Enabling next level research on roots: Automatizing Minirhizotron Image Acquisition and Analysis (NextMR-IAA) for Research and Agricultural Management

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ABSTRACT

Non-invasive imaging technologies continue to rise in use; innovation of root and rhizosphere imaging devices has however not kept pace. The lack of automated, high-resolution root imaging and analysis hampers our scientific understanding and prevents application of minirhizotrons in agricultural and environmental settings. Two complementary automatic minirhizotron systems for applied and research purposes were developed, the latter featuring an unprecedented position-accuracy and image-quality. Two pipelines based on CNN models allow for feature extraction for research and practical applications (e.g. fertigation scheduling), respectively. NIR-wavebands for soil water content estimation were tested. Our technological innovations will make rooting information widely accessible.

Keywords: Automation; Sustainable Agriculture; Breeding; C sequestration; CNN; Minirhizotron Imaging; Root Ecology; Root Traits.

1. INTRODUCTION

Non-invasive root imaging technologies are key to expand our understanding on the ‘hidden half’ of plants and ecosystems. Minirhizotron (MR) imaging is widely used in situ. In particular, MRs are used to continuously measure root system development and turnover in time and space, and root interactions with pests and mycorrhizal fungi. While taking few images is relatively easy, achieving high temporal resolutions, and extracting quantitative data related to environmental parameters requires substantial efforts. Despite increasing interest in the fields of agronomy, breeding, forestry, and ecology, the technological advances in MR systems and integrated image analyses solutions remain limited yet—preventing automation and thus wide use. Therefore, rapid and robust image capturing and analysis solutions are highly required. Additionally, sensing the soil environment based on spectral features provides an add-on to understand root response and functionality under variable soil conditions. While plant phenotyping *in situ* has been mainly focused on shoot traits such as yield, shoot vigour and canopy temperature, the root compartment has received far less attention despite its importance. The main reason for this imbalance is the inherent difficulty to access root traits non-destructively—as they are hidden in the soil.

The proposed technology has a great potential to benefit society both directly via farmers, and indirectly via enabling root researchers & crop breeders.

- The agronomy sector can implement precision farming (‘Agriculture 4.0’) based on real-time root system development,
- Researchers will use the technology to develop better model of soil carbon (C) sequestration—for climate change predictions and mitigation,
- Breeders can select more resilient crop genotypes—taking suitable root system traits for target environments into account.

The main innovation of the project: Setting a cornerstone for automatically retrieve non-invasive, reliable estimations of root growth patterns in real-time and at high spatial and temporal resolutions.

We were able to build two prototypes of MR devices. One device is at the research level and a second device is a cost-efficient model allowing for a wider deployment in agricultural settings. Focusing on the ability to measure root length under field conditions, we developed two complementary imaging pipelines—automatically identifying root attributes using Convolutional Neural Networks (CNN). In addition, we were able to quantify the water content of the subsurface with the help of hyper-spectral imaging.

2. STATE OF THE ART

Commercial minirhizotron devices are either camera or scanner-based RGB systems of limited resolution, positioned and operated manually, and are available at high costs [1]. The realized progress is limiting the wide use of MR. Several MR-prototypes showcased advanced features including automation [2,3]. However, none of the designs were implemented by the market, potentially due to limited usability including image blur, distortion and coloration (using mirrors due to narrow MR tubes), and low positioning accuracies. There are prototype MR systems that are using wavelengths beyond the visible spectrum (UV to 950 nm [2,3]) to detect differential reflectance of roots but they are not foreseen yet to monitor environmental parameters.

Despite various efforts in the last decade, root image analysis is still based on manual overlays drawn by humans. Although automatic root segmentation and classification is the goal, working solutions have been realized only for very specific situations [3]—potentially due to the heterogeneous soil ‘background’ and root ‘aging’. In addition, inconsistent illumination caused by light source, soil property changes, and artefacts (scratches, water condensation) make automated MR image analysis challenging. CNN are deep learning models achieving excellent performance for complex computer vision tasks in image-based plant phenotyping [4,5]. However, CNN models were, to the best of our knowledge, not yet used on image analysis of root grown under field conditions.

