SELECTED STUDIES (2014-2021)

THE EFFECTS OF ANALEMMA WATER ON PLANTS AND SOIL

RESEARCH REPORT

ANALEMMA

The cherry tomato study (2021)

In 2021, a series of experiments was performed on cherry tomato plants grown under different watering regimes with the purpose of assessing the **effects of Aňalemma Water on plant physiology and soil microbiome**. The most interesting results of this study are presented in the following sections.

Terrestrial plants are tightly linked to soil microorganisms (bacteria and fungi). The importance of soil microbiome is highlighted by the fact that plants invest somewhere between 11 and 40% of their photosynthetically fixed carbon as well as 10–16% of their total nitrogen into compounds used by the microorganisms surrounding their roots. In turn, bacteria and fungi actively participate in organic matter decomposition, thus releasing nutrients and furthering plant growth.
On the other hand, some pathogenic microorganisms (fungi) pose serious threat to plant health. Infestation of crops with pathogenic fungi can have disastrous effects on food production and, consequently, on global economy.

In this study, cherry tomato plants were treated with different watering treatments, including Aňalemma Water. **Significant effects of Aňalemma Water were found in several chemical parameters related to the nitrogen cycle in the soil**, linking Aňalemma Water with factors connected to soil fertility and productivity.

Additionally, **Aňalemma Water caused a reduction in a genus of pathogenic fungi, and an increase in bacterial diversity.** These results provide valuable insights into the effects of Aňalemma Water on soil fertility and biodiversity and light our path toward future research.

The cherry tomato study (2021)

EXPERIMENTAL DESIGN

Plant growth conditions: The study took place in a climate-controlled greenhouse located in Schiedam, The Netherlands. On Day 1, the soil was distributed over a concrete surface and larger chunks where crushed to achieve a more even grain size. The soil was randomly distributed into 24 plastic plant pots with a diameter of 39 cm and a height of 36 cm. The plates contained a central hole for drainage. The 6 week-old cherry tomato plants (grown by Kwekerij Poot, Schiedam, The Netherlands) were repotted from their original 10 cm pots into the 24 larger pots at random. The potted plants were kept in an isolated part of the greenhouse, not easily reachable by third parties. The study setup was randomized, with the 24 plants randomly divided into 6 groups (4 plants per treatment), designated to receive 6 different watering treatments. During the first 14 days, all plants received only Rainwater, which was dosed using an automated system. Each plant received 100 mL of rainwater at 7 a.m. CEST.

Water treatments: Two types of water were tested in this treatment, named Water 1 and Water 2. **In the following text, Aǹalemma Water will refer specifically to Water 1**. Coherent water was prepared about a month prior to beginning of the study and stored in standard water jerrycans. On Day 15, the plants belonging to the same treatment group where placed next to each other to allow easier treatment and measurements. The treatments were as follows: (1) Rainwater (2) Rainwater + organic fertilizer, (3) Aǹalemma Water, (4) Aǹalemma Water + organic fertilizer (5) Water 2, (6) Frequency water. In treatments 2 and 4, approximately 250-300 g of organic fertilizer (Fa. Orgapower, Amersfoort, NL) was used per plant. From Day 15 onward, each plant received 400 mL of the assigned water daily at 7 a.m. CEST using the automated dosing system.

INSTITUTIONS

Water & Light B.V. (Amersfoort, The Netherlands)

Kwekerij Poot (Schiedam, The Netherlands)

Chemical composition of soil

ANALYSIS OF CHEMICAL COMPOSITION

On Day 2 of the experiment, a baseline sample of the soil used in this study was collected and delivered to the Groen Agro Control laboratory (Delfgauw, The Netherlands). At the end of the growth period (Day 118), soil samples were collected from the 24 pots in which cherry tomato plants were grown under specific watering regimes. Soil samples were delivered to the Groen Agro Control laboratory (Delfgauw, The Netherlands). Chemical composition was measured for all soil samples.

RESULTS

Since more than 20 parameters were measured using 7 different groups of soil samples (Baseline at Day 2 + 6 treatments at Day 118), a large dataset was obtained. For simplicity, the data shown here was reduced to only the most relevant information. The chemical parameters measured for three groups, Baseline, Aȟalemma Water and Rainwater, are shown in different graphs in **Figure 1**. The measuring units of each parameter are listed in **Table 1**.
The majority of parameters remained largely unchanged between the Aȟalemma Water and Rainwater group. However, several parameters pointed toward interesting changes ocurring in these two groups. Firstly, **iron content was higher in Aȟalemma Water group compared to control**. Next, interesting differences were found in parameters linked to availability and utilization of soil nitrogen. These results are shown individually in **Figure 2**. **Total nitrogen and Nitrogen delivery capacity were significantly higher in the Aȟalemma group compared to Rainwater.** Total nitrogen is the major indicator of soil fertility and quality in an agricultural ecosystem, while the nitrogen delivery capacity is the amount of nitrogen from organic matter that can be made available to plants over a longer period of time.
Nitrogen is an essential plant nutrient, but the majority of soil nitrogen appears in different organic forms which plants are unable to take up into their cells. However, plants can readily take up mineral forms of nitrogen, such as nitrates. Soil microorganisms convert organic forms of nitrogen to mineral forms through a process called mineralization, which involves decomposition of organic matter.

INSTITUTIONS

Groen Agro Control (Delfgauw, The Netherlands)

Normec Groen Agro Control

Chemical composition of soil

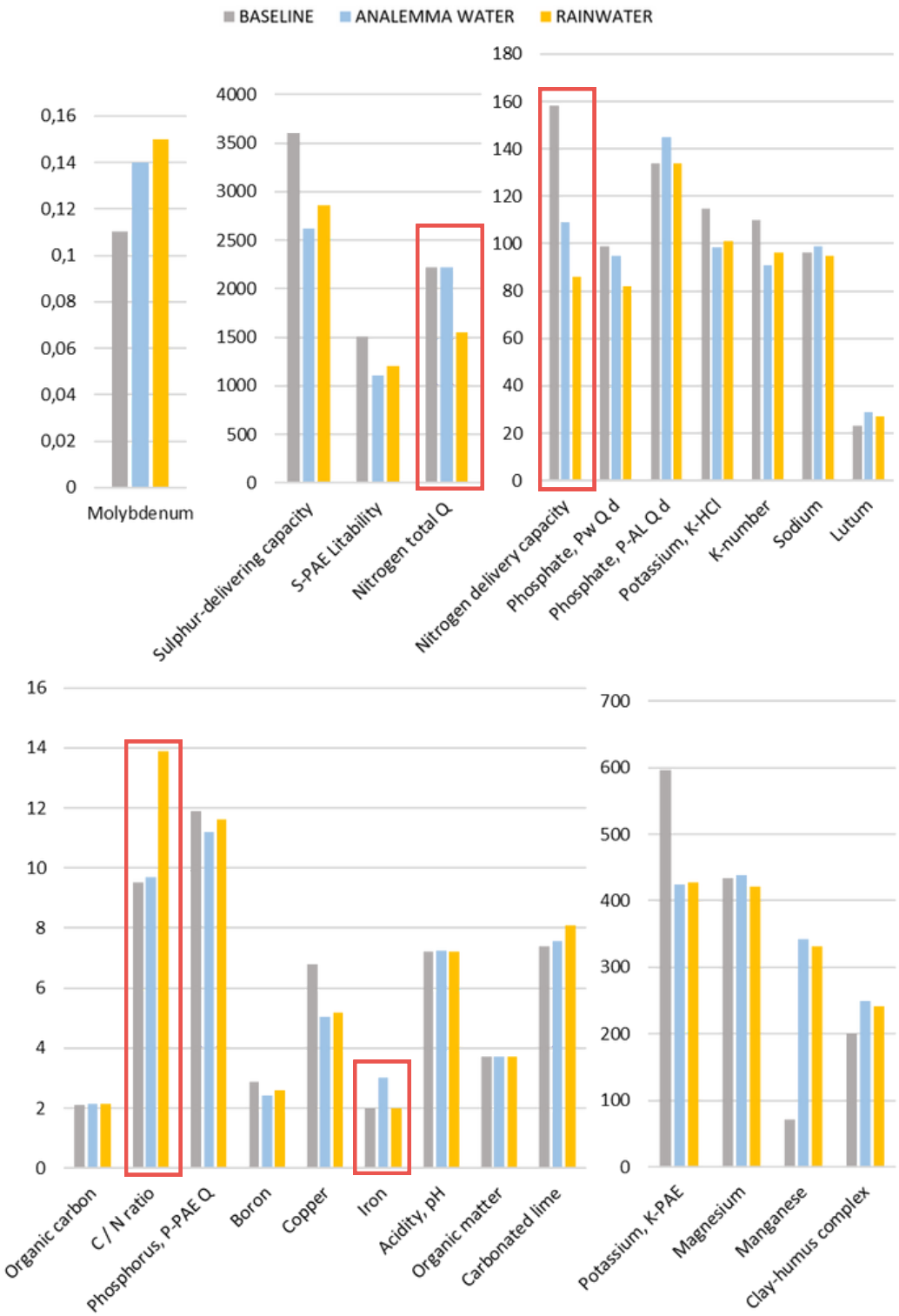

Figure 1. Mean values of chemical parameters measured for three groups of soil samples: Baseline, Aṅalemma Water and Rainwater (Control). Baseline samples were obtained on Day 2, and treated samples on Day 118 of the experiment (n=4). The measuring units are listed in **Table 1**. The parameters are grouped according to similarity in value. Red boxes indicate parameters with differences between Aṅalemma Water group and Rainwater group.

