Enhancing the bioreceptivity of ceramic RainReefs

Minor research project
Urban Reef
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Dave Matser
6215165

Supervisor:
Dr. Pierre Oskam

Examiner:
Dr. Jaco Appelman

Utrecht University

URBAN REEF

Contents

Abstract

The company Urban Reef designs 3D-printed ceramic reef-like structures that can be placed in urban areas and provides habitat for different species, thereby enhancing biodiversity and ecosystem services. The goal of the company is to increase the bioreceptivity of these reefs to promote the growth of algae, mosses, and other organisms. To do so we first conducted a field study to explore the natural succession of the reefs and the impact of temperature and humidity on this succession. Results show that a higher humidity results in an increase in algae biofilm. There was no correlation between temperature and algae growth. After a year, moss growth was still not visible on the outdoor reefs. To enhance the bioreceptivity of mosses, we studied the use of supplemental layers, buttermilk, vegan yogurt (Alpro), and beer. The buttermilk and Alpro showed promising results, but more research is necessary to draw clear conclusions. Additionally, we used an algae biofilm layer to enhance the natural succession. This proved to be a promising method with signs of moss growth within 5 weeks. The information from this study can be used by Urban Reef to improve production methods and accelerate the process of natural colonialization onto the reefs.

Layman's summary

Due to an increase in urbanization, cities are becoming bigger and more compact. Nature is replaced by concrete buildings, thereby causing biodiversity loss and habitat fragmentation. This is a bad development because nature provides us with ecosystem services, which we are ultimately dependent on. For example, these ecosystem services can help with the reduction of greenhouse gases, absorption and filtration of water, reduction of noise, and lowering of temperature. Therefore, it is important that we re-introduce nature into the urban areas.
Urban Reef is a company that aims to create nature-inclusive cities with the design of reef-like structures that can be placed in urban areas to provide shelter and habitat for different kinds of organisms. The reefs are made from 3D-printed ceramics and are designed to optimize bioreceptivity. Bioreceptivity means that a material can provide a habitat for living organisms. The primary organisms that colonize the reefs can create new conditions for other organisms to settle, this is called succession.
The goal of this study is to enhance the bioreceptivity and natural succession of the reefs and thereby accelerate moss growth. We did this by first performing a field study where natural succession is analyzed and the effect of temperature and humidity is studied. The results show that high humidity has a positive effect on natural growth. The temperature does not show any correlation with the growth. Secondly, we studied the effect of a supplemental layer of buttermilk, vegan yogurt (Alpro), and beer. Buttermilk and Alpro seem to have had a positive effect on the growth, but this needs some further investigation. Finally, we studied the effect of the addition of an algae layer on the growth of mosses. This showed to have a positive effect where mosses started to grow within five weeks and therefore can be recommended to be used in the future by Urban Reef.

1. Introduction

This study is performed on behalf of the company Urban Reef and is part of the minor research project of the master Bio-Inspired Innovation. Urban Reef is a start-up company that aims to create bio-inspired reef-like structures that can be used in urban environments to create green and living facades. The reefs will create a habitat for different organisms to thrive, thereby increasing the biodiversity of a city and providing ecosystem services to make the city more climate adaptive. This is important since due to climate change, cities are more prone to natural disasters and need to become more climate adaptive.

The 3D-printed reefs are made with fired ceramics and, for now, the reefs are placed at the end of a downspout so water can be collected inside the reefs. The reefs are therefore called RainReefs. The porous ceramics will absorb a part of the water, creating a moist environment on the outside. The goal of the company is to optimize the bioreceptive properties, to enhance the colonization of microorganisms, algae, lichens, and bryophytes on the reefs. The design is still in progress and eventually, more surface roughness will be added to the design. In this study, we will not be able to change these intrinsic properties, and therefore we will only be focusing on the extrinsic factors that can enhance bioreceptivity.

The two main questions of the company are:

1. What is the optimal microclimate on the reef (temperature and humidity) for algae and mosses to settle and grow?
2. How can we enhance/accelerate the settlement of organisms with the use of a specific nutrient layer or biofilm?

The next chapter will give some background information on the importance of green facades in cities and the concept of bioreceptivity. Then, the experimental setup and results are explained. In chapter five the results are discussed, and we finish with recommendations for the company in chapter six.

2. Background

Currently, we are living in an era called the Anthropocene, where humans have a dominant influence on climate and ecosystems. One of the manifestations of this era is the spreading, expanding, and densification of urban areas (Apfelbeck *et al.,* 2020). By 2030, more than 60% of the world's population is expected to live in cities (Figure 1) (Bolund & hunhammar, 1999). This current form of urbanization negatively influences the climate, the native ecosystems and it creates a separation between people and nature.

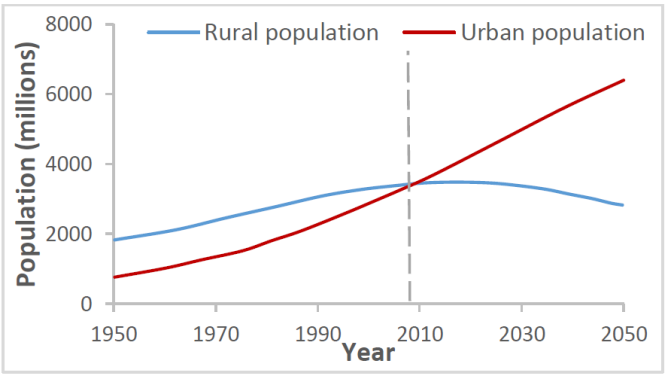

Figure 1. The (expected) urban and rural populations from 1950 to 2050 (adapted from united nations 2009).

2.1 Urbanization

The process of urbanization does not only warm the globe through CO_2 emitting activities, but it also heats the local climate and alters the weather conditions. Urban areas replace the rural landscape with stony materials that seal the soil and heat the environment. The sealed soil inhibits the absorption of rainwater, creating more floodings and reducing the land's natural ability to cool (Hamstead, 2021). In addition, since natural vegetation, which has a large effect on the regulation of the microclimate by evapotranspiration, is now replaced by concrete, the air temperature increases and the humidity decreases (Zoulia, Santamouris and Dimoudi, 2007). Concrete also has a lower albedo than vegetation, causing cities to absorb 80-85% of the incoming solar radiation. This, in combination with industrial and transportation waste heat emissions, causes heat accumulation and less cooling effect at night in urban areas compared to the surrounding landscape. This phenomenon is called the Urban Heat Island (UHI) effect (Figure 2) (Wong *et al.,* 2010).

The heat created by these urban areas creates more fuel for heat waves and droughts. Furthermore, due to the high amount of concrete and stone, cities are prone to flooding. Considering the current climate change, the occurrence and impact of such disasters are increasing, making the cities particularly risky places to live (Iwaniec *et al.,* 2021).

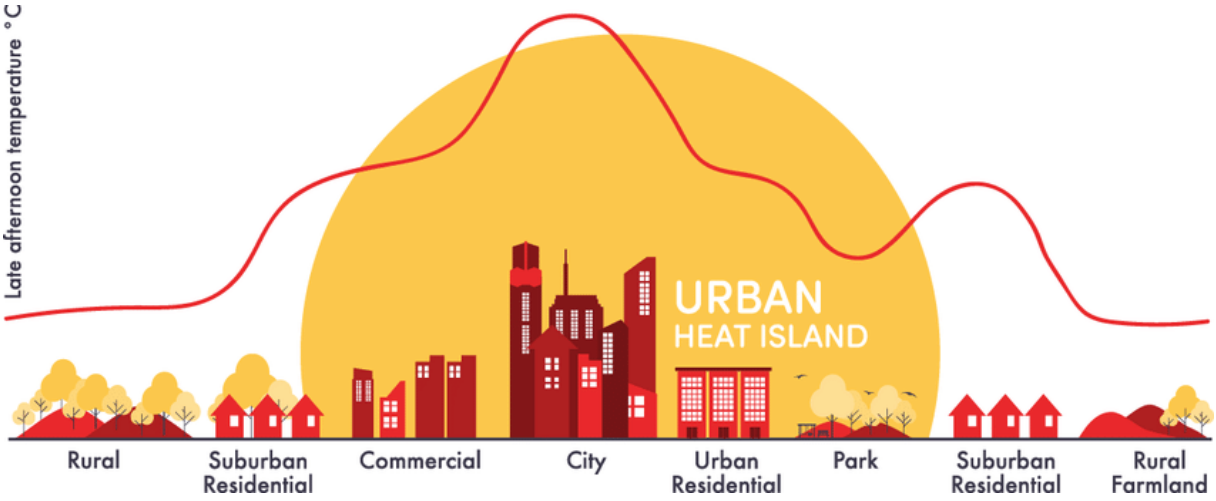

Figure 2. Visualization of the urban heat island effect with the variation of the late afternoon temperature from rural to urban areas. Source: World Meteorological Organization.

Another problem of urbanization is the causation of habitat loss and fragmentation. Among other forms of habitat loss caused by humans, urban development causes some of the greatest extinction rates and eliminates many native species. Furthermore, urbanization is lasting longer than other types of habitat loss, preventing stages of recovery and succession (McKinney, 2002). Although urban environments can provide habitats for a variety of species, a less diverse animal community is found compared to the natural environment. The animals that do live in cities are under anthropogenic stresses that differ from the stresses in their natural environments. This causes adaptations in their behavior causing increasing numbers of wildlife-human conflicts. Therefore, an increase in biodiversity is not only good for a healthy ecosystem, but it can have direct effects on human well-being and health (Apfelbeck *et al.,* 2020).

Finally, urbanization causes a separation between people and nature. Since most of the human population is living in urban rather than rural environments, there is a decline in experiences with nature. This causes a reduced knowledge of and support for environmental problems. Furthermore, living in the city is also associated with conditions such as obesity, stress, poor mental health, and a decline in physical activity, since well-being is associated with exposure to nature (Cox *et al.,* 2018). Studies have shown that visiting a garden will lower blood pressure and help with recovery from stress (Ulrich *et al.,* 1991).

2.2 Climate adaptive cities

Although people experience a greater gap between nature and their local environment, we are still ultimately dependent on it. Nature provides us with benefits, called ecosystem services (ES), that can contribute to climate adaptivity, public health, and quality of life. The ES are divided into four categories (Carpenter, Bennett & Peterson, 2006): (1) Supportive services; like nutrient cycling and soil building, are necessary to produce the other ecosystem services, (2) Regulating services; for example, climate regulation or pollination, (3) Provisioning services; providing products like food and fresh water and, (4) Cultural Services; for recreation, relaxing and well-being (Figure 3) (Ni, 2016).

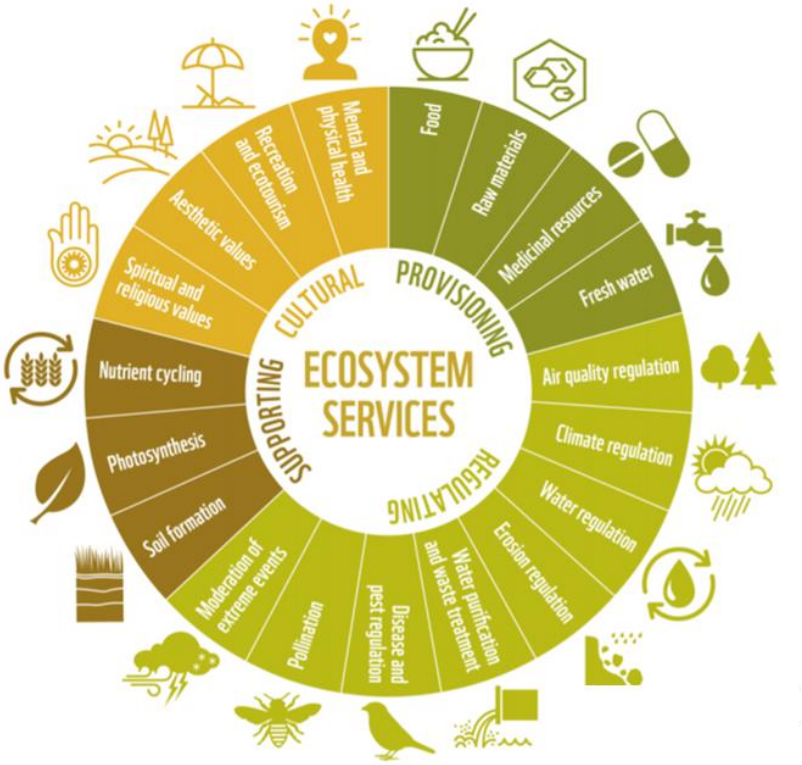

Figure 3. visualization of the four different ecosystem services and their examples (Ni, 2016).

These ES can for example help with the reduction of greenhouse gases, production of oxygen, filtration of water and air, absorption of water, reduction of noise, and lowering of temperature (bolund & hunhammar, 1999). The incorporation and preservation of urban biodiversity can sustain the ES and therefore help to make cities more climate adaptive and can help to increase the health and quality of life (Apfelbeck *et al.,* 2020).

