WATERAGRI

D5.3: Data Collected from Case Study Sites

Version 1*

M36/April 2023

WP5 Case studies

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Abstract:	Deliverable D5.3 "Data Collected from Case Study Sites" is the final deliverable in WP5 "Demonstration Case Studies". After Deliverable D5.1 "Description of Case Study Sites" and Deliverable D5.2 "WATERAGRI Solution Test Findings", this third deliverable in WP5 describes the main findings from testing WATERAGRI solutions at the Case Study sites. Deliverable D5.3 is linked to Milestone M12 "WATERAGRI Solutions tested".

***"This is the first version of the deliverable, and it presents preliminary results as for the M36 (April 2023). The information contained in this version is subject to change, and final results will be presented in the updated version of the deliverable in M42 (October 2023). The European Commission is not responsible for any use that may be made of the information contained in this deliverable."**

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CI	Classified, information as referred to in Commission Decision 2001/844/EC	
CO	Confidential, only for members of the consortium (including the CE)	

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4	TEKNOLOGIAN TUTKIMUSKESKUS VTT Oy	VTT	FI
5	DEBRECENI EGYETEM	UNIDEB	HU
6	ALCHEMIA-NOVA GMBH	ALCN	AT
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19	AB GARDSTANGA NYGARD	GN	SE
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WATERAGRI

List of Abbreviations and Acronyms

BOD	Biological oxygen demand
COD	Chemical oxygen demand
CST	Capillary suction time
CWSI	Crop water stress index
DET	Dewaterability Estimation Test
DO	Dissolved oxygen
DOC	Dissolved organic carbon
EC	Electrical conductivity
GIS	Geographic Information System
HGS	HydroGeoSphere
LSD	Least significant difference
NH_4-N	Ammonium nitrogen
NO_3-N	Nitrate nitrogen
PDAF	Parallel Data Assimilation Framework
SFCW	surface flow constructed wetland
SSM	Surface Soil Moisture
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorus
UAV	Unmanned aerial vehicle
VRI	Variable Rate Irrigation
WR	Water Retainer

1 Introduction

Deliverable D5.3 "Data Collected from Case Study Sites" is the final deliverable in WP5 "Demonstration Case Studies". After Deliverable D5.1 "Description of Case Study Sites" and Deliverable D5.2 "WATERAGRI Solution Test Findings", this third deliverable in WP5 describes the main findings from testing WATERAGRI solutions at the Case Study sites. Deliverable D5.3 is linked to Milestone M12 "WATERAGRI Solutions tested". Figure 1 shows the location of the 10 WATERAGRI Case Study sites.

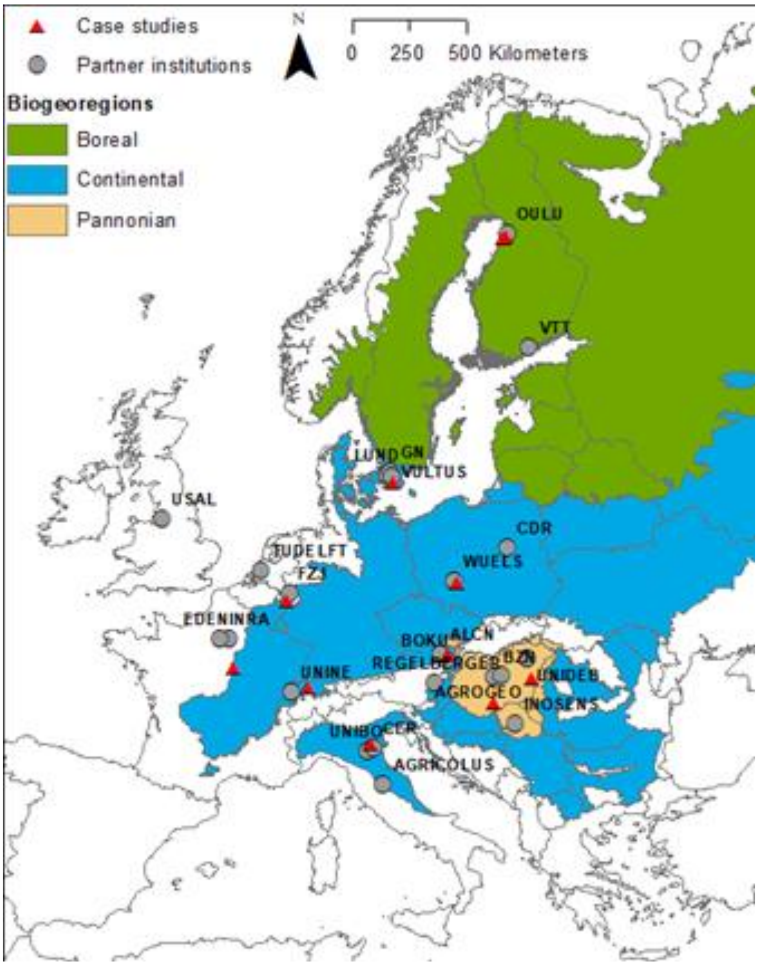

Figure 1: Location of WATERAGRI case studies (red triangles).

2 Methodology

In Deliverable D5.1, for each case study site, the following information was provided:

- The general description of the site (including climate, land use, soil/s, farming, drainage and irrigation practises, etc.),
- The WATERAGRI solutions at the site and the partners involved,
- The data availability,
- The implementation and research plan.

Following this, in Deliverable D5.2, the implementation of the WATERAGRI solutions that are tested at the case study sites are reported, i.e., for each case study site, the following information was provided:

- A short general description of the site including the WATERAGRI solutions at the site,
- The updated implementation and research plan,
- Updated information on data availability and data provision, and
- A photo documentation on implemented WATERAGRI solutions.

In this final deliverable of WP5, Deliverable D5.3, we present now for each case study:

- the main results for each WATERAGRI solution tested,
- the link to deliverables in which detailed results from the experiments are presented (if applicable), and
- key findings for each solution tested as well as for each case study site.

Experimental results that are not reported in deliverables, are presented in the appendix.

The first version of Deliverable D5.3 contains the results from testing the solutions at case study sites until the growing season 2022. A second version of Deliverable D5.3 will be prepared in October 2023 to allow incorporating results obtained in the growing season 2023. Results and key findings are thus marked as [*preliminary*] or [*final*] whether they will be updated with results obtained in the growing season 2023 or not.

3 Results

3.1 Case study and solution overview

Table 1 shows the overview of the 10 WATERAGRI case study sites including the updated list of main contact persons and their email addresses.

Table 1: Overview on WATERAGRI case study sites and updated main contacts (new contacts shown in blue colour).

	Site	Partner	Main contact	Email
	Boreal zone			
1	Finland I – Municipality of Tyrnävä	UOULU	Björn Klöve Ali Torabi Haghigh Kedar Ghag	bjorn.klove@oulu.fi Ali.TorabiHaghighi@oulu.fi Kedar.Ghag@oulu.fi
2	Finland II – Municipality of Ruukki	UOULU	Hannu Marttila	hannu.marttila@oulu.fi
	Continental zone			
3	Sweden – Gårdstånga Nygård	GN ULUND	Gustaf Ramel Linus Zhang	gustaf.ramel@gardstanga.se linus.zhang@tvrl.lth.se
4	France – Auxerre	INRAE	Raymond Reau Laurette Paravano	raymond.reau@inrae.fr l.paravano@yonne.chambagri.fr
5	Germany – Selhausen	FZJ	Harrie-Jan Hendricks Franssen Richard Hoffmann	h.hendricks-franssen@fz-juelich.de r.hoffmann@fz-juelich.de
6	Poland – Lower Silesia	UPWr	Wieslaw Fialkiewicz Arkadiusz Glogowski	wieslaw.fialkiewicz@upwr.edu.pl arkadiusz.glogowski@upwr.edu.pl
7	Switzerland – Seeland	UNINE	Philip Brunner Qi Tang	philip.brunner@unine.ch qi.tang@unine.ch
8a	Austria –Obersiebenbrunn	BOKU	Christine Stumpp	christine.stumpp@boku.ac.at
8b	Austria – Mistelbach	BOKU ALCN	Christine Stumpp Eriona Canga	christine.stumpp@boku.ac.at eriona.canga@alchemia-nova.net
8c	Austria –Gleisdorf	TBR ALCN	Martin Regelsberger Eriona Canga	martin@regelsberger.at eriona.canga@alchemia-nova.net
9	Italy – Bologna	UNIBO and CER	Attilio Toscano Stevo Lavrnic Francesco Cavazza Stefano Anconelli	attilio.toscano@unibo.it stevo.lavrnic@unibo.it cavazza@consorziocer.it anconelli@consorziocer.it
	Pannonian zone			
10	Hungary – Nyírbátor	UNIDEB	Attila Nagy	attilanagy@agr.unideb.hu

Table 2 shows the overview of the WATERAGRI solutions including the updated list of main contact persons and their email addresses. The WATERAGRI solutions are grouped into 3 groups:

1. Framework Modelling
2. Innovative and Sustainable Water Retention Solutions
3. Nutrient Recovery Solutions

Table 2: Overview on WATERAGRI solutions and updated main contacts (new contacts shown in blue colour).

Framework Modelling (Group A):	**Main contact**	**Email**
Framework (**A1**; UNINE)	Philip Brunner	philip.brunner@unine.ch
Integrated physically-based terrestrial system models (**A2**; FZJ);	Harrie-Jan Hendricks Franssen	h.hendricks-franssen@fz-juelich.de
Decision support system (**A3**; AGRICOLUS)	Diego Guidotti	d.guidotti@agricolus.com
Water-vapour sorption isotherm and water retention characteristics model (**A4**; USAL)	Yu Wang	y.wang@salford.ac.uk
WebGIS for zoning landscape matrix (**A5**; AGRICOLUS);	Diego Guidotti	d.guidotti@agricolus.com
Serious gaming (**A6**; TUDELFT)	Aashna Mittal	A.Mittal@tudelft.nl
Innovative and Sustainable Water Retention Solutions (Group B)	**Main contact**	**Email**
*Farm constructed wetlands** for water retention (**B1**; ULUND);	Rolf Larsson	rolf.larsson@tvrl.lth.se
Remote sensing pipeline (**B2**; VULTUS);	Per Karlsson	per.karlsson@vultus.se
Irrigation management and agrometeorological monitoring solutions (**B3**; AGRICOLUS);	Diego Guidotti	d.guidotti@agricolus.com
Precision irrigation system (**B4**; AGRICOLUS);	Diego Guidotti	d.guidotti@agricolus.com
Enhanced Water Retainer product and concept (**B5**; BZN);	Nora Hatvani	nora.hatvani@bayzoltan.hu
Biochar for water retention (**B6**; ALCN),	Eriona Canga	eriona.canga@alchemia-nova.net
Tracer methods (**B7**; BOKU);	Christine Stumpp	christine.stumpp@boku.ac.at
Dewaterability estimation test apparatus (**B8**; USAL)	Yu Wang	y.wang@salford.ac.uk
Nutrient Recovery Solutions (Group C)	**Main contact**	**Email**
*Farm constructed wetlands** for nutrient recovery (**C1**; ULUND),	Rolf Larsson	rolf.larsson@tvrl.lth.se
Drainage systems (**C2**; ALCN),	Eriona Canga	eriona.canga@alchemia-nova.net
Bio-based nutrient-collecting membranes (**C3**; VTT),	Mona Arnold	Mona.Arnold@vtt.fi
Biochar adsorbents for nutrient uptake (**C4**; ALCN);	Eriona Canga	eriona.canga@alchemia-nova.net
Microfluidics (**C5**; EDEN).	Abhilash Venkateshaiah	abhilash.venkateshaiah@eden-microfluidics.com

* In the context of WATERAGRI we use the term Farm Constructed Wetlands (FCW) to refer to free water surface (FWS) wetlands, also known as surface flow constructed wetlands.

Table 3 shows the comparison of the final terminology of WATERAGRI solution in the factsheets, see Deliverable D6.1 "System Design and Construction Guidelines", compared to the original proposal.

Table 3: Final terminology of WATERAGRI solution in the factsheets compared with the terminology used to the original proposal.

WATERAGRI solutions in factsheets (D6.1)	***Responsible Partner***	***WATERAGRI solutions in original work description***
AgriLemma Serious Game	TUDELFT	*Serious gaming* (**A6**; TUDELFT)
Biomembranes	VTT	*Bio-based nutrient-collecting membranes* (**C3**; VTT)
Data assimilation	FZJ	*Integrated physically-based terrestrial system models* (**A2**; FZJ)
*Farm constructed wetlands** for nutrient *retention*	ULUND	*Farm constructed wetlands* for nutrient recovery (**C1**; ULUND)
*Farm constructed wetlands** for water retention	ULUND	*Farm constructed wetlands* for water retention (B1; ULUND);
Filter drain pipe	ALCN	Drainage systems (**C2**; ALCN), *related also with: Biochar adsorbents for nutrient uptake* (**C4**; ALCN)
Irrigation management platform	AGRICOLUS	*Decision support system* (**A3**; AGRICOLUS), *WebGIS for zoning landscape matrix* (**A5**; AGRICOLUS); *Irrigation management and agrometeorological monitoring solutions* (B3; AGRICOLUS); *and Precision irrigation system* (B4; AGRICOLUS);
Microfluidics	EDEN	*Microfluidics* (**C5**; EDEN).
Multilayers filter system	ALCN	Drainage systems (**C2**; ALCN), related also with: Biochar for water retention (**B6**; ALCN), and *Biochar adsorbents for nutrient uptake* (**C4**; ALCN)
Remotely Sensed Data	VULTUS	*Remote sensing pipeline* (B2; VULTUS)
Tracer Methods	BOKU	*Tracer methods* (B7; BOKU)
Water Retainer	BZN	*Enhanced Water Retainer product and concept* (B5; BZN)
Water Retention Characteristics	USAL	*Water-vapour sorption isotherm and water retention characteristics model* (**A4**; USAL) and *Dewaterability estimation test apparatus* (B8; USAL)

* In the context of WATERAGRI we use the term Farm Constructed Wetlands (FCW) to refer to free water surface (FWS) wetlands, also known as surface flow constructed wetlands.

3.2 Case study 1 - Municipality of Tyrnävä (Finland 1)

3.2.1 General overview

Table 4: General overview – Case study 1

Case study	WATERAGRI solutions	Partner involved
Finland I 	Physical modelling (HydroGeoSphere) (A2) Remote sensing pipeline (B2) Irrigation scheduling (B3)	Leader: UOULU Participants: UNINE, FZJ, VTT and UOULU

3.2.2 Main results for WATERAGRI solutions tested

Physical modelling (HydroGeoSphere) (A2) [*preliminary*]

- Various water management options for controlled drainage systems were tested using a scenario approach. In this approach, a base case model with no tile drainage network is compared to simulation results ofs the commonly applied agricultural tile drainage practices: traditional and controlled drainage systems. Other scenarios also consider active drainage experiments, in which control structures are adjusted according to prevailing soil moisture conditions, and irrigation experiments using the tile drainage system as irrigation infrastructure.
- A three-dimensional model (6.75 ha) of the Tyrnävä potato field with a typical agricultural soil profile of the Tyrnävä region is built using fully-integrated physically-based code HydroGeoSphere (HGS). The model simulations were conducted in a transient state and included essentials for the boreal region winter processes, such as snow melting. The model was validated using measured groundwater levels and soil moisture time series. The controlled tile drainage system is incorporated into the model as one of the modeling scenarios.
- The modeling results reveal that the soil water conditions in the field system are responsive to the implemented drainage system (Figure 2) making fully integrated numerical modeling a viable tool to investigate how the field may react to various water management options. The simulations revealed that the traditional tile drainage significantly and permanently lowers the groundwater levels, whereas the use of the control structures gives the potential to sustain a higher water table during dry periods. The accurate simulations require detailed information on the field boundary conditions exerted by a surrounding open ditch network and shallow groundwater and representation of a hydraulic connection between the open ditch system and tile drainage outlet.
- Detailed results on physical modeling using HydroGeoSphere for case Tyrnävä case study are presented in Deliverable D6.2 "Model-based Assessments".

- Active drainage management incorporating weather forecasts **will be** evaluated as a part of the data assimilation framework and presented in **D7.5: Data Assimilation System for Physically Based Models.**
- The potential application of a water retainer in the potato field **will be** assessed in **D7.3**: A**ssessment of Soil Water Retention Solutions.**

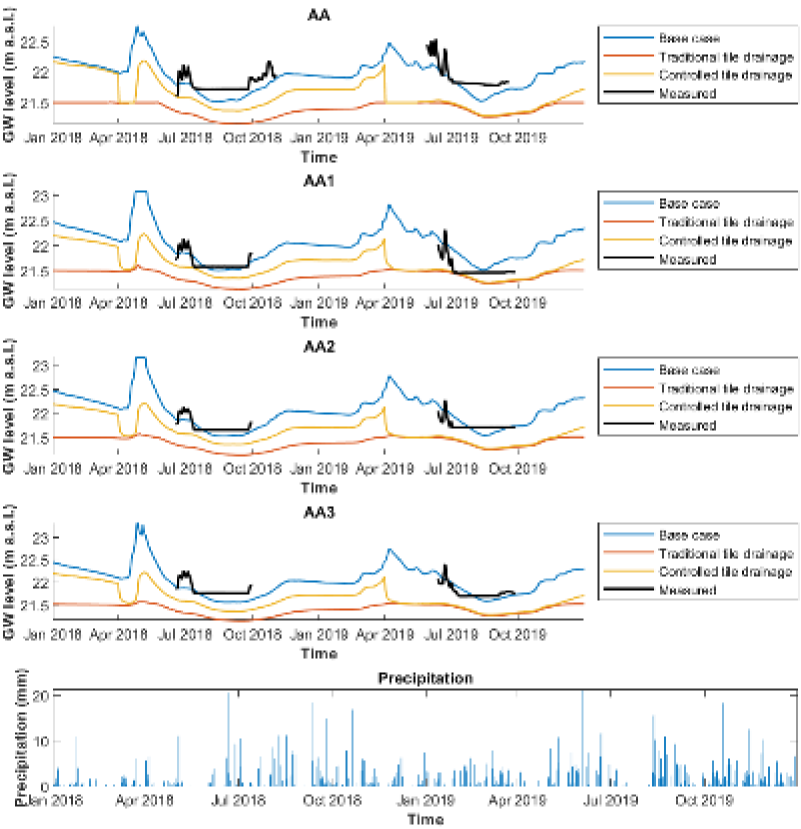

Figure 2. Simulation results for three modeling scenarios: the base case with no tile drainage system, the scenario with the traditional tile drainage system, and the scenario with the controlled tile drainage system at control well AA and three monitoring locations AA1-AA3.

Remote sensing pipeline (B2) [*final*]

- The assessment of crops using remote sensing was done to evaluate irrigation and crop management strategies (presented in deliverable D3.1 Assessment of Use of Remotely Sensed Vegetation to Improve Irrigation under Section 6.1 Satellite images of vegetation and soil with potential use for irrigation support).
- Remotely sensed data (crop water stress index, CWSI) showed water stress in 2004, 2006, and 2008 for the observed periods 2001 - 2020.
- The remotely sensed climatological variables (e.g., precipitation, land surface temperature and evapotranspiration) showed significant impact on the annual crop yield in the region.

Irrigation scheduling (B3) [*preliminary*]

- Results from a water balance simulation showed that, irrigation is needed to mitigate summer drought in certain years. However, drainage and removal of excess water is essential in all years.
- We evaluated the use of surface water and groundwater for irrigation during summer drought which is discussed in deliverable D3.1 (Assessment of Use of Remotely Sensed Vegetation to Improve Irrigation, in see Section 3 The use of surface and groundwater for irrigation).
- A novel approach was discussed to provide potential irrigation demand and drainage for better crop growth. The IoT based approach uses estimates of daily evapotranspiration (ET0), Plant growth stage constants (Kc), rainfall forecasts, and near real time soil moisture values from the field (see deliverable D3.1 under section 3.4.2).

3.2.3 Key findings

Key findings for each solution tested [*final*]

- HGS modeling is a viable tool to investigate how the field may react to various water management options. The use of the control structures gives the potential to sustain a higher water table during dry periods.
- Remotely sensed data (CWSI) showed water stress in 2004, 2006, and 2008 for the observed periods 2001 - 2020. The method can be used to plan irrigation.

3.3 Case study 2 - Municipality of Ruukki (Finland 2)

3.3.1 General overview

Table 5: General overview – Case study 1

Case study	WATERAGRI solutions	Partner involved
Finland II 	Physical modelling (HydroGeoSphere) (A2) Water quality and Tracers (B7)	Leader: UOULU Participants: BOKU, VTT and UOULU

3.3.2 Main results for WATERAGRI solutions tested

Simplified models (Drainmod) (A1/A2) [*final*]

DRAINMOD software was built and calibrated to all 6 field blocks in the Ruukki site. The model was run for 3 years data from the site and in-situ measured meteoritical, hydrological, and soil parameters were used in the model. Measured water table data was used to calibrate the model output. The model was successfully built but calibration was challenging.

Main results:

- DRAINMOD was able to predict the annual and monthly water tables but failed to produce a dynamical function of the water table variation, on a daily level.
- The problems to include a regional main drainage system to the model seems to be the main challenge in DRAINMOD calibration.

Controlled drainage assessment [*preliminary*]

- Water quality processes were evaluated from peat fields with different controls (peat depth, controlled drainage). The result show that:
- The N load in subsurface discharge waters increased upon increasing peat depth. The load of TN was also moderately high in periods when there is no vegetation cover. The proportion of NO3-N fluctuated under different conditions and was the main driver for TN load/concentration changes. The discharge concentration of NH4-N and Norg mostly stable and low.
- The total P load was low, compared to thick peat and even mineral soils. Because the coarse silty and clayey mineral subsoil likely effectively adsorbed P in the seepage water, the retention being more effective the thicker the mineral soil horizons are before the pore water reaches the drainage pipe. However, the proportion of dissolved PO_4-P increased upon increased peat depth.

- The load and discharge concentration of total organic carbon (TOC) was also found higher for thicker peat plots.
- High concentrations of sulphur and acidity in the discharge water indicated the oxidation of sulfidic materials in the subsoil.

3.3.3 Key findings

Key findings for each solution tested [*preliminary*]

- Drainmod modelling is not suitable for cultivated peatland sites with controlled drainage due to the significant influence of large open drains in the area.
- The water quality result suggests that N, dissolved P and TOC loads transported through subsurface drainage pipes from a shallow peat field are lower than from a thick peat soil where the drainage pipes have been installed in the peat.
- Our results indicate that peat cover can mitigate the negative environmental impacts of AS soils by hindering the oxidation of sulfidic material, thus preventing the formation of acidity, but the required thickness of the peat cover to achieve this effect should be studied in greater detail.

3.4 Case study 3 - Gårdstånga Nygård (Sweden)

3.4.1 General overview

Table 6: General overview – Case study 3

Case study	WATERAGRI solutions	Partner involved
Sweden 	Wetlands (B1+C1) Water Retainer (B5) DET apparatus (B8)	Leader: GN Participants: ULUND, USAL, BZN and GN

3.4.2 Main results for WATERAGRI solutions tested

Wetlands (B1+C1) [*final*]

Below is a summary of the outcomes of field investigations with water samplings related to wetlands coupled with agricultural application with respect to sediments, nutrients and pollutants and their impacts, both positive and negative. The water sampling program is carried out at the Gårdstånga Nygård, constructed wetland, next to the experimental field. The layout of the water sampling is presented in Figure 3, where 11 selected sampling points are marked. As described earlier, this small wetland system was constructed in connection with the start of the WATERAGRI project. The wetland gets its water mainly from upstream of the small stream (partly seen in the figure) and the inflow comes in via sampling point “Inlet” near the eastern corner. The wetland lake has an outgoing discharging point (Outlet) via a cylinder formed weir at the western part close to sampling point “Outlet”. In addition, there are two sampling points directly in the stream as well (points upstream (USR) and downstream (DSR)).

The sampling program can be divided into three categories, namely physical, chemical/mineral, and others related to water quality aspects in order to detect any variations and/or relations between wetland system to agricultural production activity in term of sediments, nutrients, pollutants and the water flux. The data sampling started at 2021-06-11 and finished at 2022-12-31, with 8 to 16 samples for each sampling date collected for laboratory analyses. Categories of samplings and analyses are listed in Table 7.

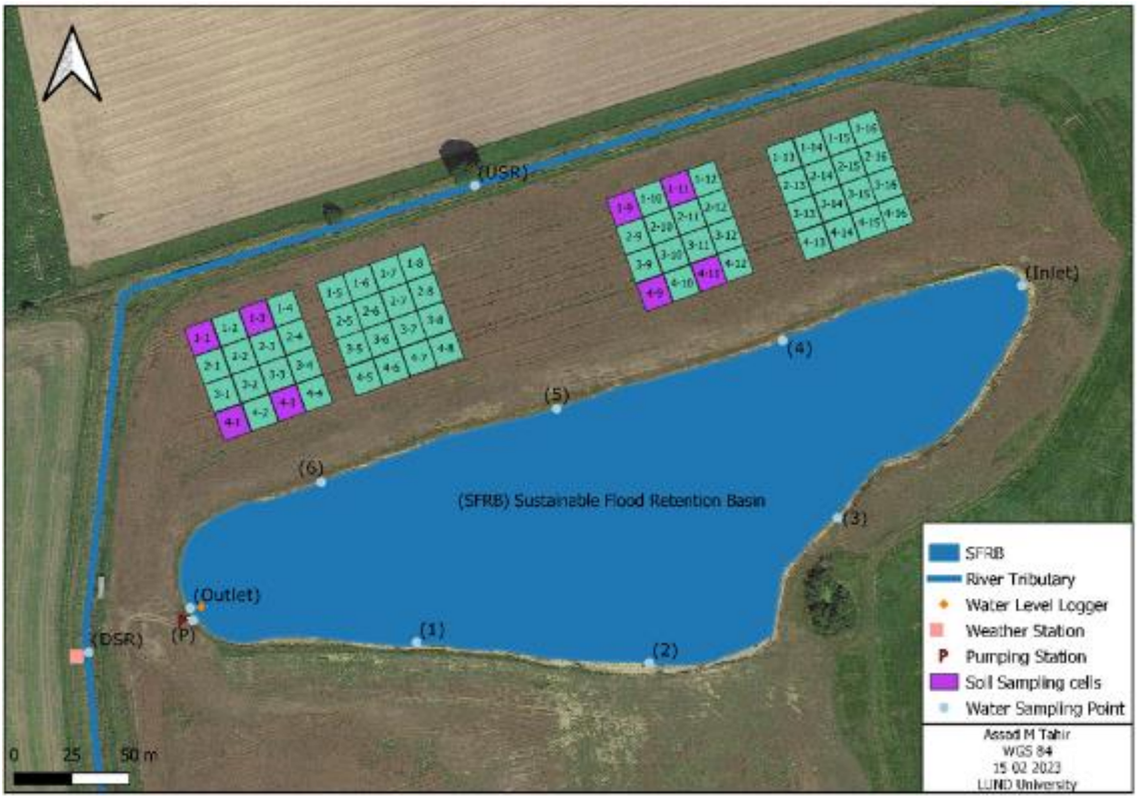

Figure 3. Water sampling points at the Gårdstånga Nygård, constructed wetland Sweden.

Table 7. Categories of samplings and analyses carried out, either on site or in laboratory.

Type of parameters	Water samplings and analyses
Physical	pH, Redox, DO, TSS, Salinity, EC, Resistivity, TDS, Turbidity
Mineral	Al, As, Cu, Ni, Pb, Zn, Co, Cr, B, Cd, Ca, Fe, Mg, Mn, Na
Organic matter and nutrients	TOC, DOC, TP, TN, NH_4-N, NO_3-N, K, BOD, COD

- For chemical elements: Al, As, Cu, Ni, Pb, Zn, Co, Cr, B, Cd, the analyses show t%hat they are either below detection level or have concentrations in general below 0.001 mg/L. For this group of 10 elements, some of them are heavy metals with large impact on aquatic environment and human life. The low concentration is a very good indication of the water quality.
- For Ca, Fe, Mg, Mn and Na, the largest variations are found in Ca, ranging from 16 to 90 mg/L. This is considered natural since the soils and water in the catchment are Ca rich in general. The second largest variation is found in Na, with an overall maximum = 32.8 mg/L. The other three minerals show a small variation interval, normally below 10 mg/L or smaller in most cases.
- For the physical parameters collected, most of them have a very stable level with minimal seasonal and spatial variations. The pH and turbidity show a clear variation pattern. pH has a max-min-mean value of 9.78 – 6.7 –7.79, showing a slightly tendency of basicity. Variations in turbidity are mostly related to climate events such as rainfall and wind, as well as agricultural activities. Results also show that the pH in summer season is higher that of winter season. For

turbidity the seasonal change is not significant but there is a clear difference in those three points close or in the river (Inlet, USR and DSR) compared to other sampling points with much larger fluctuations over the whole period.

- For the third group with nutrients and other indicators distributions and variations, COD and BOD5 are among the most significant. For COD it is noted a smooth decreasing trend both spatially from upper stream to downstream as well as temporally during the sampling period. On the other hand, no clear trend is found for the case of BOD5. The variation pattern of most other parameter is smaller compared to minerals. All the values found are within the safety intervals for these indicators.
- Detailed results on Gårdstånga Nygård case study are presented in Deliverables D4.1 and D4.2.

Water Retainer (B5) [*final*]

An experimental field with 64 cells, each 4x4 m, has been established at Gårdstånga Nygård (Figure 4). The Water Retainer has been applied on 16 cells during two growing seasons, with 16 control cells. Two more variables have been applied, P /no P, and irrigation/no irrigation, resulting in 8 possible combinations. In 2021 the crop was spring barley, while in 2022 the crop harvested was winter wheat. The harvests for both years have been analysed by Hushållningssällskapet (the Rural Economy and Agricultural Society).