Chemical composition of soil

A parameter referred to as the carbon to nitrogen ratio (C/N ratio) provides information on how easily nitrogen can be released from organic matter. A low C/N ratio indicates high levels of nitrogen in organic matter, and a high level of decomposition and mineralization. **Here, the C/N value of soil treated with Aňalemma Water was significantly lower than in soil treated with Rainwater (Figure 2), indicating higher mineralization rates.**
These results indicate that Aňalemma Water has a strong impact on the nitrogen cycles which underlie soil fertility. In the future, it will be exciting to broaden these findings with more in-depth research on the effects of Aňalemma Water on plant physiology and crop yield, as both are affected by changes in nitrogen availability.

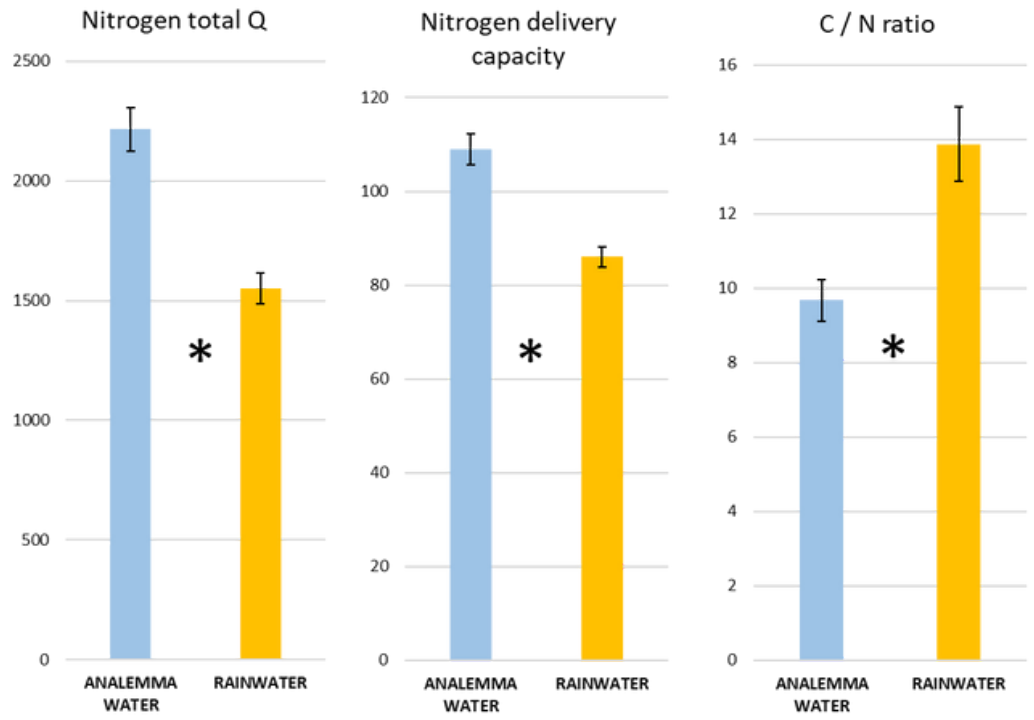

Figure 2. Differences in total nitrogen, nitrogen delivery capacity and carbon to nitrogen ratio (C/N ratio) between soil watered with Aňalemma Water and Rainwater (Day 118). The measuring units of each parameter are listed in **Table 1**. The bars show mean ± SD values (n=4). Asterisks indicate statistical significance (Student's T test, $p < 0,05$).

Chemical composition of soil

Table 1. The measuring units of 24 chemical parameters measured in the study, describing the values showed in **Figures 1** and **2**.

PARAMETER	UNIT
Organic carbon	% C
C / N ratio	-
Phosphorus, P-PAE Q	mg P/kg
Boron	mg B/kg
Copper	mg Cu/kg
Iron	mg Fe/kg
Molybdenum	mg Mo/kg
Acidity, pH	
Organic matter	%
Carbonated lime	%
Nitrogen delivery capacity	kg N/ha per year
Phosphate, Pw Q d	mg P2O5/L
Phosphate, P-AL Q d	mg P2O5/100 g
Potassium, K-HCl	mg K2O/100 g
K-number	-
Potassium, K-PAE	mg K/kg
Magnesium	mg MgO/kg
Sodium	mg Na/kg
Manganese	mg Mn/kg
Clay-humus complex (CEC)	mmol+/kg
Lutum	%
Nitrogen total Q	mg N/kg
S-PAE Litability	mg S/kg
Sulphur-delivering capacity	kg S/ha per year

Effects on fungal properties

ANALYSIS OF FUNGAL COMPOSITION

At the end of the growth period (Day 118), soil samples were collected from the 24 pots in which cherry tomato plants were grown under specific watering regimes. Soil samples were delivered to the Groen Agro Control laboratory (Delfgauw, The Netherlands). Analysis of fungal properties of the soil consisted of measuring the value known as colony forming unit per gram of ground (CFU/g). The CFU value refers to the number of viable microbial cells in a sample.

RESULTS

The results of the analysis are presented in **Table 2**. While several groups of fungi were identified on the genus level (denoted by the name of the genus followed by "sp."), others were grouped together as "other molds". Both the total number of fungi and the total number of other molds followed a similar pattern, with highest number detected in soil treated with Rainwater and the lowest number in soil treated with Rainwater supplemented with fertilizer, followed by Aňalemma Water (**Figures 3** and **4**). Since fungal communities in the soil and their relationships with different plants are highly complex, these results do not offer immediate conclusions. However, by looking at individual genera (**Figure 5**), it appears that the fungi from the genus *Cladosporium* were less abundant in soil treated with Aňalemma Water than Rainwater. This is interesting with regards to the known pathogenic nature of different species from this genus. *Cladosporium* fungi can cause black point in cereal species, they cause scab in *Cucurbita* species, and brown leaf spots in tomato. In humans, they mainly cause allergic reactions which sometimes lead to asthma. **The reduction in soil *Cladosporium* after Aňalemma Water treatment points to a beneficial role of Aňalemma Water in maintaining a healthy soil microbiome**. These results are an important step in the direction of further and more specific analyses of the effects of Aňalemma Water on the fungal properties of soil.

INSTITUTIONS

Groen Agro Control (Delfgauw, The Netherlands)

Normec Groen Agro Control

Effects on fungal properties

Table 2. Fungal CFU/g for all watering conditions and individual plants. Red text color indicates values above the upper detection limit (5000). Blue text color indicates values below the lower detection limit (100). For easier reading, different value categories were highlighted as follows: 10-1000 CFU/g representing a low amount of fungal cells (highlighted in yellow), 1000-100 000 CFU/g representing an average amount of fungal cells (highlighted in green), and more than 100 000 CFU/g representing a high amount of fungal cells (highlighted in red).