The most important method to increase ES in cities is by making cities greener. This can be done by preserving the natural habitat instead of removing natural vegetation during construction. The native animal biodiversity, that is present around the city, can be increased within the city with the revegetation of native plant species and to allow natural ecological succession. This will not only enhance diversity but also help to reduce the diversity of nonnative species, that are for example present in gardens (McKinney, 2002).
Furthermore, new green areas should be introduced. Since there is a limited amount of space in urban areas, the current focus is on the greening of the walls and roofs of buildings. The greening of the façades is called a vertical greening system (VGS), where a climber plant is directly attached to the wall (Wong *et al.,* 2010) or a living wall system, where the wall itself contains a water and nutrients layer for the plants to grow on (Köhler & Manfred, 2008). The VGS has already been implemented a lot, nevertheless, it has shown very high maintenance efforts and costs. The living wall system, on the other hand, is a relatively new idea that can be further explored in the building industry (Cruz & Richard, 2016).

2.3 Bioreceptivity

A living wall system is based on the bioreceptivity of the building materials. The bioreceptivity, as defined by Guillitte in 1995, is the ability of a material to provide colonization for living

organisms. The bioreceptivity depends on the environment, the properties of the organism, and the properties of the material itself (Sammartin *et al.*, 2021). Furthermore, bioreceptivity is dynamic as the material can chemically and physically change over time. Therefore, bioreceptivity can be divided into four categories: (1) Primary bioreceptivity is when organisms colonize the material, but the properties of the material remain very similar to the initial state. (2) When the characteristics of these properties change due to the colonization of organisms or environmental factors the term “secondary bioreceptivity” is used. (3) If any human activity affects the material and modifies the initial or secondary characteristics, it is called tertiary bioreceptivity (Guillitte, 1995). (4) Quaternary bioreceptivity is when a new material, like coating, is added (Figure 4) (sammartin *et al.,* 2021).

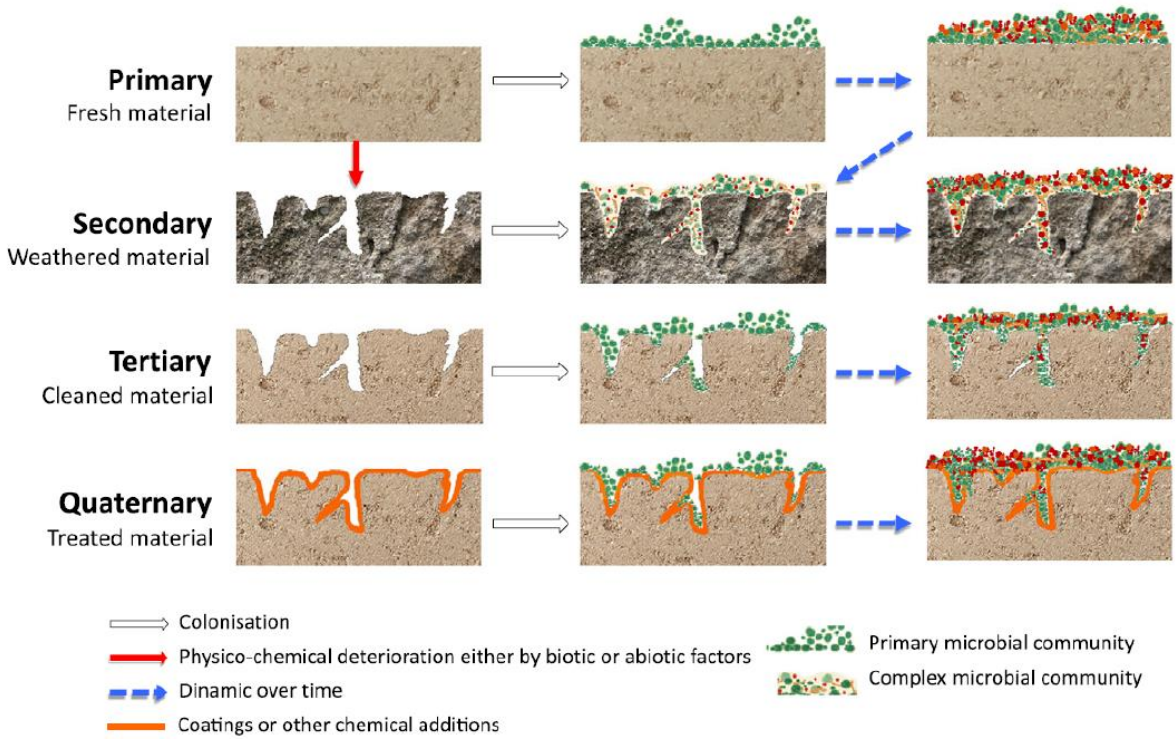

Figure 4. Visualization of the four bioreceptivity categories. The arrows indicate the changes over time (sammartin *et al.,* 2021).

The bioreceptivity of a material can be determined by intrinsic factors and extrinsic factors. For example, roughness, porosity, color, and mineralogical composition will be intrinsic factors, whereas architecture, temperature, and humidity on the material surface and added materials are extrinsic factors (sammartin *et al.,* 2021). Intrinsic factors are usually well studied under laboratory conditions, and those studies showed that bioreceptivity is primarily seen by a high surface roughness and porosity. The surface texture is especially important for pioneering organisms because the spores can settle better and are protected from weather forces (Guillitte). Extrinsic factors, on the other hand, are rarely considered (sammartin *et al.,* 2021).

Importantly, bioreceptivity is a relative concept, not an absolute one. The absence of colonization on a material does not mean that the material is not bioreceptive, but it means

that it is not bioreceptive to organisms present in that surrounding area at that moment (sammartin *et al.,* 2021).

2.4 Succession

Like any other ecosystem, the colonization of material arises through ecological succession. This means that a species replaces or succeeds another species over time in a specific order. First, primary succession occurs when the first pioneer species start from scratch. These organisms can change the chemical environment creating new conditions for other species to colonize the spot. These new organisms will form a mutualistic relationship or can replace the pioneer species (Connell & Slatyer, 1977).

On building materials, photoautotrophs, like green algae and cyanobacteria, are the pioneer species (Greengrass, 2020). These species can fix atmospheric carbon with their photosynthesis, which makes them able to grow on inorganic materials. After this settlement, the heterotrophic bacteria arise, because they can feed on the dead biomasses of the autotrophs (Curz & Richard, 2016). The bacteria and algae that are now present on the rocks get into a mutualistic relationship with each other, creating a self-sustaining matrix, called a subaerial biofilm (SAB). The biofilm settlers produce and secrete extracellular polymeric substances (EPS), creating a mucilaginous envelope around the biofilm. This will help the bacteria to retain water, gain resistance against environmental conditions, and further adhesion onto the substrate. Furthermore, it will facilitate the different bacterial species to get into symbiosis with other species, creating an ideal pioneer situation (Figure 5) (Gorbushina, 2007).

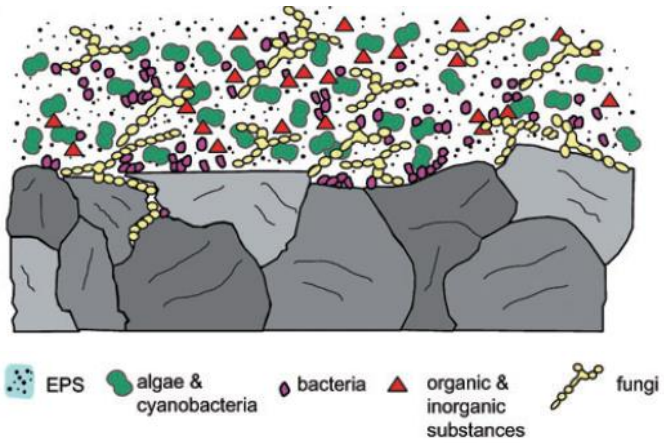

Figure 5. schematic visualization of subaerial biofilms and their interaction. Microorganisms are embedded in EPS and create a microbial ecosystem including both photoheterotrophic and photoautotrophic organisms. (Picture adapted from Gorbushina, 2007).

2.5 Algae, Cyanobacteria, and Mosses

This study focuses on the bioreceptivity of algae, cyanobacteria, and mosses and therefore we will elaborate on these pioneer species a bit further.

One of the main SAB organisms is cyanobacteria, e.g., *Arthrospira platensis*, due to their ability to both photosynthesize and fix atmospheric nitrogen. Cyanobacteria have developed protection mechanisms against desiccation and solar radiation. Furthermore, when sufficient water is present, algae can become part of the SAB organisms. SAB mainly comprises green algae, e.g., *Chlorella Vulgaris* (Gorbushina, 2007; Hauer *et al.,* 2015; Crispim & Gaylarde, 2005).

The natural succession will continue and in a more advanced state mosses can arise and get into this symbiotic relationship with the SAB (Gorbushina, 2007). Besides providing nutrients, this biofilm also reduces the pH value, which is crucial for mosses to settle (Manso *et al.*, 2014). Mosses are called non-vascular plants since they have no internal transportation system. Neither do mosses have roots. They have a small structure called rhizoids which is used for attachment purposes only and not for water and nutrient uptake. Instead, mosses use their leaves to absorb moisture with nutrients from the air. Therefore, the rhizoids of the mosses don't have to go deep, and this makes mosses a perfect pioneer species for a rocky environment (Mahrous *et al*., 2022; Chen *et al.,* 2011).

3. Methodology

3.1 Overview experiments

3.1.1 Field study: observing natural succession.

First, we studied the natural succession on a RainReef that is placed outside. Since our RainReefs are coming directly from the kiln, we assume that no other colonization has taken place that can have altered the structure in any way. As described earlier, we are therefore studying the primary succession of the reefs. The reefs are connected to a downspout on the north and south side of the building Blue City in Rotterdam. By measuring the local climate of these reefs, we can analyze the perfect circumstances for algae and mosses to grow. After three months of monitoring, we expect to see an increase in algae growth on the reefs (Hyp. 01).

3.1.2 Accessibility method: The addition of a nutrient layer.

Secondly, we used a moss cultivation method that is used by gardeners to enhance the growth of mosses (Moran, 2019; Li, 2021). In this study, we call it the accessibility method because if it works, commercial buyers of the RainReefs can apply this method themselves very easily. The method entails using buttermilk, vegan yogurt, or beer as a nutrient layer. The supplements will also lower the pH of the reefs, which is favorable for the mosses. This should enhance the quaternary succession of moss growth, and therefore, we expect to see a growth of mosses one month after the addition of the supplement layer (Hyp. 02).

3.1.3 Creating biofilms: Accelerating succession.

Finally, we stimulated the succession route by creating a biofilm of algae on the substrate and found if this biofilm stimulates the growth of mosses. To create such a biofilm of algae we used three different supplements; algae with water, algae with agar, and algae with agar and a hydrogel (Balasubramanian *et al.,* 2021). Furthermore, we tested both *Chlorella Vulgaris* and *Arthrospira platensis* green algae. We expect that the algae layer enhances the growth of mosses, so moss growth should be visible one month after the addition of spores (Hyp. 03).

Table 1. Overview of the different experiments.

Experiments:	Phase 1	Phase 2	Hypothesis	Result
Part 1: Field study	No supplements, study of natural succession.		Hyp. 01	Confirmed
Part 2: Accessibility method	Buttermilk + mosses		Hyp. 02	False
	Alpro + mosses			False
	Beer + mosses			False
Part 3: creating biofilms	Chlorella + Water	Chlorella + Agar + mosses	Hyp. 03	Confirmed
	Chlorella + Agar			
	Chlorella + Hydrogel			
	Spirulina + Water			
	Spirulina + Agar			
	Spirulina + Hydrogel			

3.2 Materials and Method

3.2.1 Materials

The RainReefs are made at UrbanReef with the use of a ceramic 3D printer
The mosses (*Leucobryum glaucum, Plagiothecium undulatum, Sphagnum magellanicum)* were ordered from www.bloemenvandegier.nl.
The algae (Chlorella Vulgaris, Arthrospira platensis) were ordered from www.etsy.nl.

3.2.2 Production of the ceramic RainReefs

The ceramic RainReefs from Urban Reef were produced with the use of a 3D clay printer (Wasp). First, 1.1 liters of water was added to 20 kilos of either new or recycled clay and mixed. The clay was put into the basin of the 3D printer and the script of the designated design was printed. After the printing, the reefs dried for about 2 weeks before firing in the kiln (Mojo Energyline). The reefs are then candled in the kiln at a maximum temperature of 100°C to make sure the reefs are completely dry. Accordingly, the Bisque firing program started with phased heating until 1070°C. This phased heating started with an 80°C increase per hour until 600°C was reached. After this, there was a maximum temperature increase per hour until 1070°C was reached. The reefs were then fired for 15 min at maximum temperature and immediately after that cooled down for about 24h. After cooling the reefs are ready to use.

Figure 6. The 3D clay printer (left) and the kiln (right).

