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Hyperspectral imaging in assessing the condition of plants: strengths and weaknesses

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Abstract. Hyperspectral remote sensing of plants is widely used in agriculture and forestry. Fast, large-area monitoring is applied, among others, in detecting and diagnosing diseases, stress conditions or predicting the yields. Using available tools to increase the yields of most important crop plants (wheat, rice, corn) without posing threat to food security is essential in the situation of current climate changes.

Spectral plant indices are associated with biochemical and biophysical plant characteristics. Using the plant spectral properties (mainly chlorophyll red light absorption and near-infrared range light reflectance in leaf intercellular spaces), it is possible to estimate plant condition, water and carotenoid contents or detect disease. More and more often, based on commonly used hyperspectral vegetation indices, new, more sensitive indices are introduced. Furthermore, to facilitate data processing, artificial intelligence is employed, i.e., neural networks and deep convolutional neural networks.

It is important in ecological research to carry out long-term observations and measurements of organisms throughout their lifespan. A non-invasive, quick method ensures that it may be used many times and at each stage of plant development.

Key words: hyperspectral remote sensing, hyperspectral vegetation indices, clonal plant, life history

1. Introduction

Hyperspectral remote sensing – HRS consists in acquiring images recorded in many narrow channels, allowing to obtain a spectral curve for each pixel (Goetz *et al.* 2007). It exceeds similar tools due to the fact that it is based on the principles of material spectroscopy, radiation transfer, imaging spectrometry and hyperspectral data processing (Eismann 2012). HRS detects minor spectral features, which can be omitted in multi-beam scanning, thus it may be better suited for estimating the quantity and quality of the tested material (Shukla & Kot 2016).

For years, hyperspectral remote sensing has been used in water resource (e.g., Govender *et al.* 2007) and forest ecosystem research (e.g., Treitz & Howarth 1999; Koch 2010) and in geology (e.g., van der Meer *et al.* 2012). Currently, hyperspectral remote sensing is most commonly used in studies on vegetation in agriculture and forest management (e.g., Koch 2010; Mahlein 2016). These works concern the detection and diagnosis of diseases/pests (e.g., Zhang *et al.* 2003; Bauriegel *et al.* 2011), plant growth monitoring and yield prediction (e.g., Freeman *et al.* 2006; Pittman *et al.* 2015), diagnostics of nutrient deficiency and stress conditions (e.g., Chaerle & Van der Straeten 2000; Tilling *et al.* 2007). On the other hand, much research is devoted to the identification and functioning of plants (e.g., Hestira *et al.* 2008; Ustin & Gamon 2010; Adam *et al.* 2010; Sid'ko *et al.* 2014).

2. Recent research trends – strengths and weaknesses of using Hyperspectral Vegetation Indices

Many studies concentrate on the recognition and tracing of progress in disease symptoms in crop plants, such as: wheat (e.g., Behmann *et al.* 2018), barley (e.g., Zhou *et al.* 2019), rice (e.g., Liu *et al.* 2008) or corn (e.g., Del Fiore *et al.* 2010). Thanks to hyperspectral imaging, pathogens may be detected in the initial phase of development, before pathological changes in plants are visible. This is very important for the production efficiency of crop plant species on which the global economy is based, such as: wheat, rice and corn.

Effort are made to increase crop efficiency without posing threat to food security (e.g., Curtis & Halford 2014). The process of early pathogen detection is demanding and usually requires different tools due to the ongoing changes in plant growth and development. Recently, hyperspectral vegetation indices (HVI) have been combined with the application of neural networks (NN) that facilitate the processing of hyperspectral data (Lowe *et al.* 2017; Golhani *et al.* 2018). Nagasubramanian *et al.* (2019), based on the advanced type of neural network – 3D deep convolutional neural network (DCNN), showed that using the learnt models, we may classify and identify plant diseases with high accuracy (e.g., 95.73% for charcoal rot of soybean).

Inteligent models that analyse thousands of spectral photos in the current time may be the future tools that soon will be used on a wide scale in the natural environment monitoring of large-area crops. However, it should be emphasized that the application of artificial intelligence in plant studies conducted in laboratory is novelty. The majority of investigations are carried out in the field conditions, where the external environment may affect the obtained results.

Field studies do not allow to control external factors that may affect the quality and accuracy of measurements, e.g. light intensity or radiation angle (Epiphanio & Huete 1995). Accurate development of data obtained from large-area research is not always possible due to problems with the classification of individual pixels in a given category, e.g. vegetation or soil. Imprecise assignment of data and analysis of soil fragments as vegetation affect the results (Carlson & Ripley 1997). In addition, the measurement value is influenced by the plant development stage, leaf optical properties or leaf position angle (e.g. Turner *et al.* 1999; Sims & Gamon 2002).