Thus, MR imaging for applied purposes needs to be low cost, robust, and both image capturing as well as basic feature extraction (i.e. root length density) needs to work automatically for a majority of crops × soils. In addition, MR systems for research purposes need to feature most accurate positioning systems and high image resolutions in VIS and NIR spectral ranges—allowing for automatic root classification, creation of super-resolution images, and environmental sensing.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

The project achieved technological breakthroughs at the level of MR hardware and automatic root image analysis—implying a significant increase of usability of future MR systems in research, and laying the cornerstone for market entry of automated MRs (AMRs) as versatile agricultural management and phenotyping tools.

Minirhizotron camera hardware

Compared to various low-cost prototypes proposed by the scientific community, the developed ‘applied’ AMR camera sets new standards in robustness at hardware costs as low as 300$—putting make-to-stock production into reach. This was achieved by combining off-the-shelf electronics with plastic housing (3D-printed) riding on a non-turntable rail guide. In contrast, innovations of the ‘research-grade’ AMR lay in precision engineering. In particular, superior image quality was achieved by using a fixed-focus 8MP-CMOS sensor for direct imaging, i.e. avoiding the standard, image-quality-reducing flat metal mirror. A superior position accuracy in the subpixel range was achieved by combining a spindle with capacitive sensors for auto calibration—proving a strong basis for tracing of individual roots and super-resolution reconstruction.

Image analysis

Though CNN for root phenotyping is evolving rapidly, existing CNN tools are applied either to root images acquired from artificial indoor facilities or rely on segmentation-based CNNs. The uniqueness of our solution is a combination of two methods both based on CNNs. U-Net is a convolutional neural network that was originally developed for biomedical image segmentation and removal of the mask; we adapted it for precise extraction of root length. The other method uses a neural network where the information of the root length constitutes the output layer directly. The accuracy of the second method is lower (5.5 mm error on average per image), but requires much less data for training compared to the first method. Improving the resolution is accomplished by applying super-resolution algorithms on sub-pixel-shifted images and merging them to create a mosaic image. Using this technique, we expect to increase MR imaging capabilities to reach stereo-microscopic resolutions.

Implications for the usability of the MR technology

The most significant breakthrough lays in coming one-step closer to develop integrated MR imaging pipelines suitable for a wide range of plants × soil conditions, one for researchers and one for agricultural applications—automatizing MR image capturing, data transfer, image pre-processing and analysis for root traits with a synchronous estimation of environmental parameters.

4. PROJECT RESULTS

‘Applied’ AMR camera system

We have developed a cost-effective automated MR system for root monitoring in applied settings—enabling continuous monitoring of root systems. The imaging components comprise of a fixed camera [5 MP, 2592×1944 px, RGB] and a LED light source. The robust mechanical components allow for linear movement only (to 1 m). All components are operated by a single board computer (Raspberry Pi). This system was installed in a MR tube, automatically capturing root images per six-hour cycle in an agricultural setting (Example: time laps video of corn roots growing under water stress).

'Research-grade' AMR camera system

A research-grade AMR camera (Fig. 1) was developed according to industrial automation standards. Combining a highly precise spindle and an anti-backlash nut with micro stepper motors allowed for subpixel movements in both linear (≤2 m) and rotational directions (360°). High accuracy is key for exact root tracing and allows implementing super resolution features beyond the native UHD resolution (8 MP, 3280×2464 px; RGB; Fig. 1A) at a later stage. A custom lighting PCB and direct imaging (no mirror) prevent reflection. The modular platform allows swapping imaging modules (multispectral module at TRL3). The system runs on low DC voltage for field use and has been tested for extended unsupervised operation. Perturbations by 'rain shadow' effects of the protruding AMR housing are minimized by guiding impinging water along the outer shell of the control unit (Fig. 1B).

