

Deliverable 6.8 Second-Year Progress Report

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1. Context of the SOILMONITOR project

To feed the world's growing population, soil resources must be exploited with greater efficiency and contaminated soils must be remediated to secure crop yields and protect our environment. This requires increasing yields and assuring the quality of soils and drinking water. Precision agriculture, which involves the use of sensors, information databases, and high-tech machinery, can improve agricultural productivity. While irrigation management is already being used successfully, no satisfactory method for managing plant nutrition exists. Addressing this problem, we propose a highly miniaturized sensor system for continuous monitoring of soil nutrients. Through time series monitoring of nitrate, ammonium and phosphate, the SOILMONITOR is aimed at contributing to a balance in the supply of soil nutrients to preserve resources, improve soil quality and reduce adverse effects on the environment, and ensure high yields and healthy products at the same time.

1.1. Nitrate pollution/State in Europe

According to a report by the EU Commission, over 60 percent of agricultural soils in the European Union are over-fertilized. The consequences are soil acidification, polluted water, reduced biodiversity and declining yields. On the other hand, up to 40 percent of applied fertilizer is lost due to erosion and nutrient leaching.

Upcoming new legislation on soil health and associated fertilization requirements are intended to improve soil quality, but pose major challenges for farmers. Despite the need for farmers to regularly record the nutrient status of soils throughout the year, there is no satisfactory solution to date. Therefore, new technical approaches are needed to help farmers analyse nutrient levels so that they can use their fertilizers optimally and comply with the regulations.

1.2. Sensor concept

The sensor (see Figure 1) is intended to remain one year maintenance-free in the soil, where it takes nutrient concentration measurements in soil water at regular intervals when there is sufficient soil moisture.

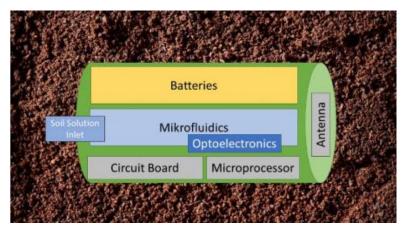


Figure 1: Schematic SOILMONITOR cartridge.

For this, soil water is drawn into a microfluidic through the ceramic of the soil-solution inlet. In the microfluidic system, the solution is stained with a nutrient-specific assay and its concentration is

determined by photometry using a light-emitting diode - photodetector pair. Once the concentrations have been determined, the mixture is pumped into a waste reservoir. The determined concentrations are to be sent to a station via a LoRaWAN network (see Figure 2). In the first development phase, nitrate is measured; the measurements are then extended to ammonium and phosphate.



Figure 2: The sensor data will be transferred via LoRaWAN directly or through a repeater into the hands of the farmer.

2. Project Scope

SOILMONITOR is a three-year EU-funded project that aims to develop and validate a prototype of this miniaturized sensor system for continuous monitoring of soil nutrients. SOILMONITOR promises seven complementary and synergistic benefits - quick and informed decisions on fertilization, higher fertilizer efficiency, soil-quality improvements, easy compliance with environmental standards, reduced management efforts, less nutrient leaching, and reduced N₂O-emissions.

The project consists of six work packages (WPs). The first two deal with technology development related to sensor system (WP1) and development of suitable chemical assays (WP2). WP3 deals with scientific validation of soil nutrient measurement by extraction of μ L soil solution volume. WP4 deals with prototype construction and validation in field tests. WP5 prepares the spin-off and post-project funding, while WP6 covers project management.

3. Areas of Impact

Within this project we develop and test a nutrient soil sensor. The soil sensor addresses three needs: First, the need for quantitative measurement of soil parameters relevant to agricultural yields; second, the direct measurement of these parameters in the soil; and third, the continuous monitoring of these soil parameters over an entire season (12 months). This includes keeping

track of periods absent of vegetation, during which nutrients are typically leached and informed decisions about the timing and amount of fertilizer application are difficult to make, yet critical.

As our solution aims to future-proof crop yields and reduce soil and water pollution, it touches on several SDGs:

- SDG 2 Zero Hunger: Ensuring crop quantity and quality increasingly depends on accurate and timely nutrient measurements due to increasing economic and regulatory pressures on farmers.
- SDG 3 Good Health and Well-Being: Nitrate-contaminated soils and waters pose a significant health risk.
- SDG 6 Clean Water and Sanitation: Nitrate leaching contributes significantly to water pollution. And finally,
- SDG 13 Climate Action: Our sensor helps reduce the impacts of climate change such as biodiversity loss, acidification and eutrophication. As acidification reduces the oceans' ability to absorb CO2, it exacerbates global warming.

Our long-term goal is to establish a company that produces the SOILMONITOR sensor on a large scale for agriculture and for soil-remediation applications.

4. Second year progress

During the second year of the SOILMONITOR project, work was carried out to integrate the sensor components into the sensor system. After completing five first-generation prototypes in July, we now finished 25 prototypes and started the first sensor tests.

