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Identification of spatial pattern of photosynthesis hotspots in moss- and lichen-dominated biological soil crusts by combining chlorophyll fluorescence imaging and multispectral BNDVI images

Andreas Kleefeld[a,1], Stella Gypser[b,*,1], Werner B. Herppich[c], Georg Bader[d], Maik Veste[b,e]

[a] *Forschungszentrum Jülich, Institute for Advanced Simulation, Jülich Supercomputing Centre, Wilhelm-Johnen-Straße, 52425 Jülich, Germany*
[b] *Brandenburg University of Technology Cottbus-Senftenberg, Soil Conservation and Recultivation, Konrad-Wachsmann-Allee 6, 03046 Cottbus, Germany*
[c] *Leibniz-Institute for Agricultural Engineering and Bioeconomy, Department Horticultural Engineering, Max-Eyth-Allee 100, 14469 Potsdam, Germany*
[d] *Brandenburg University of Technology Cottbus-Senftenberg, Numerical Mathematics and Scientific Computing, Platz der Deutschen Einheit 1, 03046 Cottbus, Germany*
[e] *University of Hohenheim, Department of Botany, Garbenstraße 30, 70599 Stuttgart, Germany*

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ABSTRACT

Although biological soil crusts can be found in open landscapes worldwide, their species composition depends on soil properties such as texture and pH, on microclimate, and their respective developmental stage. In addition, local variations in water holding capacity and/or chemical properties of soils influence the formation of spatial patterns and different types of biocrusts on the landscape level. For the evaluation of biocrusts functions and their impact on soil carbon pools, the analysis of the interrelationship between photosynthetic activity and the variations of spatial distribution pattern and types of biocrust is indispensable. For this purpose, an image processing approach was applied that combined chlorophyll fluorescence analyses and multispectral BNDVI to comprehensively characterize the spatial patterns of photosynthetic hotspots in biological soil crusts. For image analysis, five biological soil crust samples with different ratios of substrate, mosses and lichens were collected on an inland dune system in Lieberose, dominated by the moss *Polytrichum piliferum*, and the lichens *Cladonia fimbriata* and *C. coccifera*. RGB-images of the biocrusts were taken with a standard consumer camera Nikon 5200, BNDVI images with a modified Canon S110 NIR camera and chlorophyll fluorescence images with a modular open FluorCAM FC 800-O/1010, respectively. BNDVI and F_v/F_m were nearly in the same range for all biocrust samples related to the total surface area. Although mosses showed higher BNDVI than lichens within the separate biocrust samples. F_0 and F_m increased with species coverage and with advancing biocrust development. Overlapping of BNDVI with F_0 and F_m images showed that not all crustal organisms contribute to BNDVI and chlorophyll fluorescence. The overlapping areas of BNDVI and F_0 ranged between 13% and 29%, that of BNDVI and F_m between 17% and 47%. Matching of RGB, BNDVI and CFI allows visualizing spatial pattern with high or low photosynthesis in biocrusts.

1. Introduction

In many ecosystems, the soil surface is settled by various species of cyanobacteria, bacteria, green algae, mosses, liverworts, lichens and fungi (Belnap and Lange, 2003). In addition, biological soil crusts (biocrusts) can be found in various open landscapes with sparse vegetation worldwide, e.g. in polar regions, coastal and inland sand dunes, grasslands and initial ecosystems (Cutler et al., 2008; Schaaf et al., 2011; Pushkareva and Elster, 2013). Comprehensive studies on structure and function of biocrusts, however, have been nearly exclusively conducted in arid and semi-arid areas (Breckle et al., 2008; Johansen, 1993; Maestre et al., 2011; Weber et al., 2016). Characteristic for biocrusts is their three-dimensional structure, which initially has been formed by microorganisms cross-linking soil particles and developing a top layer on the soil surface. The thickness of the crusts can vary from a few millimeters to several centimeters. The species composition depends on microclimatic conditions and the development stage of biocrusts (Büdel and Veste, 2008;). For early stages, green-algae and cyanobacteria are particularly characteristic. In contrast, mosses dominate the cryptogamic communities in mid-succession, while soil lichens occur only in late successional stages (Eldridge and Greene, 1994; Gypser et al., 2015a).