3.2.3 The outdoor measurements

The two outdoor RainReefs were placed on 13/06/2022 on the north side and 30/06/2022 on the south side of Blue City in Rotterdam. The reefs were connected to a downspout, thereby collecting rainwater inside the reefs. Every week measurements were performed to document the temperature and relative humidity with the TROTEC BC06, the material moister with the Stanly STHT77030, and the natural growth of different locations on and around the reefs with photos. The measurements were done on the positions on the reefs as visualized in supplement figure S1.

3.2.4 Accessibility method

In total 15 grams of the three different mosses were taken and the mosses were carefully rinsed before being added to the blender (KichenBrothers Power Blender KB677). After this, 200 ml of either the biological buttermilk (1), Alpro soja yogurt (2), or Bavaria beer (3) (see appendix X) was added together with 200 ml water. The mixture was blended for 30 seconds at the lowest speed to create a homogeneous solution but to avoid the complete destruction of the mosses. The three different moss-supplement mixtures were then applied on the RainReefs with the use of a brush. On the fourth RainReef, no mosses or supplements were added.

The reefs were placed in the greenhouse with growing lights (red LED with 620 - 630 nm16 blue LED with 460 Nm), a set temperature of 19 C regulated with the ITC-308-WIFI Thermostat, and a set humidification of 78.2 % RH regulated with the IHC-200-WIFI measurement tool. The temperature and humidity are fluctuating around these settings and are recorded with the INKBIRD app. The RainReefs are filled with water every day with an automatic sprinkler and are watered with a plant sprayer from the outside on weekdays. The experiment ran for 6 weeks and pictures were taken twice a week. After this time, the experiment was repeated.

Figure 7. The greenhouse (left) and the setup of the experiments (right).

3.2.5 Cultivation of algae

The algae were filled into a large glass container without a lid and placed in the greenhouse under the growing lights. The cultures were mixed often by carefully tilting the container. The pH of the culture was checked from time to time with pH strips and should be around a pH of 9. After the algae have reached a deep green/blueish coloration or a pH above 12, the algae were diluted to about twice the volume. To do so, nutrient mix 1 for *Arthrospira platensis* or nutrient mix 2 for *Chlorella Vulgaris* (See appendix Figure S1 & S2) was dissolved in water to a dosage of 20g per liter. The nutrient medium is then stirred and sat for one hour to make sure it has the same temperature as the algae culture. Then, the nutrient mix is carefully added to the culture.

3.2.6 Applying the algae on the RainReefs.

Both the *Chlorella Vulgaris* and *Arthrospira platensis* algae were applied on the reefs with 3 different media.

Agar: One liter of the medium was prepared by mixing 30g agar, 0.33 ml nutrient mix 1 (*Arthrospira*) or 2 (*Chlorella*), and 1580 ml of water in the blender. The mixture was cooked for 5 minutes and cooled down to 35 °C. Per reef, 50 ml of medium 1 was mixed with 50 ml of the *Chlorella* or *Arthrospira* algae culture and then applied on the reefs with a brush.
Hydrogel: One liter of the medium was prepared by mixing 30g agar, 0.33 ml nutrient mix 1 (*Arthrospira*) or 2 (*Chlorella*), and 1580 ml of water in the blender. The mixture was cooked for 5 minutes and cooled down to 35 °C. Then 250 ml of 2% sodium alginate was added. Per reef 50 ml of the medium was mixed with 50 ml of the *Chlorella* or *Arthrospira* algae culture and applied onto the reef with a brush. In the meantime, 2.5 g calcium chloride was mixed with 250 ml water and inserted into a spray bottle. The calcium chloride solution was sprayed over the algae medium on the reefs.
Water: The algae were filtered out of 500 ml of the *Chlorella* or *Arthrospira* algae culture and were applied onto the reefs with a brush.
To stimulate the growth of algae, the reefs were placed in a plastic container inside the greenhouse with a growth light and water in it. The algae grew for 20 days and pictures were taken twice a week.

Figure 8. Experimental set-up of the Algea experiment.

3.2.7 Addition of Mosses onto the Algae

After 20 days of algae growth, spores of the mosses were added to the reefs by hand. Since the algae provided a sticky layer, the mosses could attach to the reefs without difficulty.

3.2.8 Monitoring and Analysis

The biological growth on the outdoor reefs was measured by taking photos every week from the same angle. The pictures could not be analyzed with a computer program due to the different weather conditions. Therefore, a scale was made from 0 to 5 with 0 = no vegetation visible, 1 = very thin layer of algae, 2 = thick layer of algae, 3 = very thick layer of algae (dark green/brown), 4 = moss growth and 5 = vascular plants. Four points on the reef were analyzed and given a number on this scale to indicate the growth on this measure location.

For the indoor experiments, moss and algae growth were measured by taking pictures with a flash twice a week at the same distance, from left-, right-, front- and backside. The pictures were then analyzed in ImageJ. First, the total green coverage on the reefs was measured by drawing an ROI around it and by measuring that surface. Then this surface was divided by the

total photographed surface of the reef. This number was then multiplied by 100 to give a percentage of growth. For the algae experiments, only the front pictures were analyzed, for the accessibility method, all sides were analyzed, and an average coverage was calculated from this. The steps were as follows:

1. Image → Adjust → Color Threshold
2. The Hue and Brightness were set to 0, the Saturation was adjusted until all green areas were covered.
3. Select this area by pressing Select.
4. Analyze → Measure → gives the total green area.
5. Now adjust the Saturation till the entire reef is covered.
6. Select the area with the wand tracing tool.
7. Analyze → Measure → gives the total area of the reef.

Percentage of vegetation coverage = $\frac{Surface\ of\ vegatation}{Surface\ of\ the\ reef} \times 100\%$

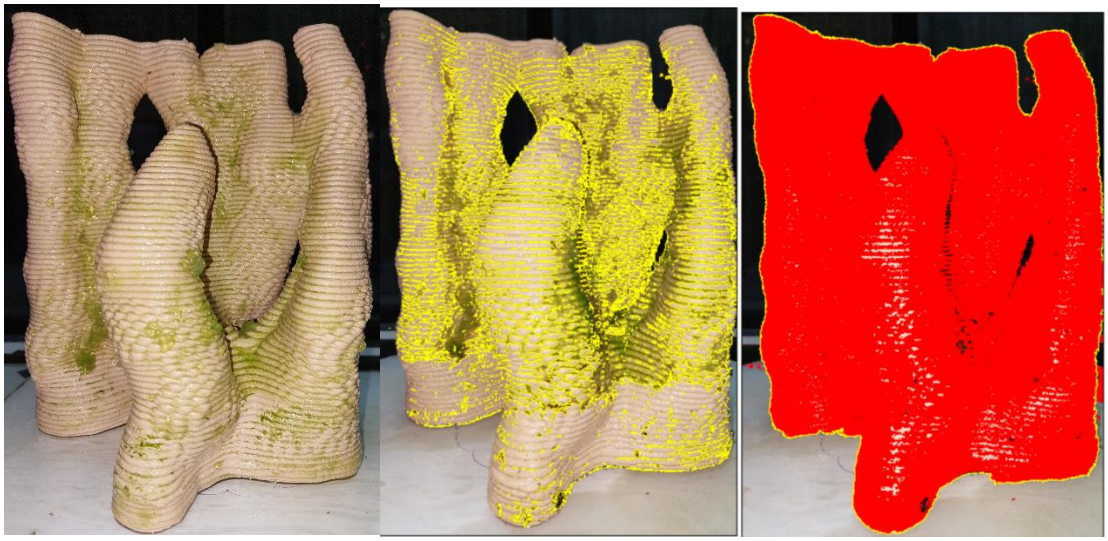

Figure 9. visualization of the analysis in Image J. a) the picture that is going to be analyzed with a layer of algae on it. b) selection of the green areas. c) selection of the entire reef.

The graphs derived from the photo analysis were mainly used to describe and analyze the data. To complement this data, a statistical analysis was performed with two-way ANOVA and a turkey posthoc test. The result of the statistical analysis is shown in the caption of the figures. Nevertheless, due to our small sample size, we do not assume that the statistical data is significant.

4. Results

4.1 Part 1: Outdoor Monitoring

First, we studied the natural succession on outdoor reefs over time. For a period of 11 weeks in February, March, and April, the temperature and humidity of different locations on the reefs were measured weekly to analyze the different microclimates. Furthermore, pictures of the reefs were taken to analyze the growth of algae, mosses, and vascular plants. The measurements were performed on two reefs, one located on the north side (BCN) and one on the south side (BCS) of BlueCity in Rotterdam.

As can be seen in Figure 10, the microclimates on the BCN-reef show overall lower temperatures and higher relative humidity compared to the surrounding air. For the BCS-reef, this difference is only visible for the humidity and not for the temperature (Figure 11). The graphs also indicate that the lower the outside humidity, the bigger the difference with the reef. Furthermore, for both BCN and BCS, the relative humidity seems to increase forward the bottom of the reef, for the temperature this gradient is less visible.

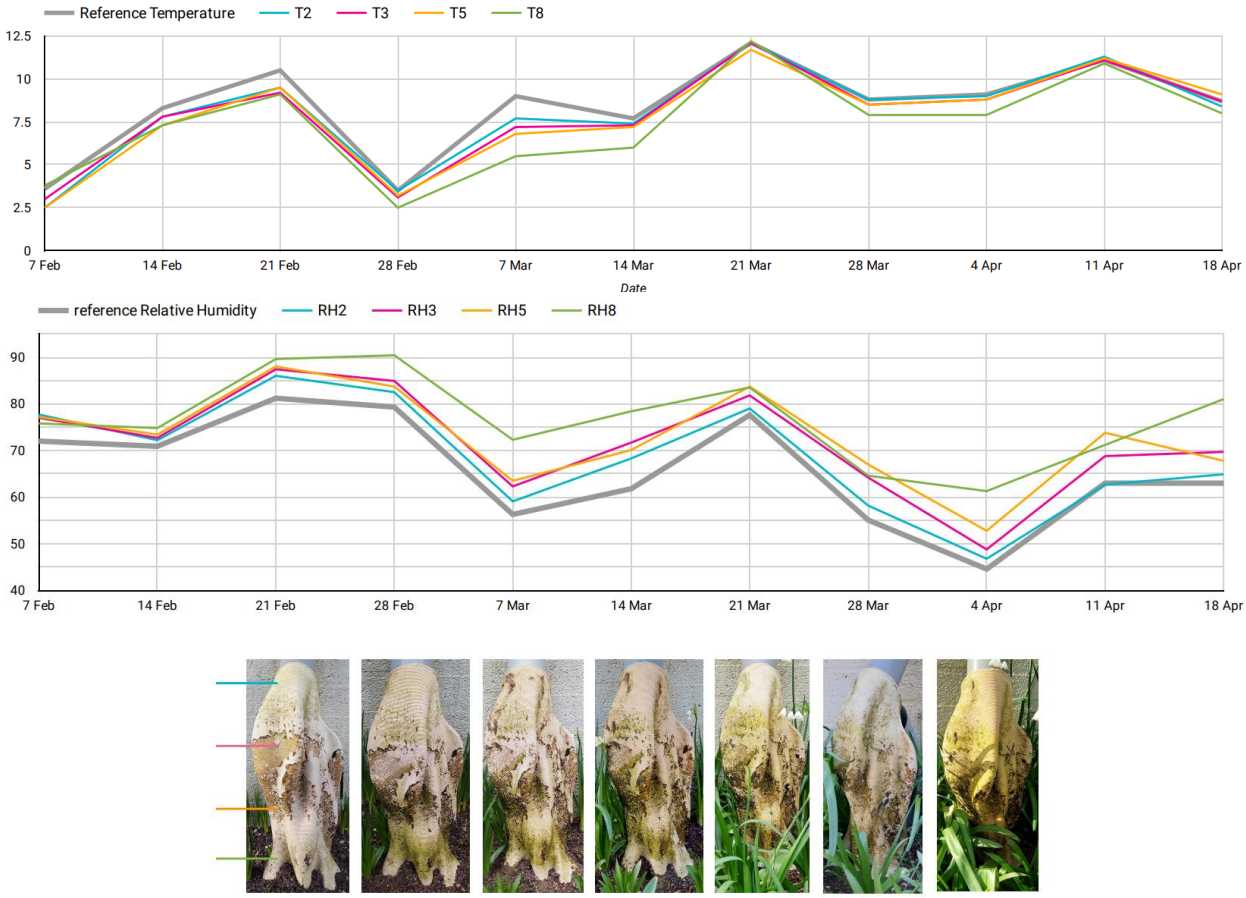

Figure 10. Measurements performed on reef BCN in 2023 over time. The reference line indicates the temperature and relative humidity that was measured in the air one meter away from the reef. The other lines (T2, RH2, T3, RH3, T5, RH5, T8, RH8) represent the temperature and humidity of the different measurement locations on the front side of the reef.

