UNIVERZA V LJUBLJANI
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OPTIČNE LASTNOSTI RASTLIN VZDOLŽ HIDROLOŠKEGA GRADIENTA PRESIHAJOČEGA JEZERA

DOKTORSKA DISERTACIJA

OPTICAL PROPERTIES OF PLANTS ALONG THE HYDROLOGICAL GRADIENT OF INTERMITTENT LAKE

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Doktorsko delo je zaključek Interdisciplinarnega doktorskega študija Bioznanosti na področju biologije. Laboratorijski del raziskav je bil opravljen na Katedri za ekologijo in varstvo okolja Oddelka za biologijo Biotehniške fakultete Univerze v Ljubljani. Priprava rastlinskih vzorcev za določanje porazdelitve elementov na tkivnem in celičnem nivoju (mikro-PIXE in LEXRF) je potekala na Katedri za botaniko in fiziologijo rastlin Oddelka za biologijo Biotehniške fakultete Univerze v Ljubljani. Meritve z XRF so bile opravljene v Laboratoriju za rentgensko fluorescenčno spektrometrijo, na Odseku za fiziko nizkih in srednjih energij Inštituta Jožef Stefan v Ljubljani. Meritve z mikro-PIXE so bile opravljene v Mikroanalitskem centru Inštituta Jožef Stefan v Podgorici. Meritve LEXRF so bile opravljene na žarkovni liniji TwinMic na Sinhrotronu Elettra v Bazovici, Italija.

Na podlagi Statuta Univerze v Ljubljani ter po sklepu Senata Biotehniške fakultete in sklepa Komisije za doktorski študij Univerze v Ljubljani z dne 19. 9. 2012 je bilo potrjeno, da kandidatka izpolnjuje pogoje za opravljanje doktorata znanosti na Interdisciplinarnem doktorskem študiju programa Bioznanosti, znanstveno področje biologija. Senat Univerze v Ljubljani je na predlog Biotehniške fakultete in študijske komisije Oddelka za biologijo odobril temo doktorske disertacije z naslovom Optične lastnosti rastlin vzdolž hidrološkega gradienta presihajočega jezera. Za mentorico je bila imenovana prof. dr. Alenka Gaberščik.

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Doktorsko delo je rezultat lastnega raziskovalnega dela. Podpisana se strinjam z objavo svoje naloge v polnem tekstu na spletni strani Digitalne knjižnice Biotehniške fakultete. Izjavljam, da je naloga, ki sem jo oddala v elektronski obliki, identična tiskani verziji.

Katja Klančnik

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IJ	sl
JI	sl/en
AI	Rastlinske vrste, ki rastejo v habitatih vzdolž hidrološkega gradienta presihajočega jezera, imajo različne optične lastnosti, ki so posledica sprememb okolja in njihove evolucijske starosti. V okviru doktorske disertacije smo merili odbojne in transmisijske spektre na ravni listov in rastlinskih sestojev. Spremljali smo morfološke in biokemijske lastnosti listov, vsebnosti silicijevih fitolitov in elementov Si, Ca, Mg ter Al. Z metodami mikro-PIXE in LEXRF smo spremljali porazdelitve in koncentracije elementov v rastlinskih tkivih in celicah. Ugotovili smo, da listi submerznih rastlin odbijajo in prepuščajo več sevanja kot listi plavajočih in emergentnih rastlin. Na optične lastnosti submerznih listov je vplivala tudi prisotnost epifitona, ki absorbira sevanje v UV, vijoličnem in modrem delu spektra. Optične lastnosti različnih tipov listov pri amfibijskih rastlinskih vrstah so se razlikovale. Variabilnost pri različnih tipih listov je bila večja znotraj posamezne vrste, v primerjavi z optičnimi lastnostmi istega tipa lista različnih vrst. Strukture na površini lista so imele na optične lastnosti večji vpliv kot biokemijske lastnosti listov. Pri travah in šaših so imele velik vpliv bodičke in silicificirane strukture na ali blizu listne površine. Pri travah so bodičke poleg Si vsebovale tudi Al, kar je povečalo odboj kratkovalovnega sevanja. Vsebnost silicijevih fitolitov in vsebnost Si je vplivala na odbojnost in prepustnost pri graminoidih, medtem ko je na prepustnost listov poleg Si vplivala tudi vsebnost Ca. Na odboj sevanja v rastlinskih sestojih je poleg lastnosti listov prevladujoče rastlinske vrste vplivala tudi struktura sestoja, zlasti njegova homogenost in naklon listov na rastlinah, ki tvorijo sestoj.

KEY WORDS DOCUMENTATION

DN Dd

DC UDK 581.5(043.3)=163.6

CX Leaf optical properties/reflectance/transmittance/hydrological gradient/silicon/phytoliths/micro-PIXE/LEXRF

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TI OPTICAL PROPERTIES OF PLANTS ALONG THE HYDROLOGICAL GRADIENT OF INTERMITTENT LAKE

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AB Optical properties of the plant species, thriving in habitats along the hydrological gradient of intermittent lake, differed due to changes of environmental conditions and their evolutionary age. In the framework of doctoral thesis we measured reflectance and transmittance spectra of plant leaves and stands. We examineded morphological and biochemical properties of leaves, silica phytoliths and elements i.e. Si, Ca, Mg and Al. Using micro-PIXE and LEXRF methods we determined localisation patterns and concentrations of elements in plant tissues and cells. Our results showed that leaves of submerged plants reflected and transmitted more incoming radiation than those of natant and emerged specimens. Optical properties of submerged leaves were also affected by epiphyton, which absorbed radiation in the UV, violet and blue ranges of spectra. Optical properties of different leaf types in amphibious plant species differed significantly. The variability of leaf optical properties of different leaf types was greater within species than of the same leaf type comparing different species. The structures at the leaf surface, if present, influenced leaf optical properties more than biochemical properties of the leaves. In the case of grasses and sedge optical properties were mainly affected by prickle hairs and silicified structures at or near-leaf surface. In grasses the prickle hairs contained besides Si also Al, which increased reflection of the short-wavelength radiation. The content of Si phytoliths and content of Si also affected the reflectance and transmittance spectra of graminoids, while the transmittance spectra were affected also by Ca content. The reflectance spectra in the plant stands were affected by leaf properties of prevailing plant species and stand structure namely stand homogeneity and leaf angle.

KAZALO VSEBINE

KAZALO SLIK

KAZALO PRILOG

Priloga: Dovoljenji za uporabo znanstvenih člankov v doktorski disertaciji

OKRAJŠAVE IN SIMBOLI

LEXRF	nizko energijska rentgensko fluorescenčna spektomikroskopija (angl. *low energy X-ray fluorescence spectromicroscopy*)
LMA	masa lista na površino (angl. *leaf mass per area*)
mikro-PIXE	protonsko inducirana emisija rentgenskih žarkov z visokoenergijskim fokusiranim žarkom (angl. *particle induced micro-X-ray fluorescence emission*)
NIR	bližnje infrardeče sevanje (angl. *near-infrared radiation*)
RDA	redundančna analiza (angl. *redundancy analysis*)
SLA	specifična listna površina (angl. *specific leaf area*)
UV	ultravijolično sevanje
UV-A	ultravijolično-A sevanje
UV-B	ultravijolično-B sevanje
XRF	rentgenska fluorescenčna spektrometrija (angl. *X-ray fluorescence spectrometry*)

1 UVOD

Življenje na Zemlji je odvisno od sončevega sevanja, ki dovaja energijo in ustvarja razmere, ugodne za življenje. Na zemeljskem površju fotoni zadenejo rastline in prihaja do interakcij sevanja z rastlinskimi organi (Larcher, 2003). Te interakcije so na eni strani odvisne od valovnih dolžin sevanja, na drugi strani pa od optičnih lastnosti rastlin, ki jih opisujemo z odbojnostjo, absorptivnostjo in prepustnostjo (Wooley, 1971; Chandrasekharan, 2005). Sprva so raziskave optičnih lastnosti rastlin potekale predvsem na ravni lista, kasneje pa je zanimanje za optične lastnosti prešlo na raven celotne rastline in rastlinskih sestojev.

V zadnjih desetletjih se je razširila uporaba slikovne spektroskopije, kjer lahko z meritvami odbojnih spektrov rastlin dobimo številne informacije o stanju vegetacije določnega območja (Nordberg in Evertson, 2003; Zarco-Tejada in Sepulcre-Cantó, 2007; Ren in sod., 2008). Tolmačenje informacij, pridobljenih na ta način, pa je močno odvisno tudi od poznavanja optičnih lastnosti na ravni lista ali posamezne rastline (Milton in sod., 2009).

Osnovnega znanja o optičnih lastnostih listov in rastlin je veliko. Izkazalo se je, da so optične lastnosti rastlin kompleksen skupek vplivov najrazličnejših fizičnih lastnosti, pa vendar se veliko raziskav omeji na delovanje posamezne ali le nekaj lastnosti hkrati. Za učinkovito ugotavljanje vplivov lastnosti listov in rastlin na njihove interakcije s sevanjem je potreben celosten pristop, ki zajema čim večji nabor možnih dejavnikov in ustvarja ustrezno zbirko informacij o interakcijah, ki bodo podpora interpretaciji podatkov, pridobljenih na višjih ravneh, npr. s slikovno spektroskopijo.

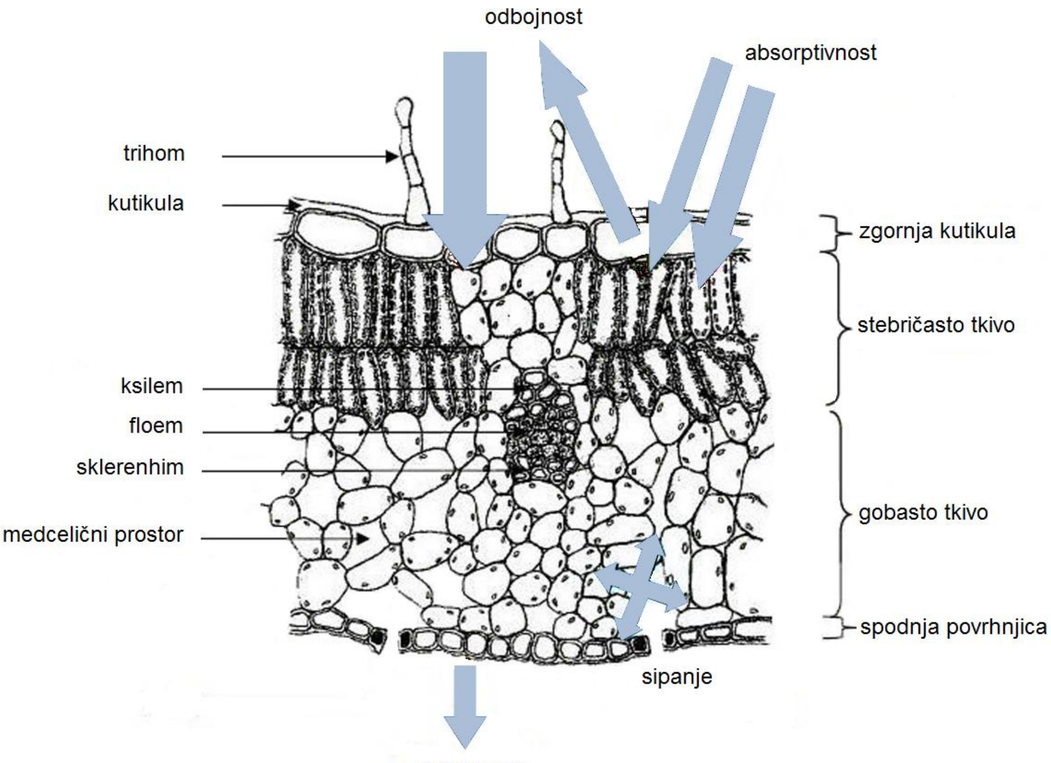
Kljub velikim obetom o uporabnosti daljinskega zaznavanja optičnih lastnosti rastlin, so meritve v bližini sestoja in na ravni lista še vedno nepogrešljive za ustrezno interpretacijo podatkov, pridobljenih z daljinskim merjenjem.

