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EVALUATION OF THE EFFECTS OF SOIL BACTERIA TREATMENTS ON FIELD CROPS BY REMOTE SENSING TECHNIQUES

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ABSTRACT

Various remote sensing (ground-based, aerial) techniques have long been used to assess the condition of vegetation. A number of vegetation indices have been generated using these methods to characterise plant physiological processes such as photosynthetic pigment levels, N content and water content. Many of the vegetation indices have been developed for natural vegetation species, but much less information is available on their applicability to agricultural crops, to assess the effects of specific treatments. Therefore the main research question of our research is whether ground and airborne remote sensing can be used to assess the effects of nutrient replenishment experiments in agricultural fields.

We applied *in vivo* field measurements (field spectroradiometer, drone mounted multispectral camera) to determine vegetation indices related to photochemical pigment composition, photochemical activity, stress resistance, nitrogen and water content of leaves. The effects of different soil bacteria treatments on barley and wheat were studied in 2019 at the Agricultural Research Institute in Kompolt, moreover, we compared the results with our previously published research on several other crops (maize, sunflower, rape, barley) carried out in 2017 and 2018.

Our results show that in spite of significant standard deviation of data in field conditions, significantly higher chlorophyll and water content, higher photochemical efficiency, and lower carotenoid content were found in wheat leaves treated mainly with stubble decomposers + soil inoculators could be characterized by, but in the case of barley, no beneficial effects of such treatments were found, which probably was due to the unusually rainy spring which brought about the rise of inland water on the experimental plot, which negatively influenced the living conditions of soil bacteria. In the case of wheat, spectral vegetation indices showed a positive effect of soil bacterial treatments already at the beginning of flowering, which is consistent with the yield, and this is similar to the results obtained for maize, sunflower, rapeseed and barley in 2017 and 2018.

Keywords: spectral vegetation indices, soil bacteria treatments, photosynthetic pigments, barley, wheat

1. INTRODUCTION

Since 1918, the Fleischmann Rudolf Research Institute in Kompolt has been involved in plant breeding and the research together with testing and optimisation of various nutrient supply systems for field crops. In addition to plant breeding activities, the research institute plays an important role in the region as an expert advisor, since its main objectives are not only basic research but applied research as well. For example, it translates the results of variety comparison trials into practical applications for farmers in the region. They help with the selection of varieties and with the development of optimal and environmentally friendly nutrient management, which can be adapted to the North-Hungarian region. The nutrient management systems aim at the adaptation of combined technologies, in which in addition to complex fertilisers, different soil microbial preparations play an important role. In 2016, our research team became involved in the study of the effects of environmentally friendly nutrient supplementation systems, focusing on the study of yield-enhancing effects of soil bacterial preparations, mainly using in vivo field measurements. The advantages of *in vivo* methods are that they do not require the destruction of plants, do not require laboratory background, allow a large number of measurements and provide rapid and realistic information on photosynthetic processes in plants, one of the most critical processes for yield, and they indicate soil and thus plant population heterogeneity at the field level.

With agricultural production becoming more and more intensive, there is a growing focus on environmentally friendly products to replace some of the artificial nutrients and chemicals. These environmentally friendly products include soil bacteria products. Bacterial fertilisers contain living micro-organisms which play an important role in soil life and in the supply of nutrients to plants. These micro-organisms are already part of soil life, but their numbers have been drastically reduced due to intensive agricultural production. Bacterial inoculation of soils is thought to improve their productivity by improving their biological condition. Soil microbes are known to play an important role in soil organic matter decomposition, humus formation and biogeochemical cycling (Kátai 2011), furthermore they facilitate the formation of nutrients that can be taken up by plants, thereby improving productivity and stress tolerance, as well as having a positive effect on soil structure. Soil bacteria preparations have been shown to be effective in a wide range of soil types, field and horticultural crops, and have been shown to increase yields and stress tolerance (Website 1).

Our work was supported by the project "Complex Development of Research Capacities and Services at Eszterházy Károly University" (EFOP 3-6-1-16-2016-00001), the main objective of which was to investigate the practical applicability of some domestic soil bacteria preparations on different crops. In Hungary, such preparations have been used since the 1960s (Manninger and Szegi 1963). Today's formulations contain several components, mainly nitrifying, cellulose-degrading and solubilising microorganisms, and biologically available mineral macro- and microelements (Biró 2003). The formulation stimulates soil life, increases the amount of plant nutrients available for uptake, which has a positive effect on the green mass yield of the plant (Makádi 2007), which is also a decisive factor for yield formation.

In this paper, we present the effects of three of the best known soil bacteria products in Hungary on some physiological parameters of the winter barley *KH Korsó* and winter wheat *Babona* (Agromag) varieties, based on the experimental results of 2019.

Phylazonit Soil Inoculator contains the following bacterial strains: *Azotobacter chroococcum, Bacillus circulans, Bacillus megaterium, Pseudomonas putida*. Their proportions have been optimised for soil inoculation, so that the use of the preparation has a beneficial effect on nutrient supply (by fixing nitrogen in soil air and mobilising bound phosphorus not available to plants) and their multiplication on the root system of the plant stimulates further root formation. The resultant denser and deeper root system can then absorb nutrients and water over a larger surface area. In addition, a thicker and stronger stem is formed, with optimum nutrient and water storage, which increases the stress tolerance of the plant (Website 9).

The bacterial strains in Phylazonite Stubble Decomposer are *Azotobacter chroococcum, Bacillus circulans, Bacillus megaterium*, which release nutrients in the tares residues, increase soil organic matter content by promoting the humus formation process, stimulate soil life and improve soil structure. The latter can save significant fuel costs due to reduced traction requirements. In addition, the water, heat and air management of the soil is improved, while its chemistry is shifted towards neutral. An important aspect of crop protection is that the amount of pathogens and pests overwintering on stubble residues is significantly reduced by the removal of stubble residues. (Website 10).