4.1. Prototype development

As mentioned earlier, our sensor measures the nitrate concentrations using a microfluidic lab-ona-chip approach (see Figure 3). We were able to develop and improve on a three-layer design that is created in-house by photolithography and assembled with additional components. The current iteration uses three PDMS-layers bonded on a glass substrate, with valves fitted on top of the microfluidic chip.

Our microfluidics use an automated protocol for the measurement procedure. This allows for measurements without human intervention between measurement cycles provided that the assay reagents and cleaning solution are sufficiently stocked.

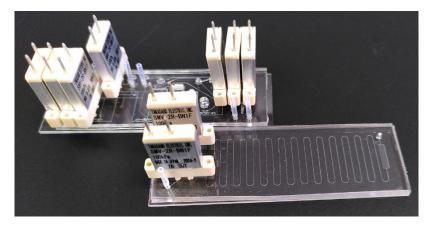


Figure 3: Microfluidic chip for our sensor system (back); microfluidic chip for mixer tests (front).

The microfluidic chip is inserted onto the circuit board, where the LED-PD pair takes the measurement (see Figure 4).

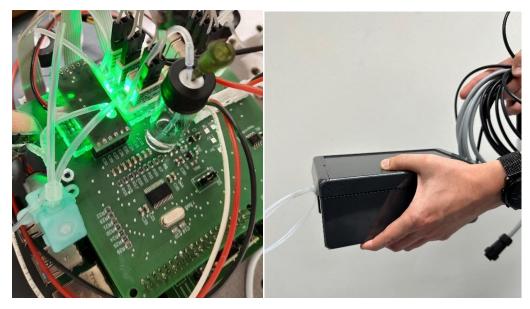


Figure 4: Microfluidic chip on circuit board during measurement (left), sensor demonstrator compared to hand (right).

We were able to successfully integrate the sensor components into a working prototype. As of now, 25 sensor prototypes were fabricated to enable pot- and field-experiments for function validation. While miniaturization efforts are still ongoing, our prototype is already able to be easily held in one hand (see Figure 4).

4.2. Work on our nutrient specific assay

The chemical assay work-package of SOILMONITOR aims to develop target-specific materials for highly sensitive, specific, and low-cost colorimetric detection of soil nutrients and to realize a reaction mechanism suitable for establishing the assay in microfluidics. For nitrate ion, an assay with floating core-shell particles with enzyme mimicking nanomaterials as core material and polymers as shell material was developed. The core material supports colorimetric detection, while the polymer material ensures specific detection of nitrate ions. Following a comparative evaluation of two different polymers, an organic ligand framework has been chosen as the optimal shell material for specific nitrate binding. Each material is synthesized in-house and evaluated individually before being assembled into the core-shell structure.

To adapt the assay to microfluidics, the reaction mechanism is developed using a pre-concentration mixture of core-shell particles along with the primary reagents, which can be stored as such within the reagent reservoir in the sensor unit. This strategy particularly reduces mixing and incubation time and speeds up the reaction. For ammonium ion detection, we decided to develop similarly fashioned core-shell material with modified nanozymes as core materials to enable simultaneous, interference-free colorimetric detection of both nitrate and ammonium. The shell framework for ammonium will be made from organic ligands that specifically targets ammonium.

In the second project year, the chemical assay for nitrate and the reaction mechanism for microfluidics integration were investigated and realized, and the design and development of materials for ammonium assay are ongoing.

4.3. Soil water extraction & pot experiments

During the joint experiments of our engineer and soil science teams, we were able to achieve soilsolution extraction for up to -60 hPa, with the exception of sand (see Figure 5).

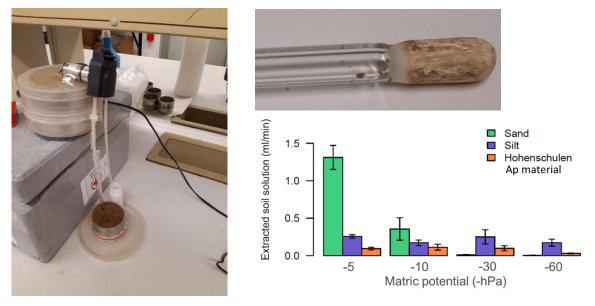


Figure 5: Soil-solution extraction setup (left); extraction unit close up (upper right); extracted soil solution in regard to matric potential (lower right).

After this break-through, we were able to start pot-experiments, extracting soil-solution from pots containing different soil compositions and fertilization levels. To create a more realistic environment, *Phacelia tanacetifolia* was grown in the pots as well. Nitrate levels in the extracted soil-solution were measured by standard laboratory methods, as well as with our microfluidic sensor system.



Figure 6: Soil-solution extraction setup; testing the impact of different soil types (left); testing the impact of different sensors (right). Pots contain different soil compositions, as well as fertilization levels, with Phacelia tanacetifolia growing in the pots.