	CFU/g	total fungi	*Penicillium sp.*	*Cladosporium sp.*	*Trichoderma sp.*	*Fusarium sp.*	other molds	yeasts	*Pythium sp.*	*Phytoph-thora sp.*
Rain water	B1	160 000	10 8000	20 000	> 5 000	< 100	52 000	<100	+	-
	B2	70 000	1 000	5 000	3 000	2 000	59 000	<100	+	-
	B7	37 000	11 000	3 000	3 000	1 000	19 000	<100	+	-
	B8	31 300	300	< 100	3 000	300	2 500	<100	+	-
Rain water with organic fertilizer	B15	4 800	8 000	< 100	4000	5 000	3 100	<100	+	-
	B16	29 400	7000	< 100	400	3 000	19 000	<100	+	-
	B23	41 000	13 000	2 000	400	2 000	24 000	<100	+	-
	B24	21 000	8 000	3 000	1 000	1 000	8 000	<100	+	-
Water 1	T3	18 000	< 100	2 000	1 000	3 000	13 000	<100	+	-
	T4	27 200	8 000	2 000	2 000	200	15 000	<100	+	-
	T5	44 300	10 000	< 100	300	3 000	31 000	<100	+	-
	T6	40 000	6 000	< 100	> 5 000	3 000	31 000	<100	+	-
Water 1 with organic fertilizer	T9	48 300	4 000	2 000	300	3 000	39 000	<100	+	-
	T10	60 000	7 000	< 100	> 5 000	4 000	49 000	<100	+	-
	T17	42 000	11 000	4 000	2 000	3 000	22 000	<100	+	-
	T18	51 000	7 000	6 000	5 000	3 000	35 000	<100	+	-
Water 2	T11	26 300	8 000	1 000	300	1 000	16000	<100	+	-
	T12	40 200	16 000	5 000	200	3 000	16000	<100	+	-
	T21	42 000	8 000	2 000	6 000	2 000	24000	<100	+	-
	T22	40 000	10 000	3 000	> 5 000	6 000	21000	<100	+	-
Frequency water	T13	57 000	15 000	2 000	1 000	1 000	38 000	<100	+	-
	T14	47 000	6 000	6 000	> 5 000	6000	29 000	<100	+	-
	T19	45 000	5 000	15 000	2 000	3 000	2 0000	<100	+	-
	T20	95 000	23 000	16 000	> 5 000	2 000	54 000	<100	+	-

Effects on fungal properties

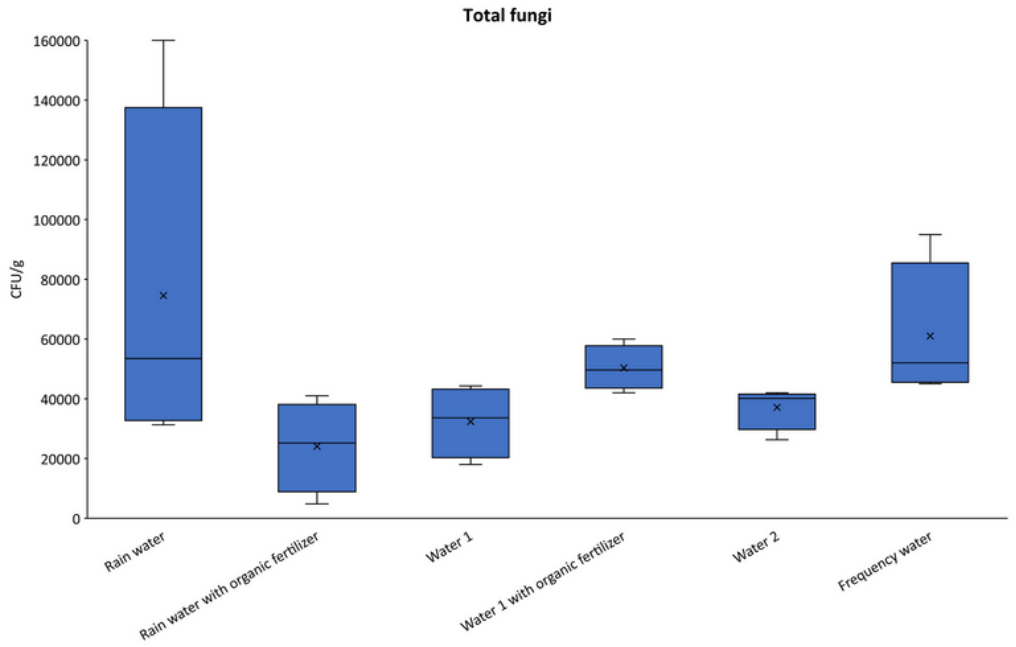

Figure 3. Total fungal community from soil samples obtained after the growth period of cherry tomato plants watered with different watering treatments. Each box plot summarizes CFU/g of four samples per watering condition.

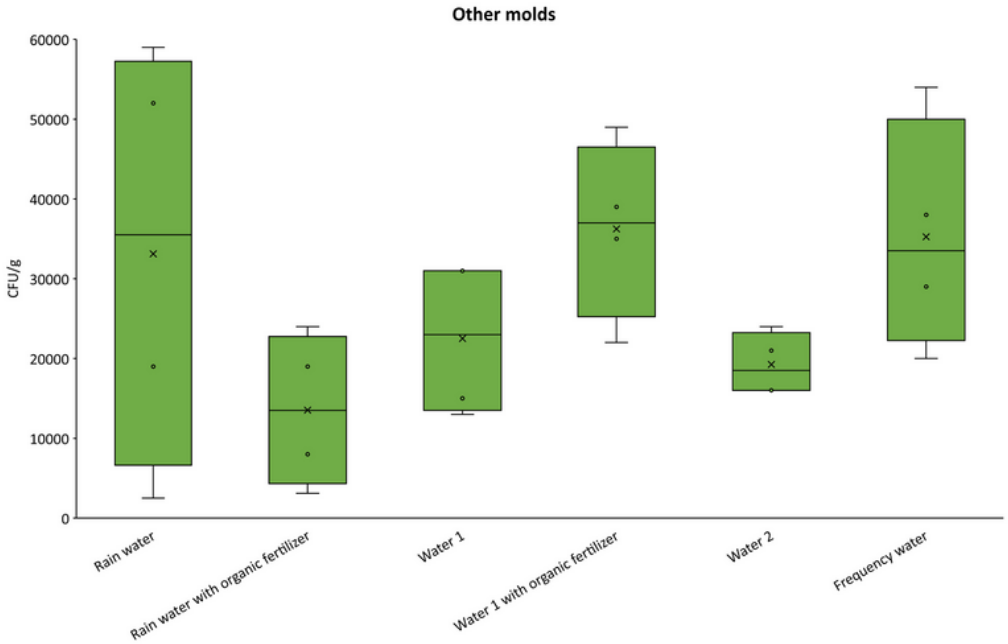

Figure 4. Total fungal community from soil samples obtained after the growth period of cherry tomato plants watered with different watering treatments. The graph shows fungal communities not characterized on the genus level but grouped together as a separate category of "other molds". Each box plot summarizes CFU/g of four samples per watering condition.

Effects on fungal properties

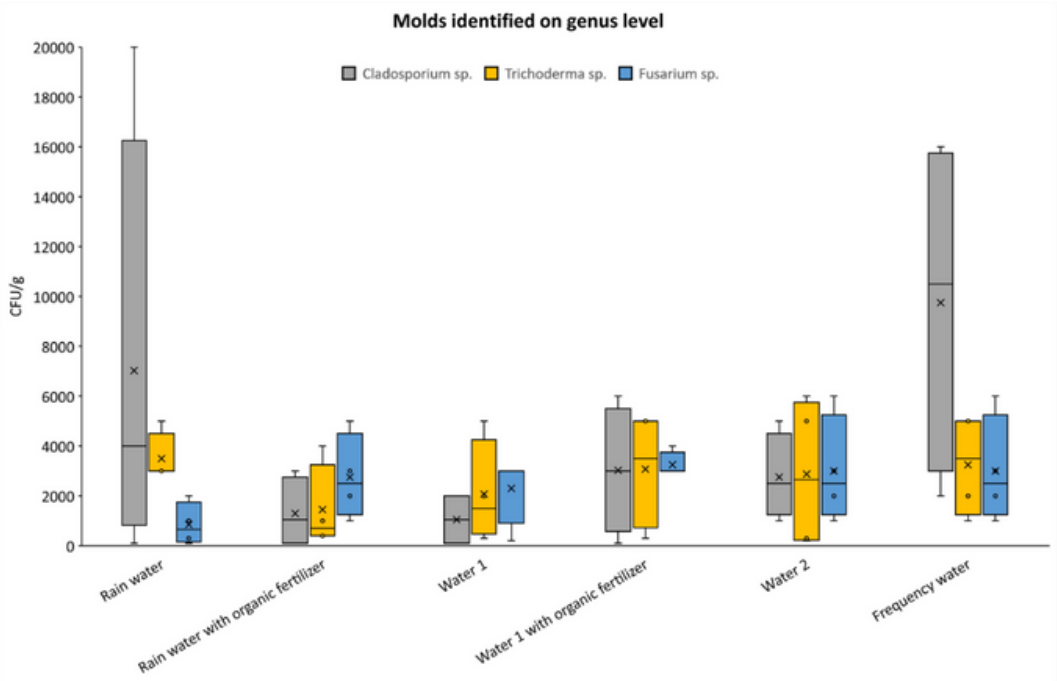

Figure 5. Fungal communities of three genera: *Cladosporium* sp. (grey), *Trichoderma* sp. (yellow), and *Fusarium* sp. (blue), measured in soil samples obtained after the growth period of cherry tomato plants watered with different watering treatments. Each box plot summarizes CFU/g of four samples per watering condition.