Phylazonite Soil Regeneration helps in the initial development of plants and in the protection of seedling plants against diseases. The bacterial strains contained in the formulation are *Bacillus megaterium, Bacillus subtilis, Pseudomonas fluorescens, Pseudomonas putida*, which produce hormones (auxin, cytokinin) to promote rooting and rapid initial development of the seedlings. Their metabolic processes produce antibiotic-like substances that inhibit soil-dwelling phytopathogenic fungi. Bacillus and Pseudomonas strains crowd out harmful microorganisms by invading their habitat. This can be particularly important if we cannot meet crop rotation requirements or if successive crops are damaged by the same pathogens. The product can also be used in organic farming (Website 11).

Since the positive effects of the combined nutrient supplementation (basal fertilizer, top dressing, soil microbial preparations) are not only reflected in yield but also in stress tolerance and photosynthetic processes at , the most critical process for yield and quality, photosynthesis, was investigated during the vegetation period, at the beginning of flowering. To this end, leaf reflectance was measured under natural light conditions using a portable field spectroradiometer. From the spectra obtained, vegetation indices were calculated that have been shown to be suitable for estimating leaf photosynthetic pigment composition (chlorophylls, carotenoids), anthocyanin and water content, carotenoid/chlorophyll ratio and indirectly nitrogen content, photochemical efficiency and stress sensitivity of leaves in several crops (Merzlyak and Giton 1995; Filella et al. 1995; Moran et al. 2000). Our results were compared with those obtained in other field crops (maize, sunflower, rape, barley) in 2017 and 2018 (Láposi et al. 2018a; 2018b; 2019).

2. MATERIALS AND METHODS

2.1. The experimental area

The research was carried out at Fleischmann Rudolf Research Institute in Kompolt. The experimental area is located on the southern side of the Mátra, between Eger and Gyöngyös, at an altitude of 125 metres above sea level. The weather is moderately warm and drought-prone, with unpredictable rainfall distribution (Holló et al. 2009). Annual precipitation is around 400 mm or less, only part of which falls during the growing season. Winters are harsh and generally snow-free, providing excellent conditions for the breeding of autumn-sown crops. The proximity of the Mátra, its rainfall and harsh winter weather make it an excellent breeding site for cultivars with high plasticity, adaptability, water conservation and resistance to abiotic stresses (Tóth 2011).

In the year of sowing there were temperatures above the average and less rainfall. The average annual temperature was 12.24 oC in 2018 and 11.64 oC in 2019, which is higher than the average of the last 70 years (9.98 oC). 408.65 mm of precipitation fell in 2018 and 381.64 mm in 2019, which is well below the average of the last 100 years (520.6 mm) (based on Tóth 2011 and our measurements). The annual rainfall distribution was not favourable for the two crops studied, as the 2-3 month period of low rainfall during flowering and bud break resulted in the plants not even reaching the average yields that are typical of Kompolt.

The soil type is predominantly non-carbonate chernozem brown forest soils, mostly formed on sedimentary andesitic detrital loess loam. The soil pH is acidic (pH 4.7-5.1), with a low calcium carbonate content ($CaCO_3$ 0%) and humus content (2.8-2.9%). The subsoil is harder and less acidic. The pH of the lower soil layers is neutral and the soil is alkaline at a depth of 130-150 cm. The humus layer is 50-80 cm thick and the humus content of the ploughed layer is 2.5-3.0%. This layer is characterised by moderate N supply, poor P supply and satisfactory K supply. The soil has unfavourable physical properties. The water table varies between about 11 m and 12 m and the amount and distribution of precipitation mainly determines the efficiency of fertilisation and yield. The soil has moderate water absorption, poor water conductivity and is difficult to cultivate. The dryness of the soil is exacerbated by high dead water content and cracking, which results in even higher water evaporation (Holló and Kádár 2003; Holló al. 2009; Tóth 2011).

2.2. Soil bacteria treatments

The study presented in this paper was carried out in 2019 on two experimental plots (wheat: plot K3 - 3.969 ha; barley: plot K9 - 9.43 ha), on two field crops (*KH Korsó* winter barley, Agromag *Babona* winter wheat). Both plots were divided into three parts. Three weeks before sowing, each plot received a basal fertilizer (250 kg/ha NPK-10:20:10). Two weeks before sowing, 15 l/ha of stubble decomposer (SD) were applied to plot 1 and plot 2. The degrading bacteria were placed directly on the chopped stalk residue and evenly mixed into the soil with it, as it is the nutrient for the bacteria. Soil inoculant (SI, 15 l/ha) was applied to plot 1 and soil regenerator (SR) 15 l/ha) was applied to plot 2 at the same time as sowing (October 2018). These preparations were also immediately rotated into the soil. The soil inoculant promotes rooting of the germinating plant, produces hormones and vitamins, and enhances nutrient availability and nitrogen fixation (website 3). The soil regenerator protects the germinating plant from fungal infections (Alternaria, Fusarium spp.) as microbes produce antibiotics, some of which are very strong competitors to pathogenic microbes, and it also promotes plant stress tolerance (Website4). In February 2019, all three treatments received 27% 150 kg/ha of calcium ammonium nitrate. The control plot (C) therefore also received base and top dressing. The preceding crop was winter rape for winter wheat and sunflower for winter barley.

2.3. Physiological measurements

The effect of soil bacterial treatments was evaluated not only on the basis of yield average and grain quality, but also on the basis of the photosynthetic pigment composition of the leaves, as this is one of the determining parameters of biomass production and provides information on plant stress sensitivity. The analysis of the pigment composition traditionally requires the preparation of leaf extracts with organic solvents and spectrophotometric determination under laboratory conditions. Recently, however, alternatives to non-destructive field pigment analysis such as near-ground and aerial remote sensing with multi- and hyperspectral cameras have been developed. They can be used to generate spectral vegetation indices that are related to a number of physiological parameters (Gitelson és Merzlyak 2004).

