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Silicified structures affect leaf optical properties in grasses and sedge

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ABSTRACT

Silicon (Si) is an important structural element that can accumulate at high concentrations in grasses and sedges, and therefore Si structures might affect the optical properties of the leaves. To better understand the role of Si in light/leaf interactions in species rich in Si, we examined the total Si and silica phytoliths, the biochemical and morphological leaf properties, and the reflectance and transmittance spectra in grasses (*Phragmites australis*, *Phalaris arundinacea*, *Molinia caerulea*, *Deschampsia cespitosa*) and sedge (*Carex elata*). We show that these grasses contain >1% phytoliths per dry mass, while the sedge contains only 0.4%. The data reveal the variable leaf structures of these species and significant differences in the amount of Si and phytoliths between developing and mature leaves within each species and between grasses and sedge, with little difference seen among the grass species. Redundancy analysis shows the significant roles of the different near-surface silicified leaf structures (e.g., prickle hairs, cuticle, epidermis), phytoliths and Si contents, which explain the majority of the reflectance and transmittance spectra variability. The amount of explained variance differs between mature and developing leaves. The transmittance spectra are also significantly affected by chlorophyll *a* content and calcium levels in the leaf tissue.

1. Introduction

Silicon (Si) is the second most abundant element in the Earth crust, and it is present in different minerals. Nevertheless, the availability of Si is highly variable in comparison to other elements [1]. As well as soil development, climate and anthropogenic influence [2], plants have important roles in Si cycling [3]. Terrestrial plants take up Si from the soil solution through the roots and deposit it in intracellular or extracellular spaces, with the formation of different shapes and sizes of silicified plant structures, known as silica phytoliths [4]. Phytoliths can occur in any plant part, although they are mainly found in the shoot epidermis and root endodermis, and in the plant vascular system. Some phytoliths might be preserved for long periods of time. As they appear in taxonomically recognizable forms, they can also have diagnostic roles, as witnesses to the past [5].

The transport of Si is either active (via ATP-based silicon transporters) or passive. Phytoliths can be deposited in intercellular and intracellular spaces, while Si that is hydrogen bonded to cellulose molecules can be found in the cell walls, as a form of silica gel. The amount of silica in a plant is species specific, and can range from 0.1% to 15% of the plant dry mass, although substantial variations of Si contents within species have been reported [2,6]. In general, monocotyledons accumulate higher levels of Si compared to dicotyledons [1,5,7]. In addition, the phylogenetic potential for the accumulation of Si and the Si composition of plants can also be affected by abiotic factors, such as the amount of water in the soil and the local climate [5,8].

Silicon is a key structural element in grasses, as it can enhance their strength and prevent lodging and shading of leaves. Silicon also has an important role in stress mitigation, through increased production of antioxidants and its binding to, and co-precipitation with, metal ions [9–11]. The amelioration of Al toxicity by Si is well established [12–15], and many studies have observed co-deposition of Al and Si in epidermal cells [10,16,17].

In grasses, Si is deposited in short cells, epidermal long cells, bulliform cells, guard cells and prickle hairs, in the form of phytoliths [5,18–20]. The silica content is particularly high in sheaths and leaves compared to other plant organs [8,21]. Although the most important task of leaves is the collection of solar energy, little consideration has been given to the possible role of such biominerals in the leaf optics and optical properties [22]. As well as Si, calcium (Ca) oxalate and Ca carbonate are frequently involved in incrustation of the leaf surface [23,24], and they are also known to be important structural elements in grasses [25]. Some studies have demonstrated that in leaves, Ca oxalate crystals and amorphous Ca carbonate act as light scatterers, to enhance the photosynthetic efficiency [22,26]. The data available in the literature show that the ecological functions of Si have generally been poorly studied, and that there are almost no data about the role of Si

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structures in the reflection and transmission spectra of short-wave or photosynthetically active radiation in plants. In the past, an idea that was proposed was that silicified structures in leaves might act as 'glass windows' that can facilitate transmission of light through the epidermis to the photosynthetic mesophyll tissue. However, measurements of light-use efficiency, quantum yield, and transmission of light through the epidermal layer have not supported these hypotheses [18,27]. Agarie and co-workers [27] compared the optical properties of leaves in Si-treated and non-treated rice plants, but their transmittance and reflectance spectra did not differ significantly in the visible regions; however, there were differences in the near infrared (NIR) regions of the spectra. On the other hand, some studies have also suggested that silica in grasses might contribute to the scattering of UV radiation [28,29].

It is generally accepted that the shape of the leaf surface relief greatly influences the surface-reflection of light, while the structure of the mesophyll affects the light penetration. We hypothesised that as well as the other leaf traits, the presence of Si structures will affect the reflectance spectra and the penetration of radiation through the mesophyll. To investigate this, we have here measured the biochemical and morphological properties and the phytolith content of five monocotyledons (four grasses and one sedge), and related these to the leaf reflectance and transmittance spectra. Using X-ray fluorescence element analysis, we have determined the total amounts of Si and Ca and the presence of Al (which might co-deposit with Si). On the basis of these data, we will be able to better understand the role of silicified structures and other leaf traits in light/ leaf interactions in monocotyledons.