- The results show no significant effect of the Water Retainer on the harvest quantity.
- For the 2021 harvest the average output was 1883 kg/ha (at 15% water content) with Water Retainer, while the corresponding number for cells with no Water Retainer was 1867 kg/ha.
- For the 2022 harvest the average output was 7262 kg/ha (at 15% water content) with Water Retainer, while the corresponding number for cells with no Water Retainer was 7368 kg/ha.
- The large difference in harvest output between the two years is mainly due to effects of heavy machinery involved in the wetland construction affecting soil structure in the experimental field in the final year.
- Detailed results on Water Retainer(B5) for case study Gårdstånga Nygård GN are presented in the Appendix (section 5.1 and 5.5) as well as in deliverable D3.2 (Assessment of Water Retention Methods).

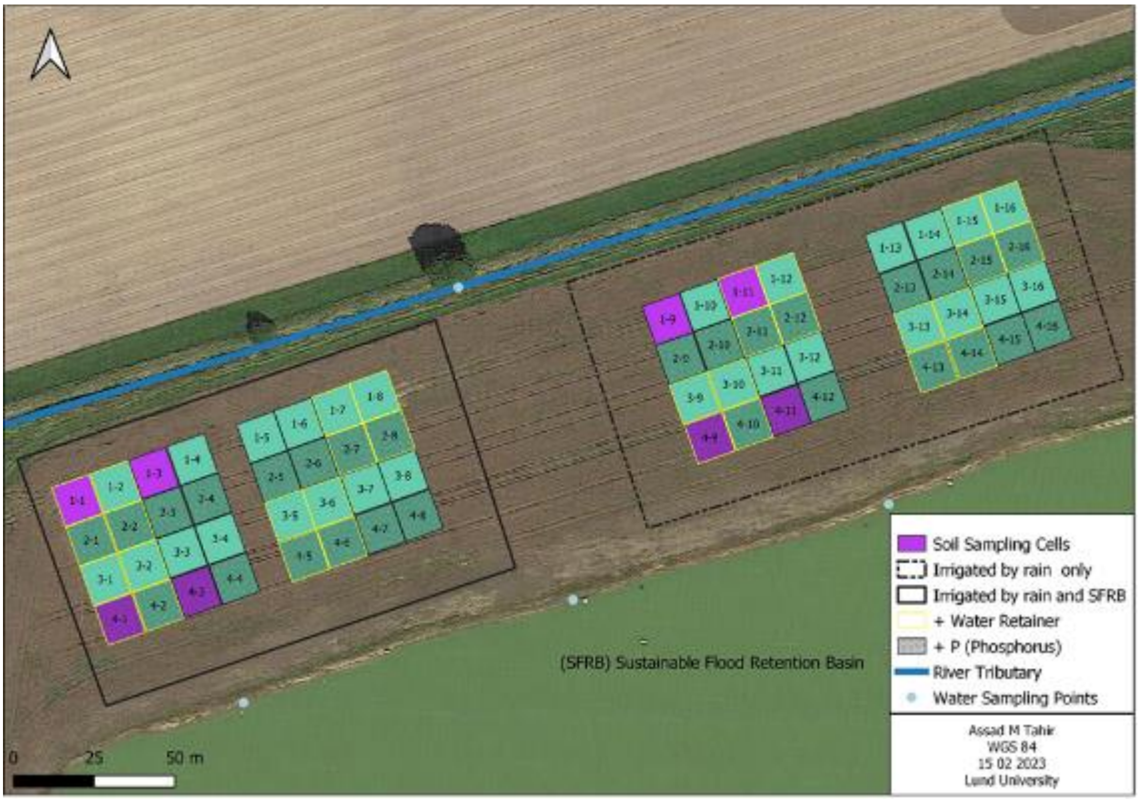

Figure 4. Experimental field at Gårdstånga Nygård, Sweden.

DET apparatus (B8) [*final*]

The Dewaterability Estimation Test device (DET), see Figure 5, which has been developed based on the principles of Capillary Suction Time (CST), can be used to quantify the ease of removing liquids from slurries and sludges. It was previously tested for evaluation of the Water Retainer and in connection with industrial food rest products, see Deliverable D3.3. At Gårdstånga Nygård a mature wetland has been dredged as part of the long-term maintenance. Samples from the sediments have been run through the DET device at USAL in order to evaluate the functionality of the device for yet another application.

- The DET device was found to perform well also for this application.
- Sediment samples were taken at three spatially separated spots. For each sample three separate tests were run with two types of filter paper. In total 3 x 3 x 2 = 18 tests were made.
- Tests show that water moves faster through the BF3 filter than through the CST filter.
- Similar variation in water front velocity through the filter for the three samples were observed for both filter types.
- The precision of the measurements was considered high with absolute deviation from mean of average velocity typically less than 5 % for all sets of three tests.
- Standard deviation of the velocity across both types of filter paper was high in comparison with what has been observed in previous tests, typically 2-5 %.
- Detailed results on DET apparatus (B8) for case study Gårdstånga Nygård GN are presented in the Appendix Chapter 5.1.

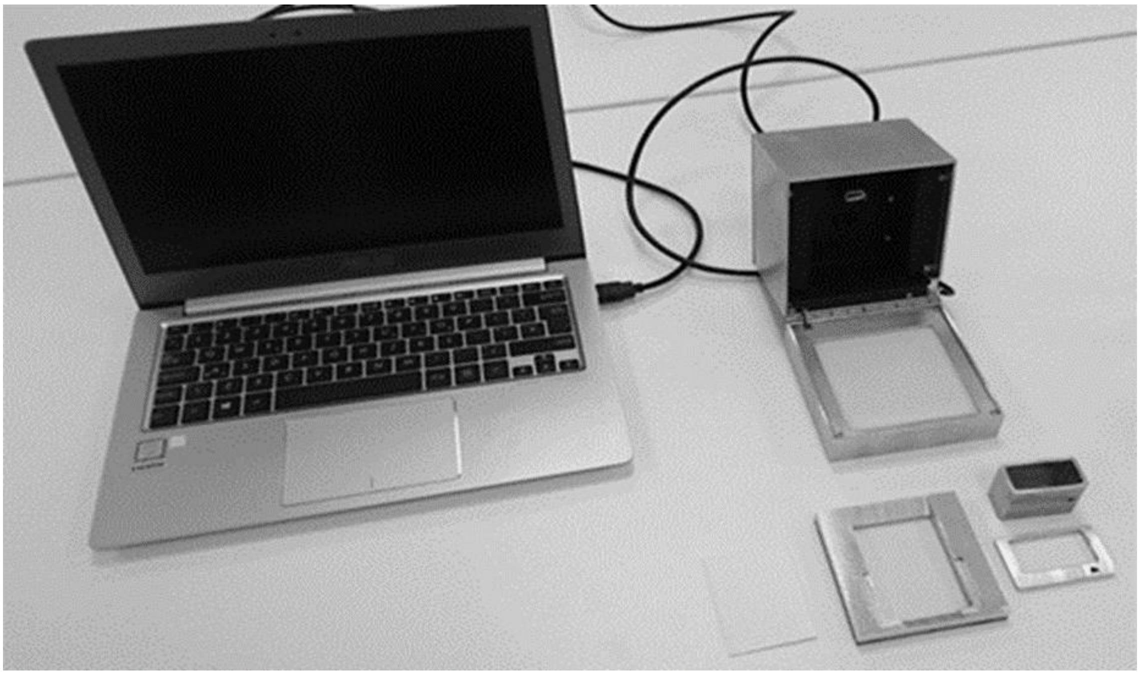

Figure 5. Components of the dewaterability estimation test (DET) with key components.

3.4.3 Key findings

Key findings for each solution tested [*final*]

- Most of the indicators based on analysis results show no significant water quality problems with low concentration and small variation intervals. The pH and turbidity show a clear variation pattern. pH has a max-min-mean value of 9.78 – 6.7 –7.79, showing a slightly tendency of basicity. Results also show that the pH in summer season is higher that of winter season. For turbidity the seasonal change is not significant but there is a clear difference in those three points close or in the river (Inlet, USR and DSR) compared to other sampling points with much larger fluctuations over the whole period.
- Nutrients and other indicators distributions and variations, the variation pattern of each parameter is smaller compared to minerals. All the values found are within the safety intervals for these indicators.
- The results from Gårdstånga Nygård show no significant effect of the Water Retainer on harvest quantity for neither of the years, 2021 and 2022.
- The Dewaterability Estimation Test device (DET) was found to perform well when tested on sediment sludge from a mature wetland at Gårdstånga Nygård.

Key findings related to the case study site [*final*]

- The sampling and analysis results from Gårdstånga Nygård show no significant effect of the water quality changes for neither of the years, 2021 and 2022.
- There was a large difference in harvest output between 2021 and 2022 at the experimental field mainly due to effects of heavy machinery, involved in the wetland construction, affecting soil structure.

3.5 Case study 4 - Auxerre (France)

3.5.1 General overview

Table 8: General overview – Case study 4

Case study	WATERAGRI solutions	Partner involved
France 	Water Retainer (B5) Microfluidics technology (C5)	Leader: INRAE Participants: BZN, INRAE and Agricultural Chamber of Yonne

3.5.2 Main results for WATERAGRI solutions tested

Water Retainer (B5) [*final*]

- The Chamber of Agriculture who followed the experiment or the farmer who carried it out did not notice any visual difference between the treated and the untreated areas. They also did not record any difference in the sunflower yield between those areas. This applies to both years of the experiment. Those results are in line with the results of the soil analyses performed by BZN on the samples taken from both areas by the Chamber of agriculture along the two cropping seasons (2021 and 2022). BZN's results are presented in deliverable D3.2 (Assessment of Water Retention Methods) and section 5.5 in the Appendix.

Microfluidics technology (C5) [*terminated*]

- No samples have been taken at the French case study site. EDEN planned to test microfluidics at the French site, which represents the continental zone. However, water sampling by EDEN was not foreseen in the budget, thus no sampling campaign has been carried out

Novo design workshops [*final*]

- Four de novo design workshops were organized with a group of seven farmers (January to June 2022) and lead to a first project for water quality related to herbicides. The control of ray grass is a key of that project, as the water catchment is mainly polluted by herbicides related to ray grass control. We started an observatory of ray-grass in wheat (June 2022): we classified 700 ha of wheat in terms of ray grass density. We will use these observations to coach farmers in a step-by-step design process to first reduce ray grass population then maintain a low ray grass seed bank and lastly use low quantity of herbicides. We will also use these observations for the project management.

Figure 6. The WR experiment field in 2021, July 20th.

Figure 7. The WR experiment field in 2022, July 27th.

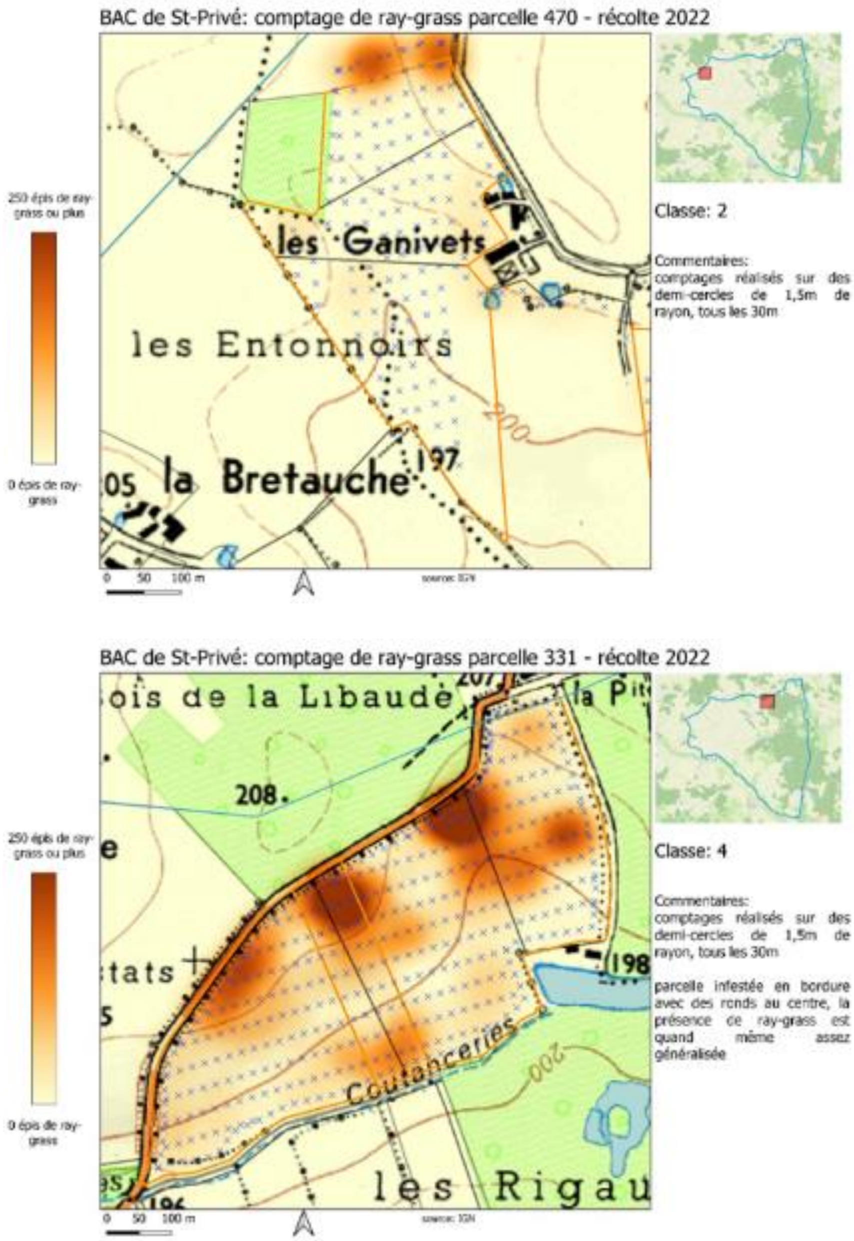

Figure 8. Map of ray grass density in a field of the water catchment area. Each blue cross represents a spot where ray grass was counted. The colour intensity represents ray grass density.

3.5.3 Key findings

Key findings for each solution tested [*final*]

- Water Retainer: No visual distinction nor crop yield difference was found between the treated and the untreated areas

Key findings related to the case study site [*final*]

- Protocols and implementation for the observatory of water were designed and tested.

3.6 Case study 5 - Selhausen (Germany)

3.6.1 General overview

Table 9: General overview – Case study 5

Case study	WATERAGRI solutions	Partner involved
Germany 	Physical modelling (A2) Remote sensing pipeline (B2) Microfluidics technology (C5)	Leader: FZJ Participants: FZJ and INRAE

3.6.2 Main results for WATERAGRI solutions tested

Physical modelling (A2) [*preliminary*]

- The automated pipeline for operational, site-specific forecasting of soil moisture, soil temperature, crop status and other relevant variables for agricultural practice is being tested for the Selhausen site (Fig. 9). The sensors installed at Selhausen host narrowband IoT modems (Wireless sensor network) that allow data transmission to a database in near real-time (Fig. 9a). Typical hydrological and meteorological observation data (e.g., soil moisture content, precipitation amount, air temperature) started in 2011 and are mostly available for 10-minute intervals and can be accessed via specific protocols for modelling.
- For modelling at the plot scale, we use the open-source code "Community Land Model" version 5 (CLM5, Lawrence et al., 2019). The plot scale model of Selhausen is contained in a single CLM5 grid cell (100x100x40 m) and considers 20 hydraulic active layers. The model is based on biophysical laws and constrained by site specific conditions (e.g., soil texture data for different layers, land use and its change from season to season) (Fig. 9 b). Vegetation and carbon and nitrogen pools are simulated prognostically after a 1000-year model spin-up which is needed to initialize the carbon and nitrogen pools. Weather data measured in the field and stored in the cloud (i.e., model factors) drive the model during the observation period. The model jointly simulates water and energy transport in the unsaturated zone, crop growth and yield, snow depth and groundwater depth, and changes in carbon and nitrogen pools.
- In addition, the Parallel Data Assimilation Framework (PDAF) has been coupled to CLM5 and CLM5-PDAF was applied for the Selhausen site. In a data assimilation approach, an ensemble of model runs is performed which should capture the model uncertainty. Measurement data from in-situ and remote (satellite) sensors are assimilated and used to correct the simulated model states in near real-time so that these are closer to the measured values, taking also measurement uncertainty into account. The data assimilation step allows an uncertainty quantification and reduces typically prediction uncertainty.

- The operational application of generating a new forecast every day is mimicked exemplarily at the Selhausen site (Figure 9c). An ensemble of 50 medium-range weather forecasts from the German Weather Service are driving CLM5-PDAF in forecast mode. This produces predictions of hydrological conditions (e.g., soil moisture contents), crop conditions (e.g., biomass, plant drought stress), energy cycles (e.g., soil temperature) and carbon and nitrogen pools and fluxes for the next two weeks.
- Detailed results on physical modelling including data assimilation for case study 5 are presented in Deliverable 6.2 “Model-based Assessment”, 7.2 “Physically based modelling outline draft“, and 7.5 “Data Assimilation framework”. Details on stakeholder feedback on physical modelling is presented in Deliverable 1.6 “Stakeholder Engagement Workshop #3”.

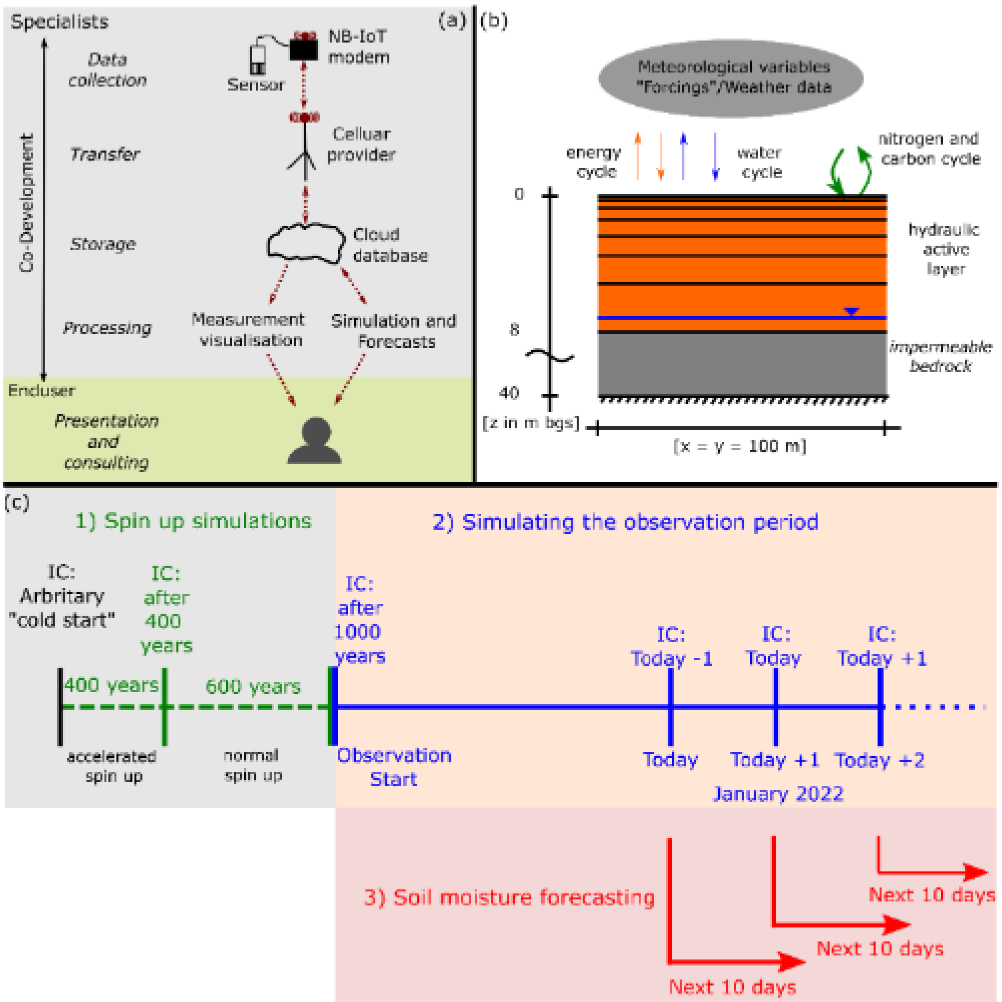

Figure 9: Automated data pipeline for operational site-specific soil moisture ensemble forecasts (Hoffmann et al., in prep.). (a) Scheme for data transmission from sensors to the end user; (b) Conceptual model for a plot-scale model of Selhausen; (c) Simulation routine for daily forecasts of the hydrologic, crop, carbon, and nitrogen conditions and fluxes for the next 10 days.

Remote sensing pipeline (B2) [*terminated*]

- VULTUS is responsible for this task. However, after some discussion it was determined that VULTUS is not responsible for purchasing sensors to collect soil moisture data. Therefore, no data has been collected by VULTUS.
- FZJ conducted drone flights at Selhausen in 2020, which provided information on the leaf area index at the site. These measurements can be used for assimilation.

Microfluidics technology (C5) [*terminated*]

- EDEN planned to test microfluidics at the Selhausen site, which represents the continental zone. However, water sampling by EDEN was not foreseen in the budget, thus no sampling campaign has been carried out.

3.6.3 Key findings

Key findings for each solution tested [*preliminary*]

Physical modelling (A2) [*preliminary*]

- The pipeline for operational site-specific soil water simulations (open-loop) is developed, while mimicking soil moisture assimilation in near real-time is ongoing and part of D7.5. However, developments are the basis for operational site-specific soil moisture ensemble forecasting (data assimilation) that allow optimal estimates of current conditions in agricultural watersheds (e.g., soil moisture, groundwater, and surface runoff). Model results can be visualized online in an easily accessible manner that could be of interest for long-term support for agricultural decision making.

Key findings related to the case study site [*preliminary*]

- We are performing near real-time modelling and soil moisture assimilation using a physically based model of the WATEAGRI Selhausen case study site. Agricultural decision-making considering the model results is mimicked for the Selhausen site. The system in its final version will provide site-specific soil moisture forecasts to stabilize yields in the face of climate variability (e.g., drought). Reliable forecasts at the plot scale are clearly required by stakeholders (workshop #3) and can help optimize irrigation schedules, for example. Therefore, the system is designed to be set up for other sites as well.

3.6.4 References

Lawrence, David M.; Fisher, Rosie A.; Koven, Charles D.; Oleson, Keith W.; Swenson, Sean C.; Bonan, Gordon et al. (2019): The Community Land Model Version 5: Description of New Features, Benchmarking, and Impact of Forcing Uncertainty. *J. Adv. Model. Earth Syst.* 11 (12), 4245–4287. DOI: 10.1029/2018MS001583.

3.7 Case study 6 - Lower Silesia (Poland)

3.7.1 General overview

Table 10: General overview – Case study 6

Case study	WATERAGRI solutions	Partner involved
Poland 	Remote sensing pipeline (B2) Optimize irrigation water (B3 and B4) Water Retainer product (B5) Dewaterability estimation test (B8)	Leader: UPWr Participants: BZN, AGRICOLUS, VULTUS, USAL, UNINE, and UPWr

3.7.2 Main results for WATERAGRI solutions tested

Remote sensing pipeline (B2) [*final*]

- The algorithm for calculation Surface Soil Moisture (SSM) index based on Sentinel-1 images has successfully been tested. The validation of algorithm showed good correlation with in-situ soil moisture measurements (Figure 10).

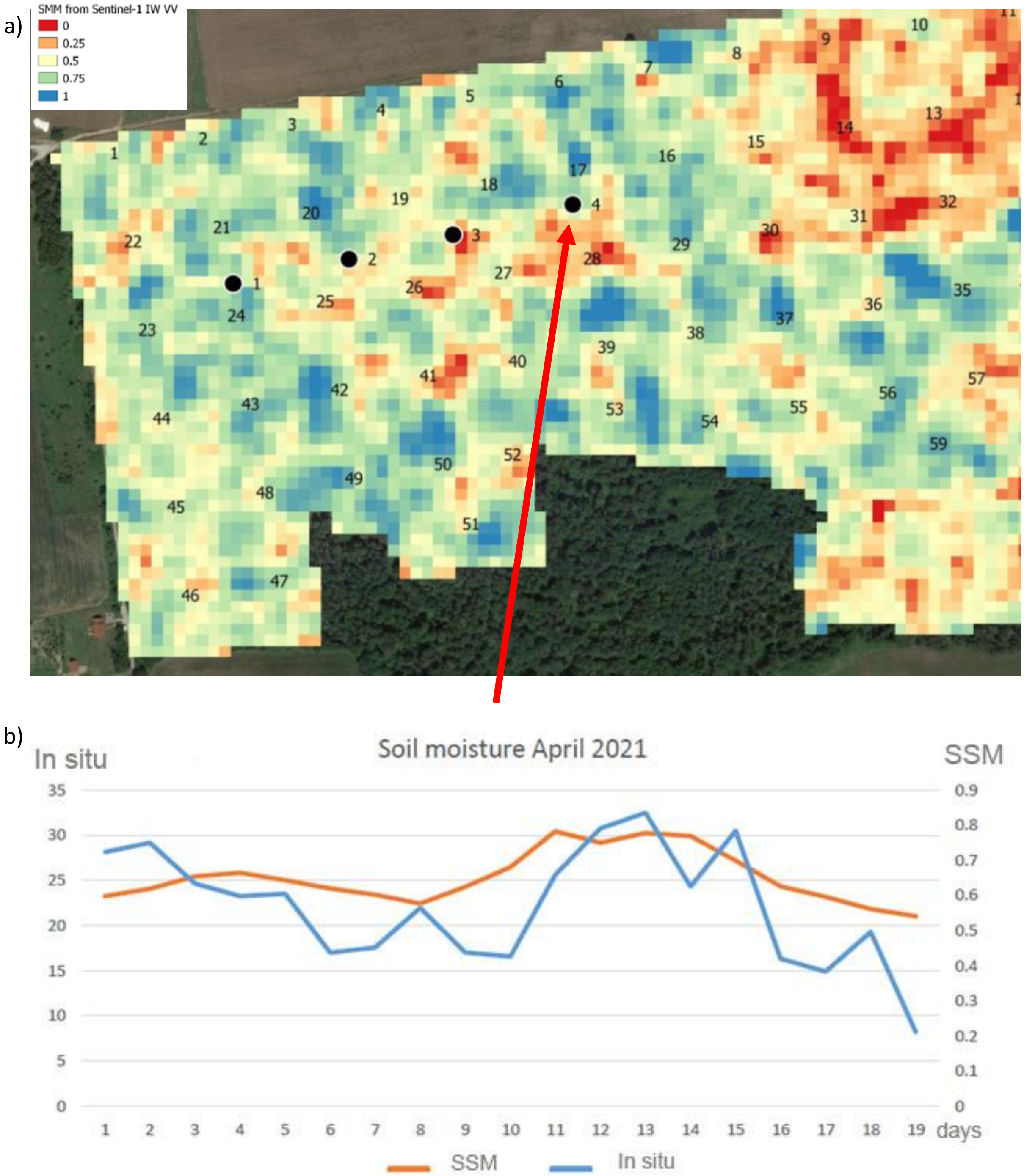

Figure 10. Distribution of SSM index in the field (a) and comparison of SSM with in-situ soil moisture measurement (b).

- Practical implementation, as well as validation of the algorithm used for automatic retrieval of phenological stages of crops of radar backscatter from Copernicus Sentinel-1 images for selected type of crops has been performed (Figure 11).

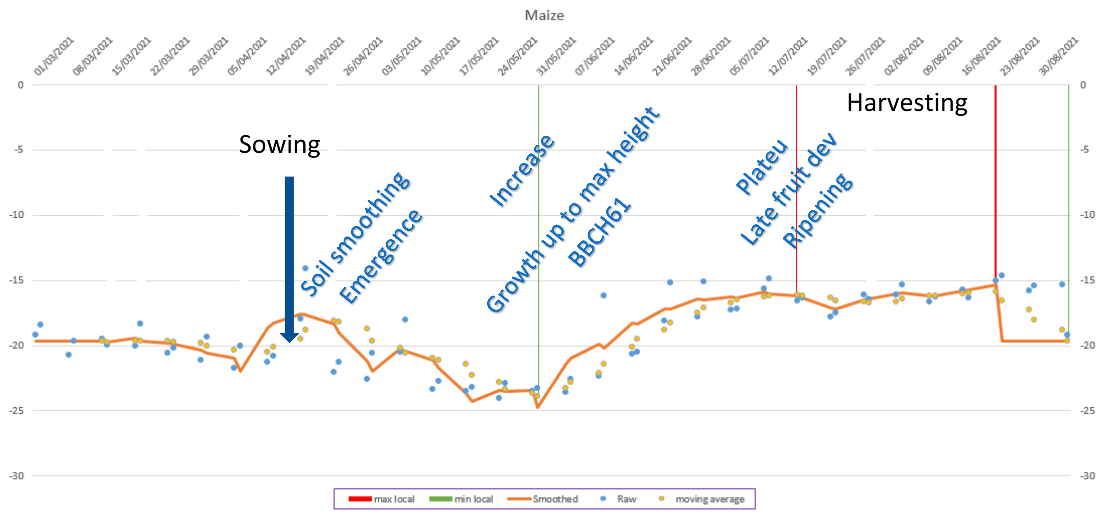

Figure 11. Example of phenological stages estimation from Sentinel-1 backscatter in VH polarization.

- Detailed results of Surface soil moisture and Crop phenology index for case study 6 - Lower Silesia (Poland) are presented in Deliverable D2.3: Remotely sensed data.