2 PREGLED OBJAV

2.1 OPTIČNE LASTNOSTI RASTLIN

2.1.1 Optične lastnosti listov

V povprečju 47 % sončevega sevanja doseže zemeljsko površje (Larcher, 2003), kjer prihaja do interakcij sevanja z vegetacijo. Ob stiku fotonov z listno površino se del teh odbije, preostanek pa vstopa v notranjost lista. V notranjosti lista se nato del fotonov absorbira, del fotonov pa prehaja skozi list in izstopa nazaj v okolico (Slika 1) (Liew in sod., 2008). Usoda fotonov ob stiku z listno površino je odvisna od njihove valovne dolžine, anatomskih in biokemijskih značilnosti lista ter vsebnosti vode v tkivih (Wooley, 1971; Yoshimura in sod., 2010; Buschmann in sod., 2012; Klančnik in sod., 2013, 2014). Tako lahko tudi pri rastlinskih organih govorimo o njihovih optičnih lastnostih, ki jih opisujejo odbojnost/refleksivnost ρ (λ), absorptivnost α (λ) in prepustnost/transmisivnost τ (λ) (Chandrasekharan, 2005). Razmerje med komponentami optičnih lastnosti opisuje enačba: $\rho(\lambda) + \tau(\lambda) + \alpha(\lambda) = 1$, kjer velja da sta odbojnost in prepustnost obratno sorazmerni z absorptivnostjo (Liew in sod., 2008; Yoshimura, 2010).



Slika 1: Interakcija sevanja z listnim tkivom (prirejeno po Liew in sod., 2008).
Figure 1: The interaction of light with leaf tissue (adapted after Liew et al., 2008).

Na listni površini prihaja do zrcalnega in razpršenega odboja fotonov. V kolikšni meri se sevanje odbija zrcalno ali razpršeno, je odvisno od vpadnega kota fotonov, strukturiranosti listne površine (kutikule, trihomov) in razlik v lomnih količnikih med mejnima plastema - zrakom in listno površino. Pri svetlečih listih prevladuje zrcalni odboj, bolj kot so listi na videz nesvetleči, večji je delež razpršenega odboja (Wooley, 1971). Razpršeni odboj se poveča, kadar so na listih prisotne fine strukture, ki so manjše od valovnih dolžin sevanja in učinkovito razpršijo zlasti sevanje krajših valovnih dolžin, kar je povezano s t.i. Reyleighovim sipanjem (Grant, 1987). Fotoni, ki vstopijo v notranjost lista, se absorbirajo na pigmentnih molekulah, preostanek fotonov pa je razpršen. Sevanje se na prehodih med vlažnimi celičnimi stenami in zračnimi prostori odbija in lomi (Knipling, 1970). Takšno sipanje svetlobe je v listih pomembno, saj podaljšuje pot potovanja fotonov znotraj lista in poveča možnost njihove absorpcije na pigmentnih molekulah. V povprečju je tako pot potovanja fotonov skozi list kar štirikrat daljša, kot če bi sevanje prehajalo navpično skozi list (Richter in Fukshansky, 1996). Površina prehodov med celičnimi stenami in medceličnimi prostori vpliva na količino sipanja fotonov znotraj lista, pri čemer sta pomembni plasti stebričastega in gobastega tkiva v mezofilu. Število plasti celic, ki sestavljajo stebričasto tkivo, se med rastlinami razlikuje in je odvisno od svetlobnih razmer (Larcher, 2003). Podolgovate celice stebričastega tkiva vsebujejo številne kloroplaste, med plastmi celic pa se ustvarjajo majhni zračni prostori, ki predstavljajo 5-20 % volumna tkiva (Woolley, 1971). Stebričasto tkivo deluje kot neke vrste sito, saj klorofil znotraj celic ni enakomerno razporejen, ampak je omejen v kloroplastih. Med molekulami klorofila tako prihaja tudi do senčenja, zaradi česar je absorpcija svetlobe v kloroplastih manjša, kot bi bila pri enaki količini klorofila enakomerno porazdeljenega v raztopini (Taiz in Zeiger, 2002). Med kloroplasti, ki predstavljajo svetlobne ponore, nastajajo prehodi, skozi katere lahko neabsorbirana svetloba prodira tudi v spodaj ležeče gobasto tkivo. To je sestavljeno pretežno iz celic nepravilnih oblik, ki jih obdaja veliko zračnih prostorov. Zračni prostori predstavljajo 50-80 % volumna gobastega tkiva, vsebnost kloroplastov v celicah pa je manjša kot v stebričastem tkivu (Woolley, 1971). Razlike v strukturi listov na zgornji in spodnji strani se odražajo tudi v odbojnih spektrih, na podlagi katerih lahko razlikujemo zgornjo in spodnjo stran lista, medtem ko je prepustnost merjena na zgornji ali spodnji strani lista bolj ali manj enaka (Wooley, 1971; Asner in Wessmann, 1997).

V splošnem so si optične lastnosti zelenih listov višjih rastlin podobne. Kljub temu pa se listi razlikujejo med sabo po zgradbi in kemični sestavi. Kompleksnost in specifične razlike v zgradbi listov se odražajo tudi v nekoliko različnih optičnih lastnostih, kar je osnova za prepoznavanje rastlinskih vrst na podlagi t.i. »spektralnih podpisov« (Knapp in Carter, 1998; Artigas and Yang, 2005; Castro-Esau, 2006).

Rastline večino kratkovalovnega ultravijoličnega (UV) sevanja absorbirajo. Voskasta kutikula, zunanje plasti povrhnjice s suberinom in fenolne snovi, prisotne v zgornjih plasteh lista prestrezajo UV sevanje, tako da največ 2-5 %, navadno pa manj kot 1 %, UV sevanja doseže globlje plasti lista (Woolley, 1971; Pfündel in sod., 2006). Zaradi velike absorpcije sta odbojnost in prepustnost kratkovalovnega sevanja majhni; odbojnost redko presega 10 %, prepustnost pa je praktično zanemarljiva (Yoshimura in sod., 2010). Izjeme so lahko listi z voski na površini, ki jim dajejo zeleno-modrikast videz. Takšni listi lahko odbijejo tudi med 20 in 35 % prejetega UV sevanja (Reicosky in Hanover, 1978; Robberecht in sod., 1980; Holmes in Keiller, 2002). Voski na površini listov sukulentnih rastlin, ki imajo nesvetleče površine, prav tako prispevajo k povečanemu odboju UV sevanja, vendar pa hkrati vplivajo tudi na zmanjšano absorpcijo sevanja v vidnem delu spektra (Mulroy, 1979; Barker in sod., 1997). Na povečan odboj UV sevanja lahko vpliva tudi prisotnost trihomov na listni površini, v kolikor ti ne vsebujejo UV-absorbirajočih substanc (Ehleringer in Björkman, 1978; Robberecht in sod., 1980).

V vidnem delu spektra se večina prejetih fotonov navadno absorbira. Absorpcija je zlasti velika v modrem (~ 450 nm) in rdečem delu spektra (~ 680 nm), najmanjša pa v zelenem delu spektra (~ 550 nm) (Klančnik in sod., 2012, 2013). Velika absorpcija v rdečem in modrem delu je odvisna predvsem od klorofilov (*a* in *b*), ksantofilov in karotenov (Knipling, 1970; Wooley, 1971; Vogelmann, 1993; Baltzer in Thomas, 2005). K absorpciji sevanja v modrem delu spektra nekoliko prispevajo tudi antociani, ki pa znatno povečujejo absorpcijo sevanja v zelenem in rumenen delu spektra (Neill in Gould, 1999; 2003). V splošnem listi odbijejo le 5-15 % sevanja v fotosintetzno aktivnem delu spektra (PAR) (Asner in Wessman, 1997; Pfündel in sod., 2006). Kot že ime pove, se absorbirani fotoni PAR porabijo v procesih fotosinteze, del fotonov pa se lahko znotraj lista pretvori v toploto ali fluorescenco (Taiz in Zeiger, 2002). Pojav fluorescence lahko prispeva k večji izmerjeni odbojnosti od dejanske, vendar pa ne presega nekaj odstotkov (Zarco-Tejada in sod., 2000). Vpliv vsebnosti barvil na odbojnost sevanja v vidnem delu spektra je osnova uporabe odbojnih spektrov pri vrednotenju stresa pri rastlinah (Carter in Knapp, 2001; Pandey in Gopal, 2011). Delovanje različnih stresnih dejavnikov na rastline se odrazi v zmanjšani produkciji fotosintetskih pigmentov in posledično povečani odbojnosti in prepustnosti v predelu PAR (Mariotti in sod., 1996; Carter in Knapp, 2001). Na podlagi optičnih lastnosti so bile razvite tudi različne metode za ocene skupne koncentracije klorofilov in razmerij med klorofili in karotenoidi (Gitelson in Merzlyak, 1994; Peñuelas in Filella, 1998; Sims in Gamon, 2002). Metode za ocene vsebnosti pigmentov na podlagi odbojnih spektrov se pogosto uporabljajo v eko-fizioloških raziskavah, saj veljajo za preporste, hitre in nedestruktivne metode (Carter and Knapp, 2001; Richardson et al., 2001; Stylinski et al., 2002).

Z daljšanjem valovnih dolžin sevanja, se v infrardečem delu spektra absorpcija sevanja znatno spremeni. V bližnjem infrardečem delu spektra (700-1300 nm) se absorpcija sevanja zmanjša na le nekaj odstotkov, na drugi strani se odbojnost in prepustnost povečata in skupaj približata 100 % (Gates in sod., 1965; Knipling, 1970; Larcher, 2003). Pri valovnih dolžinah okoli 1000 in 1200 nm, se odbojnost in prepustnost nekoliko zmanjšata na račun povečane absorpcije v vodnih molekulah v listih (Allen in Richardson, 1968; Gao in Goetz, 1995; Baldocchi, 2010). Ostale sestavine tkiv, kot so celuloza, lignin in proteini, pa absorbirajo pri valovnih dolžinah daljših od 1500 nm (Baldocchi, 2010). Razlike v vsebnosti vode in strukturiranosti mezofila pogosto botrujejo največjim razlikam med spektralnimi krivuljami različnih listov prav v predelu infrardečega sevanja (Asner in Wessman, 1997; Asner 1998; Artigas in Yang, 2005).

