and a strepto-like formation were found on the treated manure (Figure 8. I, J). Similarly, to the other carbon sources, no growth was found inclusion into the reactors and treatment with the combined inoculant (Figure 8. K, L). No growth or bio formations were observed in any of the control (abiotic) reactors (Figure 8. M, N, O).

Figure 8. Collection of SEM images over the three different carbon sources, straw, digestate and manure, between the biotic and abiotic treatments. A, B: Straw from Biotic straw reactor. C, D: Straw pre-treatment. E, F: Digestate from biotic digestate reactor. G, H: Digestate pre-treatment. I, J: Manure from biotic manure reactor. K, L: Manure pre-treatment. M: Straw from abiotic straw reactor. N: digestate from abiotic digestate reactor. O: Abiotic manure reactor

Dunite from the different reactors, both biotic and abiotic were also analysed using SEM. The biotic straw reactor had a large biofilm formation on one of the dunite grains as well as single celled organisms directly on the surface of the grain (Figure 9. A, B, C). The straw control had no visible growth on the dunite grains (Figure 9. D). Grains from the digestate reactors also had visible growth, that appeared to be hyphae formation rather than biofilm formation (Figure 9. F), although it is not entirely clear what surface the majority of the formation is attached to. Etch pits were also observed on grains from these reactors, indicating weathering of the mineral surface (Figure 9. E). Though the angle of the image makes it difficult to tell if there is any growth inside the pits. Digestate control had no such visible etching pits or growth (Figure 9. G, H). Grains from the treated manure had some visible growth but much smaller than the two other reactors (Figure 8. I, J). Manure control was free of any visible growth (Figure 9 K, L). The untreated dunite grains and the grains from the control reactors had a similar topology, with some smaller “dust” particles on the surface. Some of the untreated grains had what looks like some form of salt formation (Figure 9. G, H,) that was

not present in the untreated dunite grains. These were revealed to be sodium salt precipitates by EDX (data not showed).