Field spectroscopic reflectance measurements were conducted (from 400 to 2500nm) on 80-100 leaves per treatment for calculating these spectral vegetation indices using an ASD FieldSpec3 portable spectroradiometer in the middle of May in 2019. Using the values at given wavelengths, we calculated the vegetation indices according to the formulas presented in Table

1., from which the leaves' chlorophyll, nitrogen, anthocyanin, carotenoids, water content, stress sensitivity, and photochemical activity can be estimated (Zarco-Tejada et al. 2005). A Spectralon reflectance standard was scanned before every plot measurement and scans were corrected for the instrument's dark current.

Table 1. Summary of applied vegetation indices and related physiological parameters determined with the ASD FieldSpecPro 3 instrument and the UAV-based multispectral sensor. (References in Zarco-Tejada et al. 2005).

Structural indices	**Formulae**	**References**
Normalized Difference Vegetation Index (NDVI)	$(R_{800}-R_{670})/(R_{800}+R_{670})$	Rouse et al. (1974)
Renormalized Difference Vegetation Index (RDVI)	$(R_{800}-R_{670})/((R_{800}+R_{670})^{0,5})$	Roujean and Breon (1995)
Enhanced Vegetation Index (EVI)	$2.5\times(R_{840-670}) / (R_{840}+(6\times R_{670}) - (7.5\times R_{450}) +1)$	Huete et al. (2002)
Soil Adjusted Vegetation Index (SAVI)	$[(R_{800}-R_{670}) / (R_{800}+R_{670}+L)] \times (1+L)$; [L=0,5]	Huete (1988)
Optimized Soil-Adjusted Vegetation Index (OSAVI)	$(1+0.16)\times(R_{800}–R_{670}) / (R_{800}+R_{670}+0.61)$	Rondeaux et al. (1996)
Leaf pigments		
Modified Chlorophyll Absorption in Reflectance Index (MCARI)	$1.2\times2.5(R_{800}-R_{670})-1.3\times(R_{800}-R_{550})$	Haboudane et al. (2004)
Carotenoid Reflectance Index (CRI)	$1/R_{510}-1/R_{550}$	Gitelson et al. (2002)
Anthocyanin Reflectance Index (ARI)	$1/R_{550}-1/R_{700}$	Gitelson et al. (2001)
Stress sensitivity – carotenoid/chlorophyll ratio		
Structure Insensitive Pigment Index (SIPI)	$(R_{800}-R_{445})/(R_{800}-R_{680})$	Peñuelas et al. (1995)
Ligh use efficiency – xanthophyll index		
Photochemical Reflectance Index (PRI)	$(R_{531}-R_{570})/(R_{531}+R_{570})$	Gamon et al. (1992)
Water content of leaves		
Plant Water Index (PWI)	R_{970}/R_{900}	Peñuelas et al. (1997)
Simple Ratio Water Index (SRWI)	R_{860}/R_{1240}	Zarco-Tejada et al. (2003)

To assess the impact of multi-hectare treatments, large amounts of data are needed and it is worth mapping the heterogeneity of the plot, for which multispectral cameras mounted on drones (UAVs) are excellent tools. Some spectral vegetation indices (NDVI, RDVI, GNDVI, SAVI) can be calculated from the spectra measured by these cameras. These spectral vegetation indices have been successfully tested in several agricultural crops (wheat - Filella et al. 1995;

Haboudane et al. 2002; maize - Gabriel et al. 2017). UAV measurements were performed with a Parrot Sequoia multispectral camera for wheat (plot K3) on 16^{th} of April in 2019 and for barley (plot K9) on 8^{th} of May in 2019. Detected spectral ranges were: Green 530 – 570 nm; Red 640 – 680 nm; Red EDGE 730 – 740 nm; Near Infrared 770 –810 nm.

The relative chlorophyll content of leaves was measured with a SPAD 502 instrument (KONICA, MINOLTA, JAPAN) at the same time and on the same leaves as for the spectroradiometer. The SPAD has a measuring surface of 0.06 cm^{-2} and calculates an index in SPAD units based on the absorbance measured at 650 nm and 940 nm, which is directly proportional to the chlorophyll content of the leaves and from which the nitrogen content can be inferred (Gitelson and Merzlyak 2004).

During the harvest (17.07.2020), in addition to the yield, the quality of the grains was also tested with FOSS Infratec 1241 grain analyser, which can determine the water and protein content (dry weight %) of both crops, as well as the gluten content (dry weight %), W-value and Zeleny index (ml) of the wheat.

In 2018, the Laboratory of Agricultural and Environmental Sciences of Eszterházy Károly University investigated the soil quality parameters of these experimental plots. Available P-forms, K and Ca content were determined by ammonium lactate extraction, while N-forms and sulphate were determined by KCl extraction.

Significant differences between treatments based on relative chlorophyll content (SPAD value), vegetation indices and grain quality were tested by one-way analysis of variance (ANOVA) and Tukey's b test (SPSS 20.0).

3. RESULTS AND DISCUSSION

3.1. Chlorophyll content of leaves

In addition to being the most important pigments in the conversion of light energy into chemical energy, chlorophylls directly determine the biomass production potential of a plant (Curran et al. 1990), indirectly provide an estimate of plant nutrient availability (nitrogen content) (Filella et al. 1995, Moran et al. 2000), and are closely related to plant stress and senescence (Peñuelas and Filella 1998, Merzyak et al. 1999). In addition to SPAD-value, leaf chlorophyll content can be characterized by several vegetation indices (Zarco and Tejada 2005).

SPAD values for both plants showed high variance and no significant difference between treatments. In the case of barley, no significant difference was detected in the vegetation indices (RDVI, EVI, TCARI, MCARI, TCARI/OSAVI, MCARI/OSAVI), although a trend was observed that chlorophyll content was highest in treatment 1 (SD+SI). (Table 2.).