Analysis of bacterial composition and diversity

Next-generation sequencing (NGS) is the latest sequencing technology used to detect nucleotide sequences of entire genomes or targeted regions of DNA or RNA. The most commonly used marker for evaluation of bacterial composition of a sample is the 16S rRNA gene. Total DNA is first extracted from a sample such as soil, followed by amplification of a specific region in the 16S rRNA gene. Next, all amplified fragments are sequenced and these sequences are then compared with a vast database of 16S rRNA sequences from known species of bacteria, also known as reference sequences.

Using this method, the samples can be screened for presence of a large number of different bacterial species (or, more commonly, groups of similar species; taxa). Additionally, the change in bacterial diversity can be analyzed between different samples.

BACTERIAL DIVERSITY

NGS provides two common types of diversity indexes: **alpha diversity and beta diversity**.

Alpha diversity tells us how many different species are detected in each sample ("species richness") and also about how abundant each species is ("evenness"). For example, it can tell us that sample A contains 5 different species of bacteria, while sample B contains only 3. Alpha diversity is greater in sample A. Alpha diversity does not look into the identity of species observed in each sample, only the number.

Beta diversity measures the change in species diversity between two or more samples. It counts the total number of species that are unique to each of the samples being compared. Beta diversity provides information about species identity.

Analysis of bacterial composition and diversity

NEXT-GENERATION SEQUENCING

At the end of the growth period, soil samples were collected from 8 pots in which cherry tomato plants were grown under different watering regimes: Rainwater Control (n=4), and Rainwater treated with Aňalemma Water (n=4). Soil samples were delivered to the BaseClear laboratory (Leiden, The Netherlands) for analysis of bacterial composition and diversity. This analysis was performed by next-generation sequencing using the 16S rRNA marker. The measured parameters were as follows: **alpha diversity** (Observed richness, Shannon's index and Simpson's index), **beta diversity** (Redundancy analysis; RDA), **association testing** (Differential Abundance Analysis; DAA) and **Linear discriminant analysis Effect Size** (LEfSE). Besides providing statistical data, these analysis indicate existence of **Key Biomarker and Signature Species** in a given microbiome dataset.

RESULTS

The NGS bacterial profiling data showed that **alpha diversity significantly increased in soil treated with Aňalemma Water compared to Rainwater Control (Figure 6)**. This indicates that the number of different bacterial taxa and their abundance was larger in soil samples treated with Aňalemma Water. Beta diversity, measured by the RDA analysis, was not significantly different between water treatments.
Using machine learning methods (DAA and LEfSe), the two groups of samples were analyzed for differences in low abundant bacteria (i.e., bacterial genera whose relative abundance is less than 2%). Although these results did not reach statistical significance, they did indicate several genera which were differentially associated with the use of specific water treatments (Table 3). These results give insights into possible specific effects of Aňalemma Water treatment on soil bacteria. **For instance, bacteria of the genus *Chelativorans*, which was associated with the Aňalemma Water treatment, were recently shown to decrease the toxicity of antimony and its uptake into rice, thus serving an ecological role in remediation of soil contamination with toxic elements**. This finding would be very interesting to look into in the future.

INSTITUTIONS

BaseClear B.V. (Leiden, The Netherlands)

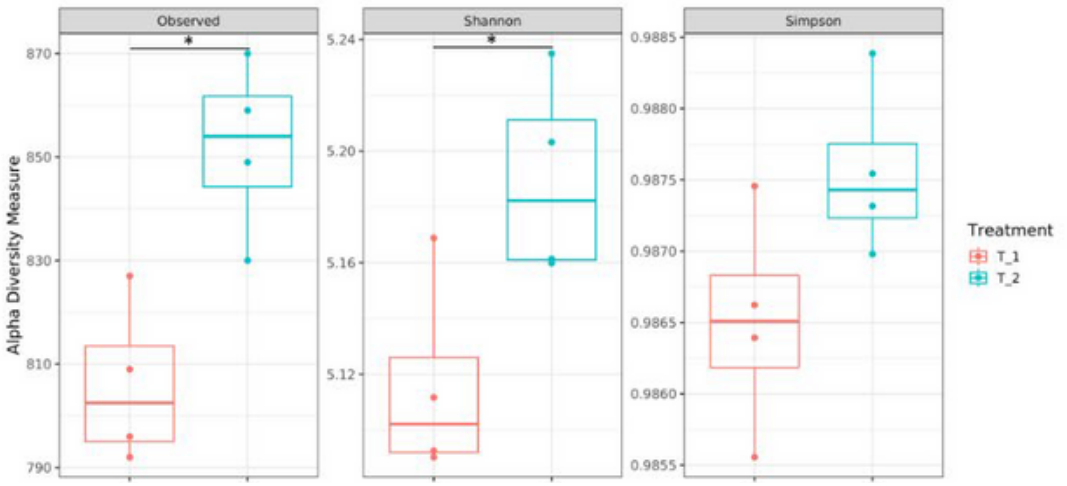

Figure 6. Results of 16S profiling. Alpha diversity was higher in soil treated with Aȟalemma Water (blue boxplots on the right) compared to Rainwater (red boxplots on the left). Alpha diversity was measured using three indices. Statistical significance was measured in Observed richness (left panel) and Shannon's index (middle panel), $p < 0.05$.

Table 3. Results of 16S profiling. Listed in the table are results of alpha and beta diversity analysis, and Bacterial Biomarker Genera (low-abundant species) differentially found in the Aȟalemma Water group using machine learning methods (DAA and LEfSe).

Compared groups	Alpha diversity, p<0.5	Beta diversity p<0.5	Bacterial Biomarker Genera
Control: Rainwater **Test Water**: Aȟalemma Water	**YES – higher in Aȟalemma Water group (Observed richness + Shannon's index)**	**NO**	Aȟalemma Water: *Thermomarinilinea*, *Chelativorans*, *Hydrogenophaga*, *Pseudoxanthomonas*

BIOPHOTON EMISSION RESEARCH (2014-2018)

The following studies were based on biophoton emission analysis of plants treated with Aṅalemma Water. Biological organisms continuously emit ultra-weak photon emission, also known as biophoton emission. Biophotons are generated via relaxation of electronic excited states in the course of oxidative metabolic processes and oxidative stress reactions which regularly occur in living organisms. This phenomenon has been observed in virtually all metabolically active systems, from the level of bacteria and fungi, across germinating seeds and whole plants, to animal tissue cultures and whole organisms, including human beings.

Because the phenomenon of biophoton emission reflects oxidative processes, either metabolic or stress-related, it can be widely used as a non-invasive tool for monitoring the physiological state of biological systems.

RESEARCH REPORT
JANUARY 2023

MEASURING BIOPHOTON EMISSION

Biophotons are emitted by organisms in the wavelength range from 200 to 800 nm. Biophoton emission analysis can be divided into two categories: **spontaneous emission** (SE), also called ultra weak photon emission (UPE), and **delayed luminescence** (DL).

Both of these properties, SE and DL, were analyzed in the following experiments using low-noise photomultiplier tubes and highly sensitive charge coupled device cameras.

Biophoton emission of tomato fruits (2018)

EXPERIMENTAL DESIGN

Plant treatment: Plants were grown in standard soil in climate-controlled greenhouse conditions. Plants were divided into two groups according to the watering treatment (**Figure 1**). One group was watered with untreated tap water (Control, n=3) and the other group with Aňalemma-treated tap water (Aňalemma Water; Treated, n=3). Plants were grown until the fruit ripening stage, producing red tomatoes.

Sampling and biophoton emission analysis: Fruit samples were harvested on 8 different days in the period between 16/07/2018 and 08/08/2018. On every harvest day, one fruit was harvested per plant according to a predefined protocol. The fruit was immediately used for biophoton emission analysis (DL and SE). After the DL measurement, the tomatoes were dark-adapted for more than 1.5 hours and subsequently their SE signals were recorded. The DL data was analyzed using 3 different models, and SE data using 2 different models. The models used for statistical analysis of biophoton emission data are discussed in **Appendix B**.

Figure 1. Plant watering treatment setup in a climate-controlled greenhouse. Tomato plants were grown until the fruit-ripening period and watered with either untreated tap water (Control, left) or Aňalemma-treated water (right). This was followed by tomato fruit harvesting and biophoton emission analysis.

INSTITUTIONS

Water & Light B.V. (Amersfoort, The Netherlands)

RESULTS

The DL and SE analyses showed that watering treatment strongly influenced the energy storage properties of tomato fruits. Using three different statistical models each with its own set of parameters, it was shown that tomato fruits of plants watered with Aȟalemma Water exhibited significantly different DL profiles than fruits of plants watered with untreated water. Statistical differences were found in the majority of parameters of all three models (Table 1). For easier visualization of these differences, two parameters, namely DL_Mean and T, are shown individually in Figure 2.