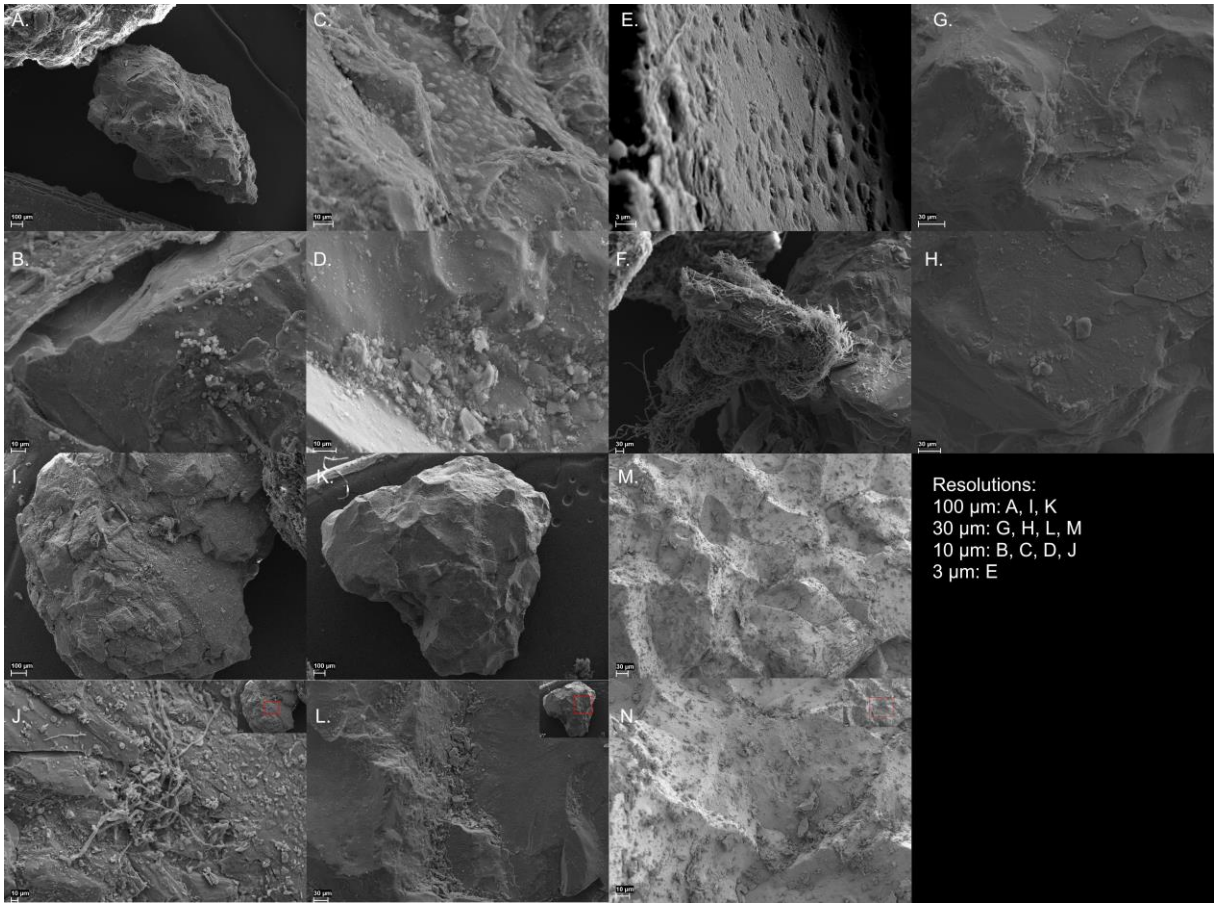

Figure 9. Dunite grains from all the different reactors. A, B, C: Biotic straw reactor. D: Abiotic Straw reactor. E, F: Biotic digestate reactor. G, H: Abiotic digestate reactor. I, J: Biotic manure reactor. K, L: Abiotic manure reactor. M, N: Dunite grains pre-treatment, meaning before being put inside the reactors.

5 Discussion

5.1 Microbial growth

The SEM pictures showed clear growth in all biotically treated reactors, with inoculant community members identifiable in the reactors (Figure 8). The extent of colonisation, biomass and biofilm formation differed significantly between carbon sources, with straw producing more biofilm formation and visible biomass (Figure 8 & Figure 9). Visual confirmation of this was given at approximately 3 weeks where there was visible growth on the surface of the straw reactors. The manure and digestate also had visible growth on the top surface inside the reactor but to a lesser extent. The SEM images and photographs suggest the

straw was the preferred carbon source, to support microbial growth and colonisation of the minerals (Figure 8 & 9).

While TIC concentrations and alkalinity demonstrated weathering was enhanced by the presence of the microbes, in all biotic reactors compared to their corresponding abiotic controls, the greater microbial growth and biofilm formation likely resulted in the increased weathering in the straw reactors (Figure 4). Quantification of microbial biomass via PLFA could further support this conclusion (data presently unavailable due to instrumental issues).

As discussed above, the straw was the preferred source of carbon, including in the digestate which contained small quantities of straw. This was visible in the SEM images, where the microbes grew most strongly around the straw in the digestate. This is likely due to the availability of labile carbon (Wang X *et al.* 2020, Numa *et al.* 2021). There could be several other factors that makes this a more viable carbon source such as nutrient content and accessibility of the surface. Digestate contains more overall micronutrients such as sulphate, phosphate, magnesium, and calcium, while straw reactors produced greater potassium concentrations (Figure 6, Figure 7) – these concentrations were likely determined by their presence in the carbon source rather than from mobilisation form mineral weathering (Table S1). This suggests that micronutrients had no apparent effect on the microbial growth, or that the controlling factors were the availability of carbon. Although assessment of the bioavailable nitrogen is necessary to confirm this conclusion (Gao *et al.* 2007, Meidute *et al.* 2008).

5.2 Weathering

The growth in the reactors was the first step in the process of inducing bio-enhanced weathering. There were clear differences between the biotic and abiotic systems. Alkalinity, conductivity, and inorganic carbon was higher in the biotic systems compared to their abiotic counterparts (Figure 2. A, B & Figure 3. B). The IC measured in the biotic systems seem to decrease at a slower pace, as evidence of the flatter curve observed in the biotic treatments compared with their abiotic controls (Figure 3. B). IC was produced during the weathering of the dunite (Meysman & Montserrat 2017), and generally decreased for all treatments through time, the inoculation of the reactors decreased the rate and magnitude of this decrease. The largest difference being between biotic and abiotic straw.