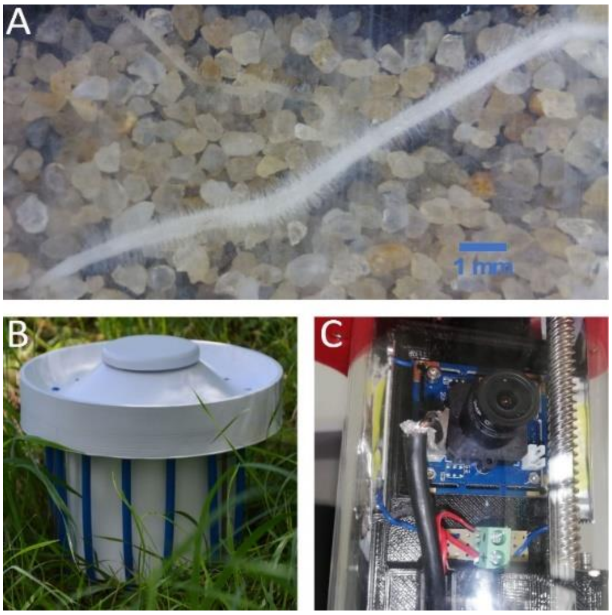

Fig. 1. Pea root with root hairs visible on UHD image of the 'Research grade' AMR (A), 'rain-shadow effect' reducing housing (B), and detail of the RGB camera module and spindle within a narrow MR tube (C).

Automatic root detection using RGB images

Using MR acquired images and Deep Learning (DL) for extracting root system architecture (RSA) information offers a compromise between costs and efficiency. Two approaches for RSA extraction on MR images were implemented. 1) Image segmentation techniques, which generate high performance results at the price of expensive and time-consuming data acquisition, making it unpractical as the process must be repeated for new plant × soil combinations. 2) A regression model, which offers simple data acquisition and scalability, at the price of less stable results. As applications such as 'growth rate measurement' require high precision, while applications such as root detection ('yes/no') do not, our proposed model enables the user to optimize cost vs. performance according to application-driven considerations. Our method combines both approaches to achieve performance stability and practicality according to the user's needs. To understand the model's limitations, we examined both edge cases: pure segmentation (Fig. 2B) or pure regression (Fig. 2C), which we later used for the proposed combined technique.

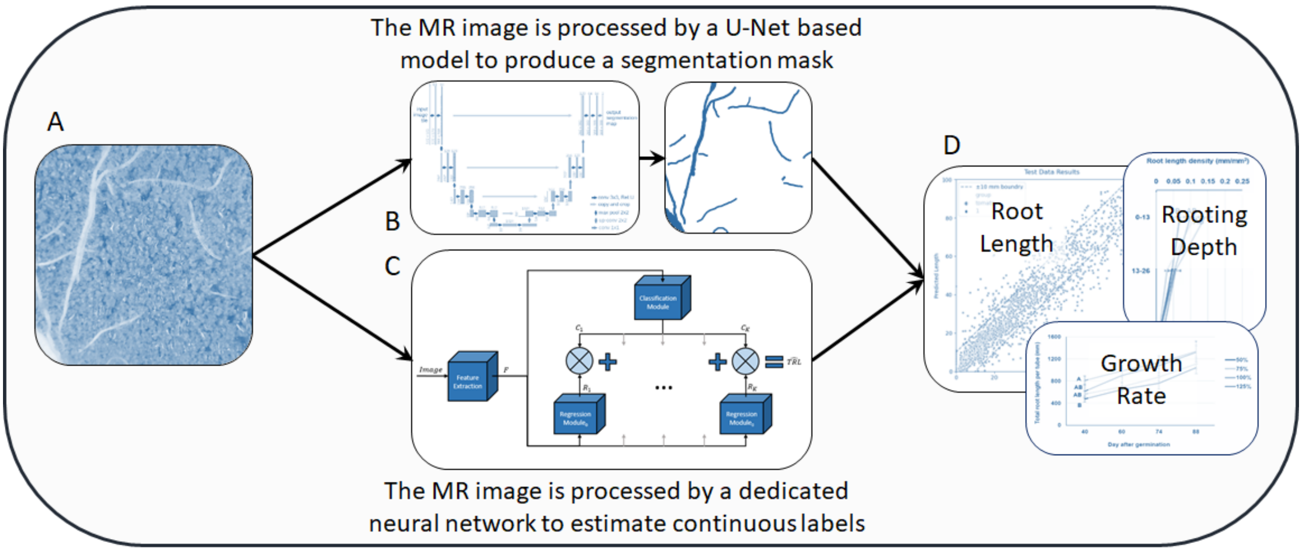

Fig. 2. Minirhizotron (MR) acquired image is used as input for two models (A), either using a segmentation driven approach generating a binary mask (B) or a regression driven approach generating a continuous label (C). Examples of root traits derived by extracting the RL_{Total} from MR images (D).