To conclude, higher average amount of delayed luminescence indicates **higher energy storage capacity of fruits produced by plants treated with Aȟalemma Water**.

Additionally, the two water treatments resulted in several differences in biophoton emission (SE). One of the parameters shown to be statistically different between treatments (SE Strength) is shown in Figure 3. Larger spontaneous biophoton emission measured in fruits of plants treated with Aȟalemma Water indicate **increased mitochondrial activity and slower biological aging**.

FOR DETAILS, SEE **APPENDIX B**

Table 1. Differences in DL parameters between tomato fruits of plants treated with Aȟalemma Water and Control. Change values (t) are shown for all parameters of the 3 models. Significant differences are highlighted in green ($p < 0.05$).

	Parameter	t-value	p-value
MODEL 1	DL_Mean	-2.69389	0.007701
	DL_SD	-2.67917	0.008035
	I0	-2.70251	0.007512
	Tau	-0.05415	0.956876
	Beta	-2.41271	0.016795
	T	1.98655	0.048425
	R1	-2.72293	0.007080
MODEL 2	Y0	-3.02229	0.002858
	A1	-2.70618	0.007433
	12	-2.62002	0.009511
	t1	0.92529	0.356001
	t2	1.01676	0.310574
	R2	-2.35394	0.019608
MODEL 3	A	-2.47811	0.014090
	B	1.07146	0.285338
	C	2.55766	0.011327
	R3	-2.68746	0.007846

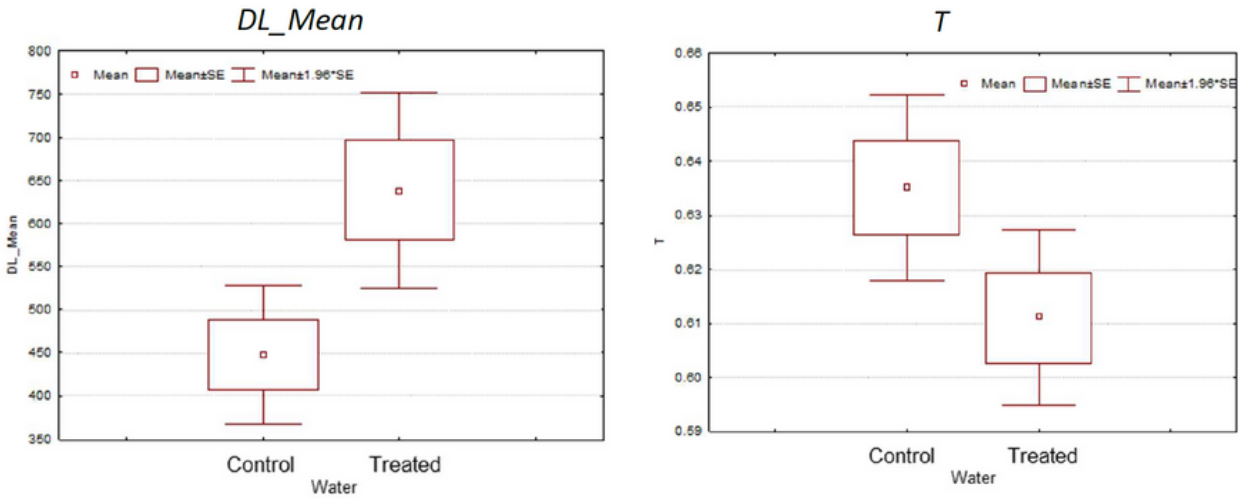

Figure 2. Means and variances for parameters DL_Mean and T of the hyperbolic decay model (Model 1) used for delayed luminescence (DL) analysis. The parameters show statistically significant differences in DL measured between tomato fruit samples of plants grown under two different watering treatments: non treated tap water (Control) and Aňalemma-treated tap water (Treated).

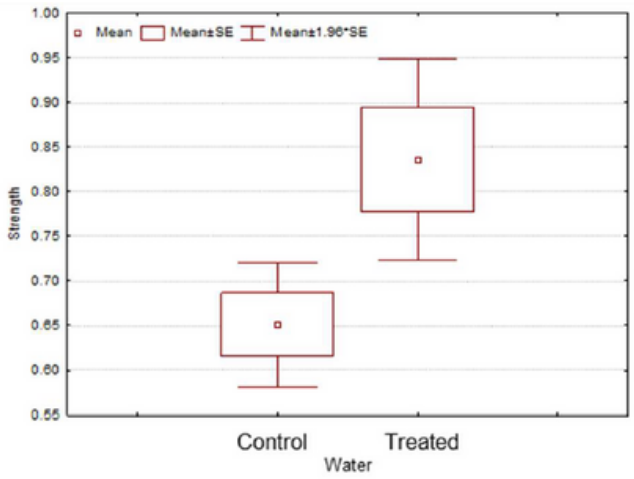

Figure 3. Means and variances for parameter Strength of the fractality model used for spontaneous emission (SE) analysis. This parameter shows statistically significant difference in SE intensity measured between tomato fruit samples of plants grown under two different watering treatments: non treated tap water (Control) and Aňalemma-treated tap water (Treated).

Biophoton emission of wheat seeds (2014)

EXPERIMENTAL DESIGN

Seed germination: Wheat seeds were placed in three square 12x12cm Petri dishes filled with 25 mL of either untreated tap water (Control), Aȟalemma Water (Treated) or Test Water 2 (Treated2) and allowed to germinate.

Biophoton emission analysis: After two days of germination, wheat seedlings were transferred to 15 cuvettes (5 for each water type). One cuvette was left empty for background measurements. Delayed luminescence was measured using a photon counting system with a multiplier tube in the spectral range of 160-870 nm. In all experiments, samples were placed in random order in the carrousel. Delayed luminescence was measured during 2 days. In total, 9 experiments were performed from March to June 2014, using 25 seeds per experiment. DL50-100 values were measured for each sample and plotted on graphs. The DL50-100 value corresponds to the mean value between the 5th and 10th second after excitation.

RESULTS

In 7 out of 9 cases, the seedlings germinated in Aȟalemma Water exhibited the highest average DL50-100 values (results of one exemplary experiment shown in **Figure 4**). This indicates that **seeds germinated in Aȟalemma Water have the highest energy storage capacity**, which is directly linked to oxidative processes underlying these highly sensitive stages of early plant life.

INSTITUTIONS

Water & Light B.V. (Amersfoort, The Netherlands)

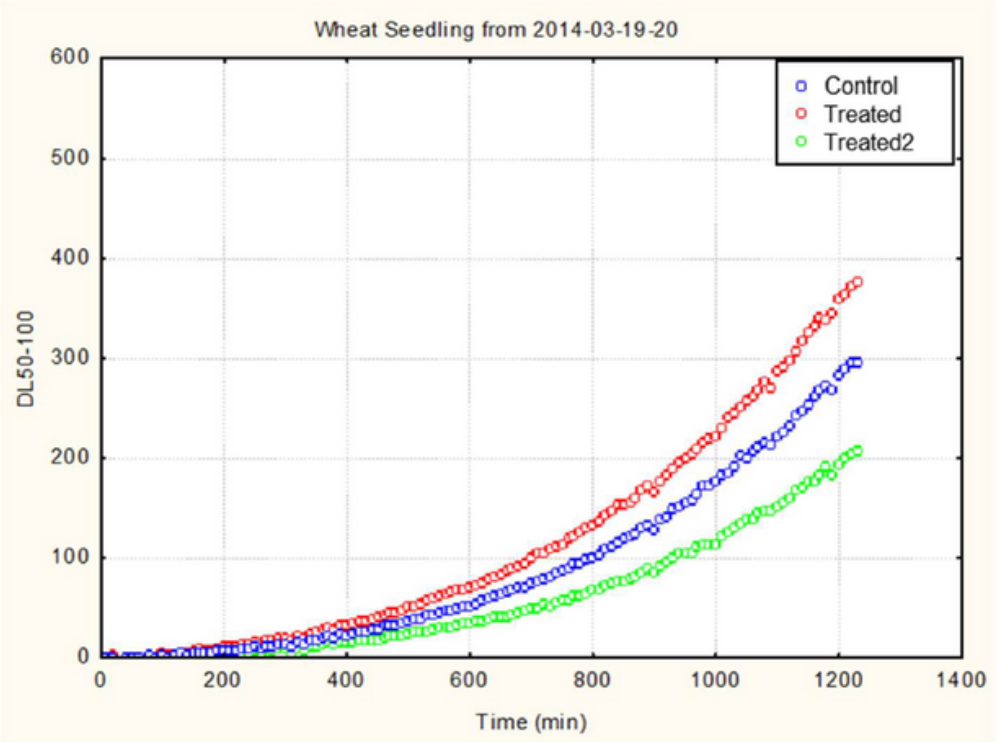

Figure 4. DL50-100 values of wheat seedlings germinated using untreated tap water (Control, blue circles), Aňalemma Water (Treated, red circles) or Test Water 2 (Treated2, green circles). Seeds treated with Aňalemma Water produced the highest DL50-100 values. This graph shows results of one representative experiment. A similar trend was observed in 7 out of 9 experiments performed during the course of the study.