The correlation between TIC and alkalinity can be used as a proxy for weathering, since alkalinity is mostly affected by inorganic carbon (Meysman & Montserrat 2017). Longer organic carbon polymers (which we expect to find in straw) have a lesser buffer capacity per mass than shorter inorganic carbons and hence have a lesser effect on the alkalinity (Song S *et al.* 2020) This is because less OH^- exist per unit of mass than for shorter bicarbonate molecules, meaning that an equal mass of bicarbonate and larger biopolymers like cellulase would differ greatly in buffer capacity (Song S *et al.* 2020). The correlation between TOC and

alkalinity differs between the biotic and abiotic system, meaning that the two groups each follow a different trend (Figure 4. A). This demonstrates the importance of separating TOC and TIC from IC to determine the weathering products.

Furthermore, there is a significant difference between the biotic and abiotic straw concentrations of magnesium, which was not seen for the calcium concentrations for straw or any other carbon source. The different carbon sources seem to have different initial levels of magnesium (Table S1), despite the low levels of Mg in the straw the inoculated straw reactors produced high concentrations in comparison to the straw control (Figure 6. B). At the final time point (day 39) there was approximately 30 mg L^{-1} difference between biotic and abiotic straw reactors, furthermore the biotic straw concentration was approximately 40 mg L^{-1} while all the rest of the systems are at roughly 10 mg L^{-1}. Since olivine is a mineral comprised of a lot of magnesium, this suggests that Mg was mobilised from the mineral, due to weathering. Potassium and calcium do not seem to differ between biotic and abiotic systems, except for biotic digestate but this quickly drops down to the same level at around day 11 (Figure 6. C).

5.3 Carbon source and weathering

A clear difference in weathering rates could be observed between the biotic and abiotic systems but also between the different carbon sources. Straw seems to have the biggest impact on weathering out of the three different carbon sources. Straw was also the carbon source produced the most growth, visible under SEM, both on the carbon source itself and on the dunite grains. Although no overall analysis was done to assess the overall abundance in all the reactors, this indicates that straw was the most successful out of the three carbon sources.

There might be several factors as to why this is the case. It might be that straw has a higher content of bioavailable carbon, meaning a higher amount of labile carbon that can more easily be used by the microorganisms (Wang X *et al.* 2020, Numa *et al.* 2021). This might also be an effect on the composition of the microorganisms, since different microorganisms prefer different carbon sources (Kramer & Gleixner 2008). Straw also has a very low initial magnesium content (Table. S1) which would motivate weathering of the olivine to supply the microorganisms with magnesium. It could also be that straw is the least compact out of the three carbon sources, meaning that oxygen is more readily available than in the manure and digestate (Garcia-Ochoa *et al.* 2010, Li Y *et al.* 2016).

5.4 Organic acids

The overall concentrations for citrate, acetate and oxalate decreased over time, but this is difficult to say for citrate and acetate since limited data points were generated (Figure 5, Figure 7. A, B, C). Straw seems to contain a high initial amount of acetate, which quickly drops off, surprisingly this was not present in the biotic straw reactors. Citrate was only measured in abiotic straw and seems to gradually disappear. In comparison, oxalate increased

for biotic straw between day 25 and 39 (Figure 7. C). However, watering was omitted for some of these reactors during the last two weeks due to clogging, this might be an effect of less volume being applied to the system and hence a lower dilution.

It is difficult in either case to find any evidence that might support increased weathering from the LMWOAs. None of the chosen acids seem to increase throughout the experiment that could explain the weathering that is seen. There might be other organic acids that could be affecting the weathering rate that are not measured as well as chelators and enzymes that might affect the weathering rate (Köhler *et al.* 2010, Xiao *et al.* 2015, Adeleke *et al.* 2017, Sokolova 2020).

It may also be the case that the effect of the organic acids is more localised, in microenvironments around microbial colonies (Hoffland *et al.* 2004, Wild *et al.* 2021), and this was indistinguishable in the leachate due to dilution. Organic acids can effect weathering locally if they are present under a biofilm attached to the mineral surface (Adeleke *et al.* 2017, Sokolova 2020, Wild *et al.* 2021). The microenvironment could have a lower pH than the rest of the grain which would impact the weathering rate of in that area (Wild *et al.* 2021). SEM images demonstrated direct growth on the dunite surface (Figure 9. B) and the presence of LMWOAs was detected in the leachate via chromatography, suggesting there may have been an affect in the microenvironments. Similarly, the effects of chelators, siderophores and enzymes go undetected and might have an effect on the higher weathering rates seen in the biotic systems.