* Corresponding author.
E-mail address: stella.gypser@b-tu.de (S. Gypser).
[1] These authors contributed equally to this project and should be considered co-first authors.

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Even if biocrusts are covering only the topsoil, they are key drivers for functional processes and the development of ecosystems (Schaaf et al., 2011). Due to their specific features, biocrusts can serve as model systems in ecosystem theory and their analysis may help to understand the interactive processes occurring during soil formation and ecosystem development (Bowker et al., 2010; Schaaf et al., 2011; Maestre et al., 2016). Biocrusts stabilize the soil surface (Breckle et al., 2008), influence the soil hydrological processes (Kidron et al., 1999; Li et al., 2016), and accumulate organic carbon (Dümig et al., 2014) and macro- and micronutrients (Brankatschk et al., 2013; Yu et al., 2016).

As rootless poikilohydric organisms, the photosynthetic activity of biocrusts strongly depends on moisture supply from dewfall, fog and/or rainfall. Differences in microclimatic conditions and water holding capacity of biocrusts, and, at least partly, in chemical properties of soils can explain the formation of spatial patterns of different biocrust types. The duration of soil surface wetness is most important for the physiological activity of biocrusts, and for the formation of different types of biocrusts on the landscape level (Veste and Littmann, 2006).

Therefore, the evaluation of photosynthesis in relation to the variation in spatial distribution patterns of the biocrusts is very important for understanding their functions. The photosynthetic activity of biocrusts can easily be monitored by chlorophyll fluorescence analyses (CFA; Schroeter et al., 1991, 1992; Veste et al., 2001; Raggio et al., 2014). Common chlorophyll fluorescence devices use fiber optics (approximately 6 mm diameter) and, thus, provide only very local information of physiological activity of biocrusts but not on its spatial variation. In contrast, current chlorophyll fluorescence imaging systems may resolve this limitation (Bauriegel and Herppich, 2014). In fact, this technique has been successfully applied to various biocrust types (Pushkareva and Elster, 2013; Gypser et al., 2016).

On the other hand, Blue Normalized Difference Vegetation Index (BNDVI, according to Van der Merwe and Price, 2015) imaging may help the analysis of spatial heterogeneity and distribution of chlorophyll (Bauriegel et al., 2011a). Consequently, this parameter was successfully applied to investigate biocrusts at different spatial scales (Burgheimer et al., 2006; Karnieli and Tsoar, 1995). Recently, modified low-cost consumer cameras proved an interesting and simple alternative for the complex multispectral monitoring of BNDVI in the field and in the laboratory. These cameras also can easily be mounted on different micro-drones to record BNDVI on the field scale (Läderach et al., 2015). These high resolution BNDVI-camera systems are also suitable for the ground-based evaluation of distribution patterns of biocrusts (Fischer et al., 2012; Gypser et al., 2016). In addition, they allow novel applications for small-scale monitoring of biocrusts.

The combination of both CFA and BNDVI may actually allow further comprehensive analyses of the spatial variations of photosynthetic activity (Bauriegel et al., 2011b) but are still lacking for biocrusts. These analyses are, however, essential to better understand the mechanisms of biocrust formation and the influence of various environmental factors (e.g. microclimate, drying, shading or surface properties of soils) on the development of various types of crusts. It was shown that photosynthesis is not uniformly distributed on the biocrust surface or over the specific crustal community (Gypser et al., 2016; Schroeter et al., 1992). The characterization and quantification of these spatial patterns is, however, still an open issue. Hence, by combination of CFA and BNDVI imaging, the presented study aims to identify the hotspots of photosynthesis and their spatial pattern in biocrusts. These hotspots are related to the specific metabolic competence characterizing the particular species determining the differing spatial distribution patterns, which, in turn, provide essential information on the respective developmental stage of biocrust. For this purpose, CFA and BNDVI images of field-sampled biocrusts, differing in their species composition were taken by different camera systems. An image processing approach was developed to combine CFA and BNDVI images and facilitate the identification of hotspots of photosynthesis.