In 2019, the measurements could not be carried out in an optimal period, as the spring was very wet, so the barley was already in a very advanced flowering stage at the time of the measurement. Accordingly, we measured a very large variation in pigment content between the individuals. In 2017, we measured a significantly higher SPAD value in barley at treatment 1 (SD+SI), which was confirmed by the chlorophyll-related vegetation indices (NDVI, RDVI, EVI) (Láposi et al 2019). In that year, we conducted our studies in an optimal season, thus confirming that not only weather conditions but also the proper phenophase of the plants play an important role in reflectance measurements (Sultana et al 2014).

Table 2. Relative chlorophyll content (SPAD-value) and spectral vegetation indices related to chlorophyll content in the leaves of barley in the middle of May in 2019 (n=80-100±SD). (Note: a, b, c index: significance groups by Tukey's-b test ($p<0.05$); n.s. – not significant by ANOVA)

Parameter	Significance	1. SD+SI treatment	2. SD+SR treatment	3. Control
SPAD-value	n.s. 321-aaa	45.45±4.64	45.05±5.64	44.07±5.73
RDVI	n.s. 321-aaa	0.475±0.082	0.473±0.051	0,453±0,085
EVI	n.s. 321-aaa	0.425±0.021	0.419±0.019	0.413±0.022
TCARI	n.s. 321-aaa	0.658±0.069	0.605±0.082	0.511±0.139
MCARI	n.s. 321-aaa	0.219±0.065	0.201±0.043	0.170±0.031
TCARI/OSAVI	n.s. 321-aaa	1.165±0.078	1.111±0.074	0.947±0.096
MCARI/OSAVI	n.s. 321-aaa	0.388±0.061	0.370±0.047	0.315±0.061

In wheat leaves, in 2019, we observed that both treatments (SD+SI, SD+SR) resulted in significantly higher SPAD value and spectral indices (RDVI, EVI, MCARI, MCARI2, VOG1). While NDMI, OSAVI, REDGE indices were only higher than the control in treatment 1 (SD+SI) (Table 3.).

Table 3. Relative chlorophyll content (SPAD-value) and chlorophyll related spectral vegetation indices in wheat leaves in the middle of May in 2019 (n=80-100±SD). (Note: a, b, c index: significance groups by Tukey's-b test ($p<0.05$); ANOVA significance: *** - $p<0.001$, ** - $p<0.01$, * - $p<0.05$)

Parameter	Significance	1. SD+SI treatment	2. SD+SR treatment	3. Control
SPAD-value	*** 312-a b b	**40.75±3.70**	**42.5±2.65**	36.5±5.05
RDVI	** 321-a b b	**0.510±0.074**	**0.493±0.099**	0.413±0.101
EVI	*** 321-a b b	**0.601±0.089**	**0.587±0.090**	0.462±0.172
MCARI1	***312-a a b	**0.160±0.074**	**0.184±0.056**	0.148±0.075
VOG1	*** 312-a b b	**1.297±0.044**	**1.317±0.061**	1.257±0.078
NDMI	* 321-a ab b	**0.217±0.037**	0.201±0.038	0.187±0.051
OSAVI	* 321-a ab b	**0.638±0.086**	0.610±0.152	0.541±0.168
REDGE	* 123-a ab b	**-0.038±0.008**	-0.034±0.013	-0.031±0.012

The NDVI index is very often used to characterize the biomass production potential of plants, but in our studies this index did not show significant differences. In their studies, Sultana and colleagues (2014) found that there was a strong correlation between yield and NDVI at maturity stage (booting and tillering), but not at stem elongation (stem elongation, anthesis, grain filling). Several studies have indicated that the time of measurement is very critical for predicting yield using spectral vegetation indices, as the correlation between them differs at different phenophases of the plant (Doraiswamy et al. 2005; Ren at al. 2008; Huang et al. 2013). Therefore, we chose the time when NDVI and EVI have seasonal maximum, so that the effect of treatments on yield can be predicted one and a half months before harvest.

In our previous studies (in case of maize in 2017 or rape and sunflower in 2018) the high level of standard deviations in the SPAD values also hid the effect of treatments (Láposi et al. 2018a; 2018b). However, chlorophyll-related spectral vegetation indices were able to indicate significant differences in the leaf chlorophyll content in these plants as well. In the case of rape, the NDVI and VOG1 values were significantly higher only in treatment 2 (SD+SR), whereas the RDVI, EVI, MCARI1, and OSAVI values were significantly higher in case of both treatments than in the control. In the sunflower leaves the VOG1 index was higher in both treatments whereas the NDVI, EVI, MCARI, TCARI, TCARI/OSAVI, and MCARI/OSAVI indices were significantly higher only in treatment 2 compared to the control (Láposi et al. 2018b). In the case of maize NDVI and TCARI/OSAVI indices (the latter of which was described for maize; Haboudane et al. 2002) were significantly higher in treatment 2, whereas the RDVI and EVI indices were significantly higher in both treatments compared to the control. (Láposi et al. 2018a). It is expected that there will be a difference between plant species as to which of the chlorophyll vegetation indices show greater sensitivity to the effects of the treatments, and thus more suitable for detecting significant differences.

Comparing the results with our previous studies (maize in 2017, rapeseed and sunflower in 2018), the SPAD value did not show a significant effect of treatments due to the high variance (Láposi et al. 2018a; 2018b). However, the spectral vegetation indices associated with chlorophyll content showed, only different indices for different plant species. For maize, the NDVI, RDVI, EVI and TCARI/OSAVI indices (the latter was described for maize by Haboudane et al. 2002); for rapeseed, the NDVI, VOG1, RDVI, EVI, MCARI1 and OSAVI indices; and for sunflower, the VOG1, NDVI, EVI, MCARI, TCARI, TCARI/OSAVI, MCARI/OSAVI indices were higher for each treatment. It is expected that there will be differences between plant species in which of the vegetation indices is more sensitive to changes in pigment content as a result of treatments.