2.1.2 Optične lastnosti rastlinskih sestojev

Sprva so raziskave interakcij svetlobe z rastlinami potekale na ravni listov. Razvoj tehnologije v zadnjih dvajsetih letih pa je omogočil merjenje optičnih lastnosti rastlin tudi izven laboratorijev. Z razvojem prenosnih spektrofotometrov je zanimanje o interakcijah med energijo sevanja in rastlinskimi organi prešlo tudi na raven celotnih rastlin in rastlinskih sestojev (Gamon in sod., 2006; Milton in sod, 2009). Zajem informacij lahko poteka na tleh ter v neposredni bližini rastline ali rastlinskega sestoja. Ta način spektroskopskih meritev lahko imenujemo terenska spektroskopija (angl. *field spectroscopy*) (Gao in sod., 2009). Drugi razširjeni način zajemanja podatkov pa poteka na daljavo, največkrat s pomočjo letal ali satelitov, redkeje s pomočjo helikopterjev ali balonov. Pri tem načinu zajema podatkov dobimo spektralne slike večjih območij, zato to metodologijo imenujemo slikovna spektroskopija (angl. *imaging spectroscopy*) (Milton in sod., 2009). Uporaba slikovne spektroskopije je v zadnjem času zelo razširjena, saj omogoča hitro pridobivanje informacij na večjem območju. Vse zmogljivejši spektrofotometri, ki dajejo natančne posnetke z veliko ločljivostjo, obetajo veliko uporabno vrednost pri različnih eko-fizioloških raziskavah, npr. mapiranju vegetacije, spremljanju sprememb pokrovnosti tal z vegetacijo, sledenje motenj vegetacije idr. (Langley in sod., 2001; Nordberg in Evertson, 2003; Zarco-Tejada in Sepulcre-Cantó, 2007; Ren in sod., 2008). Vendar pa je tudi terenska spektroskopija pri tem nepogrešljiva zlasti za umerjanje senzorjev in pridobivanje osnovnih informacij, ki so ključnega pomena za pridobivanje in tolmačenje podatkov na višjih ravneh, kot je slikovna spektroskopija (Milton in sod., 2009).

Optične lastnosti cele rastline in rastlinskih sestojev niso odvisne le od lastnosti listov, temveč tudi od zgradbe rastline oz. sestoja (Ross, 1981; Yoder in Pettigrew-Crosby, 1995). Zgradba rastline zajema lastnosti, ki določajo njeno velikost, obliko in izgled. Od zgradbe sestoja je odvisen tudi prispevek tal k odboju sevanja (Asner, 1998; Gao in Zhang, 2006). Zaradi hkratnih vplivov mnogih lastnosti so interakcije med sevanjem in rastlinskimi sestoji lahko zelo kompleksne.

Gosti rastlinski sestoji absorbirajo 60-90 % sevanja, 10-30 % ga odbijejo, le 5-10 % sončevega sevanja pa prehaja skozi sestoj do tal (Ross, 1981; Larcher, 2003). Od celotne količine absorbiranega sevanja, ga 80-90 % absorbirajo listi, preostalo pa drugi nadzemni rastlinski organi. Pri tem ima pomembno vlogo orientacija in naklon listov, njihova oblika in velikost ter optične lastnosti listov (Ross, 1981; Asner, 1998). Tudi stebla absorbirajo sevanje v sestoju in v predelu PAR lahko absorbirajo do 95 % prejetih fotonov. Vpliv stebel na absorpcijo sevanja je pomemben zlasti v sestojih z manjšim indeksom listne površine (Asner in Wessman, 1997).

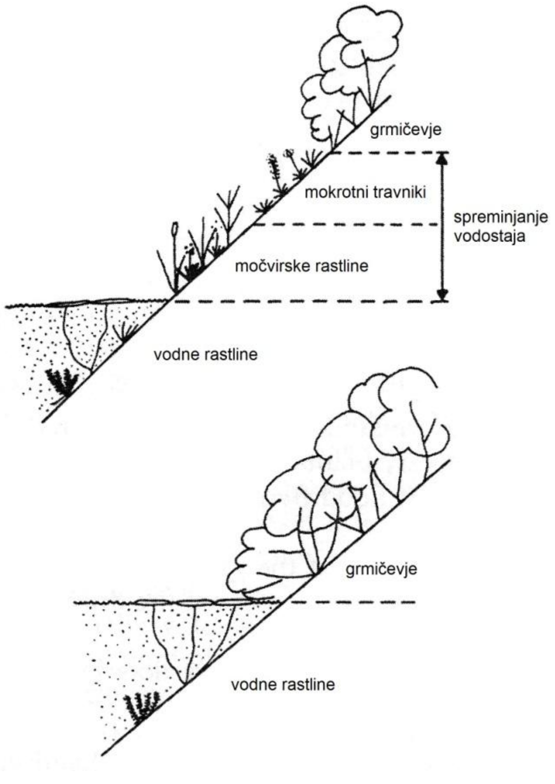
Spektralne meritve pri rastlinskih sestojih v naravi se v veliki večini nanašajo na merjenje njihovih odbojnih spektrov. Odboj sevanja v UV delu spektra je v sestoju navadno majhen (<10 %) (Gates in sod., 1965). Prav tako je v vidnem delu spektra odbojnost vitalnega sestoja majhna in ne presega 20 % (Gilmore in sod., 2008). Povečan odboj v vidnem delu spektra lahko kaže na prisotnost stresa pri rastlinah (Filella in Peñuelas, 1999; Mutanga in sod., 2003; Imanishi in sod., 2004), prav tako je povečan odboj in poenotenje odboja v tem delu spektra lahko posledica senescence sestoja (Gao in Zhang, 2006). V predelu NIR in IR se od rastlin odbije okoli 60 % vpadnega sevanja (Gilmore in sod., 2008). Nekatere študije so dokazale korelacijo med odbojnostjo v predelu NIR in biomaso rastlin (Hardisky in sod., 1986; Gross in sod., 1993; Zhang in sod., 1997).

2.2 PRESIHAJOČE JEZERO

Presihajoče jezero je poseben tip ekosistema, ki ni ne pravo jezero in ne pravo mokrišče. Poglaviten dejavnik, ki pogojuje njegovo zgradbo in delovanje je spremenljiv vodni režim z izmenjavo mokrih in suhih obdobij (Gaberščik in Urbanc-Berčič, 2003). Presihanje oblikuje edinstvene fizikalne in kemijske razmere, ki naredijo te ekosisteme drugačne tako od kopenskih kot stalnih vodnih okolij. Posredno in neposredno vpliva na oblikovanje vrstne sestave življenjskih združb, primarno produktivnost, kroženje snovi in dostopnost hranil v sistemu (Mitsch in sod., 2009).

Raznolike hidrološke razmere opazno vplivajo na biogeokemično kroženje snovi v mokriščih. Kadar so tla nasičena z vodo nastanejo redukcijske razmere, ki povzročajo spremembe v dostopnosti in koncentracijah hranilnih snovi, esencialnih za rastline (Cronk in Fennesy, 2001; Pezeshki, 2001). Mikroorganizmi imajo ključno vlogo pri dekompoziciji in kroženju hranil tudi v mokriščih. Zaradi specifičnih razmer je dekompozicija v mokriščih navadno upočasnjena oz. se spreminja glede na prisotnost vode. Ob prehodni izsušitvi jezera se ob povečani prisotnosti kisika v tleh razgradnja znatno pospeši (Boulton in Brock, 1999; Gaberščik in Urbanc-Berčič, 2003). Ob ponovni namočitvi pa prihaja do sproščanja hranilnih snovi, kar omogoča bujno rast rastlinam (Gaberščik in Urbanc-Berčič, 2003). Tudi rastline lahko ugodno vplivajo na kroženje snovi v sistemu. Prehajanje kisika iz korenin v rizosfero namreč pospeši dekompozicijo organskih snovi v prezračenih tleh v bližini korenin (Cronk in Fennesy, 2001).

Spreminjajoči se hidrološki režim je gonilna sila procesov v presihajočih jezerih, hkrati pa predstavlja omejujoč dejavnik za organizme. Rastline v spremenljivem okolju imajo številne prilagoditve in življenjske strategije, ki jim omogočajo preživetje v poplavljenih ali z vodo nasičenih tleh (Cronk in Fennesy, 2001). Strpnost organizmov na spremembe vodostaja vpliva na razporeditev rastlinskih vrst vzdolž gradienta med vodo in kopnim. Pojavnost rastlinskih vrst je odvisna od morfoloških in fizioloških prilagoditev, ki omejujejo izgubo kisika zaradi poplavljanja, na drugi strani pa omogočajo preživetje v primeru izsušitve (Rudolf, 2008). Pri mokriščih, kjer ni večjih sprememb vodostaja, je meja med uspevanjem vodnih in kopenskih rastlin jasna. V presihajočih sistemih pa spremembe vodostaja oblikujejo več con pojavljanja rastlin. Med vodnimi in kopenskimi rastlinami se pojavijo še močvirske rastline in rastline mokrotnih travnikov (Slika 2), tako je vrstna pestrost združb v sistemih s spreminjajočim se vodostajem lahko večja (Keddy, 2000).



Slika 2: Cone pojavljanja rastlin vzdolž gradienta med vodo in kopnim v mokriščih s spreminjajočim in stalnim vodostajem (povzeto po Keddy, 1990a, b).
Figure 2: Plant zones along water-dryland gradient in wetlands with fluctuating and stabilizing water levels (adapted after Keddy, 1990a, b).

Presihajoče Cerkniško jezero je poznano v svetovnem merilu, saj predstavlja *locus typicus* za presihajoča jezera in kraška polja. Presihanje in polnjenje jezera omogočajo posebne klimatske, hidrološke in geomorfološke značilnosti območja (Gaberščik in Urbanc-Berčič, 2003). Jezero se običajno pojavi dvakrat letno, spomladi in pozno jeseni oz. pozimi, presahne pa najpogosteje med avgustom in oktobrom. Cerkniško polje je napolnjeno z vodo povprečno dobrih devet mesecev letno, preostali del leta pa je kraško polje suho (Krajnc, 2003). Hidrološka variabilnost območja Cerkniškega polja z izmenjavo poplav in suš pogojuje raznolikost habitatov in združb. Življenjski cikli organizmov so povezani s periodičnostjo spreminjanja vodostaja. Občasne nepredvidljive spremembe, ki ne sovpadajo s sezonsko dinamiko organizmov, pa dodatno vplivajo na pojavljanje rastlinskih vrst (Gaberščik in sod., 2003). S skrajnostjo vodnih razmer Cerkniško jezero predstavlja odličen modelni sistem, ki omogoča opazovanje prožnosti različnih rastlinskih vrst v času in prostoru, kot tudi rastlinskih sestojev v habitatih vzdolž hidrološkega gradienta.