2. Materials and methods

2.1. Study sites

The plants were sampled in the area of an intermittent lake, Lake Cerknica (45°46'15''N, 14°21'20''E), which appears at the bottom of a karst field known as Cerkniško polje. The depression of Cerkniško polje is filled with water twice a year, in spring and in late autumn or early winter, while its dry period usually starts in late spring [30]. These water-level fluctuations create a variety of habitats with diverse communities. The majority of the shallower areas in Lake Cerknica are colonised by the cosmopolite common reed, *Phragmites australis*, and other wetland species, while areas where the flooding occurs at the beginning of the growth season only are overgrown with mire and wet grassland species, with the prevailing communities of *Deschampsio-Plantaginetum altissimae* and *Molinietum caeruleae* [31].

2.2. Plant material

The leaves of the grasses (*Phragmites australis* (Cav.), *Phalaris arundinacea* L., *Molinia caerulea* L., *Deschampsia cespitosa* L.) and sedge (*Carex elata* L.) were collected at different phenological stages in the summer of 2012. The developing leaves of *M. caerulea*, *D. cespitosa* and *C. elata* were sampled in June 2012, while the mature leaves were sampled in July 2012. For *P. australis* and *P. arundinacea*, the mature leaves were also sampled in June 2012, in addition to the developing leaves. We sampled the first developing leaf from the top that had already unrolled, and the oldest fully developed vital leaf from the same plant. Leaves of *P. australis* were sampled the same way in July 2012, to study possible seasonal changes in the phytolith contents. The measurements were carried out on ten replicates of leaves for each plant species, twice in the season.

2.3. Measurements of spectral reflectance and transmittance

The optical properties of the leaves were measured on the day of sampling. The leaf reflectance and transmittance were measured at the wavelengths from 280 nm to 880 nm, at approximately 0.3-nm intervals, using a portable spectrometer (Jaz Modular Optical Sensing Suite) fitted with an integrating sphere (ISP-30-6-R) and an optical fibre (QP600-1-SR-BX), all provided by the same producer (Ocean Optics, Inc., Dunedin, USA). The reflectance and transmittance spectra were measured for the same part of the leaf. At some wavelengths, the relative reflectance and transmittance might be overestimated, due to chlorophyll fluorescence [32].

The measurements of reflectance followed the procedures in Klančnik et al. [33]. Adaxial leaf surfaces were illuminated with a UV-VIS-NIR light source (DH-2000, Ocean Optics, Inc., Dunedin, USA). The spectrometer was calibrated to 100% reflectance using a white reference panel (Spectralon®, Labsphere, North Sutton, USA).

For the measurements of the transmittance spectra, the samples were positioned under an integrating sphere in such a way that the abaxial leaf surface was exposed to the sampling port of the integrating sphere. The adaxial leaf surface was illuminated by the above-mentioned light source. The spectrometer was calibrated to 100% transmittance with a light beam that passed directly into the integrating sphere interior.

2.4. Morphological and anatomical analysis

The specific leaf area (SLA) was determined as the leaf area per unit of dry mass ($cm^2\ g^{-1}$). The leaf anatomical analysis was carried out on transverse sections. The leaf thickness and the thickness of the cuticles, epidermis and total mesophyll were measured at 100× magnification, using an Olympus CX41 microscope equipped with an Olympus XC30 digital camera and CellSens software (Olympus, Hamburg, Germany). The density and length of the leaf stomata and prickle hairs on the upper and lower leaf surfaces were also determined.

2.5. Biochemical analysis

The contents of chlorophyll *a*, chlorophyll *b* and the carotenoids were determined according to Lichtenthaler and Buschmann [34,35]. Plant material was homogenised in 100% acetone and centrifuged (1359*g*, 4 °C, 4 min). The absorbance levels of the samples were measured at the wavelengths of 470 nm, 645 nm and 662 nm, using a UV/VIS spectrometer (Lambda 25, Perkin–Elmer, Norwalk, USA). The chlorophyll and carotenoid contents were expressed as weight per area of sample ($g\ m^{-2}$).

The content of the anthocyanins was determined as previously described by Drumm and Mohr [36]. Extracts of anthocyanins were prepared using methanol: 12.1 N HCl (99:1; v/v). The absorbance levels of the extracts were measured at 530 nm, and the content of pigment was calculated relative to the sample area (relative units per cm^{-2}).

The total methanol soluble UV-B and UV-A absorbing compounds were extracted from the plant material with methanol: distilled water: 12.1 N HCl (79:20:1; v/v/v) [37]. The absorbance levels of the extracts were measured in the spectral ranges of 280–315 nm, and 316–400 nm. The extinction values were integrated for each UV region, and are expressed relative to the sample area (relative units per cm^{-2}).

2.6. Phytolith extraction

Phytoliths were extracted from the leaves using the wet oxidation method described by Piperno [5], with some modifications.