6 Conclusion

All the biotic reactors produced visible growth and displayed higher rates of weathering compared to their controls. This was evident through the higher TIC and alkalinity levels that occurred in the biotic systems. Even though all the biotic reactors had higher rates of weathering compared to their control, biotic straw had by far the highest (magnitude at 6 weeks) compared to both its control and the other biotic reactors. The biotic straw reactors also had the most amount of visible growth (biofilm and single cells), both on the carbon source itself and on the dunite grains. This together with higher levels of magnesium suggests that straw was the most successful out of the three carbon sources for promoting microbial weathering of the dunite.

This may be because of the higher labile carbon present in the straw reactors. Micronutrients like potassium, calcium, phosphor, or sulphates did not have had any major impacts on the growth. It could be an effect of better aeration as the straw is less compact than manure and digestate

Information gathered from this experiment would suggest that bio-enhanced silicate weathering could be a good candidate for the development of NETs to mitigate rising CO_2 levels. Providing greater capacity for both long-term and short-term storage of sequestered carbon.

6.1 Further research

Even though overall the experiment was successful there are still some aspects that could be improved upon. Enzymes and chelators which have been mentioned previously might play an important role in the weathering effects that was seen throughout the experiment. Carbonic anhydrase (CA) and urease, for example, might both have a positive effect on the weathering rate (Xiao *et al.* 2015). Chelators like siderophores would also be beneficial to measure since these could interact with the minerals directly and their general abundance in this system is unknown. Similarly other organic acids (both aliphatic and aromatic) that could have an affect on weathering (Sokolova 2020).

Furthermore, it would also be interesting to assess the general abundance of the different microorganisms in the reactor – either by PLFA, qPCR, or shotgun metagenomics. To confirm the trends visible in the SEM data.

Growing the organisms by themselves in the different carbon sources might also be beneficial since it would give insight into which role the different organisms might fill. It might be that only one of the organisms is causing the increased weathering and the rest are simply growing mostly on the carbon sources. Having control reactors with only dunite, without a carbon source, could give insight into how much the carbon source themselves affect the weathering rates.

Testing different components like basalt, concrete or even steel slag could provide insight into how a system like this could handle other minerals or waste products. There are also other types of waste materials that could be used as carbon sources, like paper pulp.

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I would like to thank my main supervisor Anna Neubeck for giving more the opportunity to work on this project and all the help I've received. It has been fun to work on a project that is somewhat outside of my comfort zone, and I feel I've learned a lot of it!

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References

Adeleke R, Nwangburuka C, Oboirien B. 2017. Origins, roles and fate of organic acids in soils: A review. South African Journal of Botany 108: 393–406.

Behera BC. 2020. Citric acid from Aspergillus niger: a comprehensive overview. Critical Reviews in Microbiology 46: 727–749.

Breitenbach R, Silbernagl D, Toepel J, Sturm H, Broughton WJ, Sassaki GL, Gorbushina AA. 2018. Corrosive extracellular polysaccharides of the rock-inhabiting model fungus Knufia petricola. Extremophiles 22: 165–175.

Cairns TC, Nai C, Meyer V. 2018. How a fungus shapes biotechnology: 100 years of Aspergillus niger research. Fungal Biology and Biotechnology 5: 13.

Calvaruso C, Turpault M-P, Frey-Klett P. 2006. Root-Associated Bacteria Contribute to Mineral Weathering and to Mineral Nutrition in Trees: a Budgeting Analysis. Applied and Environmental Microbiology 72: 1258–1266.

Diels L, Van Roy S, Taghavi S, Van Houdt R. 2009. From industrial sites to environmental applications with Cupriavidus metallidurans. Antonie van Leeuwenhoek 96: 247–258.

Donnini M, Frondini F, Probst J-L, Probst A, Cardellini C, Marchesini I, Guzzetti F. 2016. Chemical weathering and consumption of atmospheric carbon dioxide in the Alpine region. Global and Planetary Change 136: 65–81.

Earl AM, Losick R, Kolter R. 2008. Ecology and genomics of Bacillus subtilis. Trends in Microbiology 16: 269–275.

Egli T, Lendenmann U, Snozzi M. 1993. Kinetics of microbial growth with mixtures of carbon sources. Antonie van Leeuwenhoek 63: 289–298.