Optimize irrigation water (B3 and B4) [*preliminary*]

- The farm does not have an irrigation system. To solve the issue of crop water stress and properly design an irrigation system, a 3-d catchment model was developed and online real-time measurements installed in the catchment for verification (Figure 12).
- Simulations of dry, normal and wet scenarios to identify vulnerable areas that require irrigation were carried out and the results were presented in Deliverable D3.1.
- The calibration of the model will be carried out in WP7 using a novel pilot point approach that allows to represent the heterogeneous structure of the modelled area.

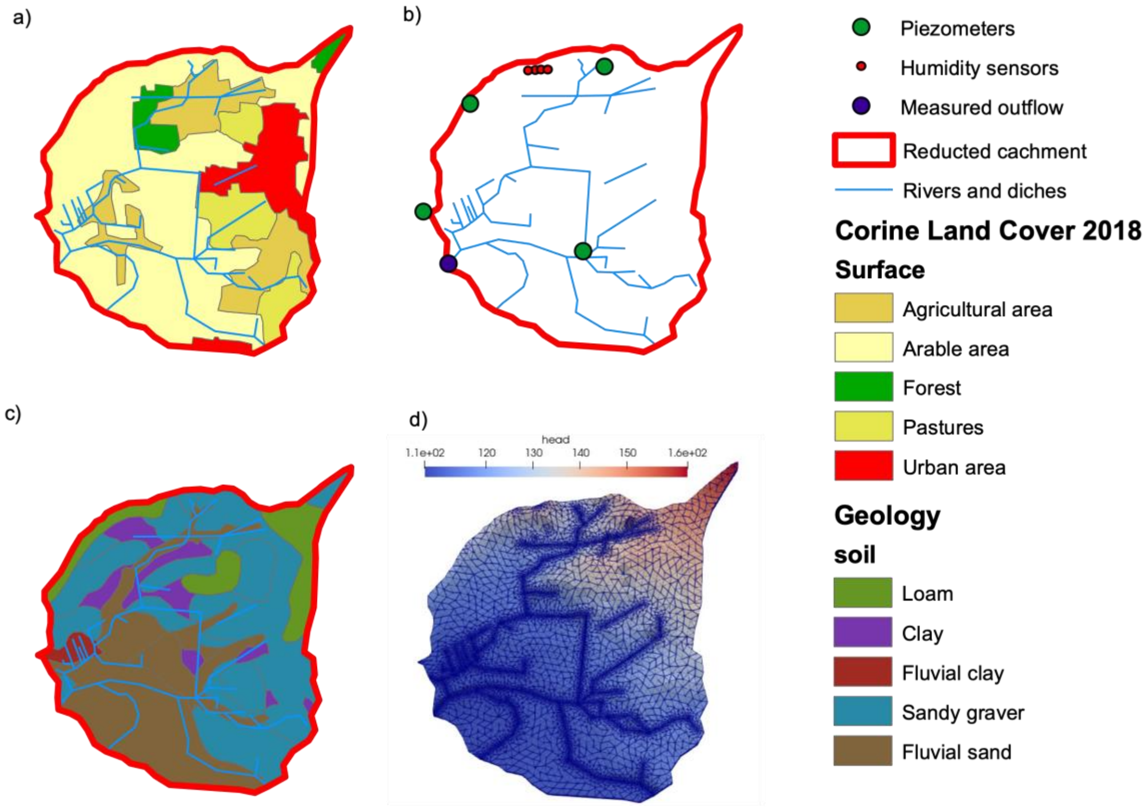

Figure 12. Maps of land cover (a), measurement network (b), soil types (c) and conceptual 3-D model of the catchment generated with HGS (d).

Water Retainer product (B5) [*final*]

- Water Retainer product was applied on 1 ha plots with oats (2021 vegetation period, sandy soil), wheat and barley (2022 vegetation period, loamy sand). The treatment resulted in 7%, 11% and 33% increase of yield compared to untreated plots, respectively.
- Simple 1-D models were made for all scenarios with and without WR application (4 with oats, 2 with wheat and 2 with barley). It was observed that WR effects on soil properties lasted for the first 3 months from application (Figure 13).

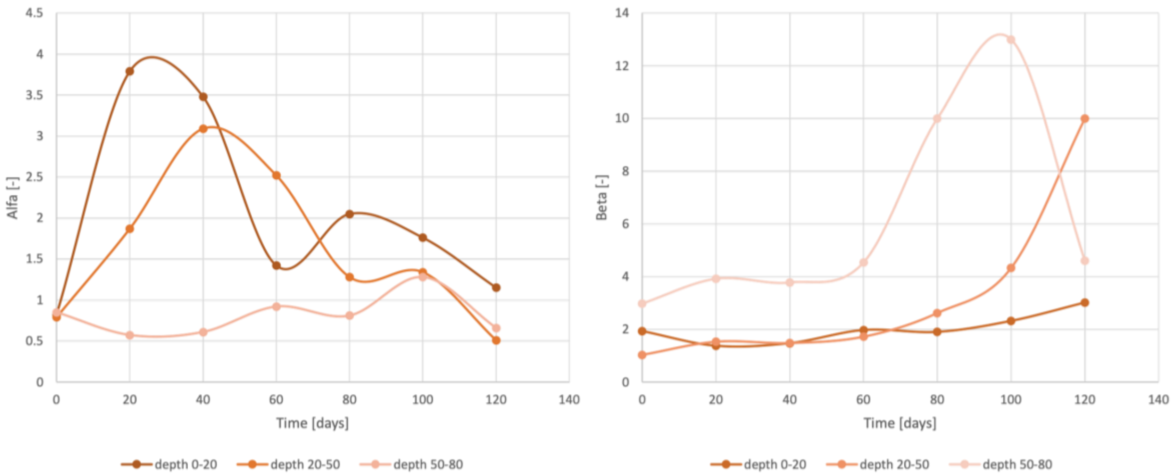

Figure 13. Variability of van Genuchten parameters alfa and beta under the influence of WR obtained from 1-D models in HGS.

- Continuous soil moisture content at different depths was measured for each variant of WR application, as well as outside the area sprayed with WR. The field water consumption method was used to assess the effectiveness of the WR. Detailed results are presented in Deliverable D3.2: Assessment of Water Retention Methods as well as in section 5.5.
- Detailed results of cost benefit analysis, water footprint assessment and life-cycle assessment related to WR applications on different crops in case study 6 - Lower Silesia (Poland) are presented in Deliverable D6.3: Assessment of Sustainability.

Dewaterability estimation test (B8) [*final*]

- Preliminary experiments were conducted on the use of DET apparatus to analyse various soil samples to assess possibility of its application in agriculture.
- To make it easier to analyse undisturbed soil samples in the DET apparatus, a PET sampler was designed and printed on 3-D printer (Figure 14).
- Results of DET experiment showed that at least 6 trials are needed to assess the representative time of water infiltration through the soil sample.
- Detailed results of DET experiments on different soils from case study 6 - Lower Silesia (Poland) are presented in Deliverable D6.3: Assessment of Sustainability.

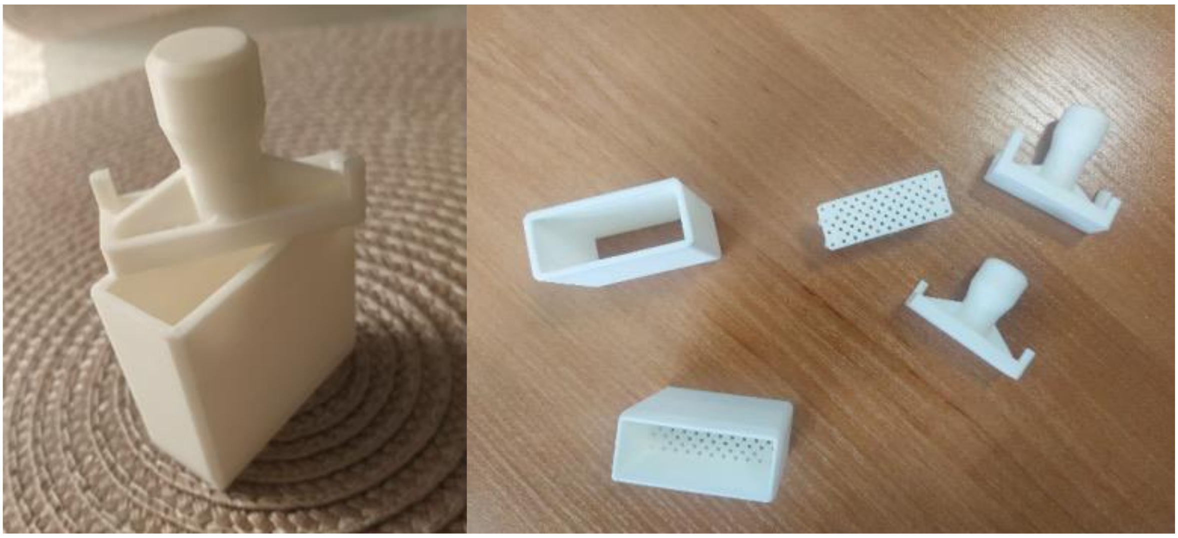

Figure 14. Prototype of soil sampling tool for taking an undisturbed soil sample.

3.7.3 Key findings

Key findings for each solution tested

Remote sensing pipeline (B2) [*final*]

- Implementation of new remote sensing methods can provide farmers with information about surface soil moisture in the field and phenological stages of crops.

Water Retainer product (B5) [*final*]

- Application of Water Retainer product over 1 ha plots without irrigation resulted in 7%, 11% and 33% increase of yield compared to untreated plots for oats, wheat and barley, respectively.

Dewaterability estimation test (B8) [*final*]

- Experiments on soil samples carried out with different methodological settings and application of soil and water mixture (suspension) give similar results with subsequent repetitions on the same soil sample. Thus DET apparatus can be a simple but time consuming tool for assessing soil characteristics to retain and release water.

Key findings related to the case study site [*final*]

- The application of Water Retainer product (B5) to increase the yield of cereals has been confirmed in field conditions.
- Usefulness of Remote sensing pipeline(B2) and Dewaterability estimation test (B8) in crop production has been demonstrated.

3.8 Case study 7 - Seeland (Switzerland)

3.8.1 General overview

Table 11: General overview – Case study 7

Case study	WATERAGRI solutions	Partner involved
Switzerland 	Physical modelling (HydroGeoSphere) (A2) Irrigation (B3+B4)	Leader: UNINE Participants: FZJ, UOULU and UNINE

3.8.2 Main results for WATERAGRI solutions tested

Physical modelling (HydroGeoSphere) (A2) [*preliminary*]

- The physically based model using the HydroGeoSphere (HGS) is complete for the Seeland.
- 3-D subsurface maps of soil hydraulic properties were recently created and will be implemented in the final model to significantly improve the physically based modelling of the vadose zone.
- Data assimilation framework for the physically based modelling HGS is in active development. The current framework allows jointly updating the state variables (e.g. heads, saturation) and model parameters (hydraulic conductivity) by assimilating the piezometeric heads and soil moisture observations.
- Synthetic modelling evaluation of soil water retention solutions are ongoing (in collaboration with UPWr and USAL).
- Detailed results of physical modelling for case study 7 are presented in Deliverable 7.5.

Irrigation (B3+B4) [*preliminary*]

- New drains are developed and included to the existing drainage system in the model as well as the test site. These new added drains in the field can be switched on/off depending on the requirement of the farmers.
- A small-scale local stakeholder workshop took place in the test site together with the largest farming association of the Seeland – Grosses Moos region and local engineering company RSW AG. Results of modelling simulations with the new drainage system were present and discussed. Suggestions were proposed and have been applied for further improving our model configuration.
- Detailed results for case study 7 are presented in Deliverable 6.2.

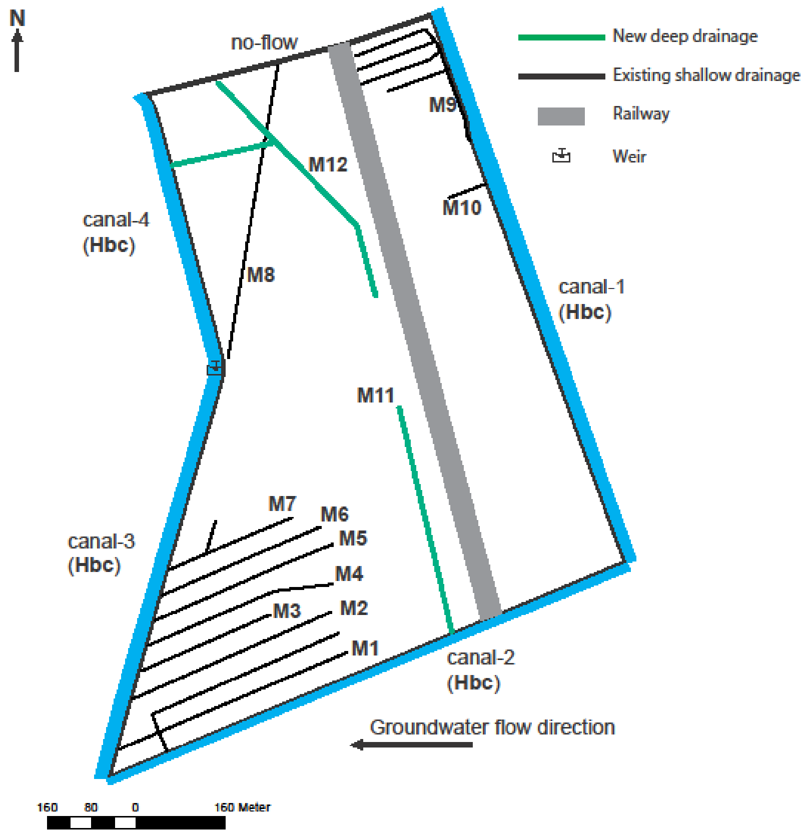

Figure 15. The drainage system in the Seeland region

3.8.3 Key findings

Key findings for each solution tested [*preliminary*]

- The developed physically based modelling framework (A2) serves as a robust tool to carry out operational simulations which allows real-time assessment.
- The proposed drainage system (B3) efficiently optimised the soil water content under different conditions (e.g., flood and drought periods).

Key findings related to the case study site [*preliminary*]

- The real-time modelling framework can integrate field data, modelling approaches and weather forecast.
- Through the active management of the surrounding canals, soil water deficit can be reduced significantly. This option can be considered in the data assimilation framework.

3.9 Case study 8 - Obersiebenbrunn/Mistelbach/ Gleisdorf (Austria)

3.9.1 General overview

Table 12: General overview – Case study 8

Case study	WATERAGRI solutions	Partner involved
Austria I - Obersiebenbrunn 	Tracers (B7) Irrigation practices (B3)	Leader: BOKU Participants: -
Austria II - Mistelbach 	Biochar for water retention (B6) Activated biochar (C2)	Leader: BOKU + ALCN Participants: UOULU
Austria III - Gleisdorf 	Drainage systems (C4)	Leader: TBR + ALCN Participants: -

3.9.2 Main results for WATERAGRI solutions tested

3.9.2.1 Case study Austria I (Obersiebenbrunn)

Tracers (B7) [*final*]

- The method allows direct quantification of average water flux in a given period in agricultural soils.
- We used oxygen and hydrogen isotopes ($\delta^{18}O$, $\delta^{2}H$) of precipitation water to study the movement of water in soils under different management practices (four tillage variants and four irrigation systems).
  - The tillage variants compared in the study were: conventional tillage (CT), reduced tillage (RT), minimal tillage (MT), no tillage (NT)

    - The irrigation systems compared were: boom irrigation (BI), consisting of a hose reel boom with nozzles, overhead sprinkler irrigation (SI), surface drip irrigation (DI), and no irrigation (NI).
- Measuring the water stable isotopes ($\delta^{18}O$, $\delta^{2}H$) in pore water has allowed tracking precipitation water from the end of November 2019 to May 2020, i.e. six months.
- The depth at which precipitation water was found indicated the flow velocity of soil water. In this case precipitation water from the end of November 2019 shaped a winter nadir, i.e. precipitation significantly depleted in heavy isotopes, between 20 and 40 cm depth.
- Soil isotope profiles showed a clear difference in flow velocity between the tillage variants. The winter nadir was found deeper in the soils managed under conventional tillage (35 cm) which indicated a higher water flow velocity than the rest of the tillage variants. The untilled soils showed the lowest water flow velocity with the winter nadir located at 25 cm depth, and reduced and minimal tillage practices had intermediate flow velocities. No differences in water flow velocity were observed between the irrigation systems.
- The peak-shift method was successfully applied to quantify average water flux in all the plots, which ranged from 3.8 to 7.6 mm/month. The water flux was included in the soil water balance as mobile soil water to calculate the evapotranspiration.
- Cumulative evapotranspiration ranged from 146 to 244 mm, and mobile soil water from 23 to 46 mm, for a period of six months.
- The increase in tillage intensity showed clear trends for evapotranspiration and mobile soil water. The more intensive the tillage, the higher the evapotranspiration and the lower the mobile soil water.
- Regarding irrigation systems, DI contributed the least to evaporation and the most to mobile soil water, while SI and BI led to higher evapotranspiration.
- This method enables to quantify average water fluxes from a single sampling campaign. This is of high importance of agricultural areas with few data available or even remote areas.
- Detailed results on Tracers (B7) for case study Austria I are presented in Deliverable D3.2. Assessment of Water Retention Methods.
- A scientific paper presenting the method and the results is under revision: Canet-Marti, A., Morales-Santos, A., Nolz, R., Langergraber, G., Stumpp, C. Quantification of water fluxes and soil water balance in agricultural fields under different tillage and irrigation systems using water stable isotopes. *Soil and Tillage Research*, *submitted*
- A simulation study is being performed to study the attenuation of $\delta^{18}O$ and $\delta^{2}H$ profiles in the unsaturated zone for different soil textures and biogeographic regions. The aim is to provide sampling recommendations such as the best time for sampling and appropriate depth intervals for sampling. So far, the profiles generated for a scenario in Vienna show soil isotope profiles that are consistent with that of our study in Obersiebenbrunn.
- Detailed results on the simulation study of soil isotope profiles in different soil textures and biogeographical regions are presented in Deliverable D7.1. Simplified models for WATERAGRI Innovations.

Irrigation practices (B3) [*final*]

- Unfortunately, as our experimental field was divided into small plots under different management practices, the surface was too small to apply *B3 – Irrigation management and agrometeorological monitoring solutions* effectively.

3.9.2.2 Case study Austria II (Mistelbach)

Bio-inspired multi-layer vertical-flow systems were constructed above ground in three IBC tanks in June 2021, in an agricultural land, each having a surface area of 1.2 m^2 and 0.65 m filter height composed of different layers of substrates. Filter 1 (Draingarden filter): a vegetated filter system (with Draingarden + biochar), Filter 2 (Biochar filter): was an unplanted filter (with biochar in the main layer); Filter 3 (Soil filter): vegetated system with local soil as reference. The surface agricultural run-off was collected from a catchment area of 30m^2 (1% slope) and directed with a 30 m long pipe in a three-way distributor, which fed each system with surface runoff.

Monitoring seasons: The systems were constructed in June 2021 and monitored for two consecutive seasons (June - August 2021; 12 May 2022 – 12 September 2022). During 2021 no data were able to be collected, due to lack of heavy rainfall that can produce enough agricultural runoff for the systems. During 2022, parameters monitored included: Tracer tests (NaCl), moisture and temperature sensors in two depths, nutrients (PO_4, NH_3-N, NO_3-N) pH, and EC. However dry periods existed also in 2022. Summarized results from monitoring season 2022, are presented below.

Biochar for water retention (B6) and nutrient uptake (C4) in a drainage system with Activated biochar (C2) [*final*]

Activated biochar ($Mg(OH)_2$ coated biochar) was tested in laboratory (batch experiments) and in field implementing two technology solutions: one for agricultural runoff (Mistelbach), the other one for subsurface drainage water treatment (Gleisdorf).

- Batch sorption experiments in laboratory showed that the coated biochar retains phosphorus and nitrogen better at high inlet P and N concentrations, probably due to $Mg(OH)_2$ coating, than at low phosphorus concentrations.
- For the results of activated biochar in field trials (Mistelbach) and (Gleisdorf), please refer to the respective key findings in each section.

Water retention (B6) [*final*]

Biochar due its high porosity, has been shown to increase soil water holding capacity (Karhu et al., 2011; Rasa et al., 2018; Yu et al., 2013). In a vertical flow system, it is not easy to assess the water holding capacity of the biochar, but comparing water moisture content between the three filters can be an indicator.

- In Mistelbach case study, monitoring started on 15.05.2022 and lasted until 15.09.2022. There were only three significant rain events in 2022 monitoring periods on days (17.5, 05.07, 28.08) where all three filters had output on the same day. The rest of the days draingarden and soil filters either had insignificant dripping after a precipitation or no water output at all. Biochar drained more water compared to other filters in general, however it has also less than five-litre output where the other filters had none.

- The data shows there was not enough rain to produce agricultural runoff in the region during the summer and there were prolonged dry and hot periods between the rain events. Since the monitoring system is designed to switch on when there is flowing water into the system, it was not possible to measure the moisture values of the filter during the dry periods.
- Data received from the moisture sensors (top 17 cm, bottom 30 cm) shows that both measurement points from the biochar filter remained humid all the time (≥100%). This percentage was calculated by the measurement range of the sensor (0-20 mA).
- The results show that moisture content of soil filter was 14%, for the upper layer of Draingarden filter was 23-26%, and for the lower layer of Draingarden moisture content varied between 19 to 28%. Biochar was the most moisture material by qualitative check, as well as from the sensor's measurement, which values indicated moisture content equal or higher than 100% .

Tracer test results (Mistelbach)

- Tracer experiments were performed on 12.05.2022. Influent consisted of 50 L of tap water spiked with 25 mS NaCl loaded in each system and followed with 50L of tap water loaded every hour, amounting in 150 L of fresh water per filter system. Effluent was monitored continuously for 47 hours with EC meter and data were stored automatically.
- Results of tracer experiments: the effluent tracer breakthrough curves demonstrated a fast peak, which is an indication of preferential flow. Accumulative NaCl tracer recovered was 90% for Soil filter, 89% for Biochar, and 115% for Draingarden.

Nutrient uptake/retention results

- Water quality monitoring: Effluent samples were collected with tipping counters and analysed for PO_4, NH_3-N, NO_3-N, pH, EC and temperature.
- Results from effluent concentrations for PO_4, NO_3-N, and NH_3-N show some variability over time; concentrations ranged between 0.06 to 3 mg/L, with an outlier for NH3 of (30 mg/L). All three filters behaved similarly for PO4 retention with effluent concentrations below 0.5 mg/L. For NO_3-N, Draingarden and biochar filter had some variability, with soil retaining better NO_3-N.
- Due to lack of precipitation events to produce enough agriculture runoff, a small dataset of results was collected that do not allow to arrive at final conclusions regarding filter performance.
- Background electrical conductivity (EC) values of the filter systems were around 0.8-1 mS/cm. Biochar filter had higher pH values (average 9.47) compared to Draingarden and soil filters (8.09 and 8.08, respectively).

Detailed result on Activated Biochar (C2), Biochar for water retention (B6) for case study Austria II, Mistelbach and case study Austria III Gleisdorf, are presented in Deliverable: D4.3. Description of Developed Drainage Technologies, and D4.5. Advanced use of biochar for nutrient retention.

3.9.2.3 Case study Austria III (Gleisdorf)

Drainage systems (C4) [*preliminary*]

The drainage system consisted of an in-pipe filter structure, inserted in the outlet of a subsurface agricultural drainage water pipe. The performance in terms of nutrient retention was investigated in a real drainage pipe in Gleisdorf at an organic farm.

- Two cartridges were inserted in the drainage pipe. Dimension of each cartridge were: 700 mm long, 74.5 mm radius, 149 mm height, and volume of 8 L. Initially both cartridges were filled with biochar, but due to limited hydraulic conductivity for this type of material, overflow occurred. Therefore, in the second trial a substrate like zeolite 4-8 mm was introduced to increase hydraulic conductivity and zeolite is also known for adsorption of ammonia (NH_4-N). The biochar used so far was fine (0-2 mm grain size), meaning limited hydraulic conductivity. Therefore, we thought to increase the grain size of the biochar as well search for biomass that contains less phosphorus to avoid previous P leaching issues from biochar. Biochar produced from cherry seeds and coated with $Mg(OH)_2$ (0–4 mm) was ordered from Sonnenerde to enhance phosphorous retention properties of biochar..
- In the third trial, the filters were filled with zeolite 4-8 mm in the first cartridge structure (deeper in the drain), and $Mg(OH)_2$ coated biochar 0-4 mm grain size produced by cherry seeds, in the second cartridge structure. The third trial operated for 110 days, and the filters were removed from the drainpipe after 32.8 m^3 of water had passed through. Overflow and subsequently sedimentation was observed on top of the filter as well as inside. The results of some sampling points showed that effluent concentrations decreased passing from influent, effluent of 1st structure, to final effluent after the 2nd structure. But this was not observed for every sampling date.
- During this first monitoring year 2021, dry periods occurred (15.07.2021 - 01.09.2021) , where no water was collected in the outlet.
- Prior to field application, the biochar was tested in laboratory to assess sorption properties of the material. Sorption curves for PO_4, NH_3-N, NO_3-N were determined with a range of inlet concentrations varying from 0 to 25 mg/L. Biochar did not reach the saturation point for these concentrations. Detailed results are given in D4.5.
- To optimize further the filter system in Gleisdorf, columns experiments are currently being carried out through a Master thesis at BOKU in collaboration with ALCN to assess nutrient capacity of zeolite and biochar under different flow rates.

Tracers (B7) [*final*]

- In the case study Austria III, we used tracer methods to compare the water flux in soils of a conventional (CON) and an organic (OR) farm. The farms were adjacent fields that had similar soil texture and cultivated the same crop (Styrian pumpkin).
- Measuring the water stable isotopes ($\delta^{18}O$, $\delta^{2}H$) in pore water has allowed tracking precipitation water from January 2022 to the beginning of November 2022, i.e. ten months.
- The CON farm had higher flow velocity than the OR farm; the precipitation from January 2022 was found deeper in the soil profile.
- CON had higher water content in the whole profile compared to OR, which also translated into higher water flux, with approximately 17.5 and 12.8 mm/month for CON and OR.

Besides the technologies, also Total Organic Carbon (TOC) was analysed in both OR and CON fields. OR fields had approximately 2% of total organic carbon in the first 30 cm, while CON had around 1.6%, with only 1.2% in the first 10 cm. From 30 cm TOC decreased in both fields.

Detailed results on tracers (B7) and Total Organic Carbon data are reported in the appendix Chapter 5.2 “Case study Austria III (Gleisdorf): Differences in soil water flux and soil organic carbon in fields under conventional and organic farming”.

3.9.3 Key findings

Key findings for each solution tested

- Tracers (Obersiebenbrunn) [*final*]: The measurement of $\delta^{18}O$ and $\delta^{2}H$ in soil pore water allowed for tracing precipitation water providing integrative information on water flow and transport. Consequently, the water fluxes were quantified in agricultural soils under different management practices. The peak-shift method proved to be sensitive enough to observe differences between the treatments. The findings provide a basis for future studies, in which the method can be applied to quantify water fluxes over shorter periods and to assess the long-term impact of treatments.
- Bio-inspired multi-layer system (Mistelbach) [*final*]: The monitoring of effluent nutrient concentrations showed that the filters could be potential solutions, but careful selection of biochar should be made because they may have phosphorus in their composition that is leached at the initial stages of operation. Batch sorption experiments in lab showed that the $Mg(OH)_2$ coated biochar retains phosphorus and nitrogen better at high inlet P and N concentrations, probably due to $Mg(OH)_2$ coating than at low phosphorus concentrations. Tracer tests with NaCl, revealed that the systems had fast peaks indicating non-homogeneous flow behaviour. The preferential flow could be attributed to the impact that ‘freeze-thaw-dry’ atmospheric conditions had on the filter systems for 2 years, which could lead to the formation of cracks or preferential pathways. Even so, the filter reduces the peak discharge, so it can be considered a good measure for temporary water retention. In terms of nutrient uptake, the data set collected is too limited to make conclusions regarding nutrient retention performance of the system. This is due to lack of agricultural runoff produced in monitoring seasons 2021 and 2022.
- Drainage system (Gleisdorf) [*preliminary*]: Zeolite 4-8 mm and $Mg(OH)_2$ coated biochar from cherry seeds can be a potential good combination to fulfil the hydraulic properties and retention properties that a filter should have. The period of exchanging filter media is to be assessed and optimized based on local conditions (inflow, fertilizer use, etc.)

Key findings related to the case study site

- Obersiebenbrunn [*final*]: The combination of agricultural management practices used demonstrates the effects of tillage practices and their combination with irrigation systems. Farming practices should be chosen on a crop-by-crop basis based on their effects on evapotranspiration and available water in the soil.
- Mistelbach [*final*]: Implementing nature-based solutions, such as bio-inspired multilayer filter system to address agricultural runoff to retain nutrients has good potential, but the systems

need further research and if upscaled, filter systems should be designed inside the land. Bioengineering techniques for real scale applications of the researched pilots are presented in D4.1 Description of Developed Wetland Technologies

- Gleisdorf [*preliminary*]: This in-pipe cartridge could provide a niche solution in addition to other forms of filter media enclosure.