Mokrišča in močvirska vegetacija so pogosto predmet raziskav v študijah o optičnih lastnosti rastlin (Artigas in Yang, 2005; Laba in sod., 2005; Gao in Zhang, 2006; Gilmore in sod., 2008; Adam in Mutanga, 2009). Prednost mokrišč je navadno ravninska topografija in pojavljanje obsežnih, homogenih rastlinskih sestojev. Velika pokrovnost posameznih rastlinskih vrst omogoča daljinsko zaznavanje (angl. *remote sensing*) vrstne sestave mokrišč (Gao in Zhang, 2006; Adam in Mutanga, 2009), spremljanje vitalnosti vegetacije (Artigas in Yang, 2005), dinamiko širjenja invazivnih rastlinskih vrst (Artigas in Yang, 2005; Laba in sod., 2005) in posredno spremljanje sprememb ter stanja mokrišč (Gilmore in sod., 2008). Ker mokrišča pogosto predstavljajo težje dostopen teren, je možnost pridobivanja omenjenih informacij na daljavo še toliko večjega pomena.

2.3 ZNAČILNOSTI RASTLIN RAZLIČNIH EKOLOŠKIH SKUPIN, KI USPEVAJO V HABITATIH VZDOLŽ HIDROLOŠKEGA GRADIENTA

2.3.1 Submerzni in natantni makrofiti

Rastline, katerih življenjski cikel je vsaj deloma vezan na vodno okolje, združujemo pod imenom makrofiti. Glede na položaj rastline v vodnem stolpcu, habitus in način pritrditve, pa makrofite delimo na več različnih podskupin (Hutchinson, 1975; Fox, 1992).

Submerzni in natantni makrofiti so vrste vodnih rastlin, katerih organi uspevajo v celoti v vodi, bodisi da so potopljeni ali pa plavajoči na vodni površini. Pri teh rastlinskih vrstah so morfološko-anatomske in fiziološke prilagoditve na vodno okolje najbolj izrazite, saj za razliko od ostalih makrofitov celoten življenjski cikel preživijo neposredno v vodi (Cronk in Fennessy, 2001).

Življenje v vodi na eni strani zahteva razvoj posebnih struktur, na drugi strani pa se določene strukture reducirajo. Pri potopljenih listih je prisotna le tanka kutikula, ki je lahko tudi povsem odsotna. Na listni površini so prisotne hidropote – predeli stanjšane kutikule za olajšano izmenjavo snovi z vodnim medijem. Listne reže, če so prisotne, so nefunkcionalne. Povrhnjica je enoplastna in pogosto vsebuje tudi kloroplaste za učinkovitejše prestrezanje svetlobe. Mezofil lista je navadno tanek in enotno strukturiran (Rascio in sod., 1999; Klančnik in sod., 2012, 2013). Značilna prilagoditev potopljenih listov je razvoj aerenhima, zračnega tkiva, z velikimi intercelularji. Obsežen sistem zračnih prostorov služi za izmenjavo plinov po rastlini, hkrati pa z zmanjševanjem specifične listne teže pripomore k povečevanju vzgona in večji plovnosti rastlinskih organov v vodnem stolpcu (Hroudová in sod., 1988; Robe in Griffiths, 1998). Obsežni zračni prostori pa lahko vplivajo tudi na optične lastnosti listov (Zimmerman, 2006).

Listi potopljenih makrofitov v vodi ne potrebujejo obsežnih opornih tkiv. Lignin je odsoten, zato so listi mehkejši in bolj elastični (Cronk in Fennessy, 2001). Potopljeni listi so pogosto trakasti ali drobno razcepljeni, kar povečuje razmerje med prostornino in listno površino (Hutchinson, 1975; Rascio et al., 1999; Germ and Gaberščik, 2003).

Za razliko od potopljenih listov imajo plavajoči listi nekoliko bolj razvita oporna tkiva, so debelejši in okrogle, ovalne ali srčaste oblike (Cronk in Fennessy, 2001). Plavajoči listi se od potopljenih razlikujejo tudi po debeli voskasti kutikuli, ki preprečuje zastajanje vode na listni površini in ščiti pred herbivori (Guntespergen in sod., 1989). Hkrati lahko kutikula poveča odboj sevanja z listne površine (Riederer in Müller, 2006).

Povrhnjica plavajočih listov je navadno enoplastna, vendar ima debele celične stene. Listne reže so dobro razvite in funkcionalne, vendar se pojavljajo le na zgornji strani lista. Mezofil lista je diferenciran v stebričasto in gobasto tkivo z aerenhimom. Prevajalna in oporna tkiva so reducirana, v listih pa so lahko prisotni kristali kalcijevega oksalata, ki povečujejo čvrstost lista. V listih vodnih rastlin pa se lahko odlaga tudi silicij, predvsem v predelu povrhnjice in ksilema (Borrelli in sod., 2011).

Svetlobne razmere v vodnem okolju so močno spremenjene v primerjavi s kopnim. Svetlobni spekter se v vodi spremeni kakovostno in količinsko (Cronk in Fennessy, makrofitov v vodnih okoljih (Madsen in Adams, 1989; Sand-Jensen in Borum, 1991).

Sevanje, ki doseže vodno površino, se deloma od nje odbije. Odboj sevanja je odvisen od vpadnega kota žarkov, od vzvalovanosti vodne površine in nadmorske višine (Cronk in Fennessy, 2001). Zaradi odboja od vodne površine so plavajoči listi v nasprotju s potopljenimi izpostavljeni povečanim jakostim sevanja. Delež sevanja, ki prehaja v vodo, se odbija ali pa absorbira na vodnih molekulah, raztopljenih organskih in anorganskih snoveh ter suspendiranih delcih (Holmes in Klein, 1987; Cronk in Fennessy, 2001).

Spremenjene svetlobne razmere v vodi se odražajo tudi v biokemijskih lastnostih potopljenih listov, ki se razlikujejo od natantnih in emergentnih listov. Absorbcija sevanja pri potopljenih listih je manjša v primerjavi s plavajočimi listi (Frost-Christensen in Sand-Jensen, 1995). Potopljeni listi navadno vsebujejo manj klorofila in karotenoidov na enoto površine, razmerje med klorofili in karotenoidi pa se poveča. Tudi število kloroplastov na enoto površine je pri potopljenih listih manjše (Ronzhina in sod., 2004; Klančnik in sod., 2012; Nedukha, 2013). Nasprotno pa Kirk (1994) poroča, da potopljeni listi lahko vsebujejo več klorofila kot odziv na slabše svetlobne razmere, v katerih rastejo v primerjavi z natantnimi in emergentnimi listi. Primerjava potopljenih in plavajočih listov plavajočega blatnika (*Nuphar luteum*) je pokazala prilagoditve celotnega fotosinteznega aparata potopljenih listov na šibkejše jakosti sevanja. Potopljeni listi so imeli značilne lastnosti listov, prilagojenih na senčne razmere, kar jim zagotavlja učinkovit potek fotosinteze tudi v slabših svetlobnih razmerah v primerjavi s plavajočimi listi in listi nad vodo (Kordyum in Klimenko, 2013).

2.3.2 Amfibijske rastline

Posebna skupina rastlin, ki se pojavlja v habitatih vzdolž hidrološkega gradienta, so rastlinske vrste z amfibijskim značajem. Amfibijske rastline so sposobne preživeti tako v vodi kot na kopnem in uspešno kljubujejo izmenjavi mokrih in suhih obdobij (Martinčič, 2002; Gaberščik in Urbanc-Berčič, 2003; Urbanc-Berčič in sod., 2005).

Amfibijske rastline so v spremenljivem okolju razvile številne prilagoditve, kot so sprememba rastne oblike, razvoj različnih tipov listov in načina reprodukcije (Braendle in Crawford, 1999). Značilna za amfibijske rastline je velika fenotipska prožnost, saj se rastline z enakim genotipom lahko v različnih okoljih fenotipsko zelo razlikujejo (Robe in Griffiths, 2000; Rezakhanlo in Talebi, 2010). Sprememba rastne oblike, ki nastane kot posledica vodnega režima, zahteva veliko fenotipsko prožnost rastline. Določene vrste amfibijskih rastlin razvijejo samo eno vrsto listov, ki pa lahko preživijo v vodi in na kopnem, pojav imenujejo homofilija. Določene rastline pa razvijejo različne liste prilagojene bodisi na vodno ali na kopensko okolje, kar imenujemo heterofilija (Braendle and Crawford, 1999). Pojem heterofilija v ožjem smislu se nanaša na en osebek in označuje pojavljanje dveh ali več različnih tipov listov na eni rastlini. V širšem smislu pa lahko označuje pojavljanje različnih tipov listov na dveh različnih rastlinah iste vrste (Hutchinson, 1975).

Razvoj večine amfibijskih rastlin se začne v vodi, nato pa sčasoma z upadanjem vodne gladine zgornji deli poganjkov sežejo nad vodno gladino in razvijejo se kopenski poganjki (Martinčič, 2002). Listi kopenskih poganjkov so navadno debelejši, imajo manj medceličnih prostorov in dosegajo večjo fotosintezo (Torres Boeger in Poulson, 2003). Listi nad vodo imajo tudi zaščitne strukture, kot so voski in trihomi, ki preprečujejo prekomerno izgubo vode (Gaberščik, 1991). Primerjava različnih tipov listov pri vrsti navadna streluša (*Sagittaria sagittifolia*) je pokazala, da potopljeni listi vsebujejo znatno manj klorofilov, razmerje med klorofilom *a* in *b* je manjše v primerjavi z listi nad vodo (Nedukha, 2013). Pri močvirski spominčici (*Myosotis scorpioides* agg.) in lasatolistni vodni zlatici (*Ranunculus trichophyllus* Chaix), se vsebnost klorofila in razmerje med klorofili *a* in *b* niso bistveno razlikovali (Germ in Gaberščik, 2003).

Heterofilne vodne rastline predstavljajo dober modelni sistem za opazovanje spreminjanja lastnosti, saj lahko znotraj ene rastlinske vrste proučujemo različne oblike listov in rastnih oblik, ki se razvijejo v različnih okoljskih razmerah (Braendle and Crawford, 1999). Na območju Cerkniškega jezera uspevajo številne amfibijske rastlinske vrste, najpogostejše so prava potočarka (*Rorippa amphibia*), močvirska spominčica (*Myosotis scorpioides*), vodna meta (*Mentha aquatica*), širokolistna koščica (*Sium latifolium*), rumeni blatnik (*Nuphar luteum*) in vodna dresen (*Polygonum amphibium*).

2.3.3 Močvirske rastline in rastline mokrotnih travnikov

Močvirske rastline, imenovane tudi helofiti, imajo prilagoditve, ki jim omogočajo preživetje v z vodo nasičenih tleh, saj so ukoreninjene v tleh, nasičenih z vodo. Njihovi asimilacijski in razmnoževalni organi pa se razvijejo nad vodno gladino. V splošnem so podobne kopenskim rastlinam, njihov koreninski sistem pa je prilagojen na vsaj občasno izpostavljenost pomanjkanju kisika (Cronk in Fennesy, 2001).