Gao L, Sun MH, Liu XZ, Che YS. 2007. Effects of carbon concentration and carbon to nitrogen ratio on the growth and sporulation of several biocontrol fungi. Mycological Research 111: 87–92.

Garcia-Ochoa F, Gomez E, Santos VE, Merchuk JC. 2010. Oxygen uptake rate in microbial processes: An overview. Biochemical Engineering Journal 49: 289–307.

Gerrits R, Wirth R, Schreiber A, Feldmann I, Knabe N, Schott J, Benning LG, Gorbushina AA. 2021. High-resolution imaging of fungal biofilm-induced olivine weathering. Chemical Geology 559: 119902.

Hoffland E, Kuyper TW, Wallander H, Plassard C, Gorbushina AA, Haselwandter K, Holmström S, Landeweert R, Lundström US, Rosling A, Sen R, Smits MM, van Hees PA, van Breemen N. 2004. The role of fungi in weathering. Frontiers in Ecology and the Environment 2: 258–264.

Isson TT, Planavsky NJ, Coogan LA, Stewart EM, Ague JJ, Bolton EW, Zhang S, McKenzie NR, Kump LR. 2020. Evolution of the Global Carbon Cycle and Climate Regulation on Earth. Global Biogeochemical Cycles 34: e2018GB006061.

Koçak B. 2020. IMPORTANCE OF UREASE ACTIVITY IN SOIL. 11.

Konstantinou C, Wang Y, Biscontin G, Soga K. 2021. The role of bacterial urease activity on the uniformity of carbonate precipitation profiles of bio-treated coarse sand specimens. Scientific Reports 11: 6161.

Kramer C, Gleixner G. 2008. Soil organic matter in soil depth profiles: Distinct carbon preferences of microbial groups during carbon transformation. Soil Biology and Biochemistry 40: 425–433.

Kretschmer M, Lieleg O. 2020. Chelate chemistry governs ion-specific stiffening of Bacillus subtilis B-1 and Azotobacter vinelandii biofilms. Biomaterials Science 8: 1923–1933.

Krevor SC, Lackner KS. 2009. Enhancing process kinetics for mineral carbon sequestration. Energy Procedia 1: 4867–4871.

Köhler P, Hartmann J, Wolf-Gladrow DA. 2010. Geoengineering potential of artificially enhanced silicate weathering of olivine. Proceedings of the National Academy of Sciences 107: 20228–20233.

Lal R, Negassa W, Lorenz K. 2015. Carbon sequestration in soil. Current Opinion in Environmental Sustainability 15: 79–86.

Li M, Cheng X, Guo H. 2013. Heavy metal removal by biomineralization of urease producing bacteria isolated from soil. International Biodeterioration & Biodegradation 76: 81–85.

Li Y, Niu W, Wang J, Liu L, Zhang M, Xu J. 2016. Effects of Artificial Soil Aeration Volume and Frequency on Soil Enzyme Activity and Microbial Abundance when Cultivating Greenhouse Tomato. Soil Science Society of America Journal 80: 1208–1221.

Meidute S, Demoling F, Bååth E. 2008. Antagonistic and synergistic effects of fungal and bacterial growth in soil after adding different carbon and nitrogen sources. Soil Biology and Biochemistry 40: 2334–2343.

Meysman FJR, Montserrat F. 2017. Negative CO2 emissions via enhanced silicate weathering in coastal environments. Biology Letters 13: 20160905.

Noack-Schönmann S, Bus T, Banasiak R, Knabe N, Broughton WJ, Den Dulk-Ras H, Hooykaas PJ, Gorbushina AA. 2014. Genetic transformation of Knufia petricola A95 - a model organism for biofilm-material interactions. AMB Express 4: 80.

Numa KB, Robinson JM, Arcus VL, Schipper LA. 2021. Separating the temperature response of soil respiration derived from soil organic matter and added labile carbon compounds. Geoderma 400: 115128.

Sokolova TA. 2011. The role of soil biota in the weathering of minerals: A review of literature. Eurasian Soil Science 44: 56–72.

Sokolova TA. 2020. Low-Molecular-Weight Organic Acids in Soils: Sources, Composition, Concentrations, and Functions: A Review. Eurasian Soil Science 53: 580–594.