Long-term biophoton emission of wheat (2014)

EXPERIMENTAL DESIGN

Plant treatment: Mature wheat seeds were harvested from adult plants grown in standard conditions. Seeds were distributed on soil and kept in standard growth conditions. Seeds were divided into two groups which differed only in the type of watering treatment. In the control group, seeds were watered with untreated tap water, and in the Aȟalemma Water group, seeds were watered with Aȟalemma-treated tap water. Germinating seeds and the resulting seedlings were grown and analyzed for biophoton emission.

Biophoton emission analysis: Ultra-weak photon (biophoton) emission was measured using low-noise photomultiplier tubes and highly sensitive charge coupled device cameras. Biophoton emission was measured at 10 different time-points over a period of 38 days. Both groups of seeds/growing plants were analyzed, using the software coupled to the measuring apparatus.

RESULTS

The spectral data obtained by biophoton emission analysis of control seeds and Aȟalemma-treated seeds showed two distinct patterns (**Figure 5**). Control seeds did not exhibit a regular variation in biophoton emission relative to time of day (**Figure 5, red line**). On the other hand, Aȟalemma-treated seeds showed a distinct semidiurnal pattern of biophoton emission (**Figure 5, blue line**). **These results indicate that treatment with Aȟalemma water affects physiological processes which underlie biophoton emission during early plant growth (e.g. oxidative metabolic processes). Aȟalemma renders these processes more periodic, following a distinct daily cycle, as evident by two peaks and then two drops in biophoton emission.** Although it is unclear how this affects the growing plant, the result strongly indicates that Aȟalemma water affects oxidation in the plant, and that this happens with respect to the natural day/night cycle.

INSTITUTIONS

Water & Light B.V. (Amersfoort, The Netherlands)

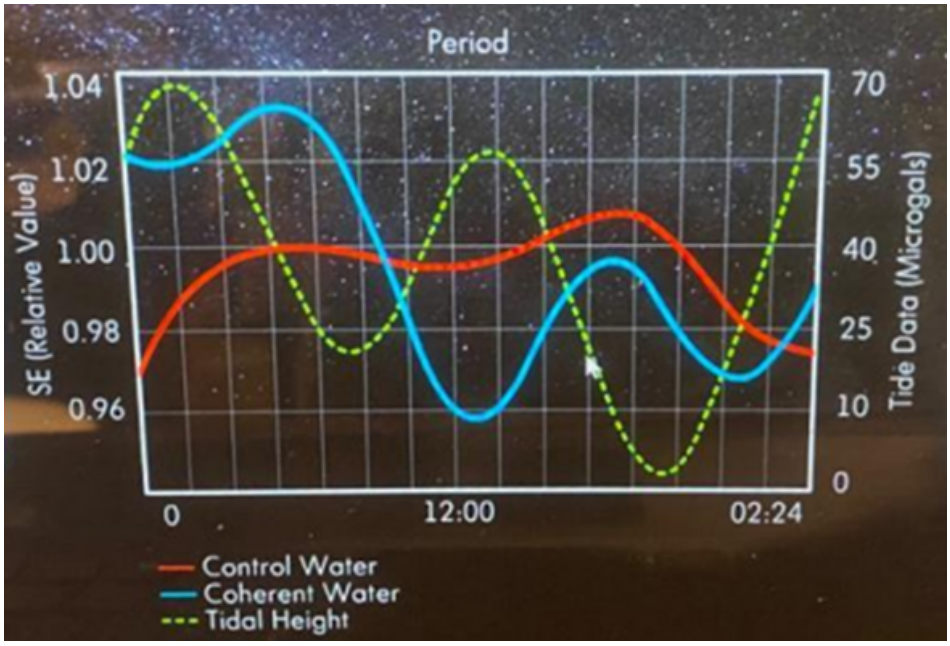

Figure 5. Biophoton emission of seeds treated with Aňalemma coherent water (blue line) vs control (red line). Aňalemma-treated seeds exhibit a semidiurnal pattern of biophoton emission synchronized with the Earth's tides (yellow dotted line).

When the researchers plotted the biophoton emission data against the Earth's tide data, it was evident that the two patterns were correlated (Figure 5, yellow dotted line), with both lines showing distinct bursts and drops relative to the daily cycle. Earth's tides are caused by the difference in gravitational forces from the Moon and the Sun on the different sides of Earth. The relative phase of the Sun and Moon cause an elastic deformation of the Earth, resulting in a diurnal and semi-diurnal periodic variation in the distance between the Earth's crust and center of mass. Corresponding to the variation in distance is the variation in the gravitational acceleration, which can be measured at any given location on Earth (referred to as tide data, expressed in microgals). **The apparent synchronized behavior of Aňalemma-treated plants with the Earth's tides indicates a strong connection between Aňalemma coherent water and the tidal forces of external planetary bodies in their natural cycles.** This correlation was, at the very least, striking, and had since then sparked numerous questions regarding the full potential of coherent water.

WHAT'S NEXT?

The results on biophoton emission were among the earliest findings on the effects of Aňalemma water on plants. The results of the long-term experiment performed on wheat are interesting with respects to the so-called circadian clock, an endogenous mechanism that plants use to adjust their growth and physiology to daily environmental cues, such as the daily rhythms of light and temperature.

A healthy plant is a well-adjusted plant. The apparent periodicity in oxidative processes of young plants, together with a possible connection with the Earth's tidal forces, opens exciting questions for further research – **can coherent water help plants become more "in tune" with natural cycles, more primed to respond to changes in their environment?** Here at Aňalemma, we are highly invested in finding the answers to these questions.

The results obtained in these experiments, together with general insights from long-term usage of Aňalemma Water in greenhouse conditions, led to the conclusion that Aňalemma Water had a highly positive effect on plant physiology and overall health.

These findings have been of utmost importance in shaping our perspective and establishing current collaborations with laboratory groups researching plant physiology.

We are currently exploring the effects of Aňalemma Water on resistance of basil plants to conditions of drought via assessing a wide array of morphological, physiological and biochemical parameters.

RESEARCH REPORT
JANUARY 2023

APPENDIX A

A1. PLANT GROWTH CONDITIONS AND TREATMENT

From Day 1 to Day 15, plants were kept in an isolated part of a climate-controlled greenhouse in similar conditions (**Figure A1**). From Day 15 until the end of the study, plants were distributed according to their designated groups to receive the specified water treatment using an automated dosing system, The exact distribution is shown in the schematic representation in **Figure A2**.