The results of studies in which populations of the same species (or the same communities) occurring in different habitat conditions were compared showed differences in ecological parameters. A spectral curve that would be universal for a species (so-called pattern) should be determined on the basis of a group of individuals living in equalized environmental conditions. Such data relating to a single individual could be analyzed in relation to higher rank units, e.g. population. Otherwise, the obtained spectral curves are a difficult to explain mixture of various dependencies.

3. Potential use of Hyperspectral Vegetation Indices in plant ecology

In ecological studies, it is important to conduct observations and measurements of organisms for a long period of their life, which is why researchers are looking for new, non-invasive tools. Hyperspectral imaging fulfills this condition. The short period of data acquisition, even in large-area field studies, enables result visualization (Jarocińska & Zagajewski 2008). The non-invasiveness of the method allows to use it repeatedly, at every stage of plant development. Thanks to this, it is possible to control the same individual during its lifetime (from the seedling, through the breeding phase to the death) in the studies on perennial plants and not to measure selected traits in random population representatives whose ages we do not know. Such a long-term study based on the individual's life history enables a better understanding of the biology of a given species and the observed relationships.

4. Individual in remote sensing and ecology

When analyzing works that apply remote sensing methods, it can be noticed that "vegetation" and "species" are the most frequent research objects. In fact, there are not many studies carried out on an individual in laboratory conditions. Distinguishing the level of generality of the conducted research is very important in the context of the interpretation of the obtained results. Each individual grows, develops and reproduces in his life, and thus, while conducting research, his current state of development is observed, which will change over the course of his life. In ecological studies, an individual is a key subject of research and should be well identified. The problem with the identification of an individual occurs in the group of clonal plants (Box 1), which includes over 80% species of vascular plants (Van Groenendael & de Kroon 1990).

Box 1. Clonal plant architecture
The clonal plant network consists of units connected with each other. A single unit (parental ramet) develops from the zygote, which reproduces vegetatively and produces daughter ramets connected to it by rhizomes or stolons. This way, a homogeneous network is formed. Each of the ramets has the ability to form all necessary structures (including leaves, roots, lateral meristems) to collect and produce resources (e.g. water, minerals, assimilates) (Gómez 2008). Single ramets are not capable of reacting independently to changing environmental conditions, but they can coordinate the response with other members of the network (de Kroon *et al.* 2005). The parental ramet transfers water and mineral salts to the daughter ramet, receiving assimilates in exchange (Stuefer *et al.* 1996). As a result, they achieve success in a heterogeneous environment (e.g. Lynch & Balmer 2004; DeWoody *et al.* 2008; Tèllez *et al.* 2008).

Folse & Roughgarden (2010) defined an individual of a clonal plant as „(…) an integrated functional agent, whose components work together in coordinated action analogous to the pieces of a machine, thus demonstrating adaptation at the level of the whole organism". Conducting research on "vegetation", "species" there is a group of individuals observed that can have different age, different level of development, and thus the obtained data are not comparable with each other.

5. Condition of plants in remote sensing and ecology

Spectral plant indices are associated with biochemical and biophysical plant characteristics. Using the plant spectral properties (mainly chlorophyll red light absorption and near-infrared range light reflectance in leaf intercellular spaces), it is possible to estimate plant condition, water and carotenoid contents or detect disease. To obtain the most precise measurements based on the present, still widely used indices (Box 2), more sensitive, modified indices are created, such as, e.g., VNIR/SWIR HSI sensor for vegetation trait mapping (Dupuis *et al.* 2019).