The high variance of the data is often due to the heterogeneity of the plots, which UAV-based multispectral imaging is an excellent tool to detect. Among the indices that can be derived from a triple-band multispectral sensor, the NDVI, GNDVI and SAVI indices are the most commonly used. In our experiments, the SAVI index was best suited to detect differences and heterogeneity between plots, better than NDVI and GNDVI. In both experiments, SAVI values were highest for treatment 1 (SD+SI) and lowest for the control plot (C), but the differences were not significant due to the large standard deviation of the data (Figure 1.).

The differences between treatments were not significant based on NDVI and GNDVI indices, but they also showed the high heterogeneity of the plots. The NDVI (Rouse et al. 1973) varied between -1.0 and 1.0. Positive values indicate more green pigment, while negative values indicate water, barren areas, ice, snow or clouds (Pettorelli et al. 2005). For cultivated crops, the general range is 0.4-0.9 (Weier and Herring 1999). The GNDVI (Gitelson et al. 1996) and ranges between 0 and 1.0 (Candiagi et al. 2015), is closely related to the amount of photosynthetically absorbed radiation, leaf area index (LAI) and biomass (Hunt et al. 2008).

Thus, it is considered to be more sensitive to leaf chlorophyll content than NDVI. The soil-adjusted vegetation index (Huete 1988) is used to eliminate soil effects in vegetation monitoring in areas where the vegetation cover is poor and the soil surface is free. The SAVI ranges from -1.0 to 1.0, with low values indicating little green vegetation. In 2019, the rainy spring resulted in inland water within the experimental plots, so the vegetation cover was not homogeneous within each experimental plot.

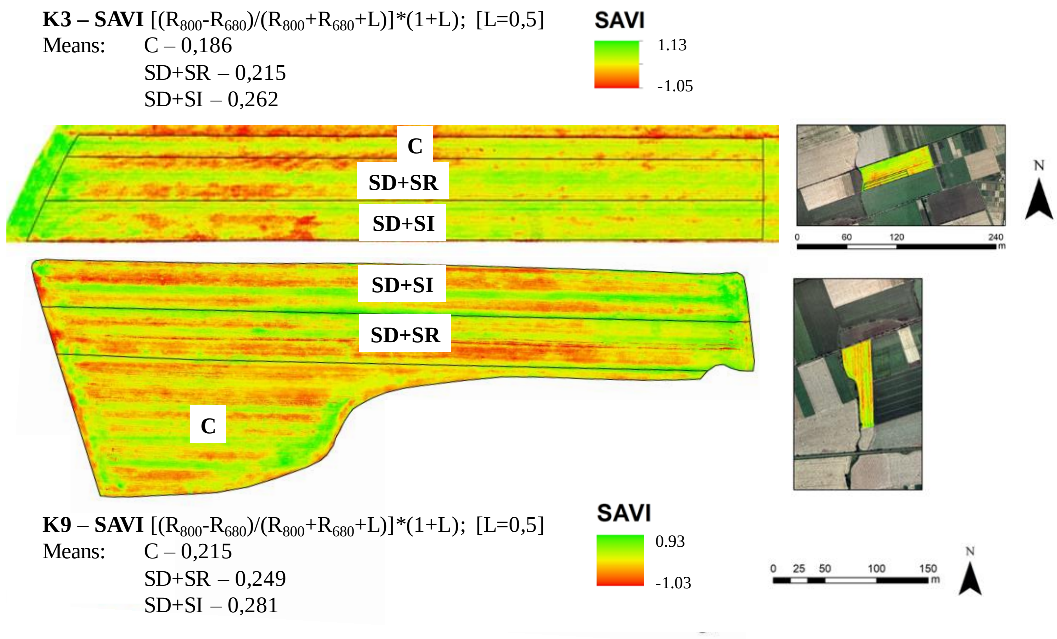

Figure 1. Chlorophyll related SAVI index of winter barley and winter wheat on K3 and K9 experimental plots in 2019 determined by UAV-based multispectral imaging.

3.2. Carotenoids, photochemical activity, stress sensitivity, water content

In addition to chlorophylls, it is important to investigate the carotenoids and anthocyanins - that protect the plant cells under stress - and the water content of the leaves. Their quantity and their relative proportions can be used to characterise the actual physiological state of plants, their susceptibility to stress and also have a significant influence on plant biomass production.

Carotenoids are thought to have a number of specific physiological functions due to their unique structural properties (Gitelson and Merzlyak 2004). For example, they are important folding elements in photosynthetic membranes, are involved in light scavenging, electron transfer, excited states of chlorophylls, and quenching of singlet oxygen and other oxygen radicals (Demmig-Adams et al. 1996). Changes in carotenoid content and composition of leaves, and even their ratio to chlorophyll, are widely used to determine the stress sensitivity of plants (Young and Britton 1990).

Anthocyanins are water-soluble, vacuolar accumulating pigments responsible for the red colour of plant tissues (Gitelson and Merzlyak 2004). Their significant accumulation is induced by high light intensity, UV-B radiation, drought, injury, bacterial and fungal infections, nitrogen and phosphorus deficiencies, and can be indicators of environmental stress and leaf senescence.

Several vegetation indices are used to characterize the amount of carotenoids (CRI) and anthocyanins (ARI), to compare photoprotective xanthophyll cycle activity (SIPI) and photochemical efficiency (PRI) and to determine leaf water content (PWI, SRWI).