V močvirjih s prosto vodno površino helofiti lahko prestrežejo svetlobo preden le-ta doseže vodno gladino, zato lahko pogosto prevladajo nad natantnimi in submerznimi makrofiti. Močvirske rastline imajo pogosto velike liste in bujno rast, saj si lahko privoščijo večje izgube vode z evapotranspiracijo. Zanje je značilno hitro širjenje s pomočjo vegetativnih poganjkov, zato lahko tvorijo obsežne monospecifične sestoje (Cronk in Fennesy, 2001). Mokrišča, kjer prevladujejo emergentni makrofiti, imajo navadno veliko primarno proizvodnjo in spadajo med najbolj produktivne ekosisteme na svetu (Klopatek in Stearns, 1978; Mitsch in Gosselink, 2007; Rocha in Goulden, 2009).

Pogosti predstavniki močvirskih rastlin prihajajo iz družin Poaceae in Cyperaceae, ki jih lahko združujemo tudi pod imenom graminoidi. Trave in ostričevke se na območju Cerkniškega jezera obsežno pojavljajo na obrobju poplavnih območij, ki so poplavljena le ob visokih vodostajih, kljub temu pa so tla večji del leta vlažna. Predstavnika, ki tvorita obsežne močvirske sestoje, sta navadni trst (*Phragmites australis*) in pisanka (*Phalaris arundinacea*), manj obsežne sklenjene sestoje pa tvorijo še različne vrste šašev (*Carex elata, C. riparia, C. rostrata*), močvirskih sit (*Eleocharis palustris*) in jezerskega bička (*Schoenoplectus lacustris*) (Martinčič in Leskovar, 2003). Na območjih, kjer sta trajanje in obseg poplav najmanj obsežna, se pojavljajo rastline mokrotnih travnikov z značilnima travnima vrstama, modro stožko (*Molinia caerulea*) in rušnato mastnico (*Deschampsia cespitosa*). Njihova rastišča so poplavljena le ob visokih vodah, vendar se hitro osušijo, v poletnih mesecih pa so tla navadno zelo suha (Martinčič in Leskovar, 2003).

Za predstavnike graminoidov je značilno, da imajo njihovi listi močno razvito sklerenhimatsko tkivo, v katerem so prisotni različni biominerali, njegova primarna vloga pa je mehanska opora listom (O'Reagain in Mentis, 1989; Motomura in sod., 2004; Hodson in sod., 2005; Motomura in sod., 2006). Najpogosteje zastopani biominerali v rastlinah so kalcijevi in silicijevi vključki (fitoliti) (Bauer in sod., 2011). Predstavniki družin trav in ostričevk so znani kot akumulatorji silicija (Si), saj njegova vsebnost lahko presega tudi 4 % suhe mase rastlin, pri nekaterih vrstah trav pa Si predstavlja celo več kot 10 % suhe mase rastlinskih tkiv (Piperno, 2006; Hodson in sod., 2005). Fitoliti se v rastlinah odlagajo v celicah ali v medceličnih prostorih, pogosto pa se Si veže v obliki silikagela na celulozna vlakna v celičnih stenah (Bauer in sod., 2011). Vsebnost Si v listih je velika v primerjavi z drugimi rastlinskimi organi. Pri travah in ostričevkah se Si pogosto odlaga v celicah opornih tkiv okoli žil, v celičnih stenah povrhnjice in mehurčastih celic, ki so značilne za liste graminoidov (Prychid in sod., 2004; Piperno, 2006; Bauer, 2011). Biominerali se lahko odlagajo tudi v različne strukture na listni površini, kot so kutikula in bodičke. Slednje so togi trihomi z okremenjeno steno, ki ustvarjajo značilno raskavo površino listov trav in travam podobnih rastlin (Jogan, 2002). Glede na to, da med travami najdemo tudi številne ekonomsko pomembne vrste, kot so žita, je vloga kopičenja Si in njegovo pomanjkanje v rastlinah pritegnilo kar nekaj pozornosti raziskovalcev (Ma in sod., 1989; Yeo in sod., 1999; Hossain in sod., 2002; Ma, 2004; Liang in sod., 2005; Liang in sod., 2007; da Cunha in do Nascimento, 2009). Na drugi strani pa je bil vpliv biomineralizacije tkiv na optične lastnosti listov bolj ali manj prezrt, kljub temu, da je delovanje rastline zelo odvisno od interakcij s sevanjem.

2.4 NAMEN DELA

Namen doktorskega dela je bil podrobneje preučiti optične lastnosti rastlinskih vrst, rastočih v habitatih, ki nastanejo vzdolž časovnih in prostorskih gradientov presihajočega jezera. Želeli smo ugotoviti, kako spremembe okolja vzdolž hidrološkega gradienta vplivajo na spremembe morfoloških in biokemijskih lastnosti listov, ki se odražajo na njihovih odbojnih in transmisijskih spektrih. Določili smo lastnosti listov, ki najbolj vplivajo na odbojnost in prepustnost na ravni lista ter na odbojnost sevanja na ravni rastlinskega sestoja. Pri rastlinskih sestojih smo ugotavljali tudi vpliv strukturnih lastnosti sestoja na odbojnost sevanja v njih.

2.5 RAZISKOVALNE HIPOTEZE

- Značilnosti rastlinskih vrst, rastočih v habitatih, ki nastanejo vzdolž časovnih in prostorskih gradientov presihajočega jezera, se odražajo v različnih optičnih lastnostih listov.
- Pri vrstah z amfibijskim značajem pričakujemo znatne razlike v spektralnih podpisih listov rastlin različnih rastnih oblik oz. listov heterofilnih rastlin.
- Lastnosti listov z značilnim vplivom na odbojnost in prepustnost se razlikujejo med vrstami in različnimi oblikami listov.
- Spremembe okoljskih razmer vplivajo na rastline, kar vpliva na absorpcijo sevanja.
- Pri odbojnosti sevanja v rastlinskih sestojih imajo ključno vlogo lastnosti prevladujoče rastlinske vrste.

3 ZNANSTVENI ČLANKI

3.1 EPIFITON VPLIVA NA KOLIČINO IN KAKOVOST SEVANJA, KI GA PRESTREŽEJO LISTI POTOPLJENIH MAKROFITOV

Katja Klančnik, David Gradinjan, Alenka Gaberščik. Epiphyton alters the quantity and quality of radiation captured by leaves in submerged macrophytes. Poslano v Aquatic Botany

Poleg anatomske in biokemijske zgradbe lista, ki določa njegove optične lastnosti, lahko na količino absorbiranega sevanja, vplivajo tudi različne snovi na listni površini, kot sta prah (Nanos in Ilias, 2007) in perifiton (Brandt in Koch, 2003). Asimilacijske površine potopljenih listov predstavljajo potencialno habitat za različne perifitonske združbe (Bécares in sod., 2008), ki jih, kadar so pritrjene na rastlinah, imenujemo tudi epifiton (Azim in sod., 2005).

Namen raziskave je bil preučiti vpliv epifitona ter anatomskih in biokemijskih lastnosti listov na optične lastnosti listov pri treh vrstah potopljenih makrofitov (*Potamogeton lucens, Potamogeton perfoliatus* in *Sagittaria sagittifolia*).

S pomočjo prenosnega spektrofotometra in integracijske sfere smo izmerili odbojne in transmisijske spektre listov v območju valovnih dolžin od 290 do 800 nm. Optične lastnosti smo izmerili na intaktnih listih, na listih z odstranjenim epifitonom z zgornje povrhnjice in v primeru prepustnosti, na listih z odstranjenim epifitonom z zgornje in spodnje povrhnjice. Epifitonu smo določili vrstno sestavo, suho maso in vsebnost klorofila *a* ter izmerili morfološke in biokemijske parametre listov makrofitov.

Na vseh rastlinah so v epifitonskih združbah prevladovale diatomeje vrste *Cocconeis placentula*. Prisotnost epifitona je vplivala na odbojnost in prepustnost listov preučevanih makrofitov. Suha masa perifitona na zgornji površini lista je razložila 15 % variabilnosti odbojnih spektrov, vsebnost klorofila *a* v listu pa je razložila še dodatnih 8 % variabilnosti. Prisotnost epifitona je imela večji vpliv na prepustnost. Suha masa epifitona na spodnji povrhnjici je razložila 24 % variabilnosti transmisijskih spektrov, 4 % pa je razložila še njegova vsebnost klorofila *a*. Prepustnost je bila odvisna tudi od vsebnosti antocianov v listih, ki so razložili 21 % variabilnosti transmisijskih spektrov.

Odstranitev epifitona z listne površine je povečala količino in kakovost odbite in prepuščene svetlobe. Vpliv je bil opazen zlasti v UV, vijoličnem in modrem delu spektra, kar kaže na pomemben prispevek epifitona s prevalujočimi diatomejami na absorpcijo sevanja krajših valovnih dolžin.

Epiphyton alters the quantity and quality of radiation captured by leaves in submerged macrophytes

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Abstract

This study aimed to investigate the effects of the epiphyton and the leaf morphological and biochemical properties on the quality and quantity of reflected and transmitted radiation in the range from 290 nm to 800 nm in submerged leaves of *Potamogeton perfoliatus*, *Potamogeton lucens* and *Sagittaria sagittifolia*. In all of these plant species, the epiphytic algal community was dominated by the diatom *Cocconeis placentula.* The presence of the epiphyton affected the amount of transmitted light to a greater extent than the amount of reflected light. Most of the variability of the reflectance spectra was explained by the epiphyton dry mass at the adaxial surface (15%). The redundancy analysis to explain the variability of the transmittance spectra showed that the epiphyton layer at the abaxial side of the leaves has a more important role then the epiphyton at the adaxial side, explaining 18% and 3% of the spectra variability, respectively. An additional 21% of transmittance spectra variability was explained by the leaf carotenoid content. Removal of the epiphyton from the adaxial leaf surface resulted in a significant increase in the leaf reflectance along the whole spectra, with pronounced differences in the UV, violet and blue ranges. The transmittance spectra measured after removal of the epiphyton also showed a marked increase in the UV, violet and blue ranges, which demonstrates the important role of the epiphyton, with the diatoms prevailing in the absorption of the short-wavelength radiation.

Keywords: Epiphyton; Optical properties; Reflectance; Transmittance; *Potamogeton*; *Sagittaria*.

1. Introduction

Solar radiation that reaches the surface of a leaf can be absorbed, reflected and transmitted. The relative levels of absorbance, reflectance and transmittance of the light depends on the physical and biochemical structure of a leaf and defines its optical properties (Wooley, 1971; Yoshimura et al., 2010; Buschmann et al., 2012; Klančnik et al., 2013, 2014). These optical properties can be species characteristic (Knapp and Carter, 1998; Castro-Esau et al., 2006) or can also vary within a species, due to phenotypic plasticity, resulting in variability caused by plant ontogenetic development and environmental conditions (i.e., the amount of nutrients, water and light) (Liew et al., 2008; Klančnik et al., 2012). The amount of radiation received by leaves can also be altered by the presence of different substances at the leaf surface, such as dust (Nanos and Ilias, 2007) or the periphyton (Brandt and Koch, 2003), and it is generally accepted that the first layer on the leaf surface strongly affects the further penetration of photons into the leaf tissue (Ehleringer et al. 1976; Holmes and Keiller, 2002; Klančnik et al., 2012; Klančnik et al., 2014).