Song S, Wang ZA, Gonneea Eagle M, Kroeger KD, Chu SN, Li D, Liang H. 2020. An important biogeochemical link between organic and inorganic carbon cycling: Effects of organic alkalinity on carbonate chemistry in coastal waters influenced by intertidal salt marshes. Geochimica et Cosmochimica Acta 275: 123139.

Song W, Ogawa N, Oguchi CT, Hatta T, Matsukura Y. 2007. Effect of Bacillus subtilis on granite weathering: A laboratory experiment. CATENA 70: 275–281.

Trenberth KE. 2018. Climate change caused by human activities is happening and it already has major consequences. Journal of Energy & Natural Resources Law 36: 463–481.

Uroz S, Oger P, Lepleux C, Collignon C, Frey-Klett P, Turpault M-P. 2011. Bacterial weathering and its contribution to nutrient cycling in temperate forest ecosystems. Research in Microbiology 162: 820–831.

Vicca S, Goll DS, Hagens M, Hartmann J, Janssens IA, Neubeck A, Peñuelas J, Poblador S, Rijnders J, Sardans J, Struyf E, Swoboda P, van Groenigen JW, Vienne A, Verbruggen E. 2022. Is the climate change mitigation effect of enhanced silicate weathering governed by biological processes? Global Change Biology 28: 711–726.

von Rozycki T, Nies DH. 2008. Cupriavidus metallidurans: evolution of a metal-resistant bacterium. Antonie van Leeuwenhoek 96: 115.

Wang W, Sun J, Dong C, Lian B. 2016. Biotite weathering by Aspergillus niger and its potential utilisation. Journal of Soils and Sediments 16: 1901–1910.

Wang X, Zhang W, Zhou F, Liu Y, He H, Zhang X. 2020. Distinct regulation of microbial processes in the immobilization of labile carbon in different soils. Soil Biology and Biochemistry 142: 107723.

Welch SA, Taunton AE, Banfield JF. 2002. Effect of Microorganisms and Microbial Metabolites on Apatite Dissolution. Geomicrobiology Journal 19: 343–367.

Wild B, Imfeld G, Daval D. 2021. Direct measurement of fungal contribution to silicate weathering rates in soil. Geology 49: 1055–1058.

Xiao L, Lian B, Hao J, Liu C, Wang S. 2015. Effect of carbonic anhydrase on silicate weathering and carbonate formation at present day CO2 concentrations compared to primordial values. Scientific Reports 5: 7733.

Zhu Y, Duan G, Chen B, Peng X, Chen Z, Sun G. 2014. Mineral weathering and element cycling in soil-microorganism-plant system. Science China Earth Sciences 57: 888–896.

Appendix

Table S1: Contents of Straw and digestate

Sample	*Ca [mg/g]*	*Fe [mg/g]*	*K [mg/g]*	*Mg [mg/g]*	*Zn [mg/g]*	*N-NH4 [mg/g]*	*P-PO4 [mg/g]*
Straw	3.15	0.13	8.70	0.45	0.00	5.37	0.50
Co-digestate	17.10	1.22	3.29	1.47	0.18	11.85	5.67

Table S2: P-values from student test performed on Alkalinity, pH, conductivity, TOC, TIC, citrate, potassium, calcium, acetate, oxalate, phosphate, and sulphate values. Values missing means that not enough data points were available to perform the test (n > 1). The p-value is rounded to 4 decimal places, meaning that a value of 0 means that the value is beyond 4 decimal places.

Parameter	Biotic Straw	Bioitc digestate	Biotic manure	Abiotic Straw	Abiotic digestate	Abiotic manure
Alkalinity	0	0	0	0	0	0
pH	0	0	0	0	0	0
Conductivity	0,0004	0,0004	0,0056	0,0037	0,0009	0
Total organic carbon	0,0001	0,0094	0,0009	0,0068	0,0206	0,0004
Total inorganic carbon	0	0	0	0	0	0
Citrate				0,023		
Potassium	0,0084	0,018	0	0,007	0,0343	0,0173
Calcium	0	0,0015	0	0	0,0006	0,0001
Acetate	0,0772	0,1385	0,2957	0,0216	0,1083	0,0807
Oxalate	0	0,002	0,0872	0	0	0
Phosphate	0,0376	0	0	0,2671	0	0
Sulphate	0,0023	0,0316	0,0001	0,0025	0,0341	0