3.10 Case study 9 - Bologna (Italy)

3.10.1 General overview

Table 13: General overview – Case study 9

Case study	WATERAGRI solutions	Partner involved
Italy 	Wetlands (B1+C1) Remote sensing pipeline (B2) Irrigation practices (B3+B4) Water Retainer (B5) Dewaterability estimation test (B8) Bio-membranes (C3) Microfluidics (C4)	Leader: UNIBO and CER Participants: AGRICOLUS, BZN, USAL, VTT, VULTUS, CER and UNIBO

3.10.2 Main results for WATERAGRI solutions tested

Wetlands (B1+C1) [*preliminary*]

- A full-scale surface flow constructed wetland (SFCW) (Figure 16) present in the Italian case study site was tested from both water quantity (water retention and groundwater recharge) and water quality (removal of nutrients and other pollutants typical for agricultural drainage water) aspect.
- The part of runoff that the system can manage to store in a year was in the range 40-50%. However, that amount is very dependent on precipitation intensity and its yearly distribution. Also, additional analysis of an intensive precipitation event showed that the system can store the first runoff produced and therefore contribute to peak flow delay.
- The SFCW in question was found to influence groundwater recharge but substantial differences were observed among points used to measure the groundwater table. Although additional studies would be needed to define the water flow routes, the recharge rate in different points did not seem to be correlated with the distance from the system.
- Monitoring of SFCW influent and effluent showed that the system acts as a sink for different agricultural pollutants. The removal of these compounds can be up to 100%, depending on the season and runoff production pattern. The higher removal was observed when the inflow to the system was well distributed over the time, ensuring high retention time.
- Detailed results on Wetlands (B1+C1) for case study 9 Bologna are presented in D3.2 and D4.1.

Figure 16. The full-scale surface flow constructed wetland at the Italian site.

Remote sensing pipeline (B2) [*final*]

- For identified fields cropped in the years from 2018 till 2021 crop's data have been collected and organized. Specifically, CER has provided the geomatic reference of the experimental field and the list of the available measurements for each field in the period 2018-2020. This helped Vultus to assess its own product re-analysing past images/data from the Copernicus satellites.
- Specific data collection procedures have been carried out for irrigated crops to provide better insights about crops development and biomass. This is the case of LAI assessment with scanning procedures detailed in Figure 17.
- Detailed results on data provision for Remote sensing pipeline for case study 9 are presented in D3.1 and D2.5.

Scanning of corn leaves previously weighed and subsequently dried in a thermo-ventilated oven at 70 ° for 72 hours.

Scanning of tomato leaves previously weighed and subsequently dried in a thermo-ventilated oven at 70 ° for 72 hours.

Figure 17: Means of validation of Remotely Sensed Vegetation Index

Irrigation practices (B3+B4) [*final*]

- Crop data have been collected from sensors, in-field measurements and experimental harvests. Data provided to assess the effectiveness of the Vultus Earth Observation products (B2) was also made available to develop B3 and B4 so that the information from satellite observation could be assimilated into the irrigation management. Furthermore, during the irrigation season 2021 some crops/irrigation methods have been selected to run IRRIFRAME water balance which has been used as benchmark for the Agricolus solution (Figure 18).
- Detailed results on data collection and provision for irrigation practices (B3+B4) development for case study 9 are presented in Deliverables D3.1. and D2.1

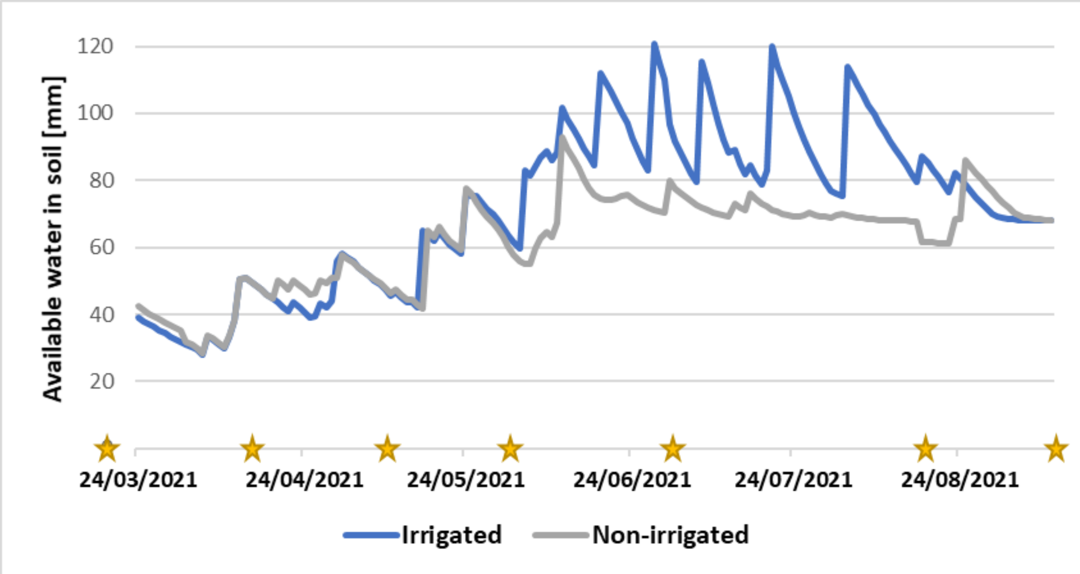

Figure 18: Output from IRRIFRAME water balance used as benchmark to assess irrigation practices.

Water retainer (B5) [*final*]

- The irrigation seasons 2020 and 2021 have been used to test Water Retainer (B5) performances. This has been done by comparing three experimental fields cultivated with corn in 2021 or soybean in 2022. The three tested thesis/scenarios are the following: (i) Irrigated (Irr): this field has been irrigated with pressurized method (roller irrigator), where timing and volume have been defined with the support of IRRIFRAME; (ii) Not irrigated (NoIrr): this field represents the baseline and is not irrigated nor treated with the WR product; (iii) Treated with water retainer (WR): this field represents the test and is not irrigated but is treated with the WR product (Figure 19).
- Main findings show that irrigated crops are always better performing in terms of yield than non-irrigated (NoIrr and WR), however some positive and significant contributions might be found by employing the Water retainer (B5) with corn but not with soybean. The mixed nature of these results highlights how trials might have been influenced by environmental settings and especially severe droughts which increased differences in soil retention capabilities between plots due to differential soil textures.
- Detailed results on data collection and provision for Water Retainer (B5) development for case study 9 are presented in Deliverable D3.2, results on Water Retainer (B5) performance for soybean in the annex in chapter 5.3. Results related to soil nutrients and microbiological measurements are presented in D3.2 (Assessment of Water Retention Methods) and section 5.5 in The Appendix.

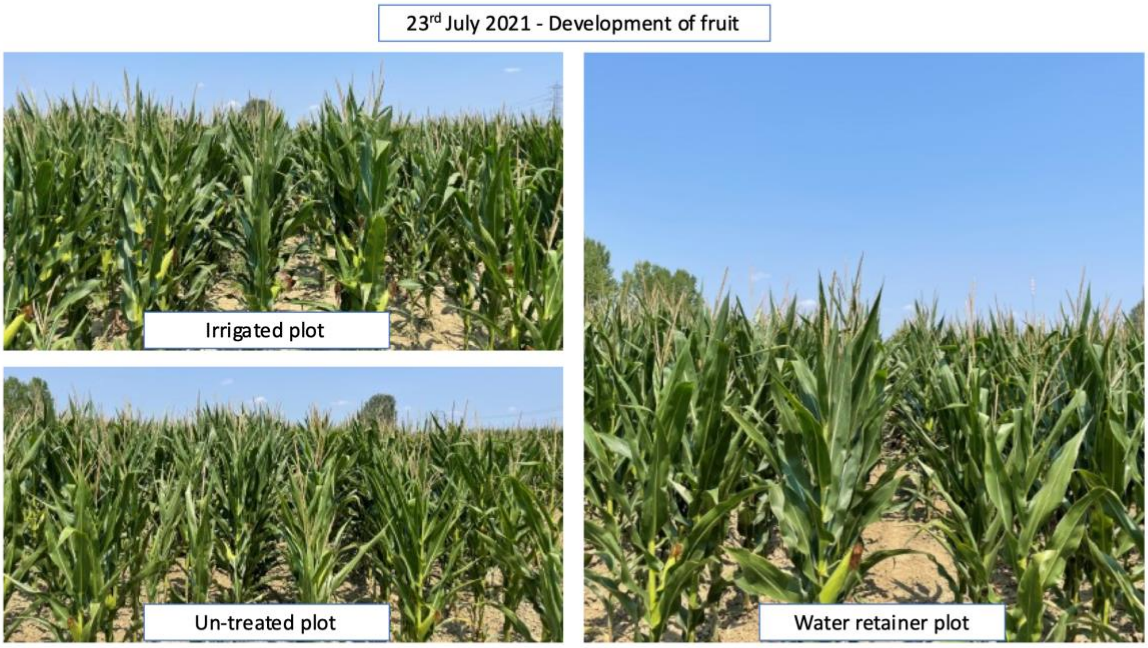

Figure 19: Crop development at the BBCH-scale (Biologische Bundesanstalt, Bundessortenamt and CHemical industry) phenophase “Development of fruit” on 23.07.2021.

DET apparatus (B8) [*final*]

- DET apparatus has been tested with agricultural soils to assess soil water content in the range between saturation and field capacity. Different dilution settings have been compared to help solution developers identify proper usage (Figure 20). Results show the capability of DET to be used with soils, but proper guidelines and manuals should be defined for effective implementation.

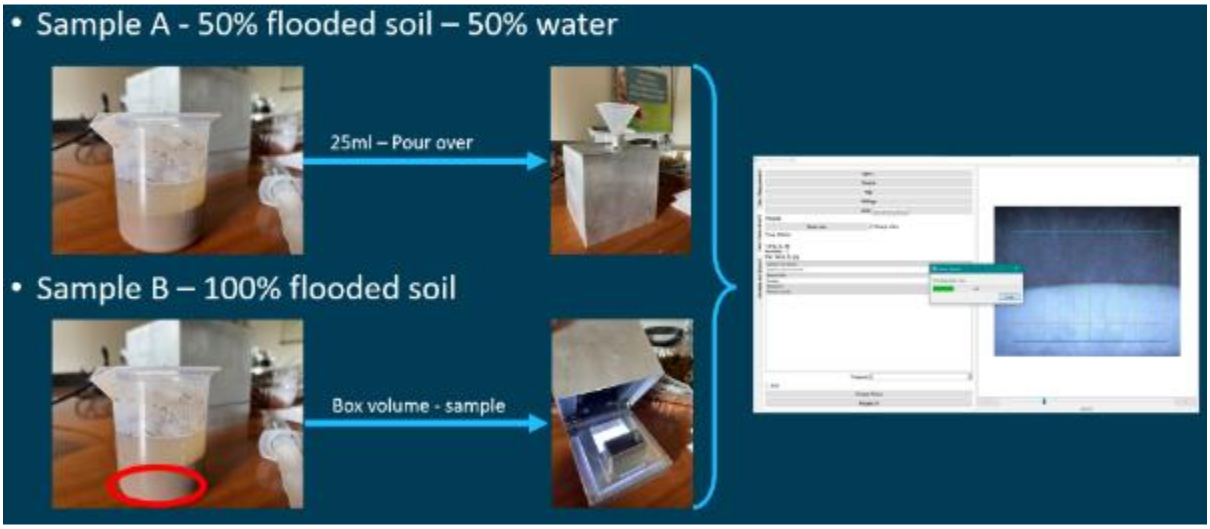

Figure 20: A scheme of DET tests performed.

Bio-membranes (C3) [*final*]

- The membrane produced by VTT was tested by the same partner in static conditions, and therefore there was a need to also test it in dynamic conditions, with a constant water flow.
- The ready material was sent to Italy where it was tested at the local case study as a part of the pilot plant (Figure 21). In total, two tests were performed, with the second one being done with a modified membrane and experimental plan according to the results of the first trial.
- The results obtained during the two trials indicated a certain capacity of the membrane to adsorb nutrient (up to approximately 15%), but also showed that the material should be modified in order to achieve higher removal rates.
- Detailed results on membrane testing at the case study 9 are presented in Deliverable D4.4.

Figure 21. Module used for Bio-membrane testing at the Italian case study site.

Microfluidics (C5) [*final*]

- In agreement with EDEN, three sampling campaigns at the Italian case study were performed - in Q4 of 2021, as well as Q1 and Q4 of 2022. Different samples of drainage and/or irrigation water were collected and sent to a laboratory chosen by EDEN for further analysis.
- The results obtained are used by EDEN to estimate if the solution is suitable for the use in agricultural setting and the results are presented in D4.6.

3.10.3 Key findings

Key findings for each solution tested [*final*]

- Wetlands (B1+C1): the experimental results have shown that farm constructed wetlands can have multiple benefits in the agricultural environment, including increased water availability and better management of intensive rain events, as well as purification of polluted agricultural streams.
- Remote sensing pipeline (B2) and Irrigation practices (B3+B4): concerning these solutions, the activities were mainly focused on data collection and provision for the development of the innovative solutions.
- Water retainer (B5): field trials revealed WR potentials to protect not-irrigated corn against droughts. The opposite can be said for soybean, where WR-treated field produced significantly less. However, the 2022 irrigating season has been particularly dry and local exogenous settings might have influenced yields. Irrigated plots were always more productive.
- DET apparatus (B8): once proper guidelines for its usage are given, DET can be effectively used for agricultural soils with potentials to gain better insights on gravitational water moment in soils, soil drainage water capabilities and hydraulic monitoring after heavy rainfall.
- Bio-membranes (C3): although the solution has a certain potential when applied in dynamic and real environmental conditions, its performance could be improved to reach higher capacity for nutrient retention.
- Microfluidics (C5): three sampling campaigns performed at the Italian case study have showed a certain presence of different elements in agricultural drainage water.

Key findings related to the case study site [*final*]

- Tests in Case study 9 highlighted how WATERAGRI solutions can help to improve water retention at the field level, gain better insights on soil water content and increase farm resilience to water related problems.
- With the adoption of nature-based solutions, biochar and membranes, nutrient recovery can be boosted with in-farm benefits related to the possibility of reducing fertilizer inputs, and with different environmental benefits (e.g., lowering nutrient pressure on natural water bodies).

3.11 Case study 10 - Nyírbátor (Hungary)

3.11.1 General overview

Table 14: General overview – Case study 10

Case study	WATERAGRI solutions	Partner involved
Hungary 	Physical modelling (A2) Irrigation practices (B3+B4) Water Retainer (B5) Dewaterability estimation test (B8) Microfluidics (C4)	Leader: UNIDEB Participants: ULUND, FZJ, BZN, USAL and UNIDEB

3.11.2 Main results for WATERAGRI solutions tested

Physical modelling (A2) [*preliminary*]

- Simulated soil moisture with depth versus time. (Figure 22 a)
- Simulated soil temperature with depth versus time (Figure 22 b).
- Detailed results on Physical modelling (A2) for case study 10 are presented in Deliverables D6.2 “Model based Assessments” and D7.5 "Data Assimilation System for Physically Based Models”.

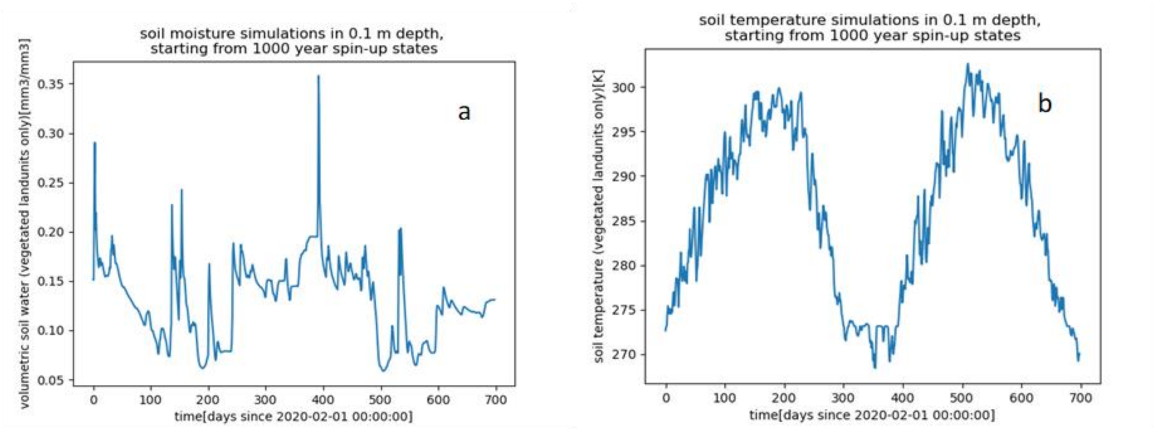

Figure 22. Simulated (a) soil moisture and (b) soil temperature at 10 cm depth

Irrigation practices (B3+B4) [*final*]

- Integration of the weather station into the Agricolus platform, resulting in precise localized weather forecasting enabling more accurate irrigation scheduling (Figure 23).
- The vegetation pattern was monitored by Agricolus platform in order to assess the homogeneity of the vegetation cover. Based on the vegetation cover and soil heterogeneity the variable rate irrigation (VRI) can be re-adjusted
- The use of spectral and LiDAR data can help farmers to detect abiotic and biotic stress as well as to improve irrigation management for increased water savings and better crop production.

- Detailed results on Irrigation practices (B3+B4) for case study 10 are presented in Deliverable D3.1: Assessment of Use of Remotely Sensed Vegetation to Improve Irrigation, Deliverable D2.5: Remote sensing pipeline and in the Appendix (Chapter 5.4.1).

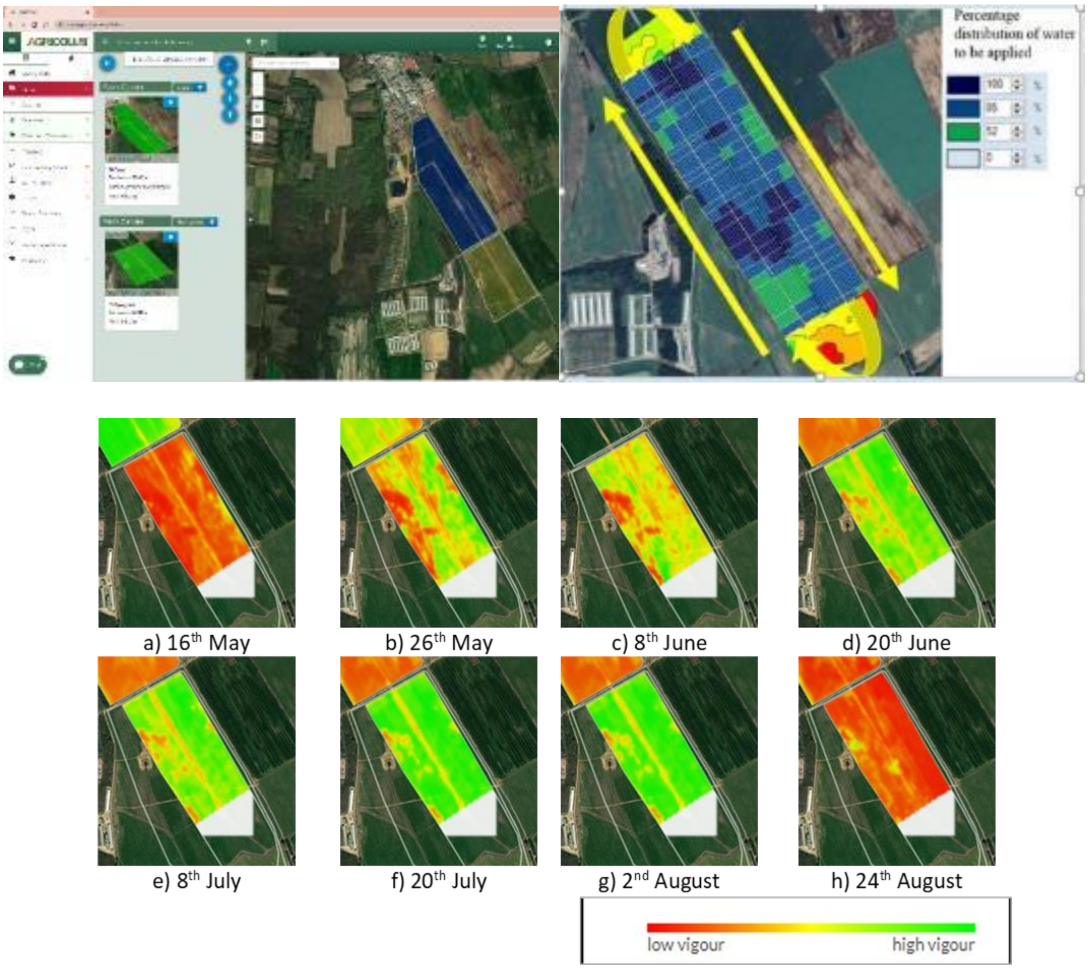

Figure 23. Hungarian case study site in Agricolus platform: NDVI images of Nyírbátor maize field at different dates in 2022.

Water retainer (B5) [*final*]

- Based on laboratory soil incubation experiments, the Water Retainer did not adversely affect soil pH, did not affect the EC value of the soil, increased the ammonium content of the soil in the weeks following its application. It was more effective in increasing soil nitrate contents when applied at 100 times dilutions.
- Based on the pot experiment: there is a difference between the effect of the tested product at different water capacity levels (Figure 24). Water Retainer resulted in higher plant biomass weight at higher water capacity level, and the applied treatments had no significant effect on plant biomass and inner parameters.
- Based on the field experiment: 100 times dilution of Water Retainer resulted in higher soil moisture level. The tested product had no significant effect on water distribution in the soil by depthFigure 24.
- Detailed results of Water Retainer application (B5) for case study 10 are presented in the Appendix (Chapter 5.4.2).

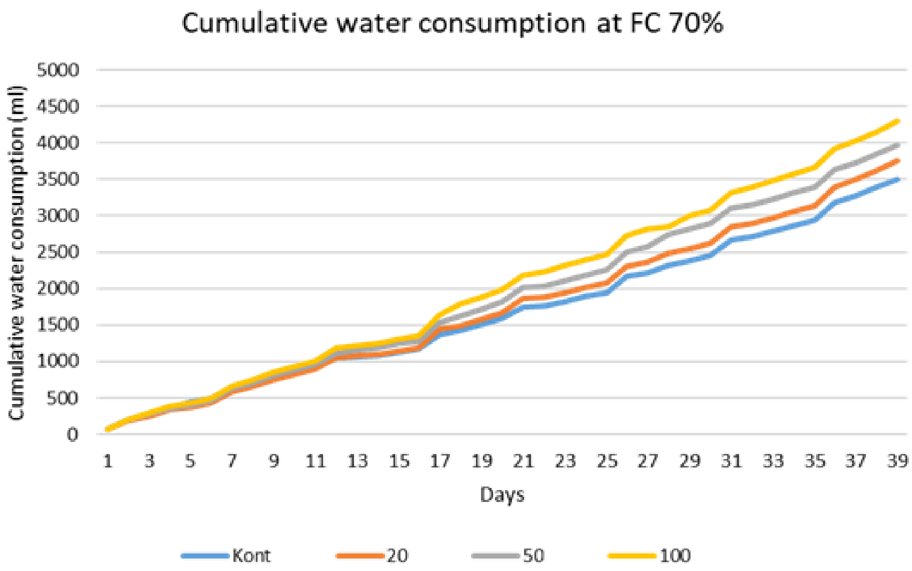

Figure 24. Cumulative water consumption at FC 70% in pot experiment (Kont-control; 20-20 times dilution of water retainer (WR); 50 – 50 times dilution of WR; 100- 100 times dilution of WR).

DET apparatus (B8) [*final*]

- Colloids from sludge suspensions can enter the filter paper, subsequently clogging the pore spaces and decreasing the velocity of the spread, resulting in long measurement times and large DET values (Figure 25).
- 1:5 soil:water ratio was found to be the best performing suspension rate for testing soil water retention parameters.
- Water Retainer has considerable effect on water retention of soil, based on DET results.
- Detailed results on DET apparatus (B8) for case study 10 are presented in the Appendix (Chapter 5.4.3).

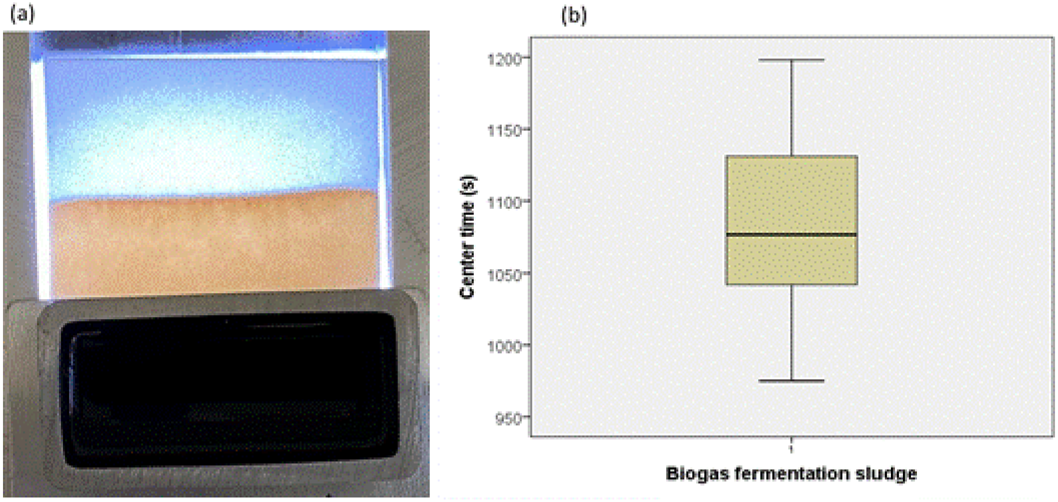

Figure 25. (a) Profile of the sludge using the DET standard filter paper; and (b) average DET value (time of spread).

Microfluidics (C4) [*final*]

- Fermented sludge and irrigation water (Figure 26) have considerable potassium, calcium and phosphorus content, and no micropollutants were detected.
- Detailed results on Microfluidics (C4) for case study 10 are presented in Deliverable D4.6. *Development of Microfluidics for Water Reuse at Farm-scale*

Fermented sludge samples after separation (10× dilution) – after microfiltration	Irrigation water + 15-25% fermented sludge water (10× dilution) - after microfiltration	Irrigation water + 15-25% fermented sludge water (10× dilution) - after microfiltration

Figure 26. Samples for analyses with Microfluidics

3.11.3 Key findings

Key findings for each solution tested [*final*]

- Physical modelling (A2): For the development of the CLM5 (1x1: 1D column) model for the Nyírbátori site, we developed the appropriate folder structure needed to build the model. The meteorological data was put into a template and converted from excel to netCDF by writing python code in jupyterlab. Domain and Surface file was created for the test area containing the coordinates and the corresponding soil and plant data. The construction of the model was successful, and during testing the model ran in the Hungarian test area. Further testing is planned to be implemented.

- Irrigation practices (B3+B4): Reveal and assimilate the meteorological datasets with numerous agro-technical, environmental parameters, including soil, hydrological, both satellite- and drone-borne spectral data, in order to perform a better farm management, providing data for better irrigation scheduling both temporally and spatially. However, import/export options of Agricolus may not fulfil the needs of qualified farmers or decision support managers with knowledge in other GIS-related solutions and claim for data assimilation of their own measurements (e.g., drones), and exporting VI data in GIS-formats would be a great option.
- Water Retainer (B5): the tested product did not adversely affect soil pH, did not affect the EC value of the soil, and increased the ammonium content of the soil in the weeks following application. The product was more effective in increasing soil nitrate contents when applied at lower dilutions, there is a difference between the effect of the tested product at different water capacity level. The product resulted in higher plant biomass weight at higher water capacity level, the applied treatments had no significant effect on plant biomass and inner parameters, lower dilution of tested product resulted in higher soil moisture level. The tested product had no significant effect on water distribution of the soil by depth.
- DET apparatus (B8): The DET is a promising tool not only for testing the dewaterability of sludges, but also for the measurement of water retention characteristics of soils. For modelling purposes, further research is required.
- Microfluidics (C4): In order to analyse the samples for analytical purposes, it was necessary to micro filter them through a 0.22µm filter. Therefore, several substances are filtered out of the samples during this level of sample filtration. This is also an interesting question because the application (for irrigation purposes) is done without filtration, only a mechanical separation is done before the application to the irrigation equipment. Therefore, testing the Solution with other sample preparation method should also be assessed.

Key findings related to the case study site [*final*]

- Soil moisture at a 10 cm depth ranges from 18 to 30%, which is typical for the region. Precipitation in July 2021 was anomalously low, making 2021 an anomalously dry year. Soil moisture simulations are lower in 2021 than in 2020.
- A LiDAR-based digital elevation model was found to provide appropriate data to identify sites affected by excess surplus water. A model concept for crop evapotranspiration estimation was developed based on vegetation indices calculated from satellite imagery.
- Based on nitrogen forms in the soil, it is recommended to use the Water Retainer product at a 100 times dilution rate.
- The applied water is suitable for irrigation purposes due to their microelement content.

4 Summary

The first version of Deliverable D5.3 presents the results obtained including the growing season 2022. Table 15 summarises the status of testing of WATERAGRI Solutions at the WATERAGRI Case Study sites. Final results are already presented for

- Case study 3 – Gårdstånga Nygård (Sweden)
- Case study 4 – Auxerre (France)
- Case study 8a+8b – Obersiebenbrunn/Mistelbach (Austria)
- Case study 9 – Bologna (Italy)

Preliminary results are presented for

- Case study 1 – Municipality of Tyrnävä (Finland 1)
- Case study 2 – Municipality of Ruukki (Finland 2)
- Case study 5 – Selhausen (Germany)
- Case study 6 – Lower Silesia (Poland)
- Case study 7 – Seeland (Switzerland)
- Case study 8c – Gleisdorf (Austria)
- Case study 10 – Nyírbátor (Hungary)

These Preliminary results will be updated using data gained during the growing season 2023. Additional data gathering and/or work is required for the following WATERAGRI Solutions:

- Integrated physically-based terrestrial system models (A2) at Case study sites Finland I, Finland II, Germany, Switzerland, and Hungary;
- Remote sensing pipeline (B2) at Case study site Germany;
- Irrigation management and agrometeorological monitoring solutions (B3) at Case study sites Finland I, Poland and Switzerland;
- Precision irrigation system (B4) at Case study sites Poland and Switzerland; and
- Biochar adsorbents for nutrient uptake (C4) at Case study site Austria / Gleisdorf.