Light conditions in the aquatic environment depend on the quality and quantity of radiation at the water surface, the absorptive properties of the water, the dissolved substances in the water, and the absorptive, reflective and scattering properties of particulate material in the water (Holmes and Klein, 1987). The light conditions determine the depth distribution and abundance of aquatic macrophytes (Madsen and Adams, 1989; Van Duin et al., 2001), and the ability of these macrophytes to capture light differs among their growth forms (Klančnik et al. 2012). The radiation environment of submerged macrophytes is very unpredictable, due to water movements, changes in water transparency, and shoot and/or leaf flexibility (Zelnik et al., 2012), and therefore they lack sun/ shade adaptations (Osmond et al., 1981; Germ et al., 2002). Compared to emergent and natant leaves, the leaves of submerged macrophytes are usually thinner, with higher specific leaf areas, and thinner cuticles and mesophyll tissue, without or with lesser stomata, and with lower contents of photosynthetic pigments (Rascio et al., 1999; Klančnik et al., 2012, 2013). The absorbance of light in submerged leaves is also lower in comparison to that in natant leaves (Frost-Christensen and Sand-Jensen, 1995). The assimilation areas of submerged leaves serve as a potential habitat for periphytic communities that can colonise different plant organs (Bécares et al., 2008). According to Wetzel (1983), the periphytic community associated to living substrata consists of algae and bacteria, which together with particulate detritus, inorganic substances (e.g., Ca_2CO_3) and different organic substances (e.g., polysaccharides, proteins) that originate from the macrophytes and the periphytic community, form the periphyton. Azim and co-workers (2005) defined periphyton associated to plants as epiphyton. The effects of the epiphyton on the radiation regime at the leaf surface depends on its amount and structure (Tóth, 2013). The thickness and structure of the epiphytic layer depends on the availability of nutrients, the macrophyte growth form, and the epiphyton architecture (dos Santos et al., 2013). The study of interactions between macrophytes, such as pondweed, and their epiphyton have shown that the epiphyton negatively affects macrophyte primary production (Tóth, 2013). A lower biomass might not only be the consequence of reduced amounts of incoming radiation, but also a consequence of the competition between macrophytes and their epiphytic algae for nutrients and dissolved carbon (Gaiser, 2008). The epiphyton can also be beneficial for macrophytes, due to its filtering out of excessive radiation. In sea grasses, the epiphyton significantly lowers the amounts of radiation in the UV-B range that is received by the host leaves (Brandt and Koch, 2003). No matter which plant resource is affected and in what way, altered growth conditions can influence the plant metabolic processes and growth (Larcher, 2003).

In the present study, we aimed to estimate the effects of the epiphyton and the leaf morphological and biochemical properties on the quality and quantity of reflected and transmitted radiation in submerged leaves of three macrophyte species, the pondweeds (*Potamogeton lucens* L. and *Potamogeton perfoliatus* L.) and arrow-head (*Sagittaria sagittifolia* L.). Particular attention was paid to diatoms, which have silica frustules and are an important constituent of the epiphyton.

2. Material and methods

2.1. Plant material

Specimens of *P. lucens, P. perfoliatus* and *S. sagittifolia* were collected along the lower reaches of the Rak River (14° 17’ 38” E, 45° 47’ 32” N). These three species thrive along the same stretch of the river, with the pondweed near the banks where the water depth is 50 cm to 80 cm, and the arrowhead in the middle of the stream where the water gets up to 1 m deep. The plant samples were placed in plastic containers with water from the river, and transported to the laboratory. All of the measurements were performed on ten parallel samples for each plant species.

2.2. Reflectance and transmittance measurements

The leaf optical properties were measured on the day of sampling. All of the reflectance and transmittance measurements were made using a portable spectrometer (Jaz Modular Optical Sensing Suite, Ocean Optics, Inc., Dunedin, USA) fitted with an integrating sphere (ISP-30-6-R) and an optical fibre (QP600-1-SR-BX). The leaf reflectance and transmittance spectra were measured over the range of 290 nm to 800 nm, with approximately 0.3-nm steps. The measurements of the reflectance followed the procedures in Klančnik et al. (2012). The total adaxial reflectance spectra were measured during illumination of the leaf with an ultraviolet-visual-near infrared (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). The transmittance spectra were measured at the abaxial leaf surface during illumination of the adaxial surface. The spectrometer was calibrated to 100% transmittance with a light beam that passed directly into the interior of the integrating sphere (Klančnik et al., 2014). Light attenuation by the periphyton is best studied using intact periphytic communities (Vermaat, 2005), and therefore intact leaves of the plants were used. The epiphyton from the adaxial and abaxial leaf surfaces was removed by gentle rubbing of the leaf surface with dry pre-weighted cotton pads. The measurements of reflectance and transmittance spectra were performed for the same leaf: (1) when it was intact; (2) after the epiphyton at the adaxial leaf surface was removed; and (3) for transmittance, after the epiphyton from both leaf surfaces was removed.

2.3. Epiphyton analysis

The epiphyton was removed from leaf surface of known area in two parallel sets of samples. Following the removal of the epiphyton (see above), the first set of cotton pads with the epiphyton were dried in a drier at 105 °C for 24 h. The epiphyton dry mass was then calculated per m^2 of the adaxial and abaxial leaf surfaces, separately. The second set of cotton pads was used to determine the epiphyton chlorophyll *a* contents using a standard methanol extraction method (Talling and Driver, 1963). The absorbances of the extracts were measured at the wavelengths of 665 nm and 750 nm with a UV/VIS spectrometer (Lambda 25, Perkin-Elmer, Norwalk, USA). The chlorophyll *a* contents were expressed per m^2 of the adaxial and abaxial leaf surfaces. The epiphyton species composition and density of diatoms at the adaxial and abaxial leaf surfaces were examined under a light microscope (Olympus CX41, Japan) fitted with digital camera (Olympus XC30, Japan), using the CellSens software (Olympus, Japan).

2.4. Anatomical analysis

The specific leaf area was determined as the leaf area per unit dry mass ($m^2\ g^{-1}$). The histological analyses of the leaves were performed on transverse sections. The following anatomical parameters were measured: thicknesses of cuticle, epidermis, mesophyll and leaf. All of these parameters were measured at 100× magnification, using the light microscope system described above.

2.5. Biochemical analysis

The contents of chlorophyll *a*, chlorophyll *b* and the carotenoids in the leaf tissues were determined according to Lichtenthaler and Buschmann (2001a, b). The absorbances of the extracts 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 per leaf area ($mg\ m^{-2}$).

The anthocyanin content was determined as described by Drumm and Mohr (1978). The absorbances of the extracts were measured at a wavelength of 530 nm, using a UV/VIS spectrometer (Lambda 25, Perkin-Elmer, Norwalk, USA). The content of the pigment is expressed in relative units per leaf area.

The total methanol-soluble UV-B and UV-A absorbing compounds were extracted according to Caldwell (1968). The absorbances of the extracts were measured in the spectral ranges of 280 nm to 319 nm (UV-B) and 320 nm to 400 nm (UV-A). The extinction values were integrated for each UV range, and are expressed in relative units per leaf area.

2.6. Statistical analysis

The normal distribution of the data was tested using Shapiro-Wilk tests. Statistical analysis of the anatomical and biochemical parameters were carried out using one-way ANOVA followed by Tukey's *post-hoc* multiple comparison tests. The measurements of the reflectance and transmittance spectra were averaged over 5-nm intervals. The significances of the differences between the reflectance and transmittance spectra of the different plant species with and without the epiphyton layer were assessed using Kruskal–Wallis tests, with Bonferroni's correction. IBM SPSS Statistics 19.0 was used for the calculations. Detrended correspondence analysis was used for the exploratory data analysis, using the CANOCO for Windows 4.5 programme package. Due to the gradient lengths obtained (<3 SD) (ter Braak and Šmilauer, 2002), redundancy analysis was used to determine whether variations in the response variables (i.e., reflectance and transmittance spectra) were related to the explanatory variables (i.e., anatomical and biochemical parameters). Forward selection of the explanatory variables was used to avoid co-linearity between variables. All of the variables used in the analysis were standardised.

3. Results

In all the three studied plant species, the epiphyton was present on both the adaxial and abaxial sides of the leaves (Table 1). In all of the samples, the epiphytic algal community was dominated by the diatom species *Cocconeis placentula* (Figs. 1, 2). On the leaves of *S. sagittifolia*, there were also cyanobacteria from the genus *Chamaesiphon*, which were much less abundant than *C. placentula*. In both of the pondweeds, the epiphyton also included a few specimens of *Tabellaria* sp., and there were numerous limestone particles, although only on the adaxial side of the leaves. The number of diatoms on the adaxial leaf surface varied significantly between the samples from the different plant species (Table 1). Diatoms were more abundant at the abaxial leaf surface in comparison to the adaxial leaf surface. In total, the greatest number of diatoms was on the abaxial surface of the *S. sagittifolia* leaves. The dry mass of the epiphyton and the amount of epiphytic chloropyll *a* from the pondweeds was higher on the adaxial leaf surface in comparison to the abaxial leaf surface; however, with *S. sagittifolia*, the amount of epiphytic chlorophyll *a* was similar on both sides of the leaves. The amount of chlorophyll *a* in the epiphyton did not correlate with the diatom cell density (Table 1).

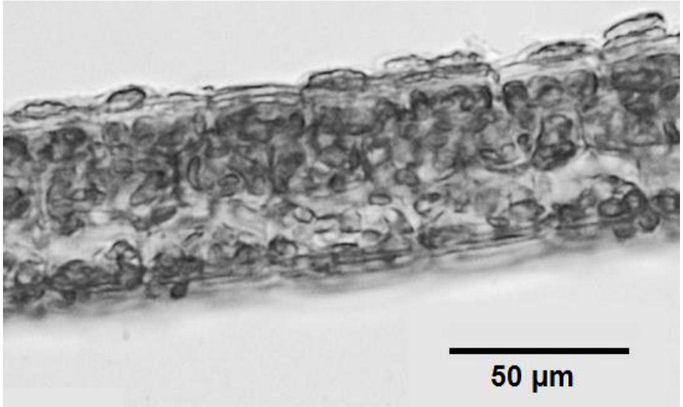

Fig. 1. Transverse section of a leaf of the pondweed *P. perfoliatus*, with the *C. placentula* diatoms on the leaf surface.

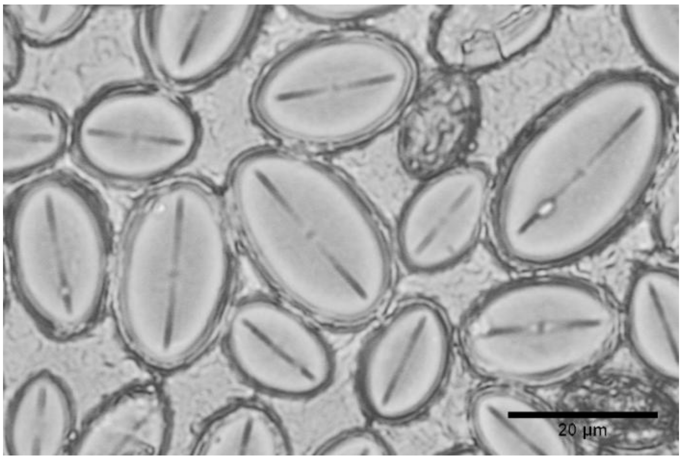

Fig. 2. Leaf surface of the pondweed *P. lucens*, with *C. placentula* diatoms.