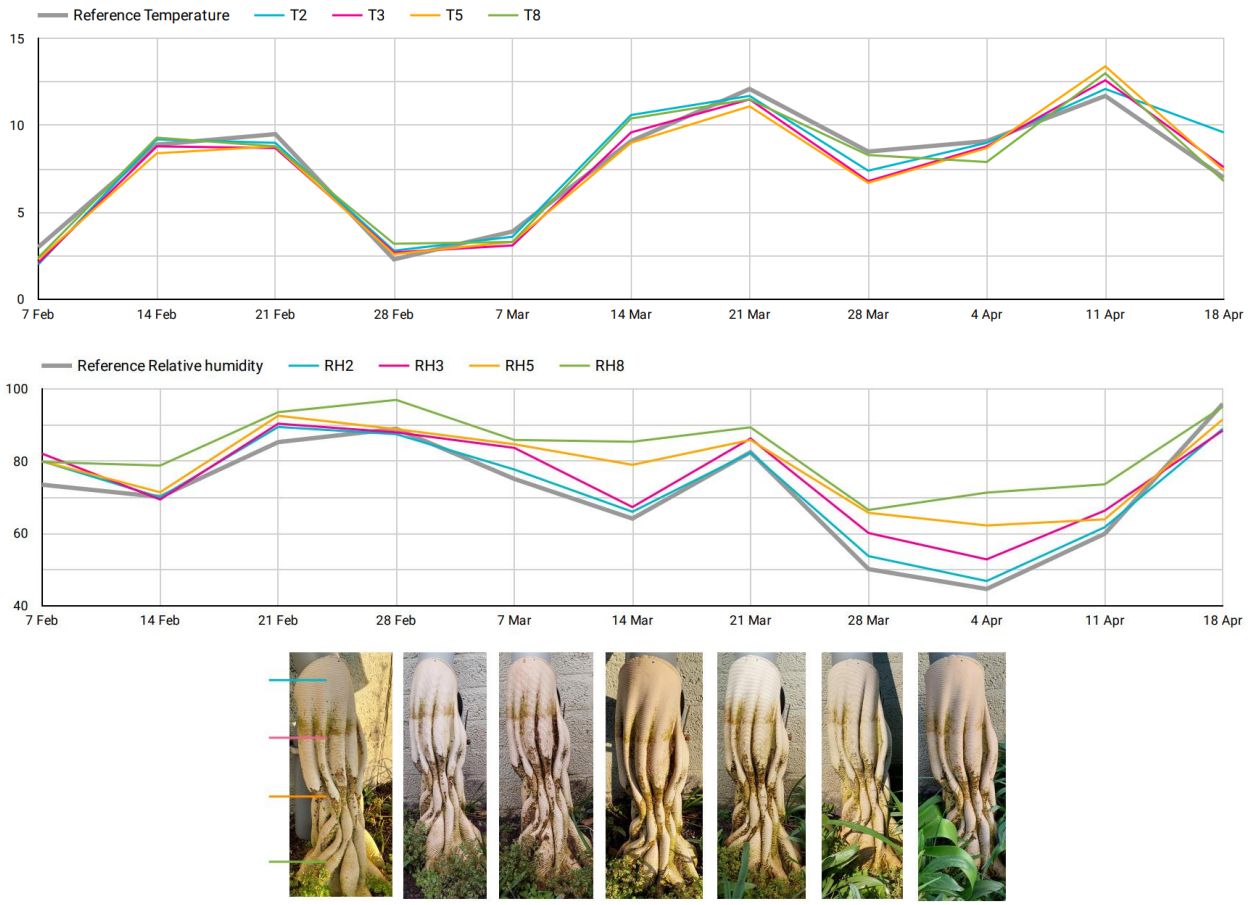

Figure 11. Measurements performed on reef BCS in 2023 over time. The reference line indicates the temperature and relative humidity that was measured in the air one meter away from the reef. The other lines (T2, RH2, T3, RH3, T5, RH5, T8, RH8) represent the temperature and humidity of the different measurement locations on the front side of the reef.

The pictures of the reefs were analyzed by indicating the vegetational coverage/growth of the different measurement locations on a scale (Table 2). A computer program could not be used due to the big changes in light and the inexact camera position for every measurement. As can be seen in the table, for BCN most growth happened at the bottom of the reef at measurement location eight. For BCS most growth appeared at measurement location five. Overall, the data shows a growth in algae on the reefs after three months and therefore we can confirm hypothesis 01 (see paragraph 3.1.1).

Table 2. Growth Index. The amount of organic growth per measurement location is indicated with the growth index. (0 = no vegetation visible, 1 = very thin layer of algae, 2 = thick layer of algae, 3 = very thick layer of algae (dark green/brown), 4 = moss growth, and 5 = vascular plants)

	BCN				BCS			
	G2	G3	G5	G8	G2	G3	G5	G8
7-2-2023	1	1	0	1	0	1	2	1
14-2-2023	1	2	0	1	0	1	2	1
21-2-2023	1	1	0	1	0	1	2	1
28-2-2023	1	0	0	2	0	1	2	1

7-3-2023	1	0	2	2	0	1	2	1
14-3-2023	0	1	2	2	0	2	2	2
21-3-2023	0	1	2	2	0	2	2	2
28-3-2023	0	2	2	2	0	1	3	2
4-4-2023	0	1	2	2	0	0	2	1
11-4-2023	1	2	3	3	0	0	3	3
18-4-2023	0	1	2	2	0	0	1	2
Average	0.5	1.1	1.5	1.9	0	0.9	2.1	1.5

Now that the amount of growth on the reefs is indicated with a number, the correlation between this growth, temperature, and humidity on the front side of the reef is analyzed. Both BCN and BCS seem to have a positive correlation between humidity and growth. The temperature, on the other hand, seems to have a negative correlation with BCN and no correlation with BCS (Figure 12, 13).

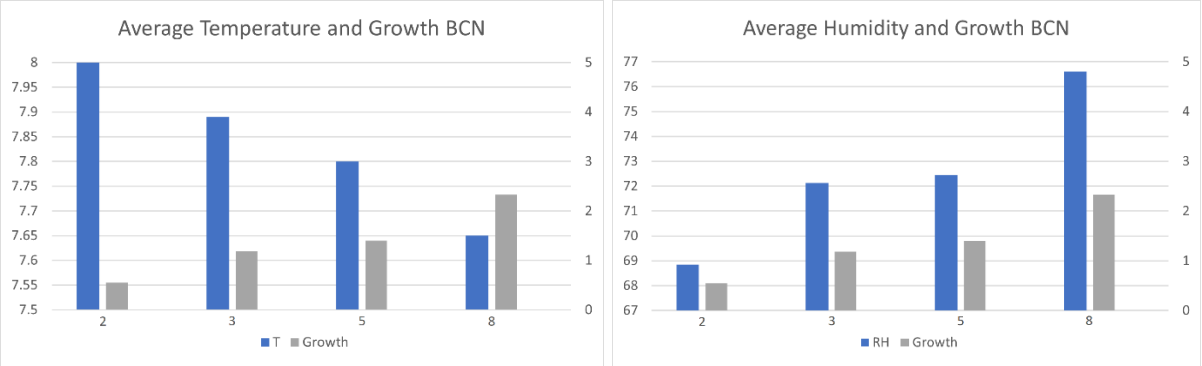

Figure 12. Relation between growth, temperature, and humidity of different measurement locations (2, 3, 5, and 8) on RainReef BCN 2023. A) Shows the comparison between the temperature and the growth (r = -0.991) B) Shows the comparison between the relative humidity and growth (r = 0.996).

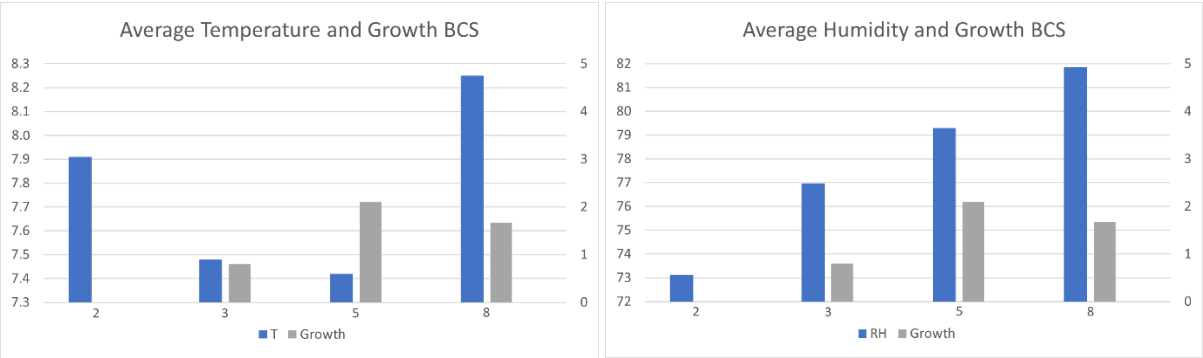

Figure 13. Relation between growth, temperature, and humidity of different measurement locations (2, 3, 5, and 8) on RainReef BCS 2023. A) Shows the comparison between the temperature and the growth (r = -0.130) B) Shows the comparison between the relative humidity and growth (r = 0.886).

Finally, we compared the temperature and humidity of the front and the back side of the reefs. Since, because of the wall we were not able to make pictures of the backside of the reef, we

cannot analyze the difference in growth on both sides. Nevertheless, it can still give us insight into the different microclimates on the reefs. As can be seen in Figure 14. compared to the frontside of the reef, the humidity is higher on the backside of the BCN reef, but lower on the backside of the BCS reef. For both reefs, the temperature is higher on the front side compared to the back side.

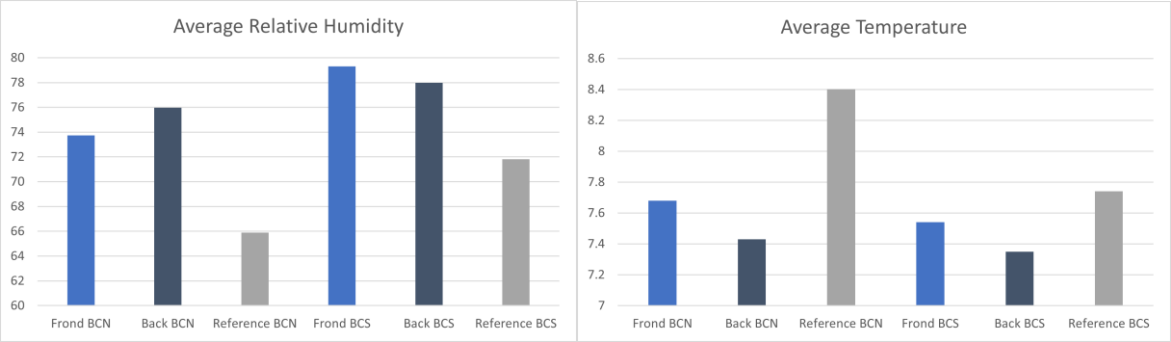

Figure 14. Comparison between the front side and backside of reef BCN and BCS. A shows the difference in relative humidity and B shows the temperature difference.

In addition, the data of previous measurements, from September, October, and November 2022, was also analyzed (Appendix B, Supplement Figure S4, S5). Overall, for reef BCN 2022, just as in 2023, the relative humidity in the air is lower than the relative humidity on the reef itself and there is a gradient visible in humidity over the reef locations. For BCS, the reference humidity is comparable to the humidity of measure location RH2 and there is no gradient visible. Comparable to the data from 2023, the temperature does not show a significant difference between the outdoor temperature and the measurements on both reefs.
We also compared the correlation between growth, humidity, and temperature for the 2022 data (Supplemented Figure S6). Comparable to the 2023 data, both BCN and BCS show a positive correlation between humidity and growth. Nevertheless, the temperature showed different results compared to the 2023 data. Where BCN 2023 had a negative correlation between temperature and growth, BCN 2022 showed no correlation. Moreover, where BCS 2023 did not correlate with temperature and growth, BCS 2022 shows a negative correlation. Finally, we compared the temperature and humidity of the front and back sides of the reefs in 2022 (Supplemented Figure S7). The BCN reef showed a very big difference in temperature between the front- and backside. For the BCS reef, this difference was smaller. For both reefs the backside was the cooler side, this is comparable to the 2023 data. For the BCN reef, the humidity was higher on the front side of the reef and for the BCS reef, the humidity was higher on the backside. This is the other way around compared to the 2023 data. This would imply that different seasons influence the results.

4.2 Part two: The accessibility method results.

Besides studying the natural outdoor succession, we tried to enhance the succession with the use of a nutrient layer that is often used by gardeners: buttermilk, vegan yogurt (Alpro), and

beer. The nutrients were applied together with mosses and were able to grow for five weeks (Figure 15). The temperature and humidity of the greenhouse were also traced during these five weeks (Appendix Figure S8, S9).