A second version of Deliverable D5.3 will be prepared after the growing season 2023 in October 2023 to allow presenting final results for all WATERAGRI Solutions and WATERAGRI Case Study sites, respectively.

Table 15: Summary of status of testing of WATERAGRI Solutions at the WATERAGRI Case Study sites (**final** = testing done, results are final; **prelim.** = preliminary results available that will be updated with data from summer 2023; n/a = solution not tested at this site).

		WATERAGRI Solutions	Physical modelling	Wetlands	Remote sensing pipeline	Irrigation management and agrometeorological monitoring solutions	Precision irrigation system	Water Retainer concept	Biochar for water retention	Tracer methods	Dewaterability estimation test	Farm wetlands	Drainage systems	Membrane	Activated biochar	Microfluidics
			FZJ	ULUND	VULTUS	AGRI-COLUS	AGRI-COLUS	BZN	ALCN	BOKU	USAL	ULUND	ALCN	VIT	ALCN	EDEN
WATERAGRI Case Study sites			**A2**	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**C1**	**C2**	**C3**	**C4**	**C5**
1	Finland I	UOULU	prelim.	n/a	final	prelim.	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
2	Finland II	UOULU	prelim.	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
3	Sweden	GN	n/a	final	n/a	n/a	n/a	final	n/a	n/a	final	final	n/a	n/a	n/a	n/a
4	France	INRAE	n/a	n/a	n/a	n/a	n/a	final	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
5	Germany	FZJ	prelim.	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
6	Poland	UPWr	n/a	n/a	final	prelim.	prelim.	final	n/a	n/a	final	n/a	n/a	n/a	n/a	n/a
7	Switzerland	UNINE	prelim.	n/a	n/a	prelim.	prelim.	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
8	Austria	BOKU/TBR	n/a	n/a	n/a	final	n/a	n/a	final	final	n/a	n/a	final	n/a	prelim.	n/a
9	Italy	UNIBO	n/a	final	final	final	final	final	n/a	n/a	final	final	n/a	final	n/a	final
10	Hungary	UNIDEB	prelim.	n/a	n/a	final	final	final	n/a	n/a	final	n/a	n/a	n/a	n/a	final

5 Annexes

5.1 Additional results from Case study Sweden

Wetlands (B1+C1)

The water sampling program is carried out at the Gårdstånga Nygård, constructed wetland, next to the experimental field. The wetland gets its water mainly from upstream of the small river (partly seen in the figure) and the inflow comes in via sampling point "Inlet" near the eastern corner. The wetland lake has an outgoing discharging point (Outlet) via a cylinder formed weir at the western part close to sampling point "Outlet". In addition, there are two sampling point directly in the river as well (points USR and DSR). It is worth to mention the facts that the wetland is only in operation in a little more than 2 years, so that some effects and impact might not be directly reflected.

Detailed wetland case results on Gårdstånga Nygård for Case study site 3 (Chapter 3.4) will be included and presented in Deliverables D4.1 and D4.2, so they are omitted here.

Water Retainer (B5)

The experimental field consists of 64 cells, each 4x4 m, 32 out of which were used for the testing of Water Retainer (WR). Two additional parameters were varied, i.e. irrigation (yes/no) and adding phosphorus as fertilizer (yes/no). With three parameters (yes/no) there were 8 different possible combinations. The experiment was designed with 4 replicas for each combination.

In 2021 spring barley (Laurate) was sown on 26 April. On 24 May Water Retainer was applied to selected cells with an intensity of 10 l/ha (200 l/ha in a 20-fold dilution). Harvesting was done on 2 September. Irrigation was applied during the growing season with 20 mm at four different occasions. In 2022 winter, wheat was sown in the preceding fall. On 9 May Water Retainer was applied to selected cells with an intensity of 10 l/ha (200 l/ha in a 20-fold dilution). Harvesting was done on 12 August. There was no irrigation in 2022.

Results 2021. The harvested amount converted to kg/ha is given in Table 16. A comparison between the 16 cells with Water Retainer and the 16 without, shows (Table 17) as follows: Average harvest (WR) was 1883 kg/ha versus 1867 kg/ha for cells without WR. The mean difference between the two groups is small and the significance level for a one-sided two-sample t-test is 0.48, based on t= 0.058 and 30 degrees of freedom. This confirms that a difference in harvest cannot be established between the two groups.

Results 2022. The harvested amount converted to kg/ha is given in Table 16. A comparison between the 16 cells with Water Retainer and the 16 without, shows (Table 18) as follows: Average harvest was 7262 kg/ha with WR versus 7368 kg/ha for cells without WR. The mean difference between the two groups is small and the significance level for a one-sided two-sample t-test is 0.41, based on t= 0.241 and 30 degrees of freedom. This confirms that a difference in harvest cannot be established between the two groups.

Table 16: Harvest (kg/ha) for all the utilised 32 cells. The situation (Yes/No) with respect to variables tested is also shown for all cells. WR = Water Retainer treatment, Irr = irrigation, P = phosphorus added as fertilizer

cell	WR	Irr	P	Harvest 2021	Harvest 2022
1	Yes	Yes	Yes	2610	7377
2	No	Yes	Yes	2236	8619
3	Yes	Yes	Yes	2453	6797
4	No	Yes	Yes	2113	6528
5	Yes	Yes	No	2922	8297
6	No	Yes	No	2824	8113
7	Yes	Yes	No	3169	6697
8	No	Yes	No	2001	7788
9	Yes	Yes	Yes	2693	8076
10	No	Yes	Yes	3180	8462
11	No	Yes	Yes	1872	6832
12	Yes	Yes	Yes	1651	6422
13	Yes	Yes	No	1988	4335
14	No	Yes	No	2055	2290
15	No	Yes	No	1497	7241
16	Yes	Yes	No	805	7115
17	Yes	No	Yes	1195	5801
18	No	No	Yes	857	7696
19	Yes	No	Yes	2710	7770
20	No	No	Yes	1790	7002
21	Yes	No	No	1807	7751
22	No	No	No	2635	7813
23	Yes	No	No	2178	7843
24	No	No	No	1722	7947
25	No	No	Yes	1395	7855
26	Yes	No	Yes	1301	8346
27	No	No	Yes	1839	8388
28	Yes	No	Yes	583	8150
29	No	No	No	573	7451
30	Yes	No	No	602	7976
31	No	No	No	1290	7864
32	Yes	No	No	1456	7437

Table 17: Harvest output (kg/ha) for the two groups of cells in 2021, i.e., 16 cells with Water Retainer (WR) and 16 cells without WR.

Water Retainer	Average harvest (kg/ha)	Standard deviation	t
yes	1883	813	0.058
no	1867	656	

Table 18: Harvest output (kg/ha) for the two groups of cells in 2022, i.e., 16 cells with Water retainer (WR) and 16 cells without WR.

Water Retainer	Average harvest (kg/ha)	Standard deviation	t
yes	7262	1427	0.24
no	7368	1032	

DET apparatus (B8)

At Gårdstånga Nygård a mature wetland has been dredged as part of the long-term maintenance. Samples from the sediments have been run through the DET device at USAL in order to evaluate the functionality of the device for yet another application.

Sediment samples were taken at three spatially separated spots. For each sample three separate tests were run with two types of filter paper. In total 3 x 3 x2 = 18 tests were made.

Results. All output data are given in Table 19. The tests show that water moves faster through the BF3 filter than through the CST filter. Similar variation in waterfront velocity through the filter for the three samples (sites) were observed for both filter types, i.e., lower velocity for site 1 and higher velocity for site 3.

The precision of the measurements was considered high with absolute deviation from mean of average velocity typically less than 5 % for all sets of three tests (Table 20). The standard deviation of the velocity across both types of filter paper was high in comparison with what has been observed in previous tests, typically 2-5%, while for instance the corresponding numbers for tests with industrial food slurries (see Deliverable 3.3) are below 1%.

Table 19: Results from DET tests on sediments from a mature wetland at Gårdstånga Nygård.

Type of filter paper	Site	Room temp	Avg Time	Min Time	Max Time	Centre Time	Std dev %	Temp.1	Temp 2
CST	1	25.00	25.90	24.00	27.00	24.99	3	28.11	28.11
CST	1	25.00	25.40	25.00	27.00	25.00	3	27.11	27.11
CST	1	25.00	30.20	30.00	31.00	30.00	1	27.11	27.11
BF3	1	25.00	21.00	20.00	22.00	21.00	2	27.11	27.11
BF3	1	25.00	20.20	19.00	21.00	20.01	3	28.11	28.11
BF3	1	25.00	22.30	22.00	23.00	23.00	2	27.11	27.11
CST	2	25.00	27.10	26.00	28.00	26.00	3	27.11	28.11
CST	2	25.00	29.20	27.00	31.00	27.00	5	28.11	28.11
CST	2	25.00	27.70	26.00	28.00	28.00	2	28.11	28.11
BF3	2	25.00	20.99	19.99	23.00	19.99	5	28.11	28.11
BF3	2	25.00	21.10	20.00	23.00	21.00	4	28.11	28.11
BF3	2	25.00	18.90	18.00	20.00	18.00	4	28.11	28.11
CST	3	25.00	22.50	21.99	23.00	22.00	2	28.11	28.11
CST	3	25.00	23.91	23.01	25.01	25.01	3	28.11	28.11
CST	3	25.00	23.00	22.01	23.99	23.00	2	28.11	28.11
BF3	3	25.00	18.00	17.00	20.00	17.00	6	28.11	28.11
BF3	3	25.00	17.20	16.00	18.00	17.00	3	28.11	28.11
BF3	3	25.00	17.50	17.00	19.00	17.00	4	28.11	28.11

Table 20: Mean average time and the deviation of average time from its mean.

Type of filter paper	Site	Mean average time (s)	Mean deviation from mean (s)	Mean deviation from mean (%)
CST	1	27.2	2.0	7%
BF3	1	21.2	0.8	4%
CST	2	28.0	0.8	3%
BF3	2	20.3	1.0	5%
CST	3	23.1	0.5	2%
BF3	3	17.6	0.3	2%

5.2 Additional results from Case study Austria III (Gleisdorf): Differences in soil water flux and soil organic carbon in fields under conventional and organic farming

Water infiltration and retention in soil under conventional (CON) and organic (OR) farming was evaluated using measurements of oxygen and hydrogen isotopes ($\delta^{18}O$, $\delta^{2}H$) of soil water and moisture contents (Tracer methods - B7). Oxygen and hydrogen isotopes ($\delta^{18}O$, $\delta^{2}H$) are environmental tracers that provide a tracer signal with every precipitation event over a certain space (Leibundgut et al., 2009). The distinct seasonal distribution of isotopic composition in precipitation may also be observed in soil profiles. Thus, it is possible to link the isotopic composition of soil water with a period (Stumpp et al., 2018). In combination with soil water content measurements, water fluxes can be quantified (Chesnaux and Stumpp, 2018) and, using a water balance approach, other fluxes can be estimated (Boumaiza et al., 2020).

The experimental fields consisted of two adjacent fields located in Gleisdorf (Styria, Austria) with similar soil texture, from sandy loam to silty loam. The conventional farm had a surface area of 7 ha and the organic farm 3.7 ha. In 2022, both fields cultivated Styrian pumpkin (Cucurbita pepo var. styriaca). On the day of sampling, the fields had been recently ploughed. The most noticeable structural changes were observed in CON, as in OR this was done partially, between rows, and crop residues and weeds were left in the field.

The sampling campaign was carried on the 3rd of November 2022. We took two soil core samples in close proximity from each field to obtain a composite sample at each depth. Soil cores were taken to a depth of 90 cm at intervals of 10 cm and stored in double Ziploc® bags. Soil samples were prepared and analysed following the procedure described in Wassenaar et al. (2008). $\delta^{18}O$ and $\delta^{2}H$ of soil water and laboratory reference standards used for the two-point calibration were analysed with a laser-based isotope analyser (Picarro L2130-i). After the analysis of $\delta^{18}O$ and $\delta^{2}H$, the soil samples were weighted and dried to determine the gravimetric water content. The total organic content was measured using the same soil samples. Two samples of 500 mg were taken from each soil sample up to 50 cm to measure total organic content (TOC). In Figure 10c the average of both samples is shown for each depth. A bulk density of 1.2 g/cm^3 and a residual water content of 0.067 cm^3/cm^3 were assumed for all the profile based on the soil texture and visual assessment to obtain an approximate volumetric water content.

A winter nadir was visually identified from temperature and precipitation data and previous patterns of deuterium in precipitation from a predicting tool (Nelson et al., 2021; https://isotope.bot.unibas.ch/PisoAI/). The winter nadir identified corresponded to the precipitation from approximately January 2022, i.e. ten months. Deuterium ($\delta^{2}H$) profiles are shown in Figure 27. Fields under CON farming showed higher water flow velocity than OR farming. The winter nadir was found at 45 and 35 cm depth for CON and OR. The higher flow velocity could be attributed to the management of the field, as it is a conventionally managed field, which is ploughed after each crop, while OR minimized tillage and integrated cover crops to protect the soil from erosion and increase the amount of organic carbon.

The soil in CON had higher water content in the whole profile compared to OR, with 176.3 mm and 127.5 mm. The high-water content and the higher flow velocity translated into higher water flux, with

WATERAGRI

approximately 17.5 and 12.8 mm/month for CON and OR. It is not clear whether the water content in the soil is a consequence of the management practice or not. Days before the sampling it rained, so the soil was very wet. Recent cracks in the soil at CON can facilitate water to infiltrate more quickly, and be retained in the soil.

As for soil organic carbon, OR fields had approximately 2% of total organic carbon in the first 30 cm, while CON had around 1.6%, with only 1.2% in the first 10 cm. From 30 cm on TOC decreased in both fields.

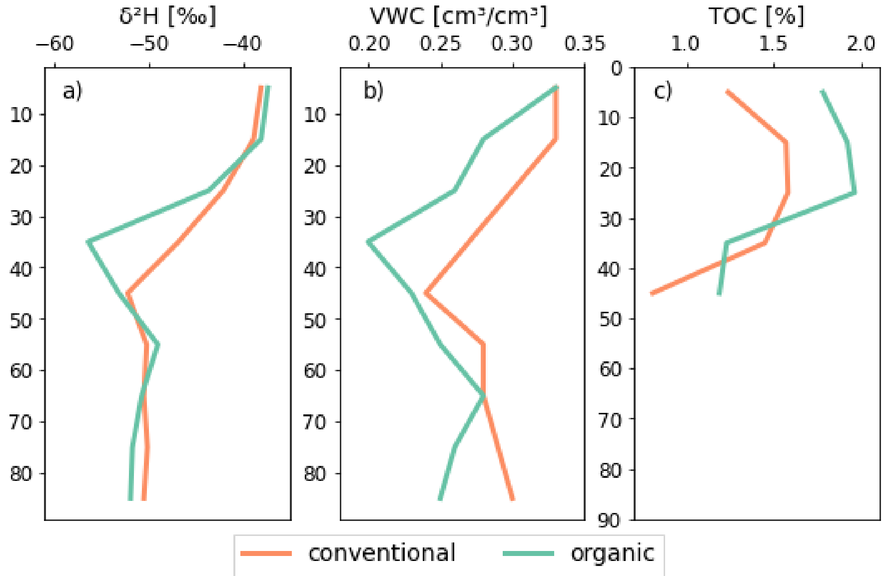

Figure 27: Profiles of a) isotope ratio of deuterium (δ²H), b) volumetric soil water content (VWC), and c) total organic carbon (TOC) of fields under organic and conventional farming in Gleisdorf (Case study Austria III).

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5.3 Additional results from Case study Italy: Assessment of WR performances for soybean in 2022

In order to assess the capabilities of the Water Retainer (WR) product, three experimental fields were cultivated with soybean (Figure 28):

- **Field 10 – Irrigated (Irr)**: this field has been irrigated with pressurized method (roller irrigator), where timing and volume have been defined with the support of IRRIFRAME
- **Field 11 – Not irrigated (NoIrr)**: this field represents the test and is not irrigated nor treated with the WR product.
- **Field 12 – Treated with Water Retainer (WR)**: this field represents the test and is not irrigated but is treated with the WR product.

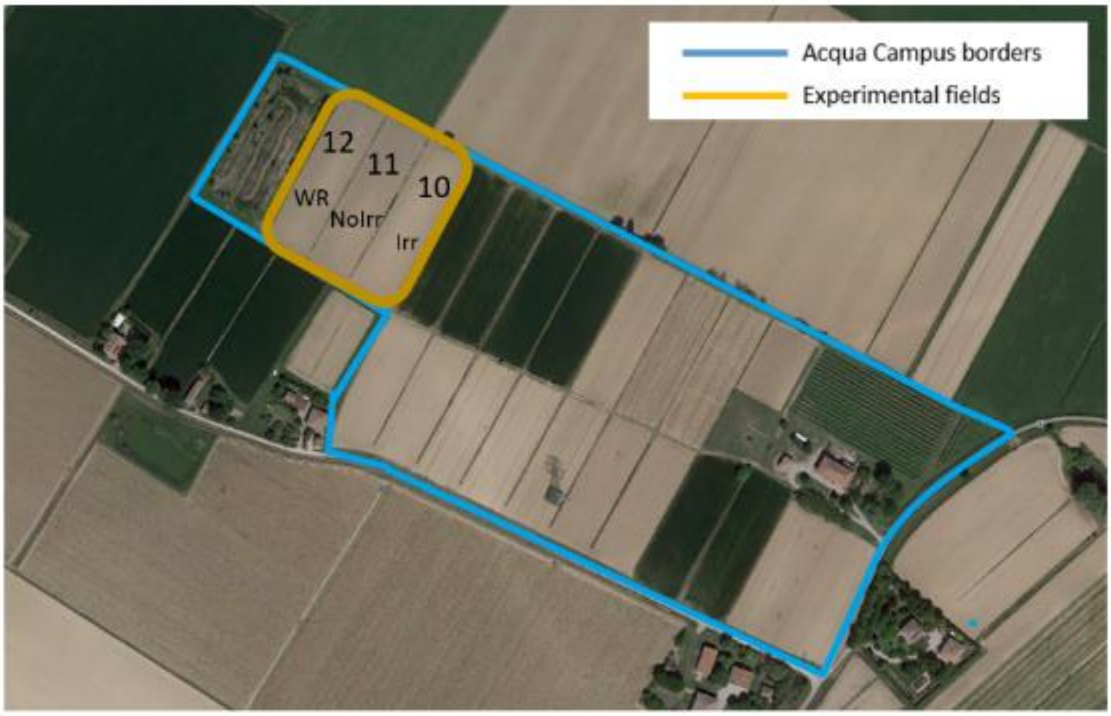


Figure 28: Experimental fields dedicated to the tests

The choice of three fields aimed to provide a complete picture of the potential for WR use in the case study area. Accordingly, not only yield from non-irrigated soybean can serve as an indicator to assess WR capabilities, but also yield from irrigated soybean can be used to assess whether the WR can be an alternative to irrigation.

Concerning crop production of seeds, data was collected during the experimental harvest taking place on 22/09/2022. The experimental harvest was conducted in random areas of 10 m^2, and the collected cobs were put in plastic boxes and moved to the labs for threshing and measurements of humidity and density. Seed production is expressed at the soybean moisture content of 14%, which is the base humidity at which soybean is marketed (Figure 29).

Analyses of yield data (Figure 29, Table 21,

Table 22) highlighted that soybean treated with WR produces around -35% less than the not treated nor irrigated area. As expected, irrigated soybean appears to be always and sensibly more productive (69% more than the test not treated nor irrigated) at the cost of irrigating.

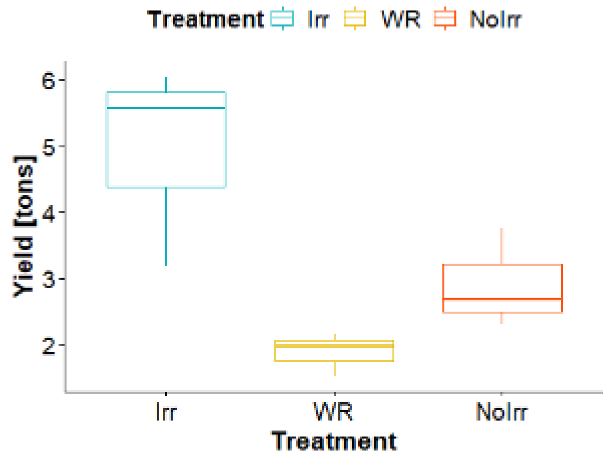


Figure 29: Boxplot for yield at base moisture (14%)

Table 21: Average soybean yield

Trial	Average yield [tons/ha]	Yield gain with respect to the test not treated nor irrigated
Irrigated	4.93	69%
Water Retainer	2.92	0%
Non-irrigated	1.89	-35%

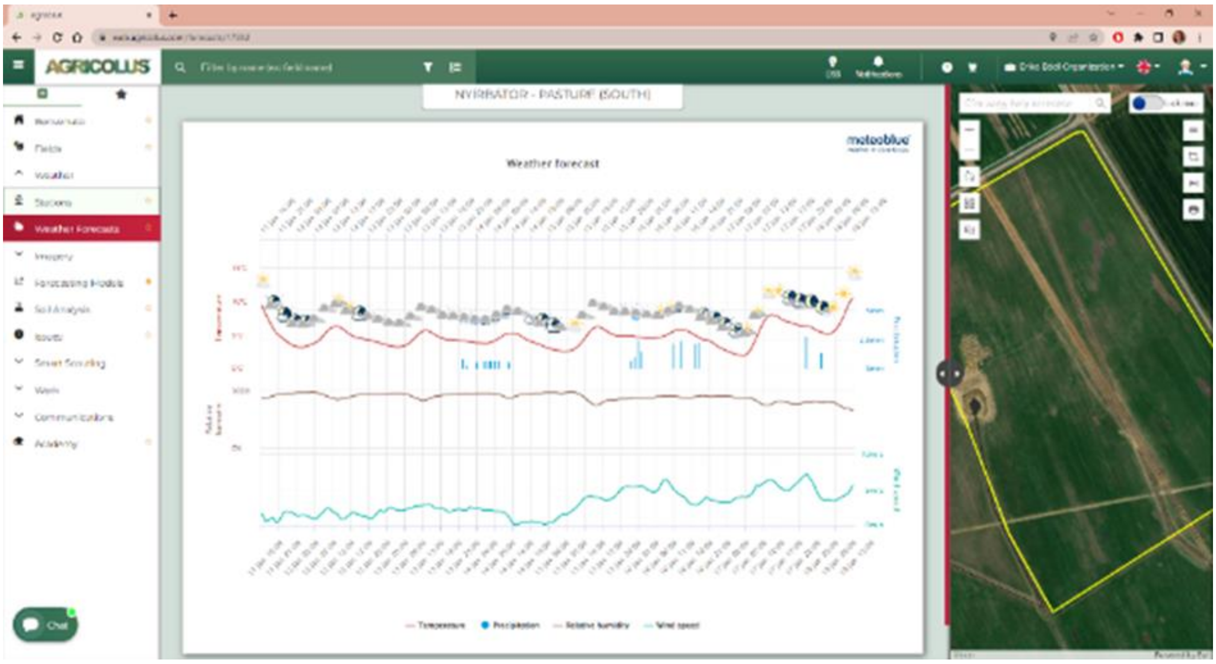
Table 22: Two-way ANOVA table estimating the effect of treatments on mean seed yield. Water Retainer and Non-irrigated trials are compared to the Irrigated field. (Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1). Comparisons are deemed statistically insignificant when Pr(>|t|) is higher than 0.1.

Yield					
	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**	
Intercept	4.93	0.58	8.50	0.000	***
WR	-3.04	0.82	-3.71	0.010	**
NoIrr	-2.01	0.82	-2.46	0.049	*
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					

5.4 Additional results from Case study Hungary

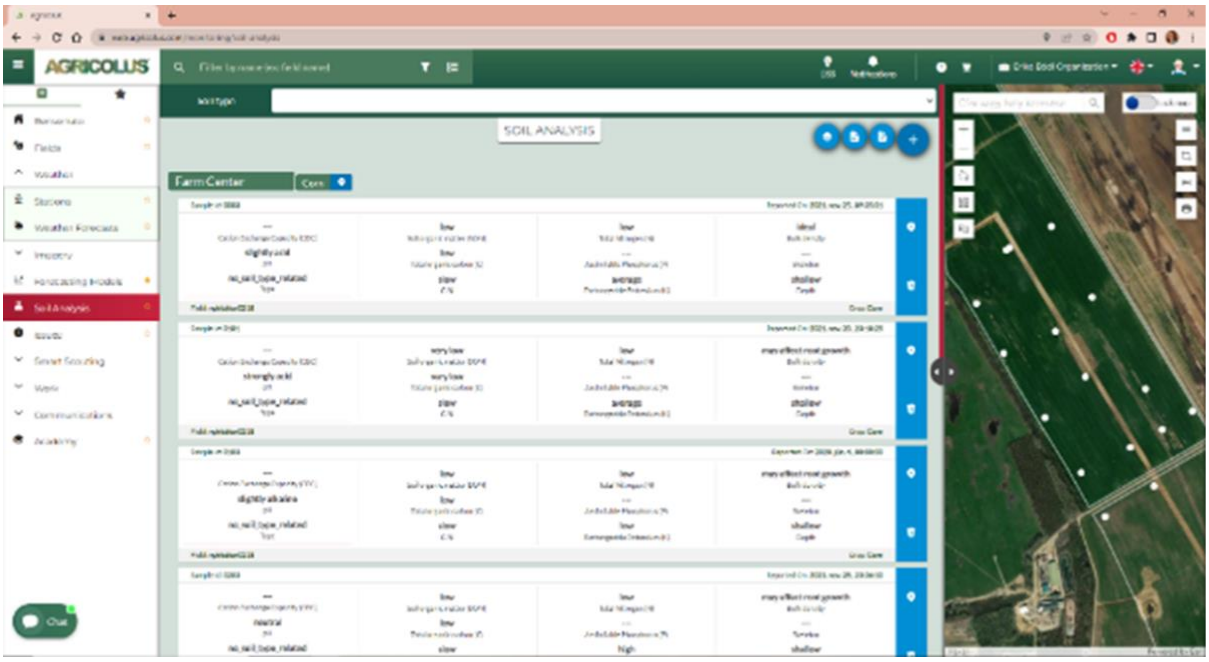
5.4.1 Result from testing [WATERAGRI solution Irrigation practices (B3+B4)]

The meteorological system part of Agricolus provides temperature (°C), precipitation (mm), relative humidity (%) and wind speed (m/s) parameters. This is performed by Davis MET meteorological stations and the related network. In Nyírbátor two Davis MET meteorological stations are installed from which one is located in the NW boundary of the irrigated field. Within the Agricolus platform one of the main menu point is Weather Forecast which provides reliable forecast for the farm unit for the next seven days. Its interface is user-friendly, clear and informative. It displays the temperature, precipitation, relative humidity, and wind speed values in graph format using symbols to aid easy understanding.



It is important to note that by using the mentioned Davis meteorological equipment with the related data logger and transport system, we can evaluate historical data in charts, and export data for further analysis. In addition, we may also access public station data from around the world, hence it can be considered as net of basic but continuously changing affecting greatly the crop. For more detailed meteorological information, the Davis met service offers an own application with several options both for viewing and data management.

Besides the metrological data, another crucial information, which can be handled in Agricolus, is the strongly spatial-related soil characteristics. Soil provides the complex biochemical-hydrological media of the root zone of the crop and without its understanding conscious farm management cannot be performed. Under the Agricolus Soil Analysis menu bar the users can upload certain soil physical and chemical data which are particle size distribution (sand, clay, silt %), pH, soil organic matter (SOM), Total Nitrogen (N), assimilable phosphorous (P), total organic carbon (C), C/N ratio, exchangeable potassium (K), cation exchange capacity (CEC), bulk density, skeleton, depth.

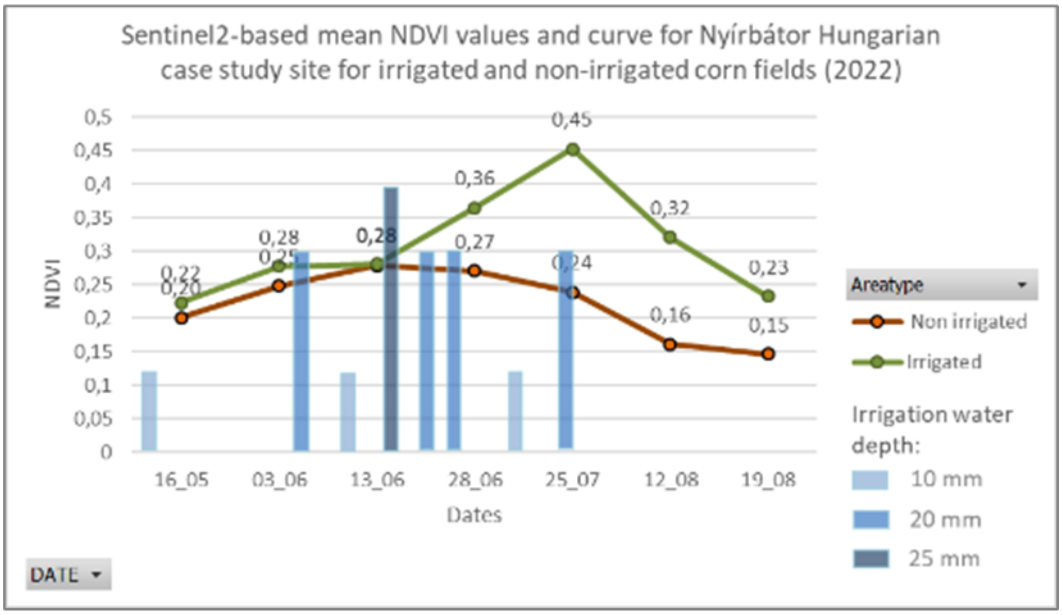


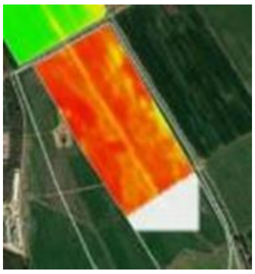
Soil parameters are measured on soil samples taken in certain depths, and then after field description and putting ID labels on, these samples are prepared and transported to laboratory for soil physical and chemical analysis. The measured parameters for each depth are collected and edited into database tables which contains X and Y coordinates and depth for each record.