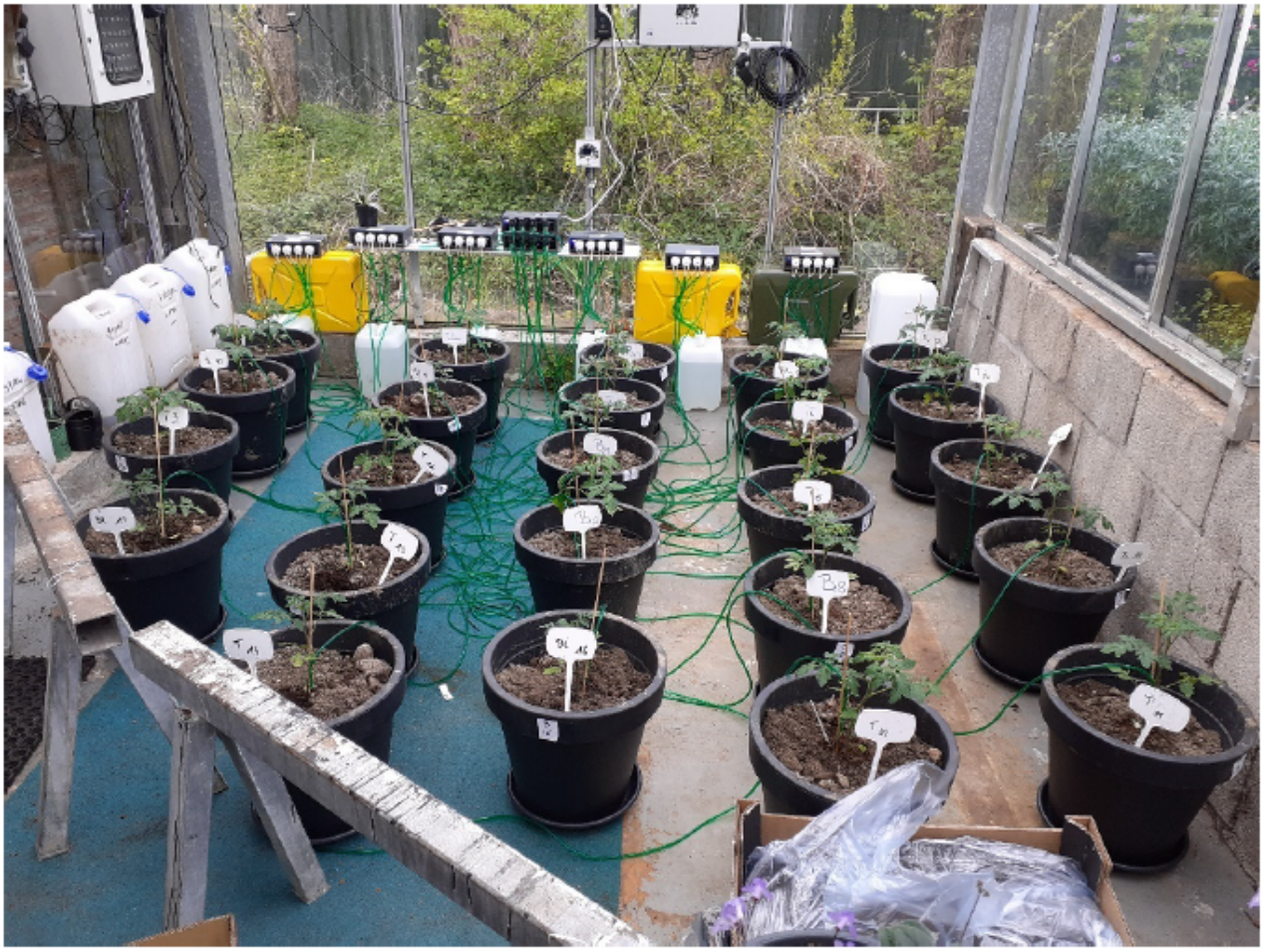

Figure A1. Plant growth setup from Day 1 to Day 15. All plants were grouped together in a climate-controlled greenhouse and watered with Rainwater using an automated dosing system.

APPENDIX A

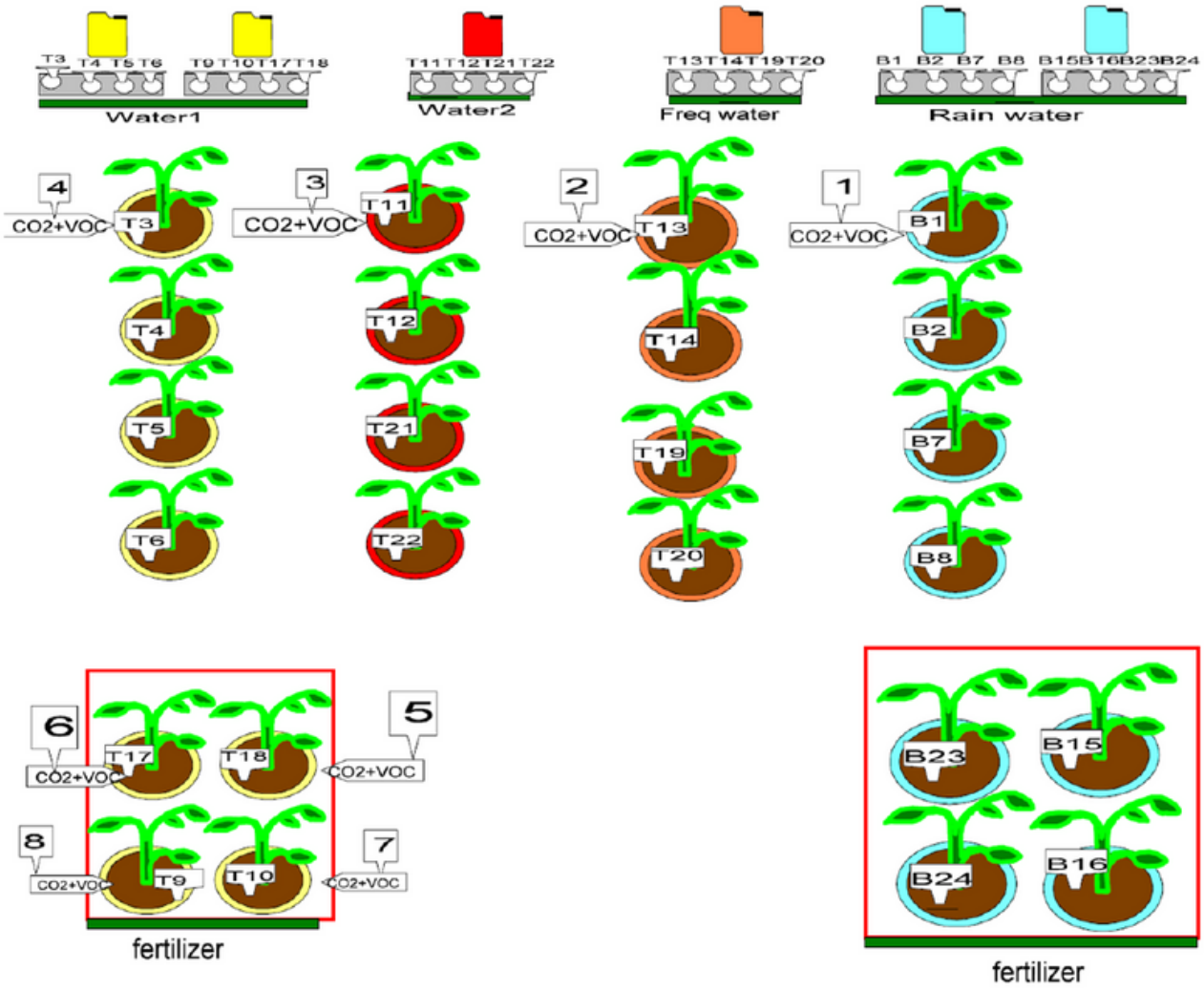

Figure A2. A schematic representation of the plant growth setup from Day 15 to the end of the study Plants were grouped according to their designated watering treatment in a climate-controlled greenhouse. Plants were watered with the specified water type using an automated dosing system.

APPENDIX A

A2. CHEMICAL COMPOSITION OF SOIL

Mean values of all 26 parameters measured in the chemical composition analysis of soils treated with different types of water are listed in **Table A1**.

Table A1. Overview of the chemical analysis showing the mean values per watering condition.

Parameter in dry soil	*Unit for condition*	*Base line measurement*	*Water I*	*Water II*	*Frequency water*	*Rain water*	*Water I + organic fertilizer*	*Water I + organic fertilizer*	*Rain water + organic fertilizer*
Organic carbon	% C	*2,1*	*2,15*	*2,23*	*2,23*	*2,15*	*2,60*	*2,50*	*2,55*
C / N ratio	-	*9,5*	*9,68*	*11,65*	*15,45*	*13,88*	*11,95*	*11,65*	*15,63*
Phosphorus, P-PAE Q	mg P/kg	*11,9*	*11,18*	*10,20*	*10,65*	*11,63*	*10,30*	*11,80*	*9,85*
Boron	mg B/kg	*2,88*	*2,43*	*2,42*	*2,30*	*2,58*	*2,25*	*2,60*	*2,58*
Copper	mg Cu/kg	*6,8*	*5,03*	*4,40*	*4,40*	*5,18*	*4,80*	*5,80*	*4,88*
Zinc	mg Zn/kg	*<0,15*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Iron	mg Fe/kg	*2*	*3*	*2*	*3*	*2*	*3*	*4*	*2*
Molybdenum	mg Mo/kg	*0,11*	*0,14*	*0,12*	*0,13*	*0,15*	*0,14*	*0,15*	*0,13*
Acidity, pH	-	*7,2*	*7,24*	*7,20*	*7,21*	*7,20*	*7,17*	*7,20*	*7,20*
Organic matter	%	*3,7*	*3,70*	*3,83*	*3,83*	*3,73*	*4,45*	*4,30*	*4,40*
Carbonated lime	%	*7,4*	*7,57*	*7,78*	*7,53*	*8,08*	*7,15*	*7,75*	*7,53*
Nitrogen delivery capacity	kg N/ha per year	*158*	*109*	*101*	*82*	*86*	*108*	*107*	*89*
Phosphate, Pw Q d	mg P_2O_5/L	*99*	*95*	*84*	*88*	*82*	*85*	*90*	*87*
Phosphate, P-AL Q d	mg P_2O_5 /100 g	*134*	*145*	*136*	*143*	*134*	*141*	*141*	*141*
Potassium, K-HCl	mg K_2O /100 g	*115*	*98,35*	*92,43*	*87,75*	*101,13*	*110,85*	*116,15*	*104,03*
K-number	-	*110*	*91*	*87*	*82*	*96*	*105*	*111*	*98*
Potassium, K-PAE	mg K/kg	*596*	*425*	*395*	*378*	*427*	*429*	*550*	*463*
Magnesium	mg MgO/kg	*433*	*438*	*394*	*394*	*421*	*428*	*502*	*412*
Sodium	mg Na/kg	*96*	*99*	*78*	*71*	*95*	*103*	*130*	*87*
Manganese	mg Mn/kg	*70,9*	*342,75*	*300,40*	*322,50*	*331,00*	*305,00*	*328,00*	*319,73*
Clay-humus complex (CEC)	mmol+/kg	*200*	*250*	*232*	*237*	*241*	*237*	*245*	*236*
Litability	%	*38,5*	*43,35*	*42,73*	*42,63*	*40,70*	*40,85*	*39,85*	*42,18*
Lutum	%	*23*	*29*	*29*	*29*	*27*	*28*	*27*	*28*
Nitrogen total Q	mg N/kg	*2220*	*2215*	*1963*	*1440*	*1550*	*2170*	*2150*	*1643*
S-PAE Litability	mg S/kg	*1511*	*1103*	*1220*	*1069*	*1200*	*1405*	*1496*	*1095*
Sulphur-delivering capacity	kg S/ha per year	*3598*	*2620*	*2887*	*2535*	*2857*	*3264*	*3489*	*2557*