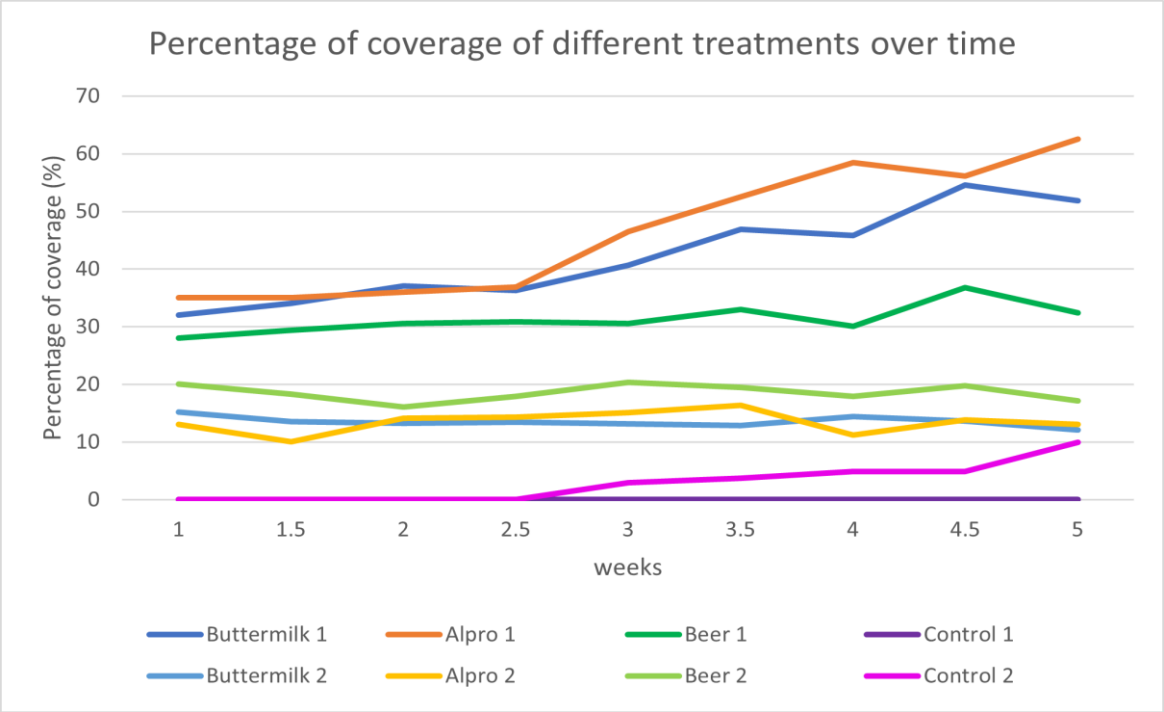

Figure 15. Percentage of coverage of different treatments over time. n = 8.

We subtracted the amount of coverage at the beginning of the experiment from the coverage at the end to visualize the absolute growth (Figure 16). Alpro from round 1 showed the highest growth of 27.49%. Buttermilk came second with 19.9% growth. Interestingly, the second round showed negative results where the mosses are washed away. The control group of the second-round experiments showed contamination of algae. The pictures were taken at the end of the experiment and visualize the difference in the amount of green area on the reefs. Due to this big difference in moss growth between round 1 and round 2, we cannot confirm hypothesis 02 (see paragraph 3.1.2).

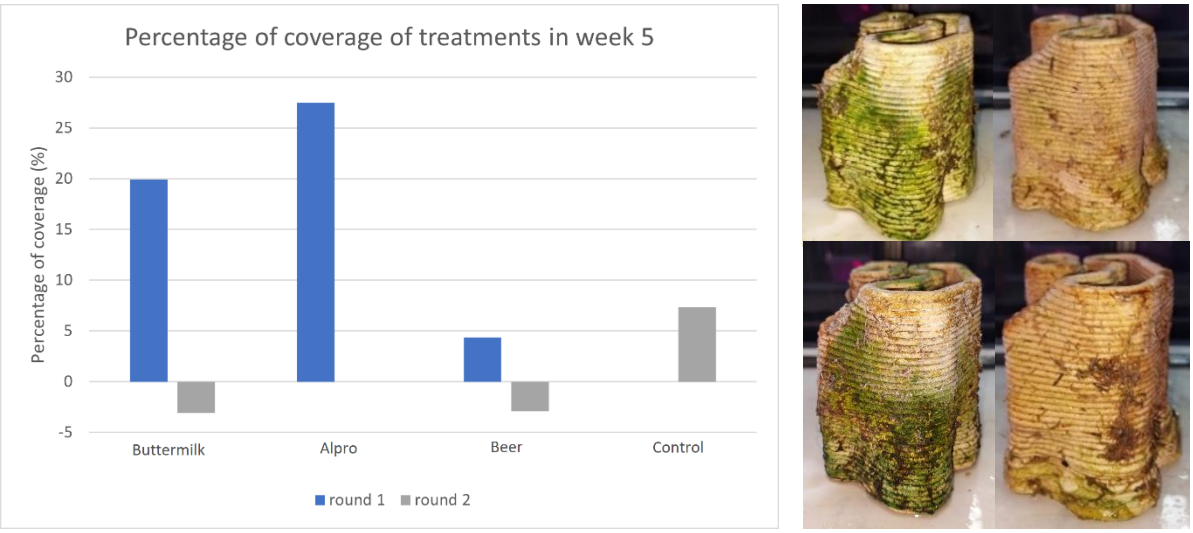

Figure 16. Comparison in total coverage week five between different experiments. A) shows the absolute growth of the different treatment groups after five weeks. B) buttermilk reef after five weeks round 1. C) buttermilk reef after 5 weeks round 2. D) Alpro reef after five weeks round 1. D) Alpro reef after five weeks round 2. n = 8.

4.3 Part three: Promoting algae growth on the reefs.

As described previously, natural colonization of substrates occurs through a succession route where micro-organisms are the first colonizers. This will create a natural biofilm that can enhance the growth of other organisms, that use the biofilm as a nutrient source. This can already be seen in the previous experiments, where algae arise first. Therefore, we want to enhance and accelerate this natural succession by adding a biofilm of algae onto the reefs and to study if this biofilm will enhance the attaching and growth of mosses.

To do this, we first analyzed which algae, *Chlorella vulgaris* or *spirulina,* in combination with which medium Agar, Hydrogel, or water, shows the highest growth on our reefs. Therefore, we applied the three different media with either *Chlorella* or *Spirulina* onto the reefs. The surface coverage percentage of the different treatments was traced for 20 days (Figure 17). After these 20 days, algae growth should be visible since these algae have a doubling time of 2-3h (Jester *et al.,* 2022; Sorokin & Krauss, 1959). Compared to all other treatments, in the chlorella + water treatment surface coverage was already visible immediately after application. This treatment also showed the highest surface coverage of 62.3% after 20 days. Spirulina with both hydrogel and agar and the control group showed almost no growth during this time.

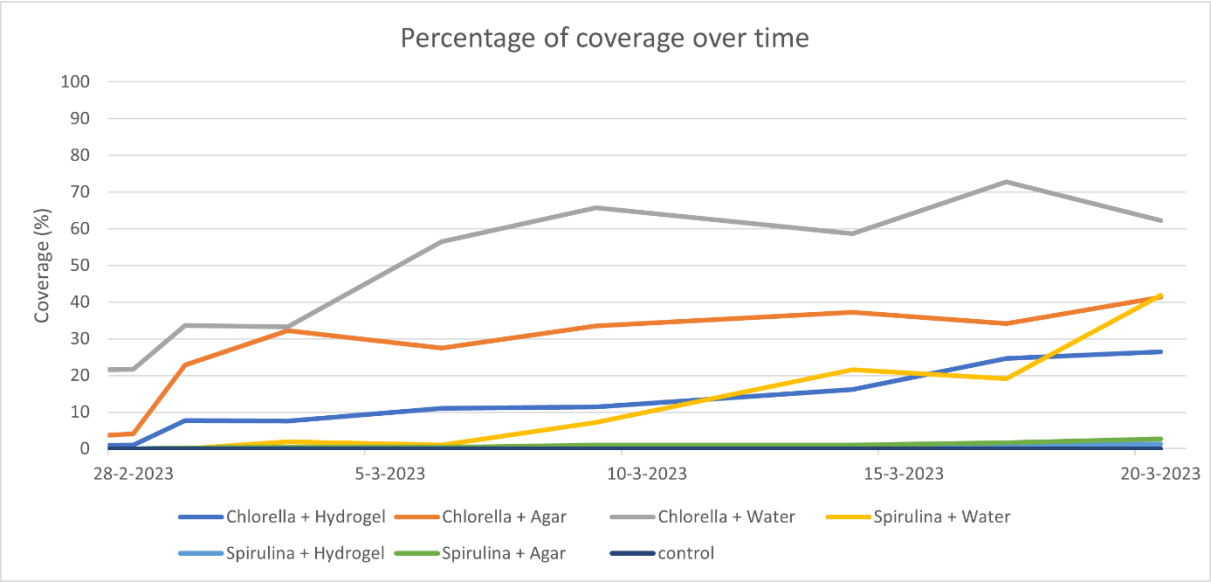

Figure 17. The percentage of coverage of the different treatments for 20 days. With n = 7.

After 20 days of growth, the total increase in green coverage percentage of the different treatments on the reefs was calculated and compared (Figure 18). Overall, Chlorella Vulgaris showed higher surface coverage compared to spirulina. For chlorella, both water (41.9%) and agar (41.3%) treatments showed similar high results. For Spirulina the water treatment (41.8%) also showed a similar amount of coverage.

Since chlorella showed overall higher results than spirulina, we decided to use these algae in the next experiment. Furthermore, we decided to go with the agar treatment. For the water treatment, we would need more algae and we didn't have the ability for that.

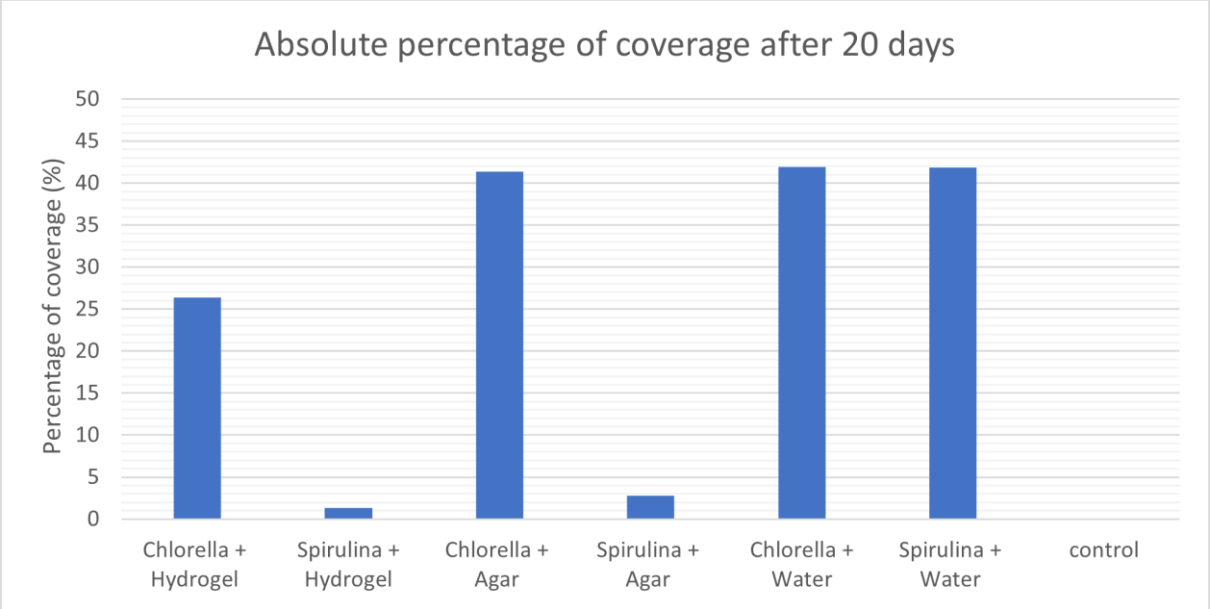

Figure 18. Percentage of coverage of the different treatments on the reefs after 20 days. The P-value of the difference between algae = 0.0356 (sign.) and the P-value of the difference between treatments = 0. 0.519 (no sign.). n = 7.

4.4 Algae and moss growth

After growing the chlorella + agar treatment for 20 days on 6 reefs, moss spores were added. The mosses were able to grow for five weeks. The control group contained algae but no moss spores. After five weeks the reefs were analyzed to see if moss growth has taken place. As can be seen in Figure 19, the green coverage was increased after 31 days.