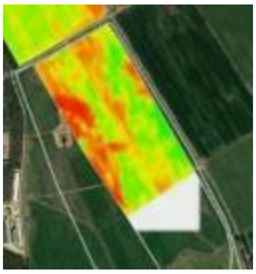
In Agricolus systems these point-feature data can be imported one by one, which makes difficult and slow the process in case of multiple depths and high-resolution data-sampling strategies. Nevertheless, the information is crucial.

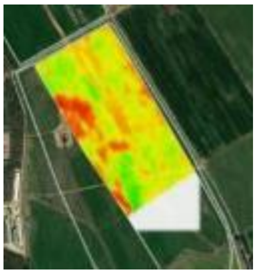
In Agricolus, Sentinel2-based imagery raster datasets are provided for the fields. It is able to visualize several vigour indices (NDVI, WDRVI, GNDVI, LAI, SAVI), chlorophyll index (TCARI/OSAVI) and water stress indices (NDMI, NMDI) . These indices are summarized in Table 1, where their Sentinel2-based expressions are presented, as well, in order to gain an insight behind the calculation methods applying the certain Sentinel2 spectral bands. These index maps are visualized to serve information on the crop and the crop field nearly real time and as a time-series view for the farmers who can make their decision on crop management according to this spatial information. This is a great tool and makes the work efficient.

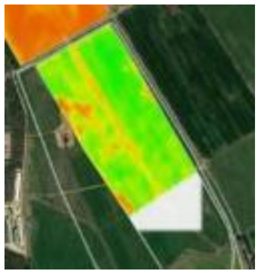
If farmer managers tend to see real time series view of their field and to reveal patterns and correlations between different factors, they need to get the original index maps containing pixel-based numerical values. In such formats, the index maps are ready for analysis. This can be carried out by using GIS solutions, and we performed an analysis with Sentinel2-based mean NDVI values and NDVI curve for Nyírbátor cornfield while non-irrigated and irrigated areas were separately handled, hence emphasize very well their status during the crop season (Figure 30a-i). In addition, we added bars referring to the amount of the irrigated water. This shows that the irrigation event number 3-4-5-6 can be considered a series of successful interventions. From 13th June, the status of the irrigated and non-irrigated crops started to separate from each other significantly thank to irrigation.

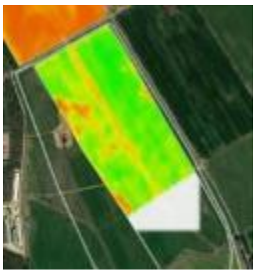


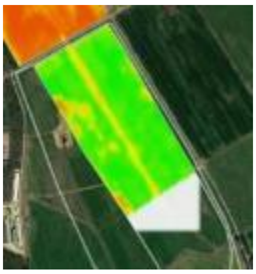

b) 16th May

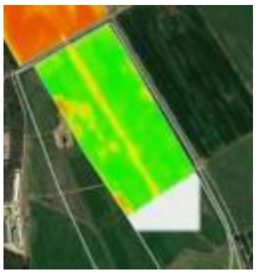

c) 26th May


d) 8th June


e) 20th June


f) 8th July


g) 20th July


h) 2nd August

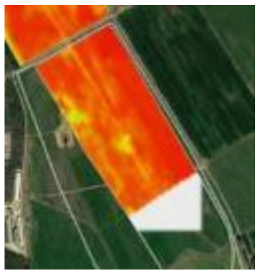

i) 24th August

Figure 30: Sentinel2-based mean NDVI values and NDVI curve for Nyírbátor cornfield (non-irrigated and irrigated areas separately handled) and the amount of irrigation for the total 8 irrigation events.

Although Sentinel2 satellites are in the position to monitor the Earth twice within 10 days, a great limitation is due to cloud cover, and as a result, makes impossible electromagnetic rays' penetration through the cloud mass, which is the main technical basis of the monitoring process. UAV-based measurement on the field can offer a great supplementary solution for this. In Nyírbátor case study site, we performed time-series spectral measurements via drones. Among the vigour indices, we managed to have NDVI values this way. We created data analysis both for non-irrigated and irrigated fields, separately (Figure 31). The curves were created from more points; however, other data did not match perfectly with the Sentinel2 survey dates.

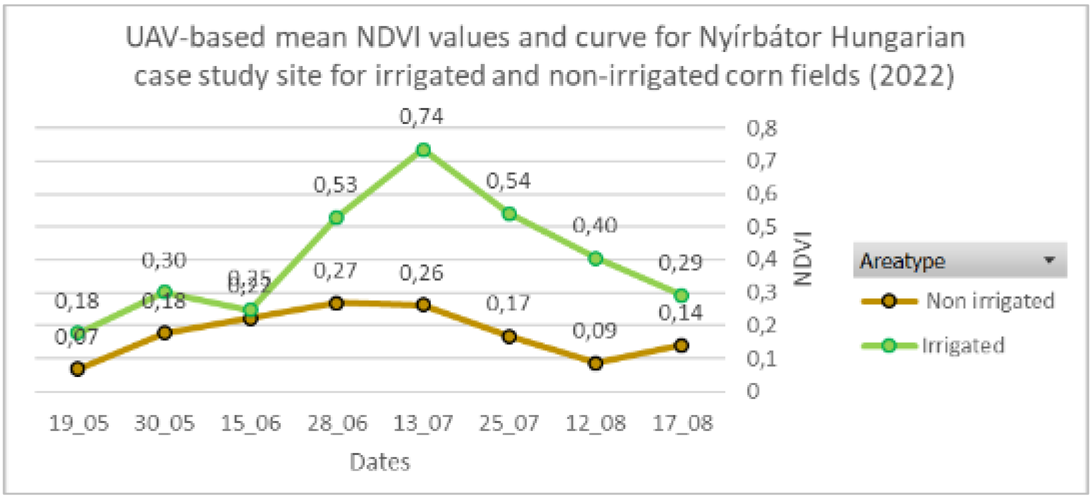


Figure 31: Drone-based mean NDVI values and NDVI curve for Nyírbátor cornfield (non-irrigated and irrigated areas separately handled).

The curve patterns show similarity, but in terms of the values slight differences are drawn. This can be explained by the different survey days and the different spatial resolution of the Sentinel2 satellite image and the UAV-borne rasters. Due to the pattern, it can be stated that the that the irrigation activity performed in early June had the greatest effect on the crop without which the crop biomass would have been depleted continuously causing crop losses and feed shortages.

In the case of detection of plant infestations, pests, these events are recorded in the Agricolus application. This option is under the Smart Scouting menu bar where Traps and Catches, Pests and Diseases, Crop Damages and Phenology option can be chosen to administrate their detections.

In the Work menu bar the certain crop operations can be set to the certain field on the specific days: sowing or transplanting, fertilization, treatments, tillage, irrigation, harvesting, canopy or soil management or other operation can be registered here.

The model concept developed in D3.1 for crop evapotranspiration estimation was also tested based on vegetation indices calculated from satellite imagery for data of 2022.

In this modelling scenario, the benefits of the high temporal resolution of MODIS NDVI and the high geographical resolution of SENTINEL NDVI indices were utilized. Despite the fact that the MODIS and SENTINEL outputs are derived from distinct sensors, a considerable link may be assumed between them in the temporal domain (See Deliverable 3.1, Chapter 7).

In the instance of the discussed modelling approach, each pixel of the satellite images was treated as a point-type entity with temporally varying environmental variables, allowing the relationship between natural and theoretical processes to be evaluated. First, we calculated the reference evapotranspiration using the Penman-Monteith method. Then different crop coefficients were assigned to different time periods based on the observed plant growth stages of the studied crop.

- Every two weeks, BBCH phenological phases were determined for the Nyírbátor field. Due to non-continuous in-situ observations, the identification of the first Kc break point is questionable, resulting in a 7-day temporal uncertainty.
- Due to the fact that the theoretical Kc sequence described in FAO-56 is unnaturally rectangular and does not reflect the natural process of canopy saturation, the break points of the series were curved using two technological approaches. The "FAO-56 curved" method involved cutting 10 days around the break points and estimating missing numbers using the spline algorithm. The alternative method was the so-called "MidPoint Spline," in which just the middle plateau was defined by the plateau's middle point and the length of the plateau phase (See Deliverable 3.1, Figure 48).

The reference evapotranspiration series was derived from the combination of in-situ sensors utilizing the Penman-Monteith method, as described in Section 6.2.4. of the Deliverable 3.1. The high spatio-temporal dataset of NDVI pixel values was computed using the median-model-based method introduced in Section 6.2.5.1. of the Deliverable 3.1. Moreover, the crop coefficient from the FAO-56 paper determines the average Kc values for maize. By fitting the unstructured statistical distribution of the entire set of NDVI values to the unstructured statistical distribution of the FAO-56 Kc series, the NDVI values can be transformed. The method is comparable to the well-known normal-score transformation in Monte Carlo simulations. The method ensures that the relative difference between the recently calculated spatial and temporal Kc values can be maintained for each pixel centre.

The water budget relative to the conditions at the beginning of the simulation is derived by combining the ETo time sequence with the currently available Kc spatiotemporal values and the irrigation data series. Figure 32 depicts the relative water balance of the region at a particular instant in time. The variable spatial distribution of relative water balance change highlights the significance of precision irrigation.

The patterns of crop evapotranspiration can be depicted using both maps and time series. Finally, for each of the three Kc, the theoretical crop evapotranspiration time series was calculated by combining the reference evapotranspiration and the crop coefficient time series. The projected ETc values appear less certain towards the Kc breakpoints.

The NDVI, NDWI, NDRE, NDREw, LAI, and NPBR 1-dimensional time series of pixel centers were established independently of the initial step for each year and parcel: NDVI, NDWI, NDRE, NDREw, LAI, and NPBR. This stage entailed: - The removal of outlier index values from the time series for each pixel center, as well as the consideration of cloud cover masks and index limit values.

- To reduce the impact of missing values, the median of the temporal pixel values was allocated to each observed time instant. The approach yields a single time sequence for each distinct spectral index, which is now regarded as indicative of the entire parcel under investigation (Figure 32).
- Estimation of the empirical Kc curves based on the spectral indices by the fitting of the index-based time series to the theoretical Kc trend. Until this moment, the index values are only known at the time instants observed. The missing values have been estimated by the application of the Piecewise Cubic Hermite Interpolating Polynomial (PCHIP) approach.

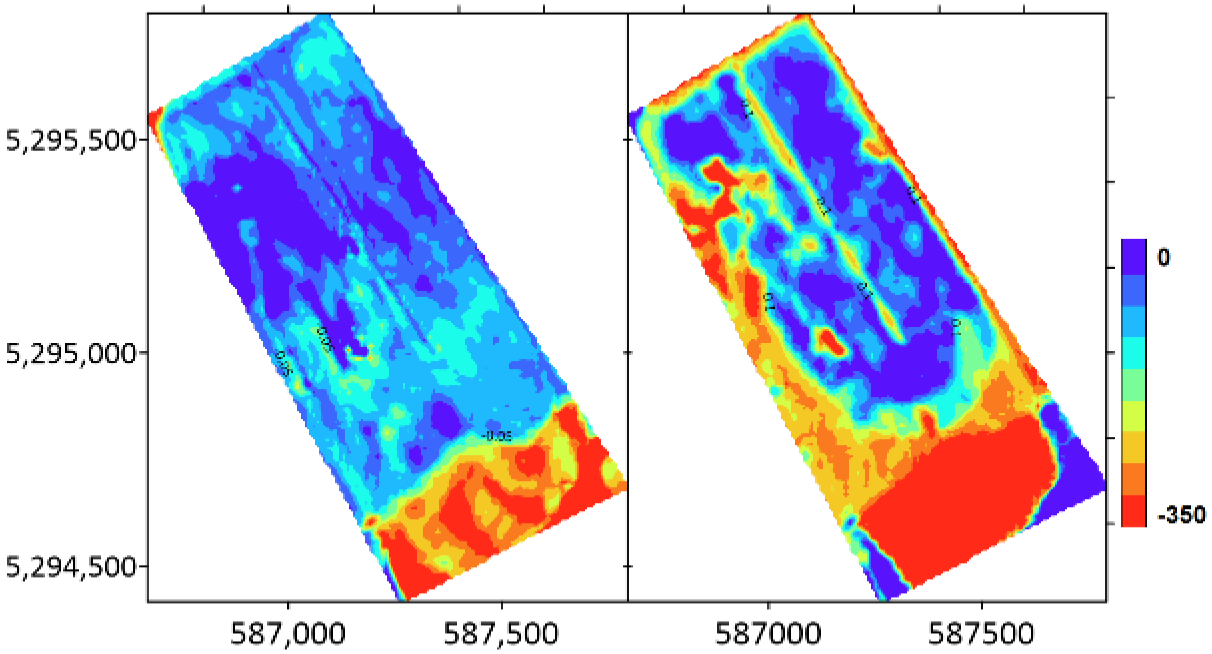


Figure 32: Comparison of the spatial pattern of estimated water balance on 10th of June 2022 (left) and 15th of August 2022. (right) on the Nyírbátor site.

5.4.2 Result from testing [WATERAGRI solution Water Retainer (B5)]

5.4.2.1 Methods

1. Level: Laboratory soil incubation experiment

Soil incubation experiment was made to study the effects of Water Retainer on soil conditions and nutrient status. Nyírbátor soil was used in the experiment (200g/tube). Beside the control, three doses of Water retainer were used (20, 50, 100-fold dilution according to the manufacturer's recommendations) at two different water capacity levels (Wc=40 and 60%) in three replicates. Soil samples were collected every two weeks in the experiment (2 months). Soil parameters (pH, EC, N forms) were measured in the soil samples.

2. Level: Small pot experiment

Small pot experiment was made to study the effects of Water Retainer on soil conditions and nutrient status in a soil-plant system. Nyírbátor soil was used in the experiment (3kg/pot). Beside the control, two doses of Water retainer were used (20, 50-fold dilution according to the manufacturer's recommendations) at three different water capacity levels (Wc=45, 70 and 90%) in three replicates. Soil samples were collected every two weeks in the experiment (2 months). Soil parameters (pH, EC, N forms) were measured in the soil samples.

3. Level: Field experiment

Field experiments were set up to study the effects of Water Retainer on soil conditions.

Water retainer was used twice in the vegetation period. The first application was made at the beginning of May and the second at the beginning of August.

The design of the experiment has taken into the manufacturer's recommendations for both application time and concentration. Water retainer was used at 25- and 50-fold dilution. Dilution was made with distilled water to ensure that the mechanism of action was not affected by the composition of the water. Soil and plant analysis were made to check the effectiveness of Water retainer during the experiment.

5.4.2.2 Results

In this chapter, focuses on the main findings and results. Detailed results and their evaluation can be found in previous research reports.

1. Results of Laboratory model experiment

Results of soil pH

During the experiment, the soil pH values are varied between 7.14 and 7.98. The pH value decreased slightly in the control and in the treated pots as well. The treatments slightly, but not significantly, decreased the soil pH at 40% water capacity compared to the control. Obtained pH values are optimal for plant growing, so the water retainer does not affect soil pH adversely.

The applied treatments had no significant effect on soil pH at 60% water capacity level compared to the control. Similar to the 40% water capacity level, soil pH decreased slightly by the end of the experiment.

Results of soil EC

The measured EC values are varied between 100 µS cm^{-1} and 242 mS cm^{-1} during the experiment. Treatments slightly increased EC values during the experiment, in all treatments but the increment was not significant in most cases. Similar tendency was observed at higher water capacity level. Water Retainer has no significant effect on soil EC.

Results of soil ammonium content

Soil ammonium content varied between 1.63 and 5.25 mg/kg. The soil ammonium content is low due to the soil type and has not been significantly affected by the treatments. In the first half of the experiment, the treatments increased the soil ammonium content compared to the control, which was reversed by the end of the experiment. The ammonium concentration in the soil showed a slightly decreasing tendency as the experiment progressed at 60% soil water capacity level. Moreover, there was no significant difference in soil ammonium content between the two water capacity levels.

Results of soil nitrate content

The nitrate content of the soil was significantly higher than the ammonium content. Ten times as much nitrate as ammonium was measured in the soil. In the control treatment, the soil nitrate content initially increased and then showed a decreasing trend. In contrast, soil nitrate contents in the treatments showed an increasing trend and exceeded the control levels from the third sampling date. The treatments had increased the nitrate content of the soil compared to the control by the end of the experiment. Overall, it can be concluded that treatments with narrower dilution rate resulted conditions that are more favourable.

Suggestion:

It is recommended to use the product in small pot experiments, where its effect on nutrient uptake by plants is measurable.

2. Results of small pot experiment

Results of daily irrigation

Daily irrigation was used during the whole experiment to maintain the adjusted water capacity level. From the daily water consumption data, the cumulative water consumption values were calculated. The lowest cumulative water consumption was obtained for the Water Retainer solution with 100 times the dilution at the 40% water capacity level, suggesting that irrigation water can be saved even in the case of insufficient water supply when using the product at this dilution.

The highest cumulative water consumption was obtained for the 100-fold diluted product solution at a 70% water capacity level. It indicates that the application of the product at this dilution rate increases the water demand but results in a significant increase in biomass mass at optimal water supply as well.

Results of microbiological analyses

1 grams of soil samples taken from the control (1.K 40) and treated (1.100 40) pots were mixed with 10 ml sterile saline solution. 10-fold serial dilutions were made in sterile saline solution and 50 µl aliquots were plated on different media in Petri dishes. Total cell number (DSM1 agar media), number of nitrogen fixing bacteria (NFX agar media), number of bacteria with ACC-deaminase activity (CNF agar media), and number of phosphorus mobilizer bacteria (Pikovskaya agar media) were determined. (These media are described in detail in section 2.1.1.2 of D3.2.) Plates were incubated at 25°C for 7 days before counting. Results are presented in Total cell numbers of soils were relatively low. The number of nitrogen fixing bacteria and number of bacteria with ACC-deaminase activity was one order of magnitude lower than the total cell number. The number of phosphorus mobilizing bacteria was low or undetectable. Generally, there was no significant difference between control and treated samples, although by the end of the experiment the number of nitrogen fixing bacteria and number of bacteria with ACC-deaminase activity was lower in the Water Retainer treated soils.

Table 23.

Total cell numbers of soils were relatively low. The number of nitrogen fixing bacteria and number of bacteria with ACC-deaminase activity was one order of magnitude lower than the total cell number. The number of phosphorus mobilizing bacteria was low or undetectable. Generally, there was no significant difference between control and treated samples, although by the end of the experiment the number of nitrogen fixing bacteria and number of bacteria with ACC-deaminase activity was lower in the Water Retainer treated soils.

Table 23: Cell counts of examined soil samples

Sampling date	Sample code	Cell count (CFU/g) DSM 1	Cell count (CFU/g) NFX	Cell count (CFU/g) CNF	Cell count (CFU/g) Pikovskaya
2021.04.28 (T1)	1.K 40	5 105	1 105	4 104	0
	1.100 40	5,8 105	1 105	0	0
2021.05.11	1.K 40	9,4 105	3,2 105	1,58 105	0
	1.100 40	9,2 105	2,26 105	1,18 105	4 103
2021.05.17 (T2)	1.K 40	1,14 106	3,4 105	6,8 104	1,6 104
	1.100 40	1,2 106	3,8 105	1,14 105	1,4 104
2021.06.05 (T3)	1.K 40	1,36 106	8,4 105	3,2 105	1,6 104
	1.100 40	1,24 106	4 105	2,4 105	1,8 104
2021.06.28 (T4)	1.K 40	1,04 106	7,6 105	4,4 105	0
	1.100 40	1,52 106	2,5 105	2,8 105	0

Results of macronutrient analyses

Soil samples for macronutrient (nitrogen, phosphorus, potassium) analysis were taken four times during the experiment, at the same dates as samples for microbiological analysis. At the first two dates samples were taken from both the plant less („soil incubation" = SI samples) and the seeded („pot experiment"=PE samples) pots. Later samples were taken only from the seeded soils.

Samples for plant analysis were taken at the 41st and 61st day of the pot experiment.

There was no significant difference in the nitrogen content of the treated and control soils in either sampling time. A slight increase in phosphorus content could be observed during the experiment. The phosphorus content did not show difference between the two groups except the last sampling time, when the treated soil had higher P content.

There was no significant difference in the potassium content between the treated and control soils, except at the first sampling time in the bare soil samples. Obviously, this difference could not be caused by the treatment as it occurred at the beginning of the experiment.

The content of all three nutrients decreased between T3 and T4 sampling time in plant samples. A slight decrease can be observed in the nitrogen as well as potassium content of soils, which could be a possible cause of this phenomenon. There is no difference between the phosphorus content of soils between the two samplings. The decrease of the quantity of plant-available phosphorus could be a possible reason which does not affect the overall P content of the soil.

The nitrogen and potassium content of the plants grown in treated soil is higher than the control ones at T3 sampling time. No such difference can be observed in T4 samples.

According to the results summarised above, the application of Water Retainer does not seem to have significant effect on the macronutrient content of soils or the macronutrient uptake of plants in laboratory pot experiments.

Results of plant analysis

Fresh or wet biomass weights were increased according to the increasing water capacity levels. Moreover, Water Retainer had an increasing effect on fresh biomass weight. 100-fold dilution resulted the highest fresh weight at 40% Wc and 50- fold dilution at 55 and 70%Wc level. Furthermore, significant differences were not obtained consequently.

Plant height was varied between 60 and 69.7 cm according to the treatment and water capacity level. Increasing water capacity levels increased the maize height in the same treatment. But we couldn't get tendentious and significant treatment effect between control and Water Retainer treatments.

Increasing water capacity level resulted increasing root length at the same treatment. With each increasing volume of the soil moisture for all the concentrations of the Water Retainer, there was an increase in the development and length of the plant root. 100-fold dilution caused the highest root length at all water capacity level, but the effect was not significant at all cases.

Chlorophyll content of leaves was varied between 1090 and 1972 µg/g. Increasing water capacity level resulted higher chlorophyll content in leaves but not at all treatment combinations. Our results pointed out that the applied treatments had no significant effect on leaf carotenoid content.

Suggestion:

It is recommended to use the product in filed experiments, where its effect on soil moisture distribution, soil parameters and nutrient uptake by plants are measurable coherently.

3. Results of field experiment

From our results it is concluded that water retention is possible and effective with the help of Water Retainer product.

It can also be seen that there was no significant difference in the control soils, so presumably all quantitative changes can be attributed to the effect of the Water Retainer. With regard to the different depths, further investigation of the 30 cm depth is necessary as this is where the changes were most likely to be cardinal.

In the light of the results of the treatments, it was concluded that the Water Retainer is capable of retaining water. However, the qualitative determination is an important step to determine whether the stored water is adding to the outdoor water capacity or to the hygroscopic (dead water) buffer.

It is necessary to repeat the experiment in order to have at least 2 years of data and to exclude the possibility of random data, and at the same time to determine the pF value, which will allow a qualitative determination.

Suggestions

- Further studies on the product are needed to establish the exact mechanism of action of the product.
- Different treatment combinations and repetitions may be recommended.

5.4.3 Result from testing [WATERAGRI solution DET apparatus (B8)]

5.4.3.1 Application of the Dewaterability Estimation Test device for fermentation sludge

In 2022 the DET device with sludge of slow settling characteristics and thus expected low dewaterability properties was tested at the University of Debrecen, Hungary. The standard CST paper (provided by Triton) was used for DET measurements, which were carried out at constant room temperature (24°C) and calibrated by using deionized water. The profile of spread was a homogenous straight line for all measurements. However, the wetted area was brown due to the particles infiltrated into the filter from the sludge. The brown wetted area of the filter paper makes it a challenge for the DET software to automatically recognize the waterfront spread. Therefore, the time of spread has to be calculated manually based on the images assessed. The DET time was 18.05±1.12 minutes, which would suggest that the dewaterability of the sludge is very low.

When using the standard CST paper with relatively large pore size, colloids from sludge suspensions can enter to the filter paper, subsequently clogging the pore spaces and decreasing the velocity of the spread, resulting in long measurement times and large DET values.

5.4.3.2 Testing aqueous suspensions of different physical soil types using the DET equipment

In the first part of the research, four different physical soil types (4 x 250 g) were selected and their physico-chemical properties were tested in the laboratory (Table 24, Table 25, and Table 26). To determine soil physical properties, saturation percentage (SP) was measured.

Table 24: Physical and chemical parameters of the four soil samples

Physical type of the soil	pH (H_2O)	EC (µS/cm)	Plasticity index according to Arany (K_A)	Hydraulical conductivity (k-index) cm^3/min
Sand soil	6.16	54.93	*	$2.31*10^{-1}$
Sandy loam soil	5.82	91.27	34.93	$1.60*10^{-2}$
Clay loam soil	6.15	160.67	40.33	$5.37*10^{-3}$
Loam soil	6.40	222.13	38.07	$5.71*10^{-3}$

*In the case of sand soils, the K_A factor cannot be measured.

The sand soil is a typical soil type of the Great Plain (mainly the Northern Great Plain and the Nyirség). According to the World Base Reference of Soil Resources (WRB), they belong to the category "Arenosols". Soils in this category are characterised by low water holding capacity and high water permeability, low nutrient content and, for all these reasons, water stress develops rapidly.

Clay loam and loam soils differ significantly from sand and chernozem soils in most of the properties studied. Both soils are characterised by a high specific conductivity, a plasticity index according to Arany and a k-factor, all of which indicate that they are soils with good water-holding capacity. Clay loam soils are characterized as fine-textured soils, with medium to high water holding capacity, medium fertility, and lower drainage rates.

Table 25: Saturation percentage of measured soils

Physical type of the soil	Saturation percentage (SP)	Category
Sand soil	-	-
Sandy loam soil	34.92	Loam soil
Clay loam soil	40.32	Loam soil
Loam soil	38.06	Loam soil

Table 26: Aggregate distribution of the four soil samples

	2 mm	1 mm	0.5 mm	0.25 mm	0.125 mm	0.063 mm	0.045 mm	< 0.045 mm
Sand soil	0.78%	0.80%	1.92%	15.69%	65.09%	14.52%	0.72%	0.49%
Sandy loam soil	6.23%	18.61%	26.70%	13.99%	9.25%	17.80%	5.64%	1.77%
Clay loam soil	20.12%	12.85%	13.20%	10.84%	10.66%	22.32%	7.61%	2.41%
Loam soil	4.91%	16.33%	20.92%	16.08%	11.96%	18.04%	8.34%	3.42%

The test results also show that we have chosen soil samples that differ significantly not only in their sampling locations but also in their physico-chemical properties.

Soil samples were dried to constant weight at 105°C and distilled water suspensions were prepared at 1:100, 1:40, 1:20, 1:10 and 1:5 soil:water ratios. With continuous stirring of the suspension, 25 ml of sample was lifted from the centre of the suspension column and then measured using the DET instrument, while the spreading curve was recorded using the DET Analyzer software. The measurements were performed in 10-15 replicates and the mean and standard deviation were evaluated by software evaluation using DET Quick software.

During the evaluation, descriptive statistics on the spreading of the soil extract were extracted from the DET Quick software: mean, minimum, maximum, mean, standard deviation. These data were further processed for statistical evaluation using R in the RStudio user environment ($p<0.05$). Statistical evaluation was performed to examine the differences between the results obtained for extracts with the same soil:water ratio and different soil physical properties, and between extracts with different soil:water ratios within each physical soil property.

The aim was to determine the optimal soil:water ratio based on existing knowledge. Our hypothesis was that, for the same soils, the spread of the moisture profile is minimal and the spread rate measured on the four soil suspensions is statistically different.

The results of the DET measurements are presented below. The evolution of the center value for different soil:water ratios is shown in Figure 33.