Box 2. Common vegetation indices in remote sensing
NDVI (Normalized Difference Vegetation Index) is commonly used in hyperspectral imaging to assess the condition of vegetation (e.g. Bhandari & Kumar 2012; Gandhi *et al.* 2015). It was first used in 1973 by Rouse. It is based on the contrast between the highest light reflectance in the near-infrared band and the absorption in the red band. ARVI (Atmospherically Resistant Vegetation Index) is an enhancement to the NDVI that is relatively resistant to atmospheric factors, for example, aerosol (Kaufman & Tanre 1992). RENDVI (Red Normalized Difference Vegetation Index) enables assessing chlorophyll content and structure of plant cell (Gitelson & Merzlyak 1994; Sims & Gamon 2002). MRENDVI (Modified Red Edge Normalized Difference Vegetation Index) is a modification of the Red Edge NDVI that corrects for leaf specular reflection (Datt 1999; Sims & Gamon 2002). REPI (Red Edge Position Index) is a narrowband reflectance measurement that is sensitive to changes in chlorophyll concentration (Curran *et al.* 1995).
To assess plant physiological condition, PRI (Photochemical Reflectance Index) is used that is sensitive to changes in carotenoid pigments (e.g., xanthophyll pigments) in live foliage (Gamon *et al.* 1992), while REP (Rep Edge Point) is strongly related to chlorophyll foliar concentration and contents (Dawson & Curran 1998).
Other indices focus on the characteristics of water absorption. Amongst the most referred indices are NDWI (Normalized Difference Water Index) is used to monitor changes in water content of leaves, using near-infrared (NIR) and short-wave infrared (SWIR) wavelengths (Gao 1996). NDII (Normalized Difference Infrared Index) is a reflectance measurement that is sensitive to changes in water content of plant canopies (Hardisky *et al.* 1983) and MSI (Moisture Stress Index) is a reflectance measurement that is sensitive to increasing leaf water content (Hunt & Rock 1989).

In case when NDVI is used as a main indicator of plant condition, the best narrow-band NDVI predictors of crop biophysical variables is identified (Thenkabail *et al.* 2010). Thanks to this, NDVI that is purposely used in wide-band analyses, may be successfully used in narrow-band analyses.

In biology and plant ecology research, quantitative data on, among others, plant size and biomass serve as the measure of plant condition (Younginger *et al.* 2017), seed germination capacity (Campbell 2017), sexual and vegetative reproduction (Aarssen 2014). Such traits are more or less directly linked to plant fitness and can be used as reliable approximation of long-term performance of individuals. However, the traits that constitute a component of reproductive expenditure cannot be assessed at any stage of the life cycle. Additionally, they cannot be used to evaluate plant condition, or 'health' status, in a given time, which is essential in the analysis of immediate effects of environmental stimuli. In such studies, an estimate that is closely linked to physiological properties of a plant is needed and this may include indices related to chlorophyll activity.

Currently, ecologists often measure photosynthetic efficiency to evaluate plant condition. CO_2 assimilation rate and chlorophyll fluorescence measurements are usually the measure of photosynthetic efficiency (Sarijeva *et al.* 2007). Different fluorimeters can be distinguished depending on the chlorophyll fluorescence measurement approach (e.g. pulse amplitude modulation (PAM) fluorimeters, the fast repetition rate (FRR) fluorimeters and advanced laser fluorimeters (ALF)), but the studied phenomenon remains the same (Sarijeva 2007). The measurement of chorophyll *a* fluorescence kinetic induction occurs under light conditions, after adaptation in the dark (Kautsky effect). Fluorescence of chlorophyll *in vivo* has a maximum emission in the red band (~690 nm) and in the far red band (735-740 nm) (Govindjee 1995; Sarijeva *et al.* 2007).

Unfortunately, reliable results can be obtained when several dozens or even several hundred individuals are tested. It is time-consuming and often impossible to do at the same time. The measurement of chlorophyll fluorescence for 50 individuals of *Hieracium pilosella* L. – our research object – using a single fluorimeter would last 91 h 40 min, assuming that: each individual produced 10 daughter ramets, we select for the measurement random 5 leaves from each mother and daughter ramet, and the measurement of one leaf lasts 2 minutes. This result does not take into account additional time for shading the plants and connecting/disconnecting the equipment.

The intensity of photosynthesis translates directly into the rate of growth that is widely recognized as an important parameter of plant life strategies (Wuyts *et al.* 2015). The rate of growth varies between species. However, it may also differ at an intra-specific level, including plastic plant responses to external factors, like nutrient availability, light intensity, competition, etc. The rate of growth is one of life history traits (sensu Stearns 1992), and thus, according to the resource allocation principle, a negative correlation with other life history traits may be expected. Therefore, chlorophyll indices, reflect not only plant condition, but they may also characterize plant strategy by manifesting the importance of photosynthesis and placing it on the trade-off continuum with other traits. If this is the case, different individuals may exhibit different values of indices not necessarily because some individuals are in better shape, but because they are at different life stages that require different partitioning of resources.

6. Research perspectives

Hyperspectral imaging, as a technique used in laboratory microscale studies, requires refinement, but has enormous potential. First of all, it is a non-invasive method, which is particularly important in regular plant condition monitoring in long-term studies. Combination of spectral imaging with quick and precise NN and DCNN analyses may enable simultaneous analyses of, e.g., species classification, plant condition evaluation and pathogen early detection. Then, at the same time, based on one set of data, we might be able to obtain results that presently require involvement of different specialists.

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