APPENDIX A

A3. EFFECTS ON FUNGAL PROPERTIES

A3.1. Generation of graphs

Graphs showing the total number of fungi (either all fungi, other molds or individual genera) were generated based on CFU/g values. When these values were higher than the detection threshold (> 5000) or lower than the detection limit (< 100), they were entered as 5000 and 100, respectively, into the calculations for the graphical representation. Although these values are not exact representations of the soil fungal composition due to detection limits, they do show trends in abundance of different groups of fungi.

A4. ANALYSIS OF BACTERIAL COMPOSITION AND DIVERSITY

A4.1. Methods

The following descriptions were provided in the scientific report delivered by BaseClear B.V..

Alpha diversity - Alpha diversity refers to the average species diversity in a habitat or specific area. Alpha diversity is a local measure. We measure alpha-diversity as the observed richness (number of taxa) or evenness (the relative abundances of those taxa) of an average sample within a habitat type.

Beta diversity - We quantify beta-diversity as the variability in community composition (the identity of taxa observed) among samples within a habitat.

RDA - Redundancy analysis (also called principal components analysis of instrumental variables) is a technique for two sets of variables, one set being dependent of the other. Its aim is maximization of the explained variance of the dependent variables by a linear combination of the explanatory variables. The principal components of a collection of points in a real coordinate space are a sequence of p unit vectors, where the i*" vector is the direction of a line that best fits the data while being orthogonaL to the first i-1 vectors. Here, a best-fitting line is defined as one that minimizes the average squared distance from the points to the line. These directions constitute an orthonormal basis in which different individual dimensions of the data are linearly uncorrelated.

Association Testing (DAA) - This section aims at detecting differentially abundant microbiome features (species/OTUs) between two predefined classes of samples, where a microbiome feature is considered differentially abundant if its mean proportion is significantly different between two conditions.

APPENDIX A

A5. REFERENCES

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APPENDIX B

Biophoton emission research (2014-2018)

B1. BIOPHOTON EMISSION OF TOMATO FRUITS (2018)

B1.1 .TECHNOLOGY

Delayed luminescence (DL) and spontaneous ultra-weak photon emission (SE) of intact tomato fruits were measured using a photomultiplier device. The device includes a dark sample chamber (9.5 cm × 15 cm × 16 cm) with a vertically positioned photomultiplier tube (PMT) (Electron Tubes Enterprises Ltd., Ruislip, UK, type 9558QB). The sample chamber was kept at 22 °C (**Figure B1**). The window opening to the PMT has a diameter of 44mm. The sensitivity of the PMT is in the range between 160 and 870 nm. The PMT was cooled to -25 °C to reduce the dark count rate to 10 counts per second. A fast preamplifier (ORTEC, USA, type 9301) was used to enlarge the photon emission signal. A PC with a counting card (National Instruments, USA, type 6602) was used for data acquisition.

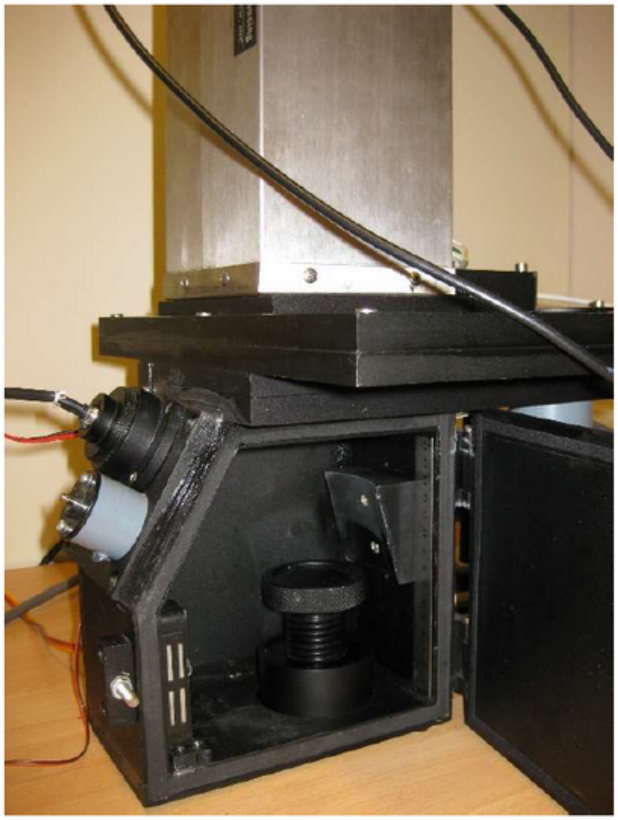

Figure B1. Photomultiplier tube and a dark chamber used for biophoton emission measurement.

B1.2. ANALYSIS

The delayed luminescence was recorded in consecutive 0.2-second time intervals for a 2-minute period, yielding a total of 600 data points. The decay curve showing light intensity decrease in time was analyzed according to three common mathematical models used by other researchers. Each model uses its own original variables (parameters).

APPENDIX B

Model 1 (hyperbolic decay model) contains the parameters Mean DL (DL_Mean), Initial intensity (IO), decay properties (Tau, Beta and T), and the goodness of fit (R).

Model 2 (double exponential model) contains the parameters: Initial intensity (y0), the properties two decays (A1, t1 and A2, t2), and the goodness of fit (R).

Model 3 (hyperbolic cosine model) contains the parameters of a decay curve that follows a hyperbolic and a shift including the parameters A, B, C, and the goodness of fit (R).

Steady state spontaneous emission of light energy (SE) was recorded in consecutive 0.05-second periods for a total of 5 minutes, yielding a total of 6000 data points. The fluctuations in photon numbers in time were then analyzed according to two models. Each model uses its own original variables (parameters).

Fractality model contains the parameters: Strength, Fano factor time curve variables (intercept and slope) and a fixed time Fano Factor (FB10).

Quantum squeezed state model contains the parameters: ABS_a, r, Theta, Phi, SSR, SSI, S.

All parameters are described in the book: Roeland Van Wijk, Yu Yan, Eduard Van Wijk (2017). Biophoton technology in energy and vitality diagnostics. A multi-disciplinary, systems biology, and biotechnology approach. Meluna, Wageningen, The Netherlands

B1.3. RESULTS

Statistical data describing the SE parameters of tomato fruits of plants treated with Aȟalemma Water or Control are shown in **Table B1**.

Table B1. Differences between SE parametres of tomato fruits of plants treated with Aȟalemma Water or Control. Change values (t) are shown for all parametres of the 2 models. Significant differences are indicated in red ($p < 0.05$).

Variable	t-value	p
Strength	-2.74058	0.006721
Intercept	0.22838	0.819597
Slope	-1.10491	0.270602
FF	0.42302	0.672762
ABS_a	-2.58665	0.010443
r	0.93003	0.353544
Theta	-0.03444	0.972566
Phi	0.03449	0.972520
SSR	-0.05630	0.955163
SSI	1.67070	0.096437
S	-2.05686	0.041074
FF10	-0.07624	0.939307

APPENDIX B

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