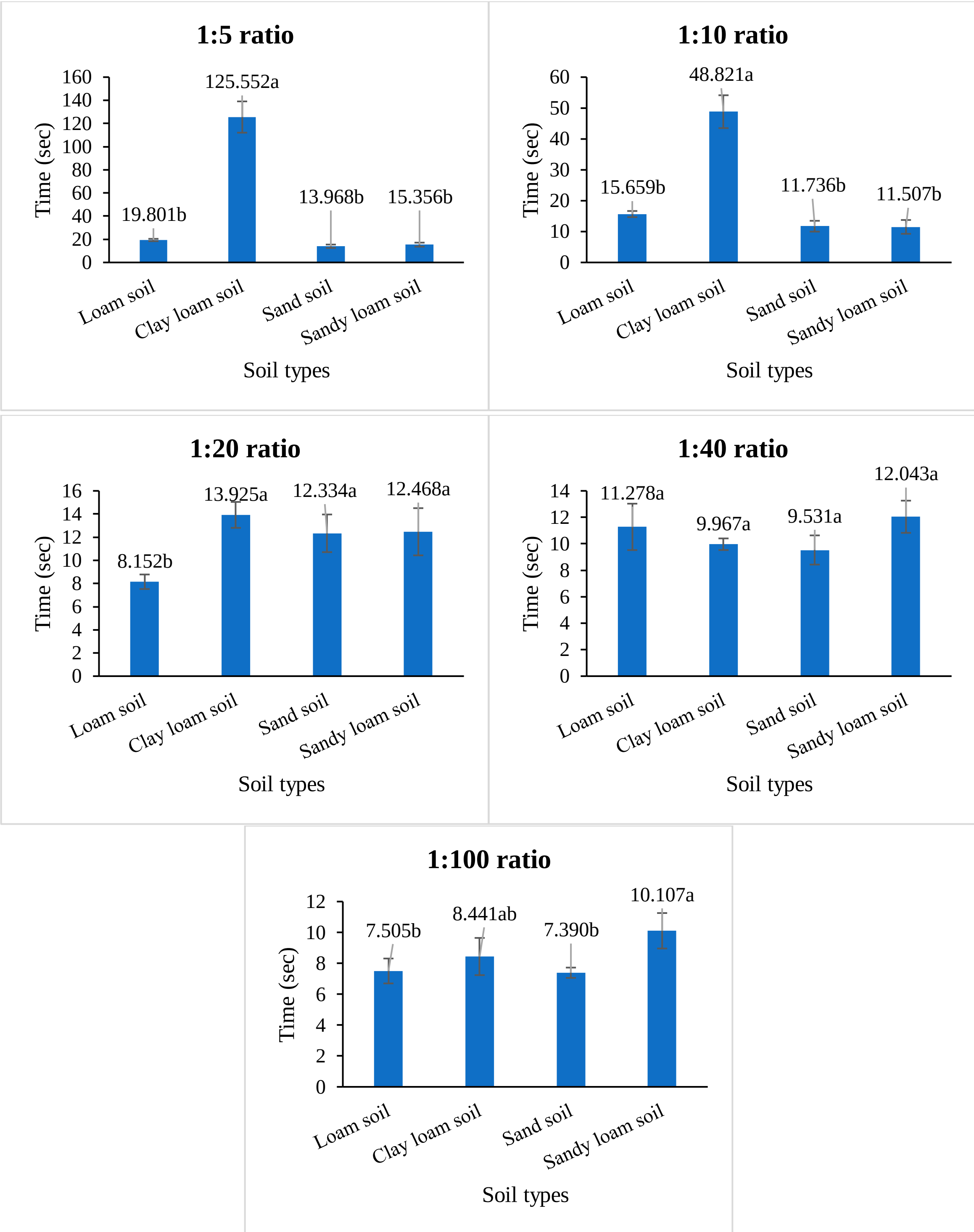


Figure 33: Changes in the center values of DET measurements of different soil types and soil:water ratios ($p<0.05$).

In Figure 33, it can be observed that the differences between the spreading times of the soils were blurred between 1:5 and 1:100, so it was necessary to concentrate on the narrower soil:water ratios in further measurements. Our results suggest that a soil:water ratio is appropriate where the variance of replicates is low within soil type and there are differences in spreading time between different soils.

In the statistical analysis, we also analysed the data (mainly the center values) for the same soil but different soil:water ratios (Table 27).

Table 27: Variation in the mean of suspensions with the same soil but different soil:water ratios ($p<0.05$).

	Sand soil	Sandy loam soil	Clay loam soil	Loam soil
1:5 ratio	13.848a	13.821a	125.552a	19.380a
1:10 ratio	11.736ab	11.507ab	48.821b	15.659ab
1:20 ratio	12.334ab	12.468ab	13.925c	8.152c
1:40 ratio	9.531bc	12.043ab	9.967c	11.278bc
1:100 ratio	7.390c	10.107b	8.440c	7.505c
LSD	**3.198**	**3.11**	**11.155**	**5.797**

Based on our measurements with DET equipment and statistical analyses, we found that the 1:5 soil:water ratio results that confirmed our hypothesis, so we worked with this soil:water ratio in the next step of the research.

5.4.3.3 Soil samples tested with the DET tool at 1:5 soil:water ratio

Five independent (random) soil samples with different physico-chemical properties were selected and subjected to the same tests as the four starting soils (Table 28 and Table 29), except that only 1:5 soil:water suspensions were prepared and tested with the DET tool. The aggregate distribution of the five „random" soil samples is shown in Table 30.

Table 28: Physical and chemical parameters of the five „random" soil samples-

Physical type of the soil	pH (H_2O)	EC (µS/cm)	Plasticity index according to Arany (KA)	Hydraulic conductivity (k-index) cm^3/min
Sand (coarse) soil	6.54	107.4	24.9	$1.21*10^{-1}$
Sand soil	6.03	55.7	*	$3.55*10^{-1}$
Loam soil	7.19	412.5	40.6	$1.72*10^{-1}$
Sandy loam soil	6.86	479.5	36.1	$1.68*10^{-2}$
Loam soil	7.08	521.5	38.2	$2.34*10^{-2}$

*In the case of Arenosol soils, the K_A factor cannot be measured.

Table 29: Saturation percentage of „random" soil samples

Physical type of the soil	Saturation percentage (SP)	Category
Sand (coarse) soil	24.90%	Sandy loam soil
Sand soil	-	-
Loam soil	40.60%	Loam soil
Sandy loam soil	36.10%	Loam soil
Loam soil	36.20%	Loam soil

Table 30: Aggregate distribution of the five „random" soil samples.

	2 mm	1 mm	0.5 mm	0.25 mm	0.125 mm	0.063 mm	0.045 mm	< 0.045 mm
Sand (coarse) soil	0.17%	1.21%	3.27%	24.43%	56.01%	13.60%	1.09%	0.23%
Sand soil	1.55%	1.64%	1.30%	6.55%	60.56%	24.84%	2.24%	1.32%
Loam soil	13.09%	17.97%	15.27%	10.84%	14.50%	24.89%	3.12%	0.32%
Sandy loam soil	19.64%	16.15%	14.56%	14.16%	18.28%	15.67%	1.42%	0.12%
Loam soil	18.45%	14.87%	14.27%	11.21%	11.36%	20.33%	7.75%	1.77%

The DET measurements and data evaluation were carried out in the same way as described in the second section, with the difference that for the independent samples only the 1:5 soil:water suspension was used (Figure 34).

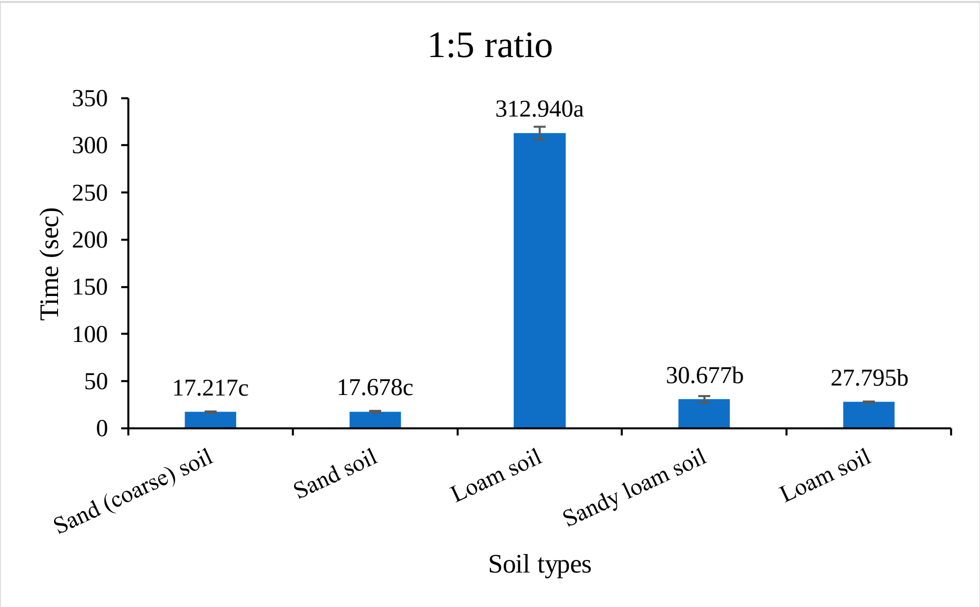


Figure 34: Changes in the center values of DET measurements for independent soil samples (p<0.05).

The differences between the spreading times of independent soil samples can be clearly observed when testing 1:5 suspensions. The highest spreading time was obtained for the loam soil, which is significantly different from the spreading times obtained for the other soil samples.

5.4.3.4 Water Retainer testing with the DET tool on two selected soil samples

Two soil samples with poor water retention capacity, based on their physical properties and literature, were selected for the Water Retainer treatments. These two soils were the Arenosol and loam soil. For these two soils, Water Retainer was applied at 20x and 50x dilutions according to the manufacturer's instructions, such that the diluted Water Retainer was used to prepare the suspension injected into the DET instrument.

Water Retainer is an organic soil conditioner. It is usually effective for up to 3 months, during which time the water retention capacity of the soil for plants is significantly increased by the use of Water Retainer. If this excellent capacity is exploited in the early stages of plant development, the developmental advantage of a better root system and improved water supply will be maintained throughout the growing season. As a result, drought can cause significantly less damage to plants. In extreme drought conditions, plants can survive up to twice as long without severe damage or stress, which will be reflected in yields, with less crop losses.

Comparing the results of the DET measurements, it can be seen that the mean times without Water Retainer were much lower compared to the two dilutions of Water Retainer (Table 31).

Table 31: Average center time of the two soil treated with Water Retainer

	Center time without Water Retainer	Center time using 20x dilution of Water Retainer	Center time using 50x dilution of Water Retainer
Sand soil	10.967 sec	19.230 sec	12.958 sec
Loam soil	12.394 sec	20.879 sec	24.058 sec

Our results also show that the centre times were significantly lower without the use of the Water Retainer compared to the use of the Water Retainer. When using 20x dilution, the spreading time increased by 8,263 seconds for sand soil, and by 1,990 seconds when using 50x dilution. In contrast, the spreading time increases were much greater for the loam soil when using Water Retainer: 8.485 seconds for the 20x dilution and 11.664 seconds for the 50x dilution.

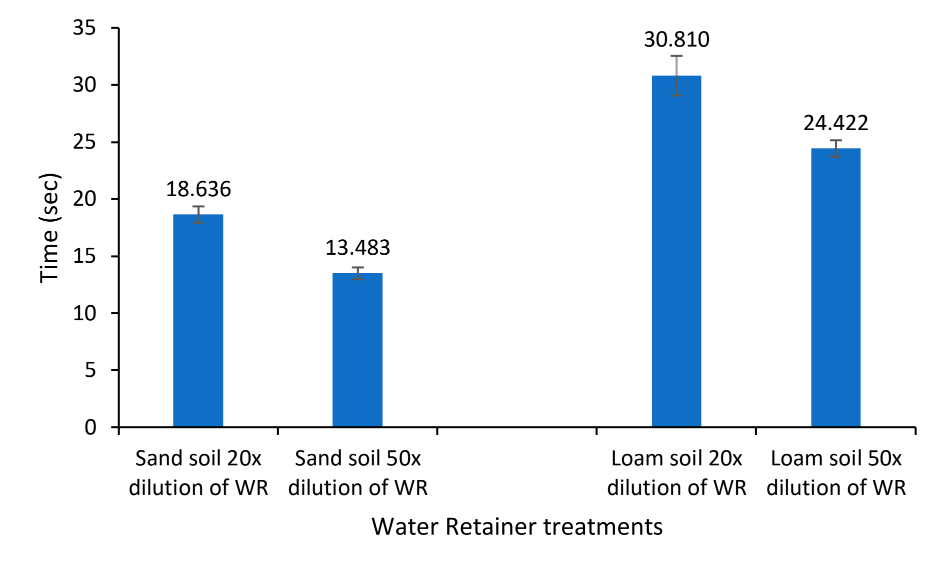


Figure 35: Changes in the average values of DET measurements of different Water Retainer treatments (p<0.05) [WR = Water Retainer].

The results have been statistically tested and analysed (Table 32, Table 33). For the 20x dilution Water Retainer treatment, the loam soil and the sandy soil belong to the same statistical group, with no significant difference (<0.05). However, for the 50x dilution Water Retainer treatment, there are significant differences (p<0.05) between the mean spreading times of the two soils tested (Table 32).

Table 32: Statistical evaluation of average spread time of the two soil treated with Water Retainer (p<0.05).

	Without Water Retainer	20x dilution of Water Retainer spread time (sec)	50x dilution of Water Retainer spread time (sec)
Sand soil	10.691 b	18.636 a	13.483 a
Loam soil	11.971 a	30.810 c	24.422 b

Table 33: Statistical evaluation of different Water Retainer treatments, but same soil type (p<0.05).

	Sandy soil	Loam soil
Without Water Retainer	10.691 c	11.971 b
20x dilution of Water Retainer spread time (sec)	18.636 a	30.810 a
50x dilution of Water Retainer spread time (sec)	13.483 b	24.422 ab
LSD	2.332 sec	2.200 sec

These results also show that Water Retainer can improve this property of soils with poor water holding capacity even when applied in such small quantities.

5.5 Additional results from Water Retainer field experiments

5.5.1 Results related to microbiological and macronutrient analysis

Determining the microbiological composition (four different types of bacteria) and macronutrient (N, P, K) content of soils was part of the field trials with Water Retainer (WR) in T3.3, T5.3, T5.4, T5.6, T5.9 and T5.10. The laboratory tests performed by BZN and the soil sampling methodology are described in detail in D3.2 (Section 2.1.1 and Annex 1 of D3.2, respectively). The WR was tested in two consecutive crop seasons (2021 and 2022). As defined in the soil sampling protocol, three soil samplings were carried out at each case study site in both years: right after WR application (T1 sampling date), in mid-season (T2 sampling date) and at harvest time (T3 sampling date).

In 2021 T1 samples showed no significant difference in cell numbers between WR-treated and control (untreated) soils. A significant difference could be observed between WR-treated and control soils in the samples taken at T2 sampling time in France and Poland. Total bacteria number and the number of bacteria with ACC-deaminase activity are lower in the WR-treated soil samples from Poland, and the number of nitrogen-fixing bacteria is lower in WR-treated soils from France. No such difference could be observed in samples from Italy and Sweden. This phenomenon is not clear and could not be unequivocally connected to WR application, as it did not happen at all sites, and not the same functional groups of bacteria were affected at the two sites where the differences were detected. As case study sites in Poland and France are larger than in the other two regions, the difference might be due to the inhomogeneity of the fields. At T3 sampling time the number of bacteria with ACC-deaminase activity was significantly lower at the control group in France. No significant difference was observed in the number of other bacteria at this site. There was no significant difference in the number of bacteria between the treated and control groups on the other three sites at T3 sampling time.

The initial (T1) macronutrient content of the soils showed a significant difference between treated and control soils in France and Poland. Nitrogen levels were higher, and phosphorus levels were lower in the treated soils in France, while nitrogen levels were higher in the control soils in Poland. No direct connection can be assumed with the WR application, as differences are inconsistent and cannot be observed in all sites. It might be due to the abovementioned larger size and the larger inhomogeneity of the fields. The results of mid-season (T2) and harvest time (T3) samples show no significant difference in macronutrient content between control and WR treated soils.

In 2022 the sampling protocol had to be slightly modified because two different crops (wheat and barley) were sown in Poland. Ten samples were taken from both wheat and barley crops (5 samples from control area and 5 samples from the area treated with WR). The timing of samplings and the processing of samples were the same as in 2021.

T1 and T2 samples showed no significant difference in cell numbers between WR-treated and control soils. In T3 sampling time the number of bacteria with ACC-deaminase activity was significantly lower in the treated soil of barley crop in Poland. The number of nitrogen fixing bacteria was significantly lower in the control soil in Italy. Based on the results of microbiological analysis, no unequivocal effect of the Water Retainer on the microbial community of the treated soils could be inferred.

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There was no significant difference in the macronutrient content of treated and untreated soils, except in Poland, where the phosphorus content of the control soils in wheat crop was significantly higher at T1 sampling time and the potassium content of treated soils was significantly higher in T2 sampling time, compared with the untreated soil in the case of barley crop. Based on the results of the two years' experiments, there is no direct connection between WT treatment and the macronutrient content of soils. The detailed numerical results of microbial and macronutrient analyses of T1, T2 and T3 soil samples are shown in Table 34 to Table 36 and Table 37 to Table 39 for 2021 and 2022, respectively.

Table 34: Results of soil samples taken at T1 sampling time in 2021.

Case Study Site (Region)	Sample code[1]	Macronutrient content[2]			Bacterial cell number [CFU/g soil] on different culture media[3]			
		Nitrogen [mg N/kg soil]	Phosphorus [mg P/kg soil]	Potassium [mg K^+/kg soil]	DSM1	NFX	CNF	Pikovskaya
Lower Silesia, Poland	RS1	469	364	115	2,60E+06	1,80E+05	1,20E+05	6,00E+04
	RS2	339	413	116	6,20E+06	1,40E+05	4,20E+05	8,00E+04
	RS3	287	273	120	7,40E+06	1,60E+05	1,00E+05	2,00E+04
	RS4	502	487	115	8,00E+05	1,40E+05	1,00E+05	8,00E+04
	RS5	559	389	117	1,60E+06	3,80E+05	1,60E+05	8,00E+04
	RS6	452	350	118	3,60E+06	3,00E+05	6,60E+05	6,00E+04
	RS7	512	373	236	8,00E+05	1,80E+05	3,00E+05	2,00E+04
	RS8	507	318	198	8,00E+05	5,20E+05	3,20E+05	2,00E+05
	RS9	482	386	117	3,00E+06	2,00E+05	3,40E+05	1,20E+05
	RS10	504	264	114	1,60E+06	1,00E+05	1,00E+05	8,00E+04
	RS11	656	286	152	1,20E+07	4,20E+05	5,80E+05	2,20E+05
	RS12	708	340	156	6,00E+06	1,60E+05	7,80E+05	8,00E+04
	RS13	637	314	115	3,40E+06	7,80E+05	5,40E+05	4,60E+05
	RS14	568	388	142	4,80E+06	1,28E+06	6,80E+05	3,20E+05
	RS15	552	316	159	4,00E+06	5,80E+05	4,00E+05	1,60E+05
	RS16	1 051	378	234	3,60E+06	8,20E+05	1,30E+06	8,00E+04
Gårdstånga Nygård, Sweden	T1	948	458	340	1,02E+06	5,20E+05	5,20E+05	0,00E+00
	T2	1 016	516	294	1,06E+06	4,40E+05	3,40E+05	2,00E+04
	U1	1 219	625	357	1,18E+06	6,80E+05	6,80E+05	2,00E+04
	U2	1 135	515	397	1,26E+06	5,00E+05	8,40E+05	0,00E+00
Auxerre, France	U1	779	374	309	2,40E+06	1,20E+06	8,00E+05	2,00E+05
	U2	866	328	302	2,20E+06	2,00E+05	1,20E+06	4,00E+05
	U3	645	324	298	7,40E+06	1,00E+06	6,00E+05	2,00E+05
	U4	720	508	233	2,80E+06	0,00E+00	6,00E+05	0,00E+00
	U5	712	391	299	1,00E+06	1,80E+06	0,00E+00	0,00E+00
	U6	724	493	276	1,60E+06	8,00E+05	8,00E+05	0,00E+00
	T1	931	336	439	2,00E+06	6,00E+05	6,00E+05	1,60E+06
	T2	1 027	361	400	1,00E+06	4,00E+05	4,00E+05	0,00E+00
	T3	788	263	288	1,40E+06	2,00E+05	0,00E+00	0,00E+00
	T4	963	281	317	6,00E+06	1,00E+06	1,00E+06	2,00E+05
	T5	1 065	296	231	4,00E+06	2,00E+05	2,00E+06	0,00E+00
	T6	871	292	279	1,00E+06	6,00E+05	4,00E+05	0,00E+00
Bologna, Italy	T1	618	118	195	1,80E+06	2,58E+06	1,64E+06	6,00E+04
	T2	716	158	191	3,40E+06	2,76E+06	2,02E+06	2,20E+05
	T3	613	179	195	2,80E+07	5,08E+06	2,66E+06	3,60E+05
	U1	560	162	153	6,40E+06	3,82E+06	2,90E+06	2,20E+05
	U2	704	130	309	2,00E+06	1,26E+06	1,32E+06	1,20E+05
	U3	628	144	272	1,60E+07	1,44E+06	1,62E+06	6,60E+05

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Table 35: Results of soil samples taken at T2 sampling time in 2021.

Case Study Site (Region)	Sample code[1]	Macronutrient content[2]			Bacterial cell number [CFU/g soil] on different culture media[3]			
		Nitrogen [mg N/kg soil]	Phosphorus [mg P/kg soil]	Potassium [mg K^+/kg soil]	DSM1	NFX	CNF	Pikovskaya
Lower Silesia, Poland	RS1	369	373	120	4,40E+05	3,40E+05	1,80E+05	0,00E+00
	RS2	430	383	116	3,20E+05	4,00E+04	1,80E+05	0,00E+00
	RS3	303	254	121	2,80E+05	2,20E+05	1,60E+05	2,00E+04
	RS4	465	434	122	6,40E+05	3,20E+05	2,40E+05	0,00E+00
	RS5	502	402	122	6,20E+05	3,40E+05	1,20E+05	1,00E+05
	RS6	425	320	117	5,00E+05	3,00E+05	2,80E+05	4,00E+04
	RS7	524	393	120	3,40E+05	1,40E+05	1,60E+05	2,00E+04
	RS8	436	305	162	3,60E+05	2,20E+05	3,60E+05	0,00E+00
	RS9	440	397	120	4,20E+05	1,40E+05	1,00E+05	0,00E+00
	RS10	589	388	119	1,00E+06	3,20E+05	2,40E+05	2,20E+05
	RS11	595	343	163	1,20E+06	5,60E+05	3,20E+05	0,00E+00
	RS12	650	396	156	1,18E+06	6,00E+05	3,00E+05	0,00E+00
	RS13	623	398	160	1,08E+06	2,40E+05	2,40E+05	0,00E+00
	RS14	588	258	161	1,54E+06	7,20E+05	3,20E+05	0,00E+00
	RS15	842	362	242	2,20E+06	1,80E+06	3,40E+05	6,00E+04
	RS16	1103	430	284	4,60E+06	8,20E+05	4,00E+05	1,00E+05
Gårdstånga Nygård, Sweden	IR1	1 129	621	205	9,40E+06	7,20E+05	3,80E+05	4,00E+04
	IR2	1 348	695	194	8,00E+06	6,80E+05	2,20E+05	0,00E+00
	A1	1 321	694	154	5,60E+06	4,20E+05	4,80E+05	0,00E+00
	A2	1 276	678	162	5,20E+06	1,60E+05	2,20E+05	0,00E+00
	B1	1 382	748	157	3,04E+07	6,00E+05	4,00E+05	0,00E+00
	B2	1 421	771	115	1,16E+07	5,60E+05	3,40E+05	0,00E+00
	B3	1 322	742	115	1,44E+06	6,00E+05	2,80E+05	2,00E+04
	B4	1 373	718	123	2,60E+06	6,00E+05	3,00E+05	8,00E+04
Auxerre, France	U1	912	724	189	3,20E+06	1,00E+06	3,20E+05	1,00E+05
	U2	990	639	162	1,00E+06	1,00E+06	2,20E+05	6,00E+04
	U3	958	617	201	2,60E+06	2,80E+05	4,20E+05	0,00E+00
	U4	838	637	164	2,00E+05	1,00E+05	4,00E+05	4,00E+04
	U5	837	627	156	1,80E+06	1,80E+05	3,20E+05	0,00E+00
	T1	896	630	200	2,00E+06	1,20E+05	2,20E+05	2,00E+04
	T2	939	643	162	1,20E+06	8,00E+04	2,00E+05	2,00E+04
	T3	1 122	683	163	2,40E+06	1,60E+05	2,60E+05	0,00E+00
	T4	1 117	712	159	2,10E+06	3,20E+05	1,80E+05	2,00E+04
	T5	1 045	675	158	1,20E+06	4,60E+05	2,80E+05	0,00E+00
Bologna, Italy	T1	607	127	238	1,84E+06	3,60E+05	2,40E+05	0,00E+00
	T2	668	224	278	1,18E+06	2,80E+05	2,40E+05	0,00E+00
	T3	624	155	241	1,60E+06	1,00E+06	3,00E+05	2,00E+04
	U1	487	135	252	1,12E+06	4,00E+05	4,00E+05	0,00E+00
	U2	644	114	240	1,40E+06	2,40E+05	2,40E+05	0,00E+00
	U3	796	223	279	1,10E+06	3,00E+05	2,40E+05	4,00E+04

Table 36: Results of soil samples taken at T3 sampling time in 2021.

Case Study Site (Region)	Sample code[1]	Macronutrient content[2]			Bacterial cell number [CFU/g soil] on different culture media[3]			
		Nitrogen [mg N/kg soil]	Phosphorus [mg P/kg soil]	Potassium [mg K^+/kg soil]	DSM1	NFX	CNF	Pikovskaya
Lower Silesia, Poland	RS1	311	310	121	4,20E+05	6,00E+04	1,40E+05	2,00E+04
	RS2	309	352	161	7,00E+05	1,00E+05	2,60E+05	0,00E+00
	RS3	455	308	119	4,80E+05	6,00E+04	1,60E+05	4,00E+04
	RS4	406	316	79	3,80E+05	4,00E+04	2,40E+05	0,00E+00
	RS5	673	330	120	7,60E+05	1,60E+05	3,60E+05	2,00E+04
	RS6	455	307	79	6,80E+05	1,60E+05	3,40E+05	6,00E+04
	RS7	585	330	121	9,80E+05	4,80E+05	5,00E+05	2,20E+05
	RS8	590	336	121	1,38E+06	5,80E+05	4,80E+05	2,00E+04
	RS9	476	405	81	3,60E+05	2,20E+05	1,20E+05	0,00E+00
	RS10	627	392	119	9,00E+05	5,20E+05	2,60E+05	4,00E+04
	RS11	529	246	81	5,60E+05	4,40E+05	2,60E+05	4,00E+04
	RS12	564	399	122	1,00E+06	6,80E+05	2,60E+05	4,00E+04
	RS13	490	300	121	5,00E+05	3,40E+05	1,60E+05	1,20E+05
	RS14	576	267	115	8,60E+05	5,20E+05	5,00E+05	4,00E+04
	RS15	925	306	202	7,60E+05	1,00E+05	3,60E+05	2,00E+04
	RS16	1003	333	157	8,40E+05	2,00E+05	3,60E+05	1,00E+05
Gårdstånga Nygård, Sweden	IR1	1285	462	158	1,58E+06	2,80E+05	3,00E+05	2,00E+04
	IR2	1356	533	159	1,72E+06	2,60E+05	2,60E+05	0,00E+00
	A1	1072	501	120	1,22E+06	1,60E+05	3,60E+05	0,00E+00
	A2	1130	424	118	1,24E+06	1,40E+05	2,60E+05	2,00E+04
	B1	1166	509	117	9,00E+05	2,00E+05	1,60E+05	0,00E+00
	B2	1332	742	119	1,08E+06	3,20E+05	4,20E+05	0,00E+00
	B3	1345	474	121	8,80E+05	3,20E+05	4,80E+05	0,00E+00
	B4	1385	518	119	1,28E+06	5,60E+05	3,20E+05	2,00E+04
Auxerre, France	U1	958	386	197	4,60E+05	4,60E+05	4,20E+05	8,00E+04
	U2	966	336	196	1,26E+06	6,00E+05	3,80E+05	0,00E+00
	U3	1014	334	157	8,40E+05	3,00E+05	3,20E+05	6,00E+04
	U4	1001	400	154	1,26E+06	4,40E+05	3,00E+05	4,00E+04
	U5	900	439	162	8,00E+05	3,20E+05	2,40E+05	1,20E+05
	U6	947	361	160	1,10E+06	5,40E+05	2,80E+05	1,00E+05
	T1	912	384	153	7,60E+05	4,60E+05	4,40E+05	2,00E+04
	T2	1030	440	161	5,80E+05	6,00E+05	3,40E+05	4,00E+04
	T3	969	430	200	7,00E+05	3,00E+05	3,60E+05	6,00E+04
	T4	986	425	157	5,20E+05	4,60E+05	5,80E+05	4,00E+04
	T5	966	464	154	7,40E+05	3,00E+05	6,40E+05	2,00E+04
	T6	1052	312	233	8,80E+05	4,00E+05	4,60E+05	1,20E+05
Bologna, Italy	T1	1016	510	162	5,60E+06	6,00E+04	1,00E+05	0,00E+00
	T2	1353	349	160	5,20E+06	1,60E+05	1,80E+05	2,00E+04
	T3	1126	481	202	8,40E+06	8,00E+04	1,60E+05	0,00E+00
	U1	1027	453	200	8,80E+06	2,20E+05	2,20E+05	0,00E+00
	U2	954	408	194	4,20E+06	1,00E+05	1,40E+05	0,00E+00
	U3	987	463	235	5,80E+06	1,00E+05	1,20E+05	0,00E+00

Table 37: Results of soil samples taken at T1 sampling time in 2022.