Not only the SIPI, which is the indicator of carotenoid/chlorophyll ratio in leaves, but also PRI that is the photochemical reflectance or xanthophyll index can signify the stress-sensitivity of plants (Peñuelas et al. 1995). PRI is closely related to the photochemical activity of leaves, inversely proportional to the amount of photoprotective xanthophylls and the intensity of their membrane protection process, namely the xanthophyll-cycle (Gamon et al. 1997). Xanthophylls can radiate excess excitation energy from the leaves in the form of heat during the conversion of violaxanthin to zeaxanthin, which can be caused by the high light intensity at noon in the summer, but high heat, drought and increased ultraviolet B radiation can also increase the intensity of the xanthophyll cycle (Demmig-Adams and Adams 1992). The carotenoid/chlorophyll ratio is in many cases more informative than the chlorophyll or carotenoid content alone since the carotenoid content increases, the chlorophyll content decreases under stress, e.g. at a high light intensity, the chlorophyll-a/b ratio also increases as chlorophyll-b is more easily damaged (Tevini et al. 1981).

The plant water index was found to be affected not only by water content but also by canopy structure (Serrano et al. 2000). In line with SRWI Zarco-Tejada and Ustin (2001) showed in a simulation study that on leaf-level variables leaf structure and dry matter content can be an influential factor, while on canopy-level LAI can be a modifier. Accordingly, the sensitivity of PWI and SRWI to the changes in leaf water content during stress can be different in the case of crops with various leaf and canopy structures.

In our experiment in 2019, for barley, we found that photochemical reflectance index (PRI) and leaf water content (PWI, SRWI) were higher in treatment 2 and SIPI index, carotenoid (CRI) and anthocyanin content (ARI) were higher in the control, but differences between treatments were not significant as for chlorophyll content (*Table 4*). In 2017, these differences were significant in the case of first treatment (Láposi et al 2018b).

Table 4. Spectral vegetation indices related to photochemical reflectance (PRI), carotenoid/chlorophyll ratio – stress sensitivity (SIPI), carotenoid content (CRI), anthocyanin content (ARI), water content (PWI, SRWI) in barley leaves in the middle of May in 2019 (n=80-100±SD). (Note: a, b, c index: significance groups by Tukey's-b test ($p<0,05$); n.s.- not significant by ANOVA)

Parameter	Significance	1. SD+SI treatment	2. SD+SR treatment	3. Control
PRI	n.s. 312-aaa	0.224±0.050	0.229±0.059	0.207±0.097
SIPI	n.s. 213-aaa	0.552±0.080	0.550±0.078	0.559±0.081
CRI	n.s. 213-aaa	2.889±0.045	2.517±0.845	2.917±0.651
ARI	n.s. 213-aaa	1.796±0.357	1.495±0.606	2.971±1.196
PWI	n.s. 132-aaa	0.980±0.028	0.983±0.031	0.981±0.031
SRWI	n.s. 132-aaa	0.870±0.035	0.904±0.025	0.901±0.048

In 2019, in wheat, photochemical activity (PRI) and leaf water content (SRWI) were highest in treatment 1 (SD+SI), while carotenoid (CRI) and anthocyanin (ARI) content were highest in the control, as was SIPI, but showed no significant difference (Table 5). At the time of measurement, the wheat leaves were healthy green and not yet showing signs of ageing, in addition, the carotenoid content was higher and the anthocyanin content lower than in the barley leaves.

Compared to our previous studies, the soil bacteria treatments resulted in higher photochemical reflectance index (PRI) (maize in treatment 1, sunflower in treatment 2, rapeseed in both treatments). Control plants were characterised by higher protective pigment concentrations and, in parallel, lower leaf water content (Láposi et al. 2018a, 2018b). Individuals of the control plot were in the least optimal condition when they were measured. Overall, the photosynthetic and other protective pigments, photochemical activity and leaf water content indicate that treatment 1 had a greater effect on barley and wheat, and treatment 2 on maize, sunflower and rapeseed compared to the control in the years studied.

Table 5. Spectral vegetation indices related to photochemical reflectance (PRI), carotenoid/chlorophyll ratio–stress sensitivity (SIPI), carotenoid content (CRI), anthocyanin content (ARI), water content (PWI, SRWI) in wheat leaves in the middle of May in 2019 (n=80-100±SD). (Note: a, b, c index: significance groups by Tukey's-b test (p<0,05); ANOVA significances: *** - p<0.001; ** - p<0.01; * - p<0.05)

Parameter	Significance	1. SD+SI treatment	2. SD+SR treatment	3. Control
PRI	* 321-a ab b	**0.309±0.065**	0.285±0.096	0.277±0.091
SIPI	n.s. 123-a a a	0.682±0.105	0.701±0.155	0.728±0.084
CRI	* 213-a ab b	5.923±1.461	5.654±1.640	**6.652±1.497**
ARI	** 123-a a a	0.276±0.461	0.747±0.560	**1.747±0.385**
PWI	n.s. 132-a a a	0.963±0.014	0.964±0.008	0.959±0.009
SRWI	** 321-a ab b	**1.080±0.028**	1.067±0.037	1.051±0.046

3.3. Yield and grain quality

At harvest, we also determined the average yield of the treated and control plots. These results confirmed what we observed when we tested photosynthetic pigments in the middle of the growing season, that soil bacterial products can enhance biomass production in plants. For barley, treatment 1 resulted in 18.9% (3.74 t ha-1) and treatment 2 in 28.9% (4 053 t ha-1) higher yields compared to the control (3 144 t ha-1). For wheat, both bacterial formulations produced similar results, with the first treatment increasing yields by 27.8% (4,029 t ha-1) and the second treatment by 27.7% (4,024 t ha-1) (*Figure 2*).