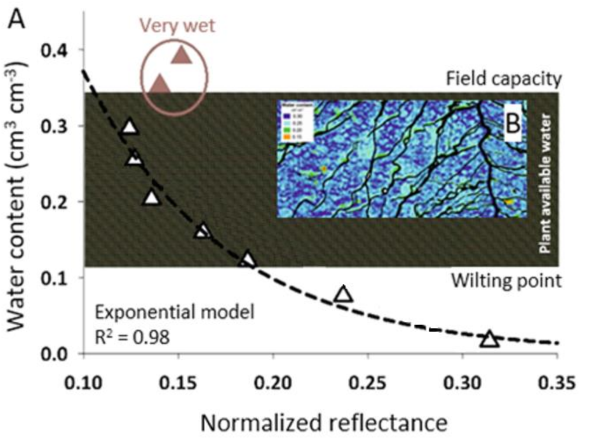

Fig. 3. Relation between spectral reflectance at 1460 nm and volumetric water content (A), and small-scale mapping of water content predicted from spectral reflectance around plant roots (black lines; inset B).

We evaluated both approaches by measuring the total root length (RL_{Total}) in each image though different models (Fig. 2D) as compared to manually accessed 'ground truth' values. Regression results currently give a mean error of 5.5 mm (test set: 5K images), with r^2 = 0.91 between the predicted and the actual RL_{Total}. Segmentation results show a mean absolute error of 2.9 mm (test set: 158 images; r^2 = 0.95).

Environmental sensing

Our results demonstrate a high correlation between water content and spectral features in the short-wave infrared (Fig. 3). The relation is particularly clear in a range between wilting point and field capacity, while the very wet end (saturation) cannot be reliably predicted. Fig. 3A shows a calibration curve of the directly measured water content and spectral reflectance at 1460 nm, which is mapped on a spectral image to infer on the water content distribution around a root system (Fig. 3B, inset). The identified wavebands allow for implementing the image-based soil moisture measurements into the advanced imaging module of the 'Research-grade' AMR system; however, the multispectral camera module is currently at early TRL3.

5. FUTURE PROJECT VISION

5.1. Technology Scaling

VSI will develop the *'Research-grade' AMR camera* to TRL8 using NextMR-IAA knowledge; VSI R&D expenditures will be used to bring the multispectral imaging module and advanced features (e.g. super-resolution) to TRL6-7 (late 2021). Additional source funding and collaborations are key to scale-up *Environmental sensing* capabilities (integrating spectroscopy), and the *'Applied' AMR camera* and related image analysis pipeline to TRL7+. Pilots must clearly demonstrate advantages for plant & ecosystem management; CNN models have to be tested using major crop×soil combinations (i.e. establishing global-databases); user interfaces (UI) must be developed to TRL8 in a participatory approach with breeders/farmers.

5.2. Project Synergies and Outreach

In Phase 2, we plan to add two core partners and 3 partners for demonstration. S4 Mobile Laboratories (USA) is a future key partner, providing unique real-time spectroscopic analysis of soil chemical signatures. A partner specializing on communication networks and distributed intelligence is decisive implementing an 'Ecosystem-of-Things' strategy (IMEC; BE). Potential partners for technology demonstration and UI development: agricultural station operators (AGES, AT; ARO, ISR), crop breeding companies (KWS, AT, Equinom, ISR), and agriculture automation companies (NETAFIM, ISR).

A multi-channel dissemination strategy will include a dedicated presence in social media and a multi-language webpage. Students will contribute a blog and tweeds. Field days and exhibitions (e.g. European Researchers' Night), webinars, and documentation packages will engage the public and stakeholders; the scientific community will be involved via Gold OA-publications and conferences.