Case Study Site (Region)	Sample code[1]	Macronutrient content[2]			Bacterial cell number [CFU/g soil] on different culture media[3]			
		Nitrogen [mg N/kg soil]	Phosphorus [mg P/kg soil]	Potassium [mg K^+/kg soil]	DSM1	NFX	CNF	Pikovskaya
Lower Silesia, Poland	RS1WU	845	781	155	7,40E+05	3,40E+05	2,80E+05	4,00E+04
	RS2WU	543	530	153	7,00E+05	2,60E+05	1,60E+05	0,00E+00
	RS3WU	579	525	122	6,60E+05	2,80E+05	2,20E+05	0,00E+00
	RS4WU	448	467	115	1,32E+06	1,80E+05	2,80E+05	6,00E+04
	RS5WU	381	395	122	5,80E+05	1,80E+05	1,20E+05	0,00E+00
	RS1WT	547	432	115	9,00E+05	2,00E+05	2,00E+05	4,00E+04
	RS2WT	527	292	116	8,60E+05	3,80E+05	2,40E+05	2,00E+04
	RS3WT	495	328	156	8,80E+05	3,00E+05	1,80E+05	0,00E+00
	RS4WT	564	352	150	4,40E+05	1,60E+05	1,00E+05	0,00E+00
	RS5WT	448	311	157	4,60E+05	2,00E+05	2,20E+05	2,00E+04
	RS1BU	598	607	160	7,40E+05	1,20E+05	1,80E+05	0,00E+00
	RS2BU	623	411	121	7,00E+05	2,00E+05	1,00E+05	0,00E+00
	RS3BU	557	370	199	7,80E+05	2,00E+05	8,00E+05	0,00E+00
	RS4BU	311	224	155	5,40E+05	1,00E+05	2,40E+05	0,00E+00
	RS5BU	232	263	118	5,00E+05	8,00E+04	1,20E+05	0,00E+00
	RS1BT	542	513	117	3,40E+05	2,00E+04	1,60E+05	0,00E+00
	RS2BT	849	414	158	7,00E+05	2,60E+05	2,60E+05	0,00E+00
	RS3BT	767	384	161	4,60E+05	1,00E+05	1,00E+05	0,00E+00
	RS4BT	550	298	154	3,60E+05	1,00E+05	2,00E+05	0,00E+00
	RS5BT	361	316	116	5,80E+05	4,00E+04	2,00E+05	0,00E+00
Gårdstånga Nygård, Sweden	A1	1356	462	164	2,60E+05	1,20E+05	2,00E+05	0,00E+00
	A2	1290	390	155	2,00E+05	2,00E+05	2,00E+05	0,00E+00
	A3	1328	329	154	6,00E+05	2,40E+05	2,00E+05	0,00E+00
	A4	1141	279	115	7,00E+05	1,80E+05	3,00E+05	0,00E+00
	A5	1363	422	161	8,40E+05	2,20E+05	1,60E+05	0,00E+00
	B1	1415	317	124	1,14E+06	2,20E+05	3,60E+05	0,00E+00
	B2	1305	433	117	1,00E+06	2,60E+05	3,40E+05	0,00E+00
	B3	1348	353	124	8,60E+05	1,00E+05	1,60E+05	0,00E+00
	B4	1309	457	163	5,60E+05	1,40E+05	2,80E+05	0,00E+00
	B5	1308	493	118	8,20E+05	1,60E+05	2,20E+05	0,00E+00
Auxerre, France	U1	890	303	203	6,80E+05	2,00E+05	1,40E+05	8,00E+04
	U2	862	402	241	5,80E+05	1,40E+05	2,00E+05	2,00E+04
	U3	911	388	161	7,00E+05	1,00E+05	1,80E+05	4,00E+04
	U4	899	307	161	1,30E+06	2,40E+05	2,00E+05	8,00E+04
	U5	1026	367	198	1,00E+06	2,20E+05	2,40E+05	6,00E+04
	T1	797	462	155	6,20E+05	3,00E+05	1,80E+05	6,00E+04
	T2	885	304	155	8,80E+05	1,60E+05	3,20E+05	1,20E+05
	T3	892	527	195	1,40E+06	3,00E+05	3,00E+05	0,00E+00
	T4	961	495	197	6,60E+05	2,20E+05	2,80E+05	0,00E+00
	T5	1335	429	265	5,60E+05	1,80E+05	2,40E+05	0,00E+00
Bologna, Italy	T1	679	367	389	3,00E+05	8,00E+04	8,00E+04	0,00E+00
	T2	847	361	284	4,60E+05	8,00E+04	4,00E+04	0,00E+00
	T3	923	403	229	2,20E+05	1,00E+05	8,00E+04	0,00E+00
	U1	674	358	240	2,80E+05	8,00E+04	6,00E+04	0,00E+00
	U2	768	389	242	2,00E+05	1,00E+05	4,00E+04	4,00E+04
	U3	930	328	204	2,20E+05	4,00E+04	2,00E+05	0,00E+00

Table 38: Results of soil samples taken at T2 sampling time in 2022.

Case Study Site (Region)	Sample code[1]	Macronutrient content[2]			Bacterial cell number [CFU/g soil] on different culture media[3]			
		Nitrogen [mg N/kg soil]	Phosphorus [mg P/kg soil]	Potassium [mg K^+/kg soil]	DSM1	NFX	CNF	Pikovskaya
Lower Silesia, Poland	RS1WU	800	380	163	6,00E+06	3,00E+05	1,00E+05	6,00E+04
	RS2WU	1006	353	181	1,24E+06	2,80E+05	4,00E+05	6,00E+04
	RS3WU	832	271	206	1,12E+06	2,80E+05	4,60E+05	2,00E+04
	RS4WU	789	247	155	8,60E+05	2,80E+05	3,00E+05	2,00E+04
	RS5WU	732	413	136	7,60E+05	1,80E+05	3,00E+05	0,00E+00
	RS1WT	760	453	187	2,18E+06	2,60E+05	3,00E+05	1,00E+05
	RS2WT	1100	331	197	1,90E+06	4,60E+05	1,20E+05	0,00E+00
	RS3WT	922	317	234	1,06E+06	3,20E+05	2,00E+05	1,00E+05
	RS4WT	816	311	193	1,44E+06	1,60E+05	4,20E+05	0,00E+00
	RS5WT	615	323	171	1,56E+06	2,20E+05	3,80E+05	0,00E+00
	RS1BU	1115	677	172	5,60E+05	2,60E+05	3,40E+05	0,00E+00
	RS2BU	758	431	177	9,60E+05	1,40E+05	3,00E+05	0,00E+00
	RS3BU	818	535	187	7,00E+05	2,40E+05	3,40E+05	6,00E+04
	RS4BU	730	417	172	1,40E+06	5,20E+05	2,80E+05	1,00E+05
	RS5BU	664	344	149	6,80E+05	2,40E+05	2,20E+05	4,00E+04
	RS1BT	896	517	248	6,00E+05	3,40E+05	3,80E+05	0,00E+00
	RS2BT	876	405	202	1,26E+06	2,40E+05	6,60E+05	4,00E+04
	RS3BT	832	433	214	1,36E+06	4,60E+05	3,00E+05	1,00E+05
	RS4BT	662	300	227	1,10E+06	3,40E+05	4,00E+05	0,00E+00
	RS5BT	517	271	189	8,20E+05	2,20E+05	2,20E+05	0,00E+00
Gårdstånga Nygård, Sweden	A1	1128	436	166	1,06E+06	1,80E+05	3,80E+05	0,00E+00
	A2	1052	346	161	5,80E+05	1,20E+05	2,80E+05	0,00E+00
	A3	1330	431	158	5,20E+05	1,20E+05	2,60E+05	2,00E+04
	A4	1299	508	158	5,60E+05	1,40E+05	2,40E+05	0,00E+00
	A5	1364	607	147	1,04E+06	2,00E+05	1,80E+05	2,00E+04
	B1	1531	122	150	5,60E+05	2,00E+05	2,60E+05	0,00E+00
	B2	1268	292	162	8,00E+05	1,00E+05	3,40E+05	2,00E+04
	B3	1351	307	167	7,20E+05	1,80E+05	4,00E+05	2,00E+04
	B4	1420	354	124	5,80E+05	2,60E+05	3,00E+05	0,00E+00
	B5	1427	348	119	1,14E+06	3,40E+05	3,60E+05	0,00E+00
Auxerre, France	U1	787	431	225	3,60E+05	8,00E+04	2,40E+05	6,00E+04
	U2	813	356	195	8,20E+05	3,00E+05	2,60E+05	4,00E+04
	U3	753	359	193	8,20E+05	8,00E+04	2,60E+05	2,00E+04
	U4	789	348	157	7,40E+05	1,60E+05	3,60E+05	0,00E+00
	U5	775	411	155	7,00E+05	3,00E+05	2,40E+05	2,00E+04
	T1	743	409	163	9,20E+05	2,80E+05	2,00E+05	6,00E+04
	T2	804	372	165	3,60E+05	1,80E+05	2,40E+05	0,00E+00
	T3	714	327	162	2,80E+05	6,00E+04	1,60E+05	4,00E+04
	T4	726	425	150	5,00E+05	1,80E+05	2,60E+05	2,00E+04
	T5	883	408	142	3,00E+05	1,60E+05	2,20E+05	4,00E+04
Bologna, Italy	T1	859	777	368	5,60E+05	4,40E+05	3,60E+05	0,00E+00
	T2	952	423	202	6,60E+05	2,00E+05	5,00E+05	0,00E+00
	T3	1012	468	201	5,40E+05	2,20E+05	4,00E+05	0,00E+00
	U1	821	546	146	5,40E+05	3,80E+05	4,80E+05	0,00E+00
	U2	921	438	158	1,00E+06	4,40E+05	5,40E+05	8,00E+04
	U3	974	567	156	6,80E+05	3,80E+05	6,60E+05	0,00E+00

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Table 39: Results of soil samples taken at T3 sampling time in 2022.

Case Study Site (Region)	Sample code[1]	Macronutrient content[2]			Bacterial cell number [CFU/g soil] on different culture media[3]			
		Nitrogen [mg N/kg soil]	Phosphorus [mg P/kg soil]	Potassium [mg K^+/kg soil]	DSM1	NFX	CNF	Pikovskaya
Lower Silesia, Poland	RS1WU	1189	341	202	7,20E+05	2,60E+05	2,60E+05	0,00E+00
	RS2WU	897	358	196	1,34E+06	3,40E+05	2,80E+05	2,00E+04
	RS3WU	895	463	201	1,14E+06	2,60E+05	3,80E+05	0,00E+00
	RS4WU	744	331	235	1,06E+06	1,80E+05	3,00E+05	0,00E+00
	RS5WU	671	388	192	5,00E+05	2,20E+05	2,20E+05	0,00E+00
	RS1WT	788	851	198	7,40E+05	2,20E+05	6,00E+04	0,00E+00
	RS2WT	1602	564	226	4,80E+05	2,40E+05	2,00E+05	0,00E+00
	RS3WT	928	487	238	1,04E+06	3,40E+05	2,20E+05	0,00E+00
	RS4WT	821	425	230	7,40E+05	3,40E+05	3,00E+05	0,00E+00
	RS5WT	650	399	194	1,04E+06	2,20E+05	3,20E+05	4,00E+04
	RS1BU	980	919	199	1,00E+06	6,20E+05	3,80E+05	0,00E+00
	RS2BU	890	646	177	6,20E+05	3,60E+05	3,40E+05	0,00E+00
	RS3BU	898	731	190	1,00E+06	2,60E+05	3,00E+05	0,00E+00
	RS4BU	744	529	353	7,20E+05	2,60E+05	4,20E+05	0,00E+00
	RS5BU	695	466	204	1,40E+06	2,40E+05	2,20E+05	0,00E+00
	RS1BT	952	777	149	1,40E+06	2,20E+05	2,60E+05	0,00E+00
	RS2BT	904	473	227	8,80E+05	2,20E+05	2,00E+05	0,00E+00
	RS3BT	799	526	244	7,00E+05	1,40E+05	2,80E+05	0,00E+00
	RS4BT	608	380	184	6,40E+05	1,20E+05	8,00E+04	0,00E+00
	RS5BT	580	406	185	5,60E+05	1,00E+05	1,40E+05	0,00E+00
Gårdstånga Nygård, Sweden	A1	1259	405	238	6,00E+05	2,20E+05	3,00E+05	0,00E+00
	A2	1287	486	154	3,80E+05	1,40E+05	2,60E+05	0,00E+00
	A3	1409	581	162	7,20E+05	1,40E+05	3,60E+05	2,00E+04
	A4	1479	467	162	1,00E+06	2,60E+05	2,40E+05	0,00E+00
	A5	1448	488	159	4,00E+05	1,60E+05	2,40E+05	0,00E+00
	B1	1251	387	117	1,00E+06	1,40E+05	3,40E+05	0,00E+00
	B2	1431	531	121	7,80E+05	2,60E+05	1,40E+05	0,00E+00
	B3	1315	412	118	1,02E+06	1,60E+05	2,80E+05	0,00E+00
	B4	1296	410	114	8,60E+05	1,60E+05	3,00E+05	0,00E+00
	B5	1621	543	122	1,00E+06	2,40E+05	2,60E+05	0,00E+00
Auxerre, France	U1	778	159	278	9,60E+05	2,60E+05	2,40E+05	2,00E+04
	U2	960	171	204	1,14E+06	2,60E+05	4,40E+05	2,00E+04
	U3	824	366	199	7,20E+05	2,60E+05	2,00E+05	2,00E+04
	U4	929	327	78	1,28E+06	5,60E+05	2,80E+05	0,00E+00
	U5	859	371	197	8,60E+05	4,20E+05	1,60E+05	0,00E+00
	T1	855	324	228	1,22E+06	2,40E+05	2,80E+05	0,00E+00
	T2	862	365	202	1,60E+06	4,00E+05	4,60E+05	2,00E+04
	T3	802	324	182	1,56E+06	4,20E+05	2,80E+05	0,00E+00
	T4	845	448	192	1,80E+06	3,20E+05	3,40E+05	2,00E+04
	T5	952	203	202	2,20E+06	8,00E+04	3,20E+05	2,00E+04
Bologna, Italy	T1	940	323	313	1,44E+06	4,40E+05	4,80E+05	0,00E+00
	T2	800	367	238	1,14E+06	4,60E+05	4,20E+05	2,00E+04
	T3	1000	360	224	1,76E+06	4,00E+05	5,00E+05	0,00E+00
	U1	838	405	227	1,64E+06	4,00E+04	3,00E+05	0,00E+00
	U2	948	329	243	1,60E+06	2,00E+05	3,20E+05	2,00E+04
	U3	1063	474	271	1,70E+06	3,20E+05	4,00E+05	4,00E+04

[1] Sample codes have been given by WATERAGRI partners responsible for taking soil samples.
[2] Total N, P, K content of the soil samples was determined by methods described in Section 2.1.1.2. of D3.2.
[3] Microbiological analyses were performed on four different culture media (DSM1: Total cultivable bacteria; NFX: Nitrogen fixing bacteria; CNF: Bacteria with ACC-deaminase activity; Pikovskaya: Phosphate mobilizing bacteria), described in Section 2.1.1.2. of D3.2.

5.5.2 Results related to metagenome analysis of soil samples

Materials and methods

Molecular biological analysis was carried out in the T1, T2, T3 soil samples described above, collected in 2021. Bacterial DNA was extracted from soil samples using the DNeasy PowerSoil Kit (Qiagen) as instructed by the manufacturer's protocol. The concentration of genomic DNA was measured using a Qubit 2.0 Fluorometer (Invitrogen) with Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific).

Bacterial DNA was amplified with tagged primers[1] covering V3–V4 hypervariable region of the bacterial 16S rRNA gene (Klindworth et al., 2013). Polymerase chain reactions (PCR) and DNA purifications were performed according to Illumina's demonstrated protocol (Part # 15044223 Rev. B). The PCR product libraries were quantified and qualified by using High Sensitivity D1000 ScreenTape on TapeStation 2200 instrument (Agilent). Equimolar concentrations of libraries were pooled and sequenced on an Illumina MiSeq platform using MiSeq Reagent Kit v3 (600 cycles PE).

For the bioinformatic analysis, in average ca. 400 000 raw sequencing reads per sample were generated, which were demultiplexed, adapter-trimmed and quality-filtered by using MiSeq Control Software (Illumina). Classification is performed using the Illumina 16S Metagenomics workflow based on the DADA2 formatted RefSeq RDP 16S v3 database (Wang et al., 2007; Alishum, 2019).

In the case of T1 sampling time, this analysis was performed separately for each individual soil sample, in order to gain information on the variability of bacterial composition. T1 samples represent the bacterial composition of the soils prior to WR application. In the case of T2 and T3 sampling times, average control and treated samples were created before the extraction of bacterial DNA by mixing individual control samples and treated samples.

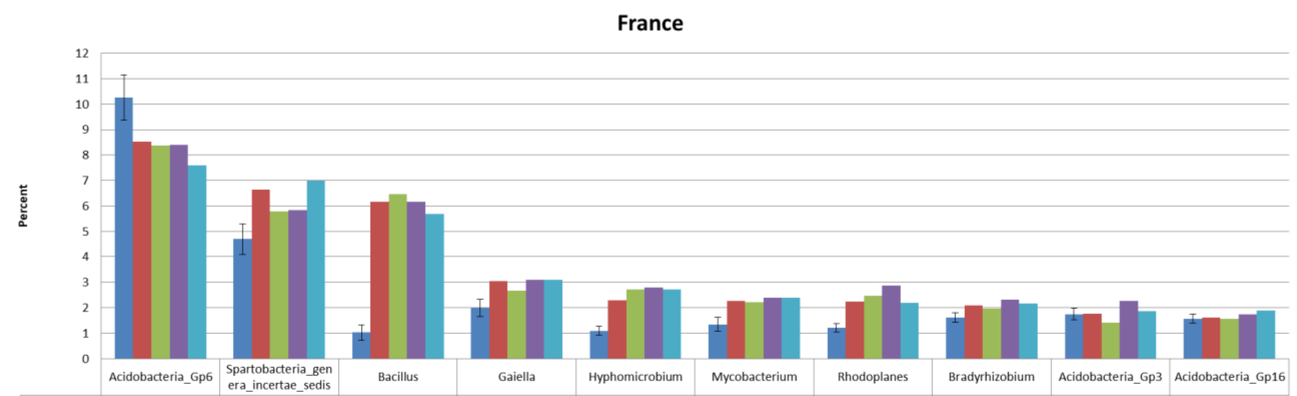
Results

The majority of bacteria detected belong to the *Acidobacteria*, *Actinobacteria*, *Firmicutes*, *Gemmatimonadetes*, *Proteobacteria* and *Verrucomicrobia* phyla. Based on the sequencing results of the individual samples, the different sites are relatively inhomogeneous in terms of microbial composition in the soil, 20-43% deviation could be detected for some genera.

Percentage share of the ten genera occurring most frequently at all sites was used for the comparison of treated and control soils. Control soil samples of T2 sampling date were used as a reference for the selection of these ten most frequent genera.

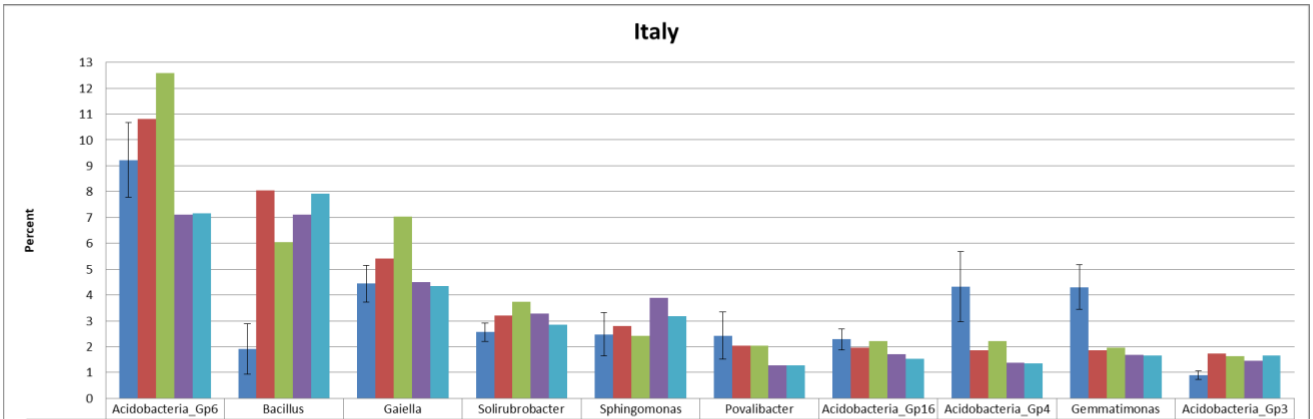
Having compared the sequencing results of treated and control soils, no difference can be observed for any of the genera in relation to the application of Water Retainer. The results are presented in Figure 36 for Auxerre/France, in Figure 37 for Bologna/Italy, in Figure 38 for Lower Silesia/Poland, and in Figure 39 for Gårdstånga Nygård/Sweden.

[1] 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTA CGGGNGGCWGCAG and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATC TAATCC



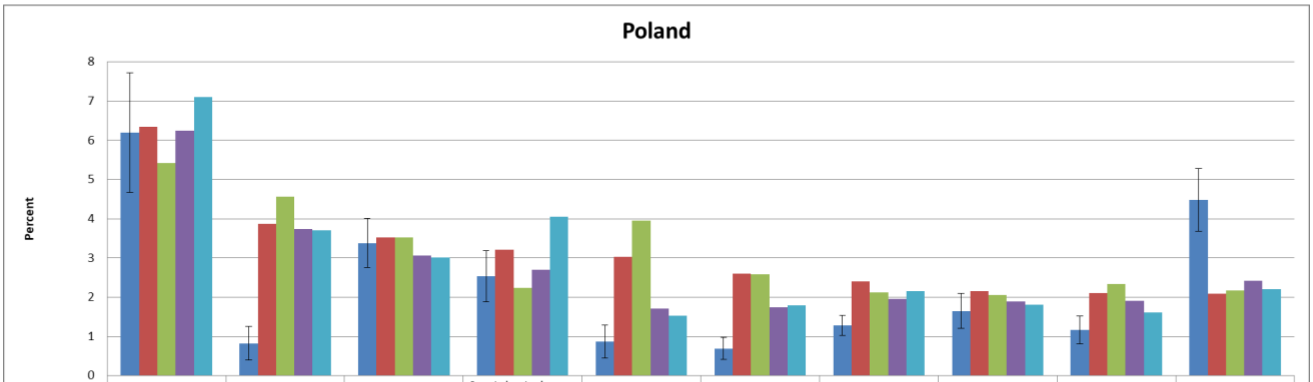
	Acidobacteria_Gp6	Spartobacteria_genera_incertae_sedis	Bacillus	Gaiella	Hyphomicrobium	Mycobacterium	Rhodoplanes	Bradyrhizobium	Acidobacteria_Gp3	Acidobacteria_Gp16
FR_T1	10,25	4,70	1,02	1,99	1,09	1,35	1,21	1,61	1,75	1,57
FR_UTC_T2	8,52	6,64	6,17	3,04	2,30	2,25	2,23	2,10	1,76	1,60
FR_TRT_T2	8,38	5,79	6,46	2,66	2,71	2,22	2,46	1,96	1,42	1,56
FR_UTC_T3	8,41	5,83	6,16	3,08	2,79	2,40	2,86	2,33	2,27	1,73
FR_TRT_T3	7,59	7,00	5,69	3,09	2,72	2,40	2,20	2,17	1,85	1,88

Figure 36: Occurrence of the ten most frequent genera in the soil samples from Auxerre/France, i.e. Case Study site 4. (FR = France, UTC = untreated control, TRT = treated, T1, T2, T3: sampling times as described in Chapter 5.5.1; FRT_1: U1-T6 in Table 34; FR_UTC_T2: U1-U5 in Table 35; FR_TRT_T2: T1-T5 in Table 35; FR_UTC_T3: U1-U6 in Table 36; FR_TRT_T3: T1-T6 in Table 36).



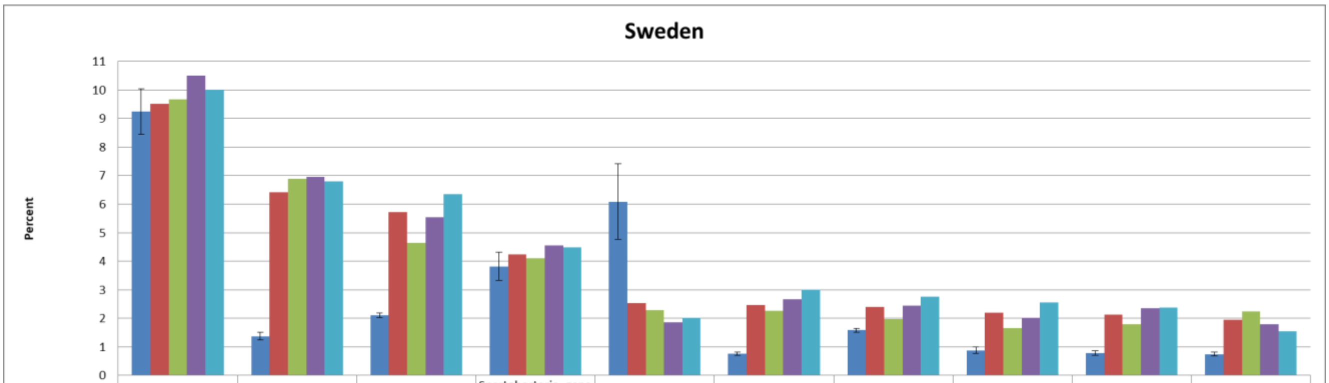
	Acidobacteria_Gp6	Bacillus	Gaiella	Solirubrobacter	Sphingomonas	Povalibacter	Acidobacteria_Gp16	Acidobacteria_Gp4	Gemmatimonas	Acidobacteria_Gp3
IT_T1	9,23	1,91	4,44	2,56	2,48	2,42	2,29	4,32	4,31	0,90
IT_UTC_T2	10,81	8,04	5,41	3,20	2,79	2,03	1,96	1,86	1,86	1,74
IT_TRT_T2	12,58	6,06	7,02	3,74	2,42	2,04	2,21	2,23	1,95	1,64
IT_UTC_T3	7,12	7,10	4,49	3,28	3,89	1,27	1,70	1,37	1,67	1,45
IT_TRT_T3	7,16	7,93	4,36	2,84	3,18	1,26	1,54	1,36	1,65	1,66

Figure 37: Occurrence of the ten most frequent genera in the soil samples from Bologna/Italy, i.e. Case Study site 9 (IT = Italy, UTC = untreated control, TRT = treated, T1, T2, T3: sampling times as described in Chapter 5.5.1; IT_T1: U1-T3 in Table 34; IT_UTC_T2: U1-U3 in Table 35; IT_TRT_T2: T1-T3 in Table 35; IT_UTC_T3: U1-U3 in Table 36; IT_TRT_T3: T1-T3 in Table 36).



	Acidobacteria_Gp6	Bacillus	Sphingomonas	Spartobacteria_genera_incertae_sedis	Arthrobacter	Streptomyces	Gaiella	Acidobacteria_Gp16	Solirubrobacter	Gemmatimonas
PL_T1	6,20	0,83	3,38	2,54	0,87	0,70	1,28	1,65	1,16	4,48
PL_UTC_T2	6,35	3,86	3,52	3,21	3,03	2,60	2,41	2,15	2,11	2,09
PL_TRT_T2	5,42	4,56	3,53	2,24	3,95	2,59	2,12	2,06	2,34	2,18
PL_UTC_T3	6,25	3,74	3,06	2,70	1,71	1,74	1,97	1,90	1,91	2,41
PL_TRT_T3	7,10	3,70	3,01	4,05	1,52	1,79	2,15	1,81	1,61	2,20

Figure 38: Occurrence of the ten most frequent genera in the soil samples from Lower Silesia/Poland, i.e. Case Study site 6 (PL = Poland, UTC = untreated control, TRT = treated, T1, T2, T3: sampling times as described in Chapter 5.5.1; PL_T1: RS1-RS16 in Table 34; PL_UTC_T2: RS1-RS8 in Table 35; PL_TRT_T2: RS9-RS16 in Table 35; PL_UTC_T3: RS1-RS8 in Table 36; PL_TRT_T3: RS9-RS16 in Table 36).



	Acidobacteria_Gp6	Bacillus	Gaiella	Spartobacteria_genera_incertae_sedis	Sphingomonas	Hyphomicrobium	Acidobacteria_Gp16	Solirubrobacter	Rhodoplanes	Arthrobacter
SWE_T1	9,24	1,38	2,11	3,82	6,09	0,76	1,59	0,88	0,79	0,75
SWE_UTC_T2	9,50	6,41	5,71	4,23	2,52	2,47	2,39	2,19	2,13	1,95
SWE_TRT_T2	9,67	6,89	4,65	4,11	2,30	2,26	1,97	1,67	1,79	2,24
SWE_UTC_T3	10,49	6,95	5,53	4,56	1,85	2,68	2,44	2,03	2,35	1,79
SWE_TRT_T3	10,01	6,79	6,34	4,49	2,02	3,00	2,76	2,55	2,37	1,54

Figure 39: Occurrence of the ten most frequent genera in the soil samples from Lower Gårdstånga Nygård/Sweden, i.e. Case Study site 3 (SWE = Sweden, UTC = untreated control, TRT = treated, T1, T2, T3: sampling times as described in Chapter 5.5.1; SWE_T1: T1-U2 in Table 34; SWE_UTC_T2: U1-U2 in Table 35; SWE_TRT_T2: T1-T2 in Table 35; SWE_UTC_T3: U1-U2 in Table 36; SWE_TRT_T3: T1-T2 in Table 36).

The genus *Gemmatimonas* is known for its ability to adapt to low soil moisture (Fawaz, 2013), so special attention was taken to the occurrence of this genus in the soil samples. However, there was no significant difference between treated and control soil samples in this regard.

Metagenomics can be a very useful method in the investigation of soil ecosystems. The continuous development of databases provides a more exact taxonomical classification. A precise, preferably GPSbased sampling is required for following the changes in the population, as well as the imminent processing of the samples following a short, proper storage period. It has to be noted that, for several reasons, these requirements could not be met in the tasks of WP5 where the application of the Water Retainer was included, thus the results of comparing the different samples from microbiological and molecular biological perspective cannot be considered as a clear conclusion.

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