Table 1 Anatomical and biochemical parameters of the *P. lucens*, *P. perfoliatus* and *S. sagittifolia* leaves. Data are means ±SD. Different letters indicate a significant difference between the species (p ≤0.05).

Leaf traits	***Potamogeton lucens*** **(a)**	***Potamogeton perfoliatus*** **(b)**	***Sagittaria sagittifolia*** **(c)**
Anatomical parameters			
Specific leaf area (m^2 g^{-1})	5.56 ±0.59 ^{c}	5.24 ±0.52 ^{c}	8.04 ±1.52 ^{ab}
Leaf thickness (µm)	52.49 ±2.51 ^{c}	51.04 ±3.25 ^{c}	168.14 ±79.01 ^{ab}
Mesophyll thickness (µm)	22.27 ±2.39 ^{c}	18.48 ±1.58 ^{c}	136.67 ±64.39 ^{ab}
Adaxial leaf surface			
Epidermis thickness (µm)	17.86 ±1.20	19.56 ±1.48	15.56 ±6.28
Diatom density (mm^{-2})	638 ±152 ^{bc}	309 ±127 ^{ac}	1182 ±470 ^{ab}
Epiphyton weigth (g m^{-2})	10.75 ±5.69 ^{c}	6.79 ±3.03	1.17 ±0.53 ^{a}
Epiphyton chlorophyll *a* (mg m^{-2})	3.86 ±1.61 ^{c}	3.61 ±2.17 ^{c}	12.00 ±6.97 ^{ab}
Abaxial leaf surface			
Epidermis thickness (µm)	12.36 ±0.70	12.99 ±1.25	15.90 ±9.43
Diatom density (mm^{-2})	1209 ±483	1229 ±371	1465 ±165
Epiphyton weigth (g m^{-2})	0.99 ±0.52 ^{c}	0.69 ±0.25 ^{c}	2.70 ±0.89 ^{ab}
Epiphyton chlorophyll *a* (mg m^{-2})	2.62 ±0.76 ^{c}	1.69 ±1.24 ^{c}	12.22 ±4.26 ^{ab}

Biochemical parameters			
Chlorophyll *a* (mg m^{-2})	163 ±20 ^{bc}	130 ±20 ^{ac}	57 ±14 ^{ab}
Chlorophyll *b* (mg m^{-2})	94 ±22 ^{bc}	70 ±21 ^{ac}	19 ±82 ^{ab}
Carotenoids (mg m^{-2})	53 ±7.3 ^{c}	49 ±6.5 ^{c}	19 ±5.1 ^{ab}
Anthocyanins (rel. units cm^{-2})	0.45 ±0.10 ^{c}	0.52 ±0.10 ^{c}	0.18 ±0.04 ^{ab}
UV-B absorbing compounds (rel. units cm^{-2})	10.48 ±2.77 ^{c}	8.73 ±1.47 ^{c}	1.42 ±0.30 ^{ab}
UV-A absorbing compounds (rel. units cm^{-2})	14.85 ±4.00 ^{c}	12.62 ±2.02 ^{c}	2.09 ±0.46 ^{ab}

The majority of the morphological and biochemical characteristics of the leaves were similar for both of the species of pondweed, where they differed significantly from the leaves of *S. sagittifolia* (Table 1). The pondweeds had very thin leaves (Fig. 1), while the leaves of the arrowhead were about three times thicker. However, the presence of abundant aerenchyma in the arrowhead leaves produced high specific leaf areas (Table 1). The amounts of photosynthetic pigments and UV-absorbing compounds were higher in the pondweed, compared to the arrowhead. These differences were the most pronounced for the UV-absorbing compounds (Table 1).

In general, between the plant species, the transmittance spectra varied to a greater extent than the reflectance spectra (Figs. 3, 4). Comparisons of the shapes of the reflectance spectra curves showed that they were similar, but with slight changes along the whole range, with the exception of the NIR region (Fig. 3a). In the samples where the epiphyton was removed from the adaxial leaf surface, the differences in the NIR were even lower (Fig. 3b). The presence of the epiphyton reduced the reflectance along the whole range. The greatest differences were obtained at the shorter wavelengths and in the green-yellow range. More differences were observed in *P. perfoliatus*, with the least in *P. lucens.*

The presence of epiphyton affected the amounts of transmitted light to a greater extent than the amounts of reflected light. When the epiphyton was removed from the adaxial leaf surface, the transmittance increased along the whole range, with the exception of the red range (Fig. 4), and the peaks of the spectral curves became more distinct than in the curves obtained when the measurements were performed with the epiphyton on the leaf surfaces. In *S. sagittifolia* leaves without the epiphyton, the increase in the transmittance in the UV, violet and blue ranges were significantly higher than in the *Potamogeton* species after the epiphyton had been removed (Fig. 4c).

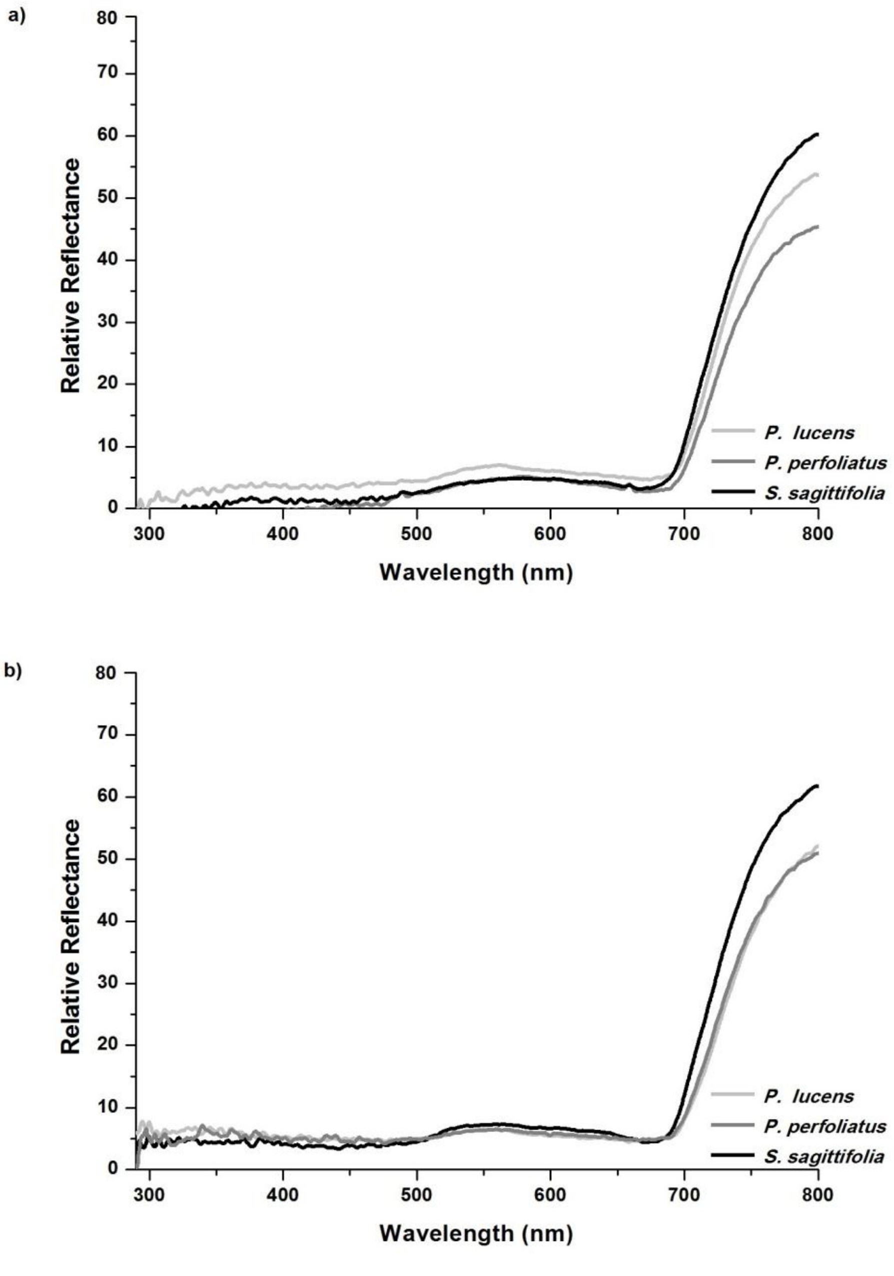

Fig. 3. Mean relative reflectance spectra for *P. lucens*, *P. perfoliatus* and *S. sagittifolia* (as indicated) for intact leaves with the epiphyton on the adaxial and abaxial leaf surfaces (a), and leaves after the epiphyton at the adaxial leaf surface has been removed (b). Data are means over 5-nm intervals (*n* = 10).