While there is still much work to be done in the next year, our pot-experiments showed great promise for soil-solution extraction and consequently nutrient measurements. We were able to extract more than enough soil solution for on-chip measurements.

4.4. Economic progress

We were able to confirm Freedom-to-Operate (FTO) by April this year, as well as filing a patent application for our sensor system, that is still pending as of now. We furthered our customer clinic and gained new insights on our future markets. As the agricultural markets are quite diverse and can be complex to understand, we want to present two exemplary business cases for the SOILMONITOR:

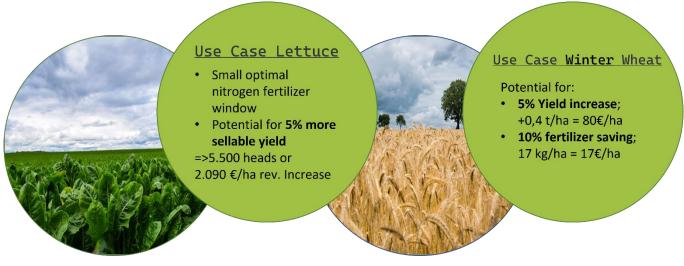


Figure 7: Use Cases Lettuce and Winter Wheat.

Lettuce is one of the prime examples for a SOILMONITOR use case. It has quick growing cycles, high yields (110.00 heads/ha), high revenues (33.440 €/ha or 0,38 €/head), but is highly dependent on nitrogen levels and has also room for improvement (only ~80% of heads are sellable). If lettuce gets too little nitrogen, it lowers yields, creates pale, yellow leaves and prolongs growth cycles and subsequently field use. If it gets too much nitrogen however, it is prone to necrosis of inner leaves, glassiness of leaves, and higher pest pressure. And if it exceeds the legal limit (3.000 mg/kg) it can no longer be sold. While potential cost savings by reduced nitrogen fertilizer use are economically irrelevant in this case, assuming 5% more heads sold (5.500) by improved nitrogen fertilization, a revenue increase of 2.090 €/ha can be achieved.

Winter Wheat is a commodity crop in terms of revenue, while the added value of daily soil nitrate data is high for high value crops as shown above, the potential benefit is much lower for commodity crops. Winter Wheat is grown between October and July, it yields around 8 t/ha and has revenues of ~200 \in /t, which results in a total revenue of 1.600 \in /ha. Nitrogen fertilization costs are ~170 \in /ha). Assuming an optimized nitrogen fertilization leads to a 5% yield increase or a 10% fertilizer saving the results on revenue are as follows:

٠	5% yield increase:	+0,4 t/ha	= 80 € /ha
•	10% fertilizer saving:	- 17 kg N/ha	a = 17 €/ha

Consequently, SOILMONITOR will target fresh produce markets at market entry, with the aim of reducing sensor costs, providing nutrient data from fresh produce to arable farming.

5. Communication Tools

In order to communicate with our stakeholders, we developed a communication strategy and a range of communication tools. These are shortly described here.

5.1.Website

The 1st version of the website was launched in December 2022. A new version was launched in April 2024 and can be reached at the following URL: <u>www.soilmonitor.org</u>. Due to its importance, the URL of the website is noted at every other dissemination tool. As we were in "stealth mode" until the first patent application for the overall system was filed, we are just now beginning to add technical content. Visit our website to experience our upcoming reveal story on our project work. From the beginning, we provided information which events we will visit. External stakeholders can contact the team directly if interested. The project website provides links to the project's social media channels to create a shared communication environment.

5.2. Social Media

By enabling communication, networking, and content sharing, social media is an important tool for disseminating information about SOILMONITOR and connecting with potential stakeholders. Due to its scientific and business relevance. We have set up a social media account for the project on LinkedIn, where we share project-related news and try to direct followers to the main project website. With the help of the platform, the project generates targeted interest from stakeholders within the project members' networks and enable direct contact with the project team.

5.3. Scientific publications

To reach related research groups and affiliates in particular, project members visit conferences and publish their research findings in professionally recognized journals. As this project targets a spin-off company, patenting is considered before publication and publications are delayed correspondingly.

5.4. Events

SOILMONITOR participates in various events in the form of conferences, webinars, technologytransfer events as well as public outreach events to reach diverse stakeholders. For example, SOILMONITOR was present with a booth at the Agritechnica 23, gave a talk at the 4th Stakeholder Forum 2024 organized by the EU Soil Observatory (EUSO), participated in three pitch sessions with investors and offered an open-lab public-outreach event at the Science Day 2024.

6. Outlook

In this exciting second project year, we completed 25 prototype systems for nitrate measurements – our SOILMONITOR-N. The third year aims at validating the SOILMONITOR-N performance in field tests. Additionally, we work on realizing a SOILMONITOR prototype that measures all three targeted nutrients: nitrate, ammonium, and phosphate. In parallel, we developed our business plan and started pitching it to potential investors. In the third year, we will continue pitching the business proposal to secure funds for the successful transition from prototype to product.