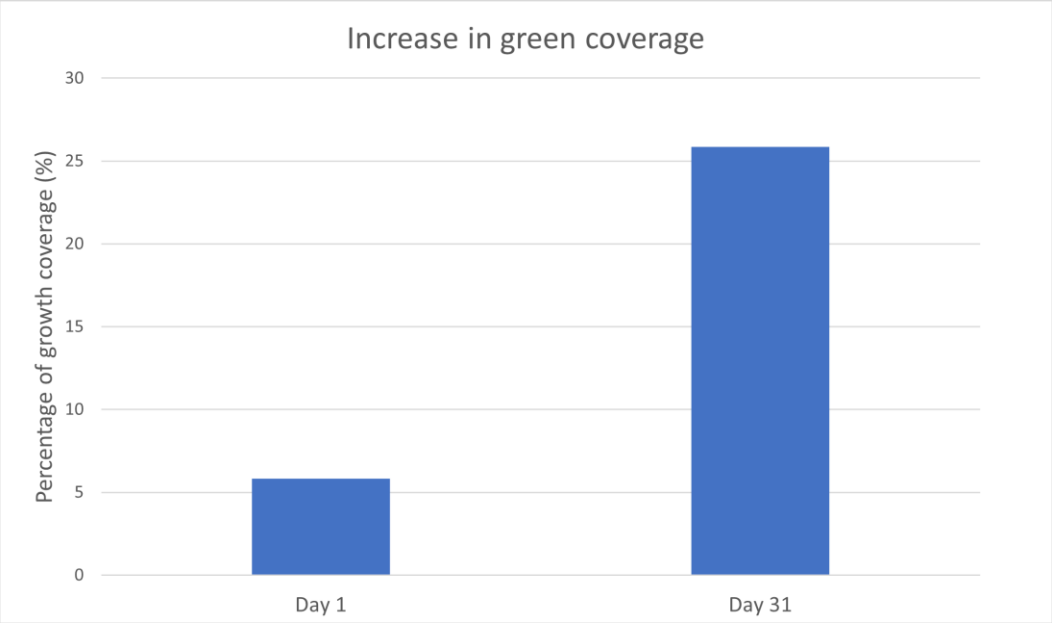

Figure 19. The increase in green coverage after 31 days. n = 6.

Since it is hard to analyze the growth of mosses with ImageJ, zoomed-in pictures were taken of the reefs as proof that moss growth has taken place (Figure 20). Therefore, we can accept hypothesis 03.

Figure 20. pictures that show the moss growth present on the reefs.

5. Discussion

The company Urban Reef designs 3D-printed ceramic reef-like structures that can be placed in urban areas and provides habitat for different species, thereby enhancing biodiversity and ecosystem services. The goal of the company is to increase the bioreceptivity of these reefs to promote the growth of algae, mosses, and other organisms. In this study, we try to enhance the bioreceptivity of mosses with the use of different supplemental layers and the addition of an algae biofilm. Furthermore, we explored the natural succession of the reefs in a field study.

A field study was conducted to investigate the natural succession of the reefs and how that is influenced by the temperature and relative humidity. Our results show that an increase in relative humidity leads to an increase in algae growth on the reefs. On the other hand, there was no correlation between the temperature and algae growth. In this study, we only focused on the growth on the front side of the reef. It would also be interesting to study the growth on the backside of the reef to better understand the effects of shade and lux intensity.
Overall, the relative humidity in this study is very high. This can be caused because the location of the reefs is in the Rotterdam docks. This means that another, dryer, location can possibly influence the results.
Some interesting result was that in 2022, the BCN reef showed higher humidity on the front side and the BCS reef showed higher humidity om the backside. This is the other way around compared to the data form 2023. This could be explained by the fact that the BCS reef is on the sunny side of the building and therefore the seasons influence the amount of lux more compared to the north side of the building.

The reefs have been outside for about a year now, and there is still no sign of moss growth. We concluded that intervention with the use of a supplement is necessary to speed up the process of natural succession. Therefore, we studied a moss cultivation method using buttermilk, Alpro, and beer as supplement layers. The experiment was performed twice, with both experiments showing different results. In the first round Alpro and buttermilk already showed moss hatching after 2.5 weeks. This would mean that the use of one of these supplements can be a good moss starter by providing nutrients and lowering the pH. Beer did not show any signs of growth. Nevertheless, in the second round, no moss growth happened at all. There are two possible explanations for this difference. Firstly, in the first round more mosses were added compared to the second round. There might be a threshold in the number of mosses that are needed to cause moss to hatch. Secondly, in week four of the second experiment, the humidifier broke down, causing the air to be less moist. Since mosses get their water from the air, this could also have influenced the hatching.
The results of this experiment are debatable because there was only a very low n value due to the limited number of reefs that were available. Furthermore, a few experiments failed since leakages arise in the reefs over time and that influenced the humidity. Nevertheless, since the first round showed promising results for Alpro and buttermilk, it might still be a usable method. More research is needed on how to enhance the success rate of this method.

Finally, we wanted to speed up the natural succession route by lubricating an algae layer on the reefs. We decided to go with a *Chlorella vulgaris* + agar biofilm, because only a small number of algae were needed, and it showed a fast rate of growth. Nevertheless, this was under controlled temperature and fixed humidity and therefore outdoor reefs might need

more resistant algae. In the future, the experiment can be repeated with other algae and/or supplements and in an outdoor environment to study the resistance.
After a biofilm growth of 20 days, the moss spores were added and were allowed to grow for a month. The control group only contained a biofilm and no moss spores. After 5 weeks, moss growth was visible on all reefs, including the control group. This means that our greenhouse is not sterile and moss spores could travel through the air. Since this is also the cause in nature, it is not seen as a problem. Because moss growth was already visible after five weeks, we could say that algae positively influence the hatching and thereby is a promising supplement that can be used by Urban Reef.

The analysis of the bioreceptivity was done by calculating the percentage of surface coverage with ImageJ. A few problems arise with this method.
First, the reefs that we used have a lot of complex shapes. This makes it hard to analyze the reefs with only one or a few photos. Some reefs in our experiments had moss growth on spots that were not visible in our pictures. Furthermore, the different shapes create shade, which is also detected by ImageJ. A solution could be to use a 3D picture, to make it possible to analyze the entire reef at once.
Secondly, with ImageJ, only the surface coverage is measured. The number of different species and the thickness of the moss layer are not considered. A new method that can also study this would be a more accurate display of the total bioreceptivity.
Finally, we could not use this method outside, due to the changes in weather conditions and therefore light intensity. Outside we had to use our own interpretation of the amount of growth by using a scale. This made the results less accurate since we did not analyze the entire reef, but only identified the amount of growth on 4 small specific spots on the frontside of the reef.

To conclude, the RainReefs of Urban Reef are already a bit bioreceptive for some algae in a natural environment, but an additional layer is needed to speed up the growth of mosses. The addition of a *chlorella vulgaris* biofilm is a promising method to enhance the succession and thereby supporting the hatching of mosses. The addition of Alpo or buttermilk might also be a good supplement to support moss growth, but for this more research is needed.

6. Recommendations

- Since we now know that humidity is correlated with the growth of algae on the reefs, we can say that a RainReef design that can collect water is a good design. More studies on the porosity of the material can lead to a bigger humidity on the outside of the reef and therefore can be an important factor to enhance the natural growth.
- To understand the influence of shade and sun intensity, the amount of growth on the backside of the outdoor reefs should also be studied.
- The use of Alpro or buttermilk can be a good accessible supplement to be used by buyers of the reefs, nevertheless, more research is necessary to understand the optimal conditions. Since it is a food supplement, more research is needed on the legislation of using this in public areas.
- The use of a *chlorella vulgaris* algae biofilm is a promising method that we would recommend urban reef to use in further studies. *Chlorella vulgaris* is proven to be a safe organism and it creates a natural succession.
- Both the supplemented layers and the algae biofilms are only studied inside the greenhouse under controlled conditions. The next step in this study could be to place reefs with supplemental layers or biofilms outside. The temperature and humidity difference in the outside world can influence the results. Furthermore, natural conditions like other plants in the surroundings or nutrients in the rainwater can also have an impact on the algae and moss growth. Furthermore, *chlorella vulgaris* might not be resistant enough to the outdoor environment and alternative algae can be studied.
- The structure of the reefs that we used in this study was very smooth which made it hard for mosses to attach. Therefore, we would recommend enhancing the roughness of the reefs in the new designs.

Overall, the use of algae as a biofilm layer to increase bioreceptivity is the most promising method that Urban Reef can help in their further design process. It is a method that enhances natural succession and colonization of mosses and other organisms, thereby fitting with their philosophy of working together with nature.

7. Acknowledgments

I have greatly enjoyed my minor research project at the company of Urban Reef. During my time I learned a lot about aspects of material research and about all the obstacles and possibilities you can encounter when starting your own company. I would like to thank my supervisor Pierre Oskam for his interesting ideas and our meaningful discussions. Furthermore, I would like to thank Max Latour for his daily support and explanations about ceramics and design. Additionally, I would like to thank Jaco Appelman for his guidance throughout the six months of research and for all the feedback that I gained during the writing of this report. Finally, I would like to thank the other interns for their support and the creation of a nice work environment. I felt very welcome and could always approach anyone with questions or thoughts.

Literature

Apfelbeck, B., Snep, R. P., Hauck, T. E., Ferguson, J., Holy, M., Jakoby, C., ... & Weisser, W. W. (2020). Designing wildlife-inclusive cities that support human-animal co-existence. *Landscape and Urban Planning*, *200*, 103817.

Balasubramanian, S., Yu, K., Meyer, A. S., Karana, E., & Aubin-Tam, M. E. (2021). Bioprinting of regenerative photosynthetic living materials. *Advanced Functional Materials*, *31*(31), 2011162.

Bolund, P., & Hunhammar, S. (1999). Ecosystem services in urban areas. *Ecological economics*, *29*(2), 293-301.

Carpenter, S. R., Bennett, E. M., & Peterson, G. D. (2006). Scenarios for ecosystem services: an overview. *Ecology and Society*, *11*(1).

Connell, J. H., & Slatyer, R. O. (1977). Mechanisms of succession in natural communities and their role in community stability and organization. *The american naturalist*, *111*(982), 1119-1144.

Cox, D. T., Shanahan, D. F., Hudson, H. L., Fuller, R. A., & Gaston, K. J. (2018). The impact of urbanisation on nature dose and the implications for human health. *Landscape and Urban Planning*, *179*, 72-80.

Greengrass, K. S. (2020). *Geodermatophilaceae biofilm formation and rock recolonization* (Doctoral dissertation, University of New Hampshire).

Crispim, C. A., & Gaylarde, C. C. (2005). Cyanobacteria and biodeterioration of cultural heritage: a review. *Microbial ecology*, *49*, 1-9.

Cruz, M., & Beckett, R. (2016). Bioreceptive design: A novel approach to biodigital materiality. *Arq: Architectural Research Quarterly*, *20*(1), 51-64.

Gorbushina, A. A. (2007). Life on the rocks. *Environmental microbiology*, *9*(7), 1613-1631.
Moran, M., 2019. *How to grow moss*. February 15. https://www.wikihow.life/Grow-Moss.

Guillitte, O. (1995). Bioreceptivity: a new concept for building ecology studies. *Science of the total environment*, *167*(1-3), 215-220.

Guillitte, O., & Dreesen, R. (1995). Laboratory chamber studies and petrographical analysis as bioreceptivity assessment tools of building materials. *Science of the total environment*, *167*(1-3), 365-374.

Hamstead, Zoé A. "How We Got Here: Producing Climate Inequity and Vulnerability to Urban Weather Extremes." *Resilient Urban Futures* (2021): 11-28.

Hauer, T., Mühlsteinová, R., Bohunická, M., Kaštovský, J., & Mareš, J. (2015). Diversity of cyanobacteria on rock surfaces. *Biodiversity and conservation*, *24*, 759-779.

Jester, B. W., Zhao, H., Gewe, M., Adame, T., Perruzza, L., Bolick, D. T., ... & Roberts, J. (2022). Development of spirulina for the manufacture and oral delivery of protein therapeutics. *Nature Biotechnology*, *40*(6), 956-964.

Köhler, Manfred. "Green facades—a view back and some visions." *Urban Ecosystems* 11 (2008): 423-436.

Li, R. (2021). Quay walls Greening with Mosses:"How can quay wall elements be designed with improved bio receptivity to stimulate high moss growth coverage that will add social and environmental values to Amsterdam citizens' wellbeing?"

Mahrous, R., Giancola, E., Osman, A., Asawa, T., & Mahmoud, H. (2022). Review of key factors that affect the implementation of bio-receptive façades in a hot arid climate: Case study north Egypt. *Building and Environment*, 108920.

Ni, T. (2016). Living Planet Report 2016. WWF.

Sanmartín, P., Miller, A. Z., Prieto, B., & Viles, H. A. (2021). Revisiting and reanalysing the concept of bioreceptivity 25 years on. *Science of The Total Environment*, *770*, 145314.

Sorokin, C., & Krauss, R. W. (1959). Maximum growth rates of Chlorella in steady-state and in synchronized cultures. *Proceedings of the National Academy of Sciences*, *45*(12), 1740-1744.

Ulrich, R. S., Simons, R. F., Losito, B. D., Fiorito, E., Miles, M. A., & Zelson, M. (1991). Stress recovery during exposure to natural and urban environments. *Journal of environmental psychology*, *11*(3), 201-230.