2. Materials and methods

2.1. Biological soil crusts

Samples of biological soil crusts were collected in an inland dune system "Lieberose Heath" (51°55′35" N, 14°20′05" E) in Lower Lusatia (Brandenburg, Germany). The Lower Lusatia region is characterized by the transitional Atlantic to continental climate with a mean annual temperature of 9.3 °C and a mean annual precipitation of 581 mm a^{-1} (1981–2010) recorded at the nearest climate station in Cottbus (DWD, 2014). In 2016, a mean annual temperature of 9.5 °C and a total annual precipitation of 632 mm a^{-1} were recorded (DWD, 2017). The crusts were taken from an inland dune with dry acidic grassland dominated by *Corynephorus canescens* and heath dominated by *Calluna vulgaris* (Ellenberg and Leuschner, 2010).

For image analyses, five biocrust samples, representing five different biocrust types with varying ratios of moss, soil lichens and bare substrate cover were collected to enable image analyses with various distribution patterns. Samples BC-M (M – moss) and BC-SM (S – substrate) were dominated by the moss *Polytrichum piliferum*. While BC-M showed high moss coverage of nearly 90%, BC-SM had an estimated surface coverage of 60%. The biocrust samples BC-LM (L – lichen) and BC-ML contained a mixture of the moss *Polytrichum piliferum* and soil lichens of the genus *Cladonia*. Both biocrust samples had a surface coverage of almost 95%, while the mosses and lichens contribute in equal parts. Whereas lichens occurred just on one half of the surface area of BC-LM, they covered the whole area in BC-ML. The surface area of BC-L was totally covered, dominated by the soil lichens *Cladonia fimbriata* and *C. coccifera*, but the crust also contained mosses of the species *Polytrichum piliferum*.

Biocrusts containing green algae or cyanobacteria were not included in this study because cyanobacterial crusts do not develop on acid sand dunes (Bettina Weber, personal communication) with a low soil pH around 4.6. Under such acidic soil conditions (pH $<$ 5), these organisms are normally absent (Brock, 1973). Even green algae biocrusts will only dominate in early successional stages (Fischer et al., 2012), which are only small areas of the Lieberose Heath. Therefore, this study focused on later successional stages with mosses and lichens, which are characteristic for the plant community with *Calluna vulgaris*. Furthermore, for the first testing of the methods, pictures of moss- and lichen biocrusts give more structures for the overlaying of the different pictures. The samples were collected by gently coring Petri dishes (10 cm × 10 cm) in the upper soil layer. Ruptures were carefully avoided to obtaining well-defined surface areas easily comparable between samples. The fact that Petri dishes were quadratic and the added marking labels facilitated matching of the respective RGB, BNDVI (red labels) and chlorophyll fluorescence (blue labels) images.

2.2. RGB-images

RGB images of the five biocrust samples were recorded with a standard consumer camera Nikon 5200 (Nikon, Tokyo, Japan) equipped with a standard 35 mm objective and a resolution of 6000 pixels × 4000 pixels.

2.3. BNDVI images

In recent years, various applications with modified consumer cameras connected to small unmanned aircraft systems were developed to determined spatial pattern of NDVI in the context of precision farming and for the detection of crop stress index in a variety of crops (Haghighattalab et al., 2016; Hunt et al., 2010; Zhao et al., 2015) and for the detection of cyanobacteria along lake shores (Van der Merwe and Price, 2015). According to the experiences by Van der Merwe and Price (2015), the BNDVI can be used in a similar manner to the "red" NDVI, as long as the sensor is used at a relatively low altitude and the