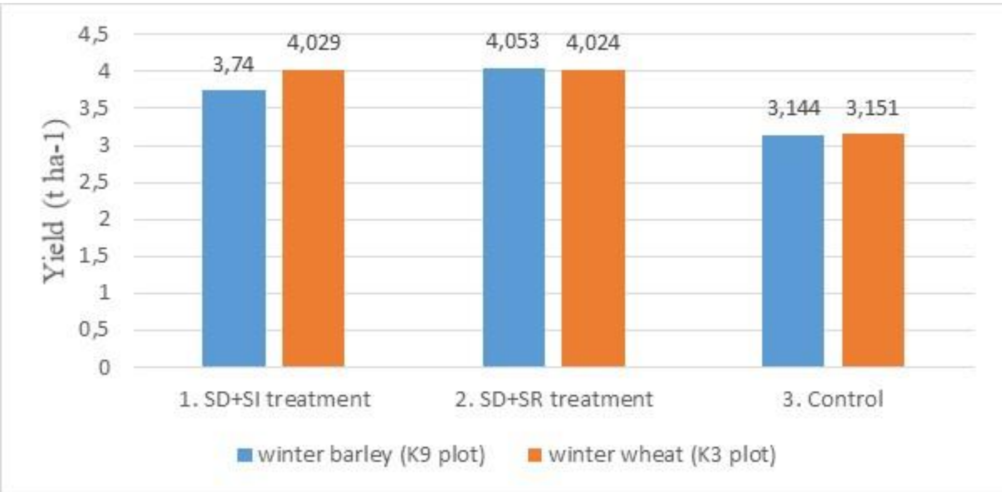

Figure 2. Yield (t ha^{-1}) of winter barley and winter wheat on K3 and K9 experimental plots in 2019 (Data source: Agricultural Research Institute of Kompolt; Harvest time: 17/07/2019).

In previous years, we have seen higher yield increases for barley. Indeed, in 2017, winter barley yields were 63% higher (2.9 t ha-1) in treatment 1 and 98% higher in treatment 2 (3.6 t ha-1) compared to the control (1.8 t ha-1), which is a huge achievement, as farmers generally reported yield increases of 20-40% for Phylazonit products in cereals (webpage 1). However, this can still be considered a very low yield average, as KH Korsó winter barley on Kompolt can achieve yield averages of 6.8 t/ha. The low yield was due to agrotechnical reasons on the one hand, and on the other hand, very little rainfall in the area between December and the end of April 2016.

The amount of precipitation during the growing season, the water content of the soil and the soil structure have a significant influence on the ability of soil bacteria preparations to exert their beneficial effects. In 2017, maize yields were only 13.7% (5.6 t/ha^{-1}) higher in treatment 1 and 15.9% (5.7 t/ha^{-1}) higher in treatment 2 than in the control plot (4.9 t/ha^{-1}). These yield averages were significantly lower than the 8 t/ha otherwise typical for maize. This could be due to the low rainfall during flowering and tasseling, which greatly influenced yields. In 2018, in rapeseed, treatment 1 increased yields by only 5.5% (1.38 t/ha^{-1}) and treatment 2 by 14.7% (1.50 t/ha^{-1}). The control plot yielded only 1.31 t ha^{-1} that year. This was also significantly lower than the 3 t/ha yield typical of Kompolt due to very low rainfall in April and May. In 2018, the yield of sunflower was increased by 18% (2.33 t/ha^{-1}) with treatment 1 and by 36% (2.56 t/ha^{-1}) with treatment 2 compared to the control (1.89 t/ha^{-1}), but still below the expected 3.5 t/ha. This was due to the drought in August and September which inhibited plant growth. Overall, the soil bacteria products were able to produce their effects despite the drought, thanks to good agrotechnical practices, timely application and application of the products and proper soil preparation.

In 2019, the Infratec Grain Analyzer was used to test the grain quality of wheat and barley, in particular the water and protein content of the two crops, and the W-value and Zeleny index of wheat, in addition to gluten content. At post-harvest, the protein, gluten and Zeleny index of wheat were significantly higher in both soil bacteria treatments, and significantly higher water and protein contents were also measured in barley treated with both soil bacteria products compared to the control (Figure 3.). This means that the treatments have a positive effect not only on yield but also on grain quality.

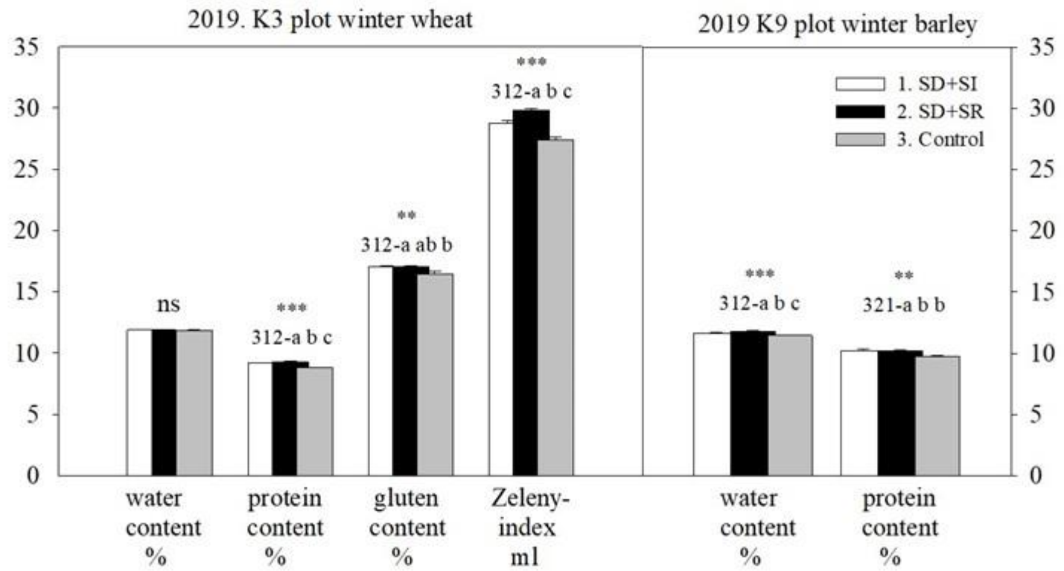

Figure 3. Quality parameters of winter wheat and winter barley after harvest (17/07/2019) (n=3±SD). (Note: a, b, c index: significance groups by Tukey's-b test ($p<0,05$); ANOVA significances: *** - $p<0.001$; ** - $p<0.01$)

3.4. Soil parameters

The nutrient-mobilizing and nitrogen-binding effects of soil bacteria are also reflected in the results of soil analysis, although a larger number of samples would be required to determine this. In 2018, the K3 plot had higher soil nitrate and nitrite contents than both treatments, while the phosphorus, phosphate and potassium contents were higher only after the 2nd treatment. In the case of plot K9, higher phosphorus, potassium and calcium contents were measured only in treatment 1 (Table 6.).