Wong, N. H., Tan, A. Y. K., Chen, Y., Sekar, K., Tan, P. Y., Chan, D., ... & Wong, N. C. (2010). Thermal evaluation of vertical greenery systems for building walls. *Building and environment*, *45*(3), 663-672.

Zoulia, I., Santamouris, M., & Dimoudi, A. (2009). Monitoring the effect of urban green areas on the heat island in Athens. *Environmental monitoring and assessment*, *156*, 275-292.
McKinney, M. L. (2002). Urbanization, Biodiversity, and Conservation. *Bioscience*, *52*(10), 883-890.

Appendix

Appendix A – Supplemented materials and methods

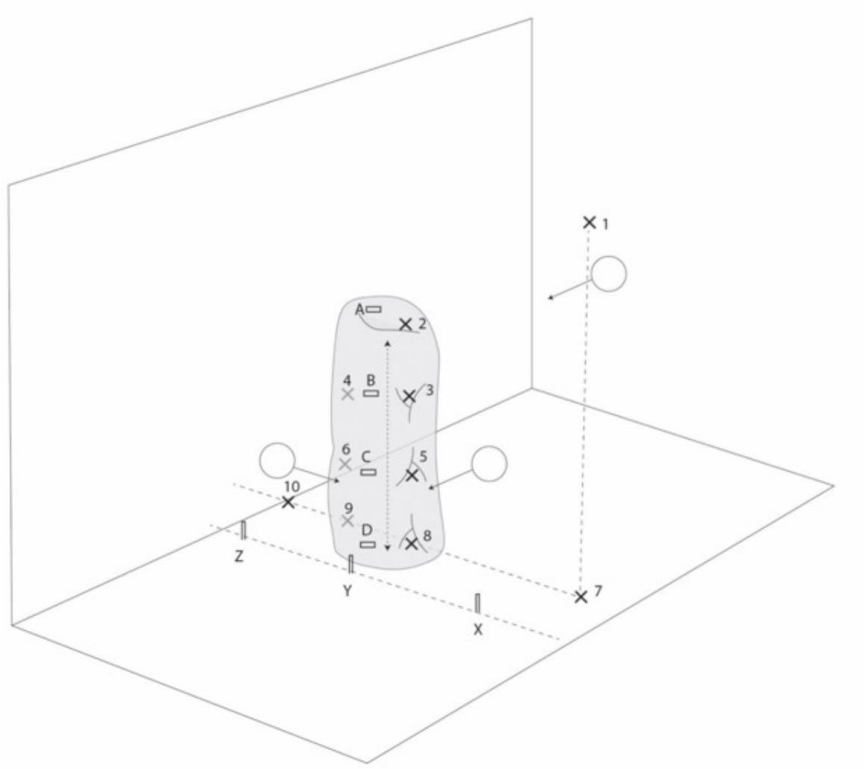

Figure S1. Visualization of the measurement locations on the outdoor reefs.

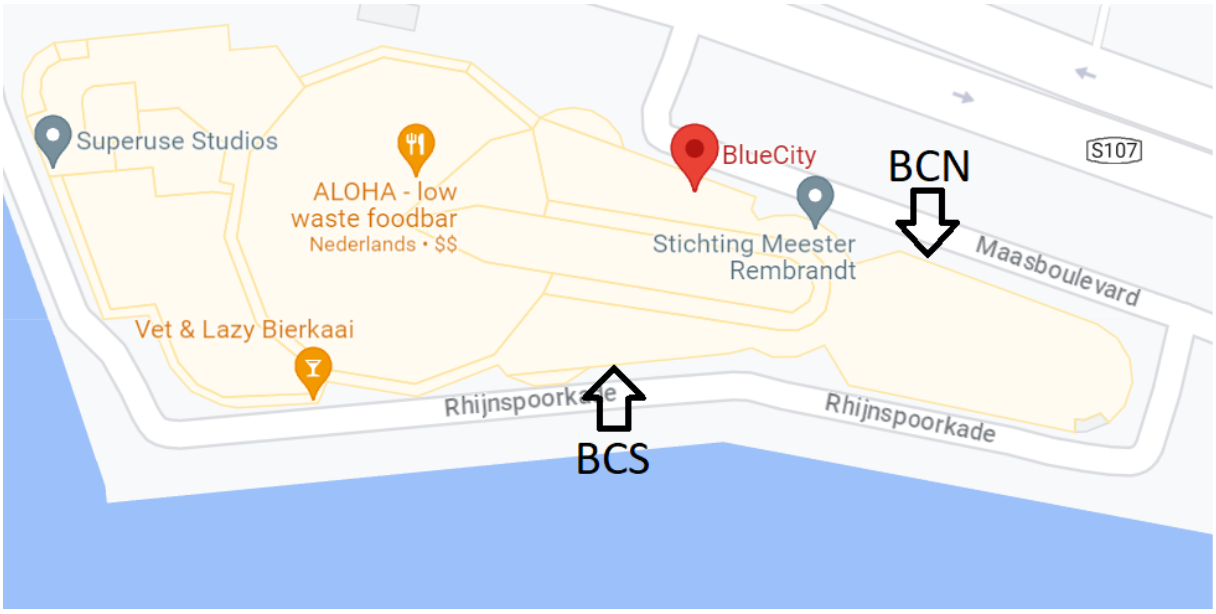

Figure S2. Map of the location of the BCN and BCS reefs at Blue City in Rotterdam.

100% PLANT-BASED. NATURALLY LACTOSE FREE

Gemiddelde voedingswaarde / Valeurs nutritionnelles moyennes	per / pour 100 g
Energie	212 kJ / 51 kcal
Vetten / Matières grasses	2,3 g
waarvan / dont verzadigde vetzuren / acides gras saturés	0,4 g
Koolhydraten / Glucides	2,1 g
waarvan / dont suikers / sucres	2,1 g
Vezels / Fibres alimentaires	1,0 g
Eiwitten / Protéines	4,0 g
Zout / Sel	0,25 g
Vitaminen / Vitamines:	
• D	0,75 µg*
• B12	0,38 µg*
Mineralen / Sels minéraux:	
• calcium	120 mg*

* = 15% van de voedingswaardereferenties / des valeurs nutritionnelles de référence

Karnemelk

Ingrediënten: KARNEMELK.

Voedingswaarde	100 ml	portie*	**	***
Energie	125 kJ / 29 kcal	250 kJ / 59 kcal	3%	
Vetten	0,1 g	0,2 g	<1%	
waarvan: verzadigd vet	0,1 g	0,2 g	1%	
enkelvoudig onverz. vet	0,0 g	0,0 g		
meervoudig onverz. vet	0,0 g	0,0 g		
Koolhydraten	3,6 g	7,2 g		
waarvan: suikers	3,6 g	7,2 g	8%	
Vezels	0,0 g	0,0 g		
Eiwitten	3,0 g	6,0 g		
Zout	0,09 g	0,19 g	3%	
Calcium	120 mg	240 mg		15%
Vitamine B2 (riboflavine)	0,15 mg	0,30 mg		11%

Portiegrootte	**1 flesje (300 ml)**	
	Per portie	**RI***
Energie	**540 kj**	**6%**
	129 kcal	
Vet	**0,00g**	**0%**
Verzadigd Vet	**0,000g**	**0%**
Koolhydraten	**10,50g**	**4%**
Suiker	**1,50g**	**2%**
Vezels	0,0g	
Eiwitten	**1,05g**	**2%**
Zout	**0,00g**	**0%**

Figure S3. Supplements used for the accessibility method.

Table S1. Nutrient mix 1 per 10l culture medium.

156g	Sodium bicarbonate
10 g	Salt
10g	Blugol hydroponic NPK fertilizer (nitrogen [N 8%], phosphorus [P 8%], potassium [K 6%] + micronutrients) + trace elements + EDTA, EDDHA)
15g	Masterblend hydroponic fertilizer ((nitrogen [N 4%], phosphorus [P 18%], potassium [K 38%], magnesium 0.2%, boron 0.02%, copper 0.4% manganese 0.2 %, molybdenum 0.01%, zinc 0.05%)+EDTA)
4g	Urea fertilizer
5g	Potassium sulfate

Table S2. Nutrient mix 2 per 10l culture medium.

156g	Sodium bicarbonate
10 g	Salt
10g	Blugol hydroponic NPK fertilizer (nitrogen [N 8%], phosphorus [P 8%], potassium [K 6%] + micronutrients) + trace elements + EDTA, EDDHA)
15g	Masterblend hydroponic fertilizer ((nitrogen [N 4%], phosphorus [P 18%], potassium [K 38%], magnesium 0.2%, boron 0.02%, copper 0.4% manganese 0.2 %, molybdenum 0.01%, zinc 0.05%)+EDTA)
4g	Urea fertilizer
5g	Potassium sulfate
10 ml	Phytoplankton solution

Appendix B – Supplemented results

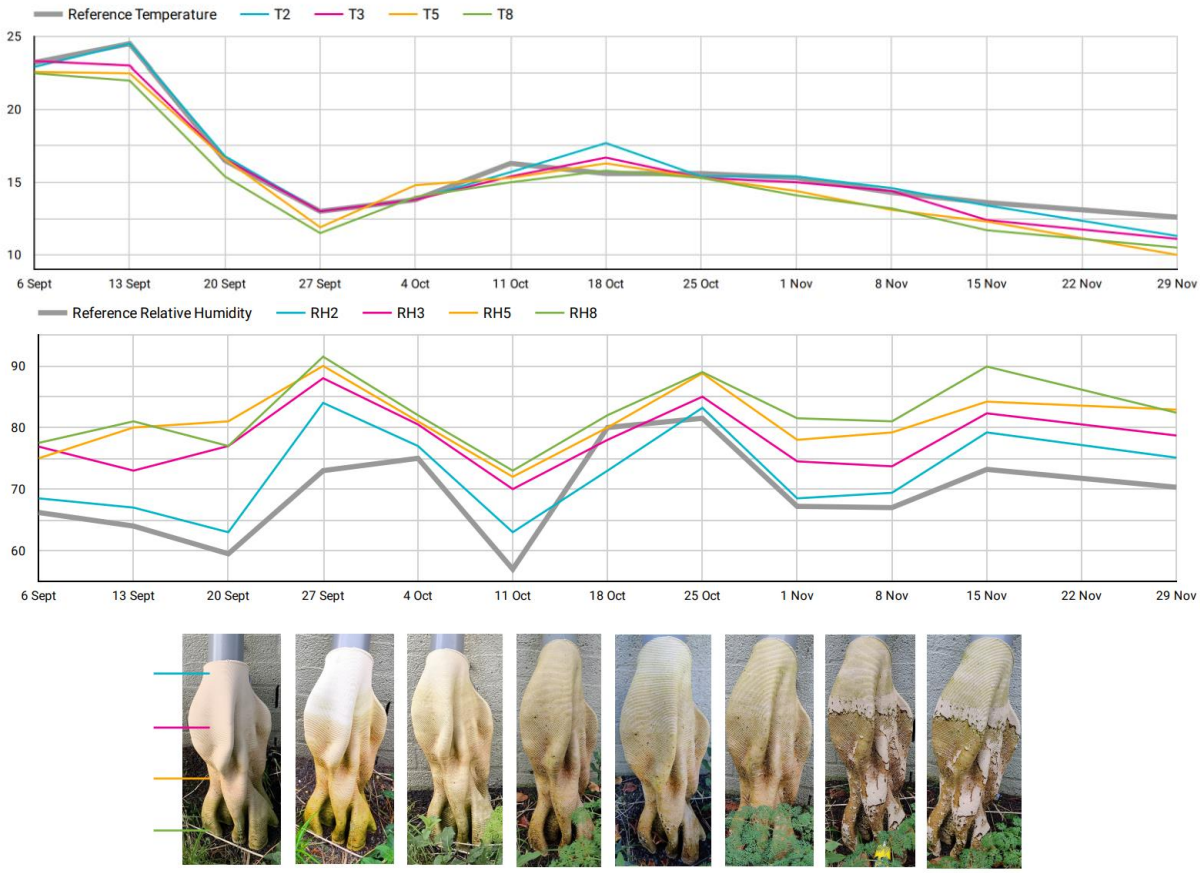

Figure S4. Overview of measurements performed on reef BCN in 2022. The reference line indicates the temperature and relative humidity that was measured in the air one meter away from the reef. The other lines (T2, RH2, T3, RH3, T5, RH5, T8, RH8) represent the temperature and humidity of the different measurement locations on the front side of the reef.

Figure S5. Overview of measurements performed on reef BCS in 2022. The reference line indicates the temperature and relative humidity that was measured in the air one meter away from the reef. The other lines (T2, RH2, T3, RH3, T5, RH5, T8, RH8) represent the temperature and humidity of the different measurement locations on the front side of the reef.