5.3. Technology application and demonstration cases

In Phase 2, we will demonstrate benefits of our technology to academia and industry using three pilot studies—tackling global societal challenges in the domains of 'Food security, sustainable agriculture and forestry' and 'Climate action, environment, resource efficiency and raw materials'.

Fertigation scheduling in agriculture – increasing yield and resource use, decreasing pollution

Plants' ability to utilize available water and nutrients is largely determined by the root density in particular soil layers. Globally, crop plants absorb on average only 50% of the water applied by conventional irrigation and about 30% of applied fertilizers; the latter frequently percolate below the rooting zone, contaminating water bodies. Using field experiments and modelling, we will demonstrate: Fertigation scheduling based on AMR data will dramatically increase the resource use efficiency of intensively managed croplands, increase yield and yield stability, and decrease (nitrate, phosphate) pollution of water bodies.

Breeding of crops for resource-efficient farming

Selection for root system architectures in crop cultivars is important for the development of resource-efficient, extensive farming systems. Root distribution largely determines plants' access to resources, e.g. phosphate in the top soil or greater water resources in deep soil layers. Using established breeding trails, we will demonstrate: AMR and automated analysing pipelines enable breeders to screen cultivars cost-effectively for root traits—allowing integrating this tool into their phenotyping toolbox. This technology application will benefit the Plant Phenotyping Research Infrastructure in Europe (EMPHASIS). The European Strategy Forum for Research Infrastructure (ESFRI) has identified 'Plant Phenotyping' as a priority.

Improving soil carbon sequestration models in the context of climate change prediction

Globally, soil organic matter contains more than three times as much C as the atmosphere. Even small changes to the soil C pool will have strong effects on the atmospheric CO_2 concentration. Using field experiments and modelling, we will demonstrate: Multispectral- and super-resolution capacities, increased monitoring frequencies and access to remote areas via automation will significantly improve the accuracy of determined root turnover rates—allowing to improve current estimates of C sequestration rates (via root litter) and to identify abiotic drivers.

5.4. Technology commercialization

The 'Research-grade' AMR is commercialized by VSI via internal R&D expenditures and will be available in early 2021. Commercialisation of the 'root and environmental sensing services' will require complementary funding sources, i.e. seed funding by international and national public R&D funds (e.g. ATTRACT Phase 2, EU; FFG, Austria), philanthropic donations, and 'impact' investment funding (e.g. Bridges, ISR), and regionalized and domain-wise market entry strategies. Spin-offs related to BGU will benefit from established business relations ('Cyber'). An integrated business model, based on AMR hardware sales/leasing combined with cloud-based analysing services will be developed to found a joint business entity.

5.5. Envisioned risks

Core technological risk in Phase 2 is the life period of cost-effective AMR systems operating constantly under field conditions; mitigation measures comprise the parallel long-term testing of different mechatronic solutions. The technology demonstrations depend largely on 1) the availability of a sufficient number of AMR devices, and 2) the weather conditions during the validation studies. Potential mitigation of the first is the temporary utilization of manual MR camera systems. As the suggested duration of Phase 2 is 2-3 years, the diversification of experimental sites across several climatic zones is key for minimizing risks.

5.6. Liaison with Student Teams and Socio-Economic Study

NextMR-IAA actively involved students at PhD, MSc and BSc levels. Pavel Baykalov (BOKU-PhD), Adam Soffer and Mor Elmakies (BGU-MSc), and Ron Gershburg and Thamara Bivchov (BGU-BSc) worked on deep learning for image analysis, Kaining Zhou (BGU-PhD) and Oliver Hoi (BOKU-BSc) on image annotation and labelling, Mads Sørenssen (BOKU-MSc) tested AMR prototypes. In Phase 2, the project will be related to the doctoral-school 'Digitalization and Innovation in Agriculture (BOKU)' and we will nominate personnel that will coordinate all student-related activities (incl. exchange); all R&D activities and case studies will comprise student teams of all levels. Our participatory approach, refining the UI with stakeholders, and experiments (using 'BACI-designs') will allow for efficient coordination with the expert-driven socio-economic study of the ATTRACT initiative.

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