Table 6. Soil parameters of experimental plots (K3 and K9) in Kompolt in 2018 (n=3±SD). (Data source: Laboratory of Agro- and Environmental Sciences, Eszterházy Károly University)

Exp. plots	**Treat-ment, 2018**	**Nitrite** (NO_2^-)	**Nitrate** (NO_3^-)	**Sulfate** (SO_4^{2-})	**Phos-phate** (PO_4^{3-})	**Phos-phorus** (P)	**Potassi-um** (K)	**Calcium** (Ca)
		mg kg-1 air dry weight						
K3 plot:	1. SD+SI	**3.6±0.0**	**14013±64**	1707±12	768±3	251±1	777±9	2867±6
	2. SD+SR	**3.6±0.0**	**7821±126**	**2341±33**	**1059±12**	**345±4**	**1119±17**	2776±12
	Control	2.3±0.0	6000±34	1658±9	851±8	278±3	399±3	2745±42
K9 plot:	1. SD+SI	**5.4±0.1**	**10007±188**	2569±49	**1493±6**	**487±1**	**392±3**	**3340±3**
	2. SD-SR	**5.8±0.1**	**10007±79**	**3105±34**	902±6	272±2	338±6	2559±27
	Control	3.4±0.1	8914±14	2927±7	835±9	294±2	336±6	2645±1

4. CONCLUSION

- Under field conditions, not only the heterogeneity of the soil but also the differences between individuals can be expected to result in large variance of data compared to controlled laboratory experiments.
- Nevertheless, in our experiments, we found that both soil bacterial products resulted in higher yields for both crops compared to the control.
- Of the two soil bacteria products used, the soil decomposer-soil regenerator (SD+SR) was the main treatment that resulted in higher yields in winter barley, than in previous years for rapeseed, sunflower and maize.
- In the case of wheat, there was no difference between the two treatments, both of them produced an increase of about 30%.
- Due to the large variance in the data, the SPAD value only showed a significant difference between treatments only for winter barley in 2017.
- However, the spectral vegetation indices showed a positive effect of the treatments in the other crops studied: at the beginning of flowering we could observed significantly higher chlorophyll and N content, more active photosynthesis, lower stress sensitivity and, in parallel, lower carotenoid/chlorophyll ratio, anthocyanin content, higher water content than in the control plants.
- Soil bacterial treatments are thought to increase the stress tolerance of plants by providing a more balanced supply of nutrients and water to the plants, and thus higher yields.
- And the nutrient mobilising effect of soil bacteria can be traced back to changes in soil composition.
- The more uniform nutrient supply has increased the homogeneity of the treated plots, as can be seen in the multispectral aerial photographs, and it is a key factor for crop safety.
- Overall, it can be said that the applied *in vivo* measurement techniques were well suited for the evaluation of the effects of the treatments under field conditions based on the data from the 3 years examined.

In the future, we plan to continue the investigation of vegetation indices applicable to each species by conducting remote sensing measurements several times during the growing season. Indeed, as the age of the leaves and the structure change, the proportion of sclerenchyma tissue increases, water content decreases and chlorophyll levels decrease, while carotenoid concentrations increase. These changes can have a major impact on spectral properties.

Vegetation indices determined by UAV-based multispectral imaging can be an important part of precision crop production as it can provide accurate information not only on the condition of plants plant stress sensitivity during the vegetation period but also on soil heterogeneity, relative nutrient deficiency, which are essential for designing modern nutrient supply.

This study has demonstrated not only the usefulness of soil bacterial treatment in arable fields, but above all the importance of a healthy soil biota in agro-ecosystems where soil depletion is well known due to monoculture, the use of synthetic fertilizers and pesticides that disrupt or destroy the microbial community of soils. Natural soil development is a long process that takes several decades, while soil degradation caused by intensive monoculture and the overuse of fertilisers and pesticides takes much less time. Chemical and biological soil degradation can be prevented through sustainable practices such as crop rotation, use of compost, green manures, soil-bacteria products, well-controlled irrigation with chemically and biologically treated wastewater, use of cover cropping and mulching, no-till or low-till techniques, limited or zero pesticide use, and sustainable pest management techniques such as buffer zones and beneficial insect use. Although the soil bacteria cultures presented here cannot be considered a magic wand, they can be very useful for soil conditioning and regeneration purposes, where the aim is to restore soil microbial processes. However, they can only be beneficial if the soil structure, oxygen content, pH and water supply are adequate.

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NYILATKOZAT

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Kelt: Gödöllő, 2022. április 5.

Hallgató

NYILATKOZAT

Az **Evaluation of the effects of soil bacteria treatments on field crops by remote sensing techniques** című dolgozat készítőjének konzulense nyilatkozom arról, hogy a Záródolgozatot/Szakdolgozatot/Diplomadolgozatot áttekintettem, a hallgatót az irodalmi források korrekt kezelésének követelményeiről, jogi és etikai szabályairól tájékoztattam.

A Záródolgozatot/Szakdolgozatot/Diplomadolgozatot záróvizsgán történő védésre javaslom / nem javaslom*.

A dolgozat állam- vagy szolgálati titkot tartalmaz: igen nem*

Kelt: Gödöllő, 2022 év április hó 11 nap

Belső konzulens

***Kérjük a megfelelőt aláhúzni!**