Table S3. Growth Index. The amount of organic growth per measurement location is indicated by the growth index of BCN and BCS in 2022. (0 = no vegetation visible, 1 = very thin layer of algae, 2 = thick layer of algae, 3 = very thick layer of algae (dark green/brown), 4 = moss growth, and 5 = vascular plants)

	BCN				BCS			
	G2	G3	G5	G8	G2	G3	G5	G8
6-9-2022	0	0	0	2	0	0	0	1
13-9-2022	0	0	1	2	0	0	1	2
20-9-2022	0	1	1	2	0	1	2	3
27-9-2022	0	1	1	2	0	1	2	3
4-10-2022	0	1	1	2	0	1	2	3
11-10-2022	0	1	2	2	0	1	2	3
18-10-2022	0	1	2	2	0	2	3	3
25-10-2022	0	1	2	3	1	2	3	3
1-11-2022	1	1	2	3	1	1	1	2
8-11-2022	0	1	2	3	0	1	2	0
15-11-2022	1	3	3	3	0	1	2	0
29-11-2022	1	3	3	3	0	1	2	0
Average	0.250	1.167	1.667	2.417	0.167	1.000	1.833	1.917

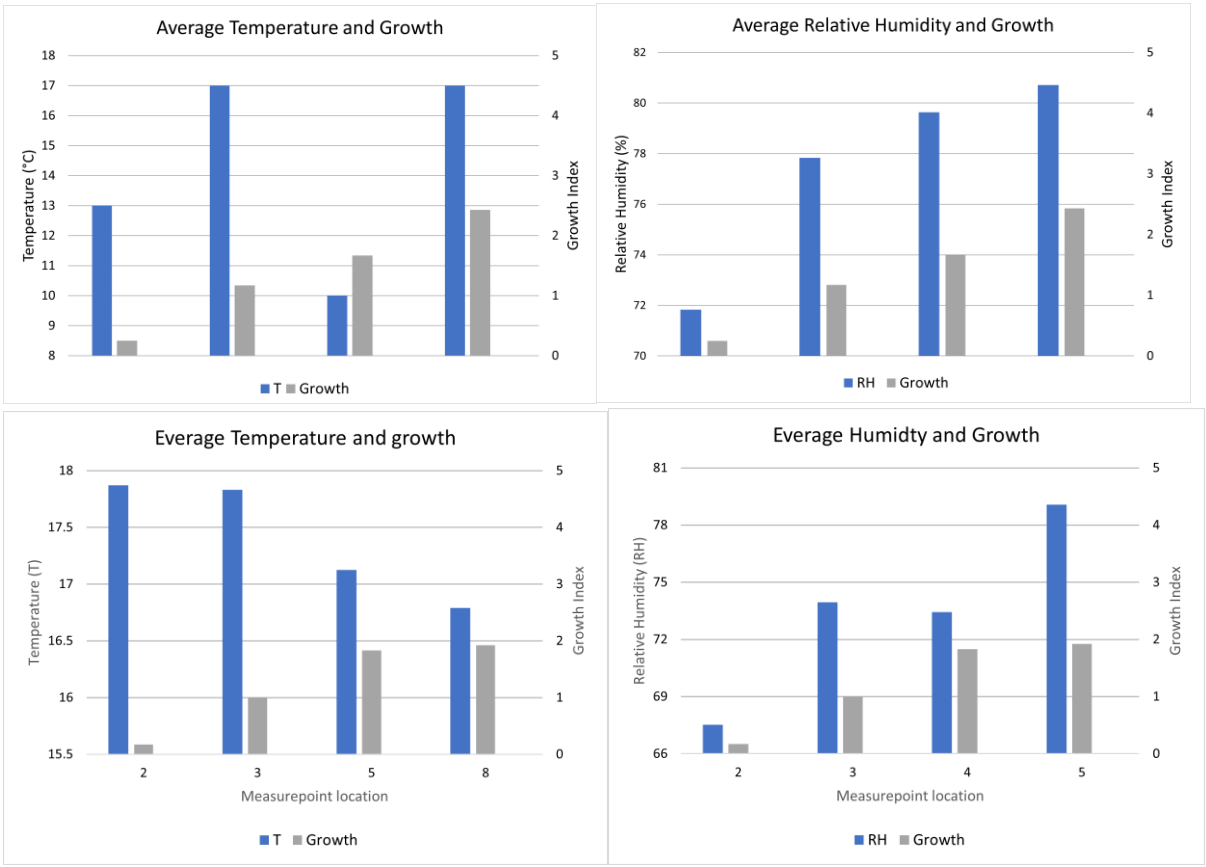

Figure S6. Relation between growth, temperature, and humidity of RainReefs 2022. A) shows the comparison between the temperature and the growth of BCN (r = 0.267) B) shows the comparison between the relative humidity and growth of BCN (r = 0.950). C) shows the comparison between the temperature and growth of BCS (r = -0.903). D) shows the comparison between the relative humidity and growth of BCS (r = 0.865).

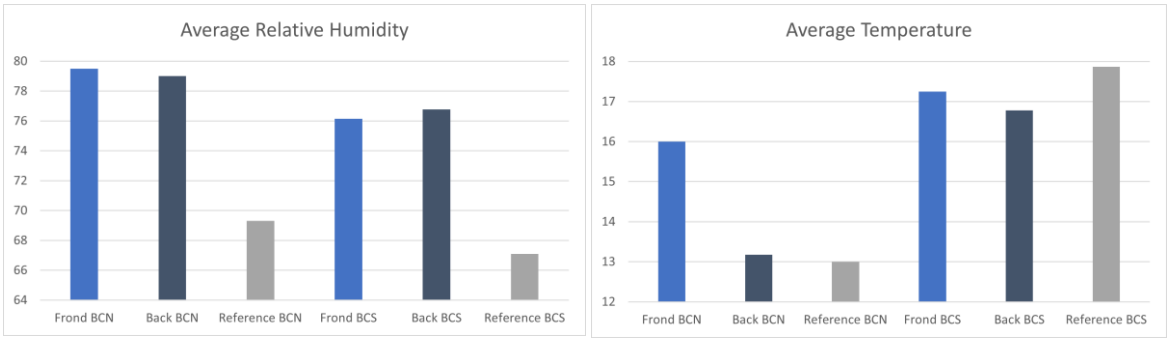

Figure S7. Comparison in temperature and relative humidity between the front and the backside of the reefs BCN and BCS in 2022.

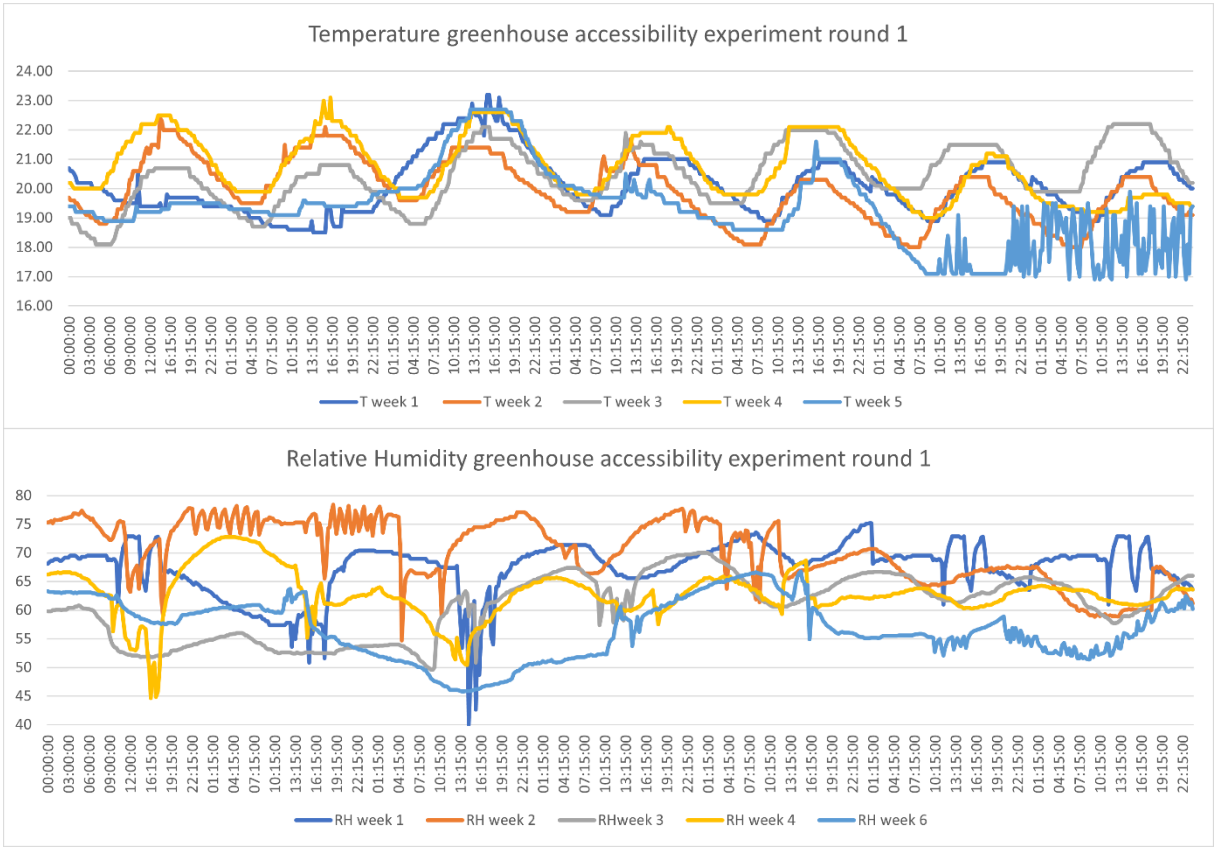

Figure S8. Temperature and Humidity of the greenhouse during accessibility experiment round 1

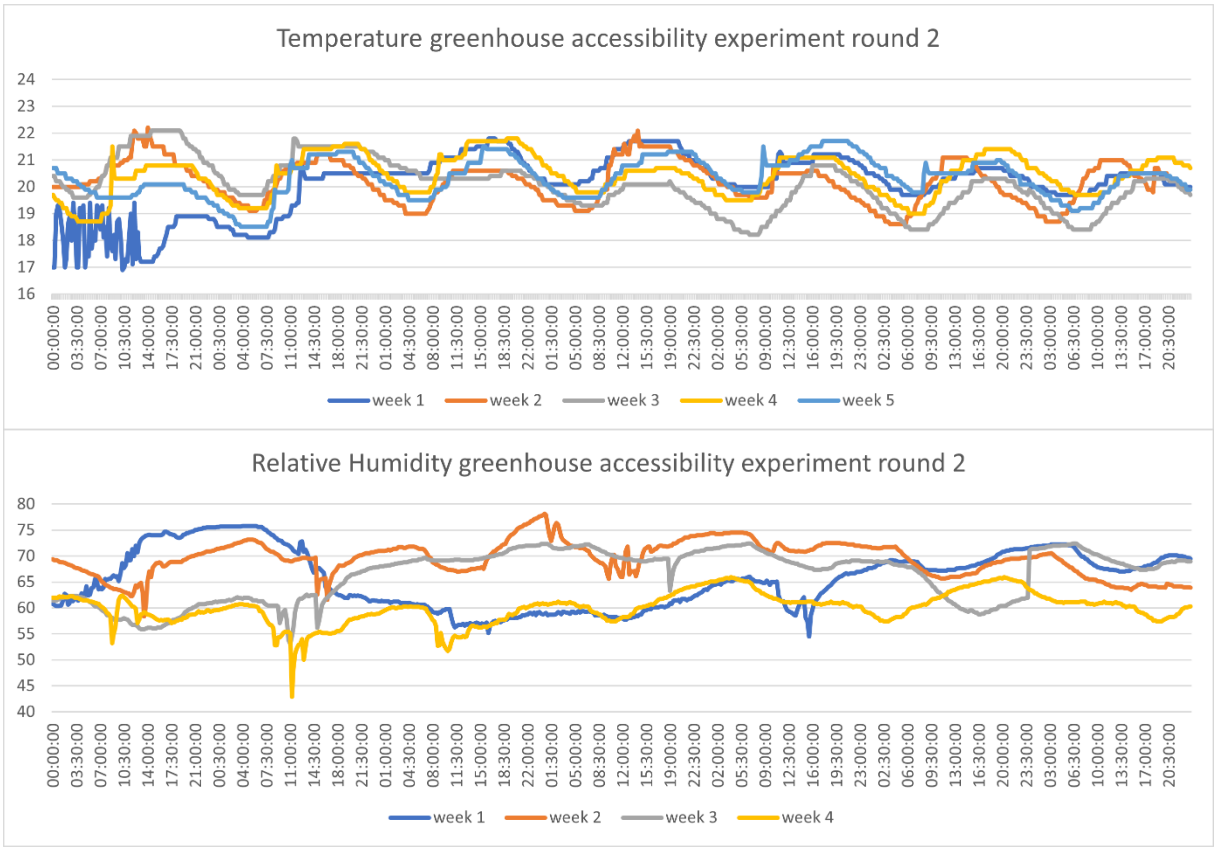

Figure S9. Temperature and humidity of the greenhouse during accessibility experiment round 2