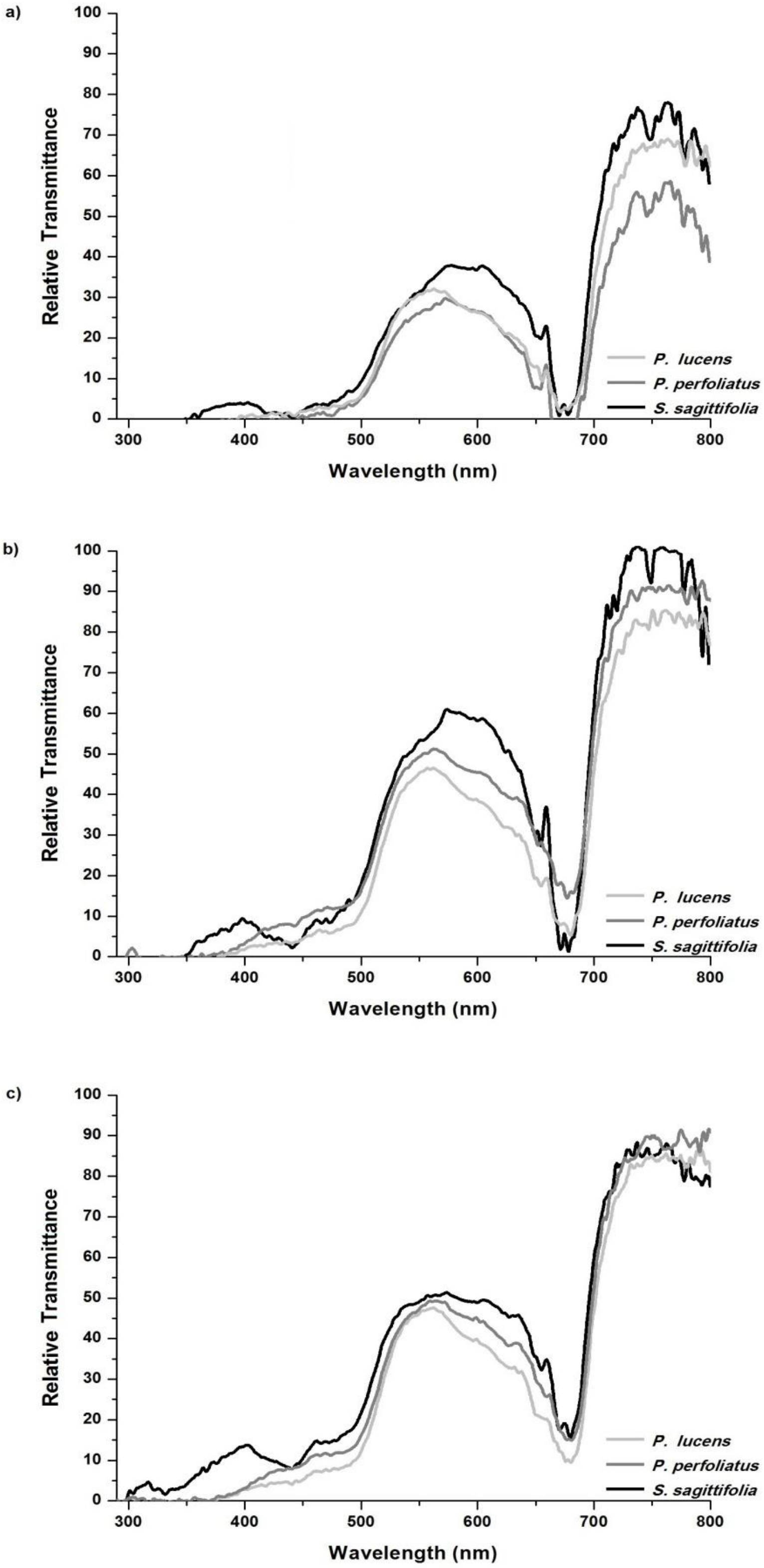

Fig. 4. Mean relative transmittance spectra for *P. lucens*, *P. perfoliatus* and *S. sagittifolia* (as indicated) for intact leaves with the epiphytic community on the adaxial and abaxial leaf surfaces (a), leaves after the epiphyton at the adaxial leaf surface has been removed (b), and leaves after the epiphyton at the adaxial and abaxial leaf surface have been removed (c). Data are means over 5-nm intervals (*n* = 10).

Redundancy analysis was performed to determine which variables explained the variability of the reflectance and transmittance spectra. Most of the variability of the reflectance spectra was explained by the epiphyton dry mass at the adaxial surface (15%, $p < 0.01$) and by the amount of chlorophyll *b* per leaf area (8%, $p < 0.05$) (Fig. 5); the other parameters examined did not have any significant effects on reflectance spectra. The dry mass of the epiphyton correlated negatively with all of the regions of the reflectance spectra, with the strongest correlation with the UV, blue, violet and NIR ranges (Fig. 5).

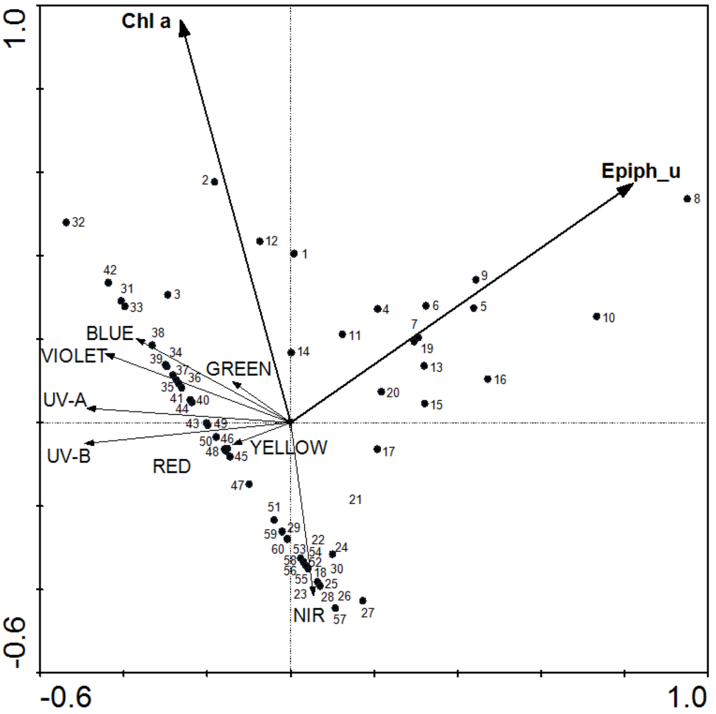

Fig. 5. Redundancy analysis ordination diagram showing the strength of the associations between the significant parameters ($p < 0.05$) and the regions of the reflectance spectra. The eigenvalues for the first two axes are 0.220 and 0.009. Chl a, chlorophyll *a* content per area; Epiph_u, dry mass of the epiphyton on the adaxial leaf surface. Samples of intact leaves with the epiphyton on the adaxial and abaxial leaf surfaces: 1-10, *P. lucens*; 11-20, *P. perfoliatus*; 21-30, *S. sagittifolia*. Samples of leaves after the epiphyton on the adaxial leaf surface has been removed: 31-40, *P. lucens*; 41-50, *P. perfoliatus*; 51-60, *S. sagittifolia*.

When the redundancy analysis was run to explain the variability of the transmittance spectra, the diatom density at the abaxial surface explained 18% of the variance ($p < 0.01$), the amount of carotenoids per leaf area, 21% ($p < 0.01$), and the diatom density at the adaxial surface, the epiphytic chlorophyll *a* content at the abaxial surface, and the epiphyton dry mass at the abaxial surface, each explained an additional 3% ($p < 0.05$) (Fig. 6). Overall, the redundancy analysis explained 48% of the total variability of the transmittance spectra. Among these parameters, the diatom density at the adaxial and abaxial surface, the leaf carotenoids, and the epiphytic chlorophyll *a* content at the abaxial surface, all correlated negatively with the visible region of the transmittance spectra (Fig. 6).

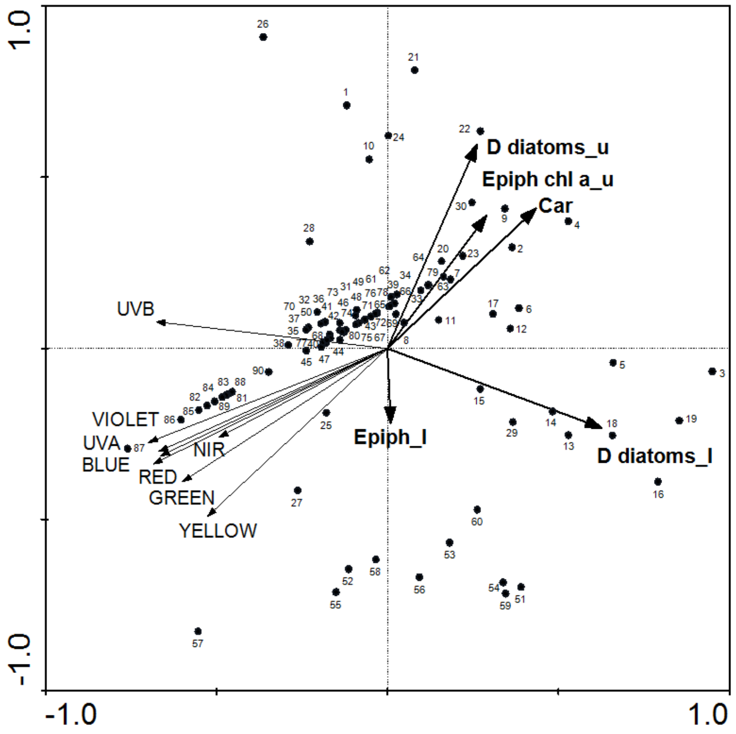

Fig. 6. Redundancy analysis ordination diagram showing the strength of the associations between the significant parameters ($p < 0.05$) and the regions of the transmittance spectra. The eigenvalues for the first two axes are 0.452 and 0.027. D diatoms_u, diatoms density at the adaxial leaf surface; D diatoms_l, diatoms density on the abaxial leaf surface; Epiph chl a_u, epiphytic chlorophyll *a* content at the adaxial leaf surface; Car, carotenoids content per leaf area; Epiph_l, dry mass of epiphyton on abaxial leaf surface. Samples of intact leaves with epiphyton on adaxial and abaxial leaf surfaces: 1-10, *P. lucens*; 11-20, *P. perfoliatus*; 21-30, *S. sagittifolia*. Samples of leaves after the epiphyton at the adaxial surface has been removed: 31-40, *P. lucens*; 41-50, *P. perfoliatus*; 51-60, *S. sagittifolia*. Samples of leaves after the epiphyton at the adaxial and abaxial surface have been removed: 61-70, *P. lucens*; 71-80, *P. perfoliatus*; 81-90, *S. sagittifolia*.

4. Discussion

The capture of light by leaves depends on the biophysical structure and the orientation of the leaves (Ustin et al., 2001). The similarity of the morphological and biochemical properties of both of these pondweeds was a consequence of their similar growth conditions, species growth strategies, and growth forms. Both of these pondweeds form dense pillar-type stands that have a uniform mass distribution along the vertical axis (Wychera et al., 1993). The *S. sagittifolia* stands are variable, as arrowhead can produce a variety of submerged, floating and emergent leaves according to the ontogenetic stage of the plant and the environmental conditions (Hroudová et al., 1988; Dorken and Barrett, 2003). Abundant submerged very flexible ribbon-like leaves develop in water that is about 90 cm deep (Hroudová et al., 1988). Abundant aerenchyma increases their buoyancy in the water column (Klančnik et al., 2013).

All of the plant species were colonised by a epiphytic community. The prevailing algal species in all of these plants was the diatom *C. placentula*. According to Hudon and Bourget (1981), diatoms of the genus *Cocconeis* belong to the group of diatoms that have motile solitary cells that lie flat on the substrate and are very tolerant to light variations. This great tolerance to light conditions was probably the main reason why *C. placentula* were found at both sides of the leaves, with greater abundance at the abaxial leaf surface. Also, the concentration of chlorophyll *a* in the epiphyton did not correlate with the epiphyton biomass. This was possibly a consequence of the ability of the diatoms to change from autotrophy to heterotrophy (Azim and Asaeda, 2005; Azim et al., 2005). Facultative heterotrophy in diatoms is an advantage that can often result in increased growth rates in comparison with their autotrophic counterparts (Pahl et al., 2010). Heterotrophy usually occurs when the light intensity is too low for photosynthesis (Tuchman et al., 2006), even though the amount of chlorophyll does not necessarily decrease (Jørgensen, 1977). Gilpin and co-workers (2004) also showed that in diatoms the relationship between chlorophyll *a* and particulate carbon is strongly non-linear, except during the exponential growth rate.

Literature data have revealed the sensitivity of some taxa of diatoms to different pHs (ter Braak and van Dam, 1989). Representatives of the genus *Potamogeton* have polar leaves that increase the availability of CO_2 for photosynthesis. The pH near the lower epidermis in *P. lucens* drops to as low as 3.5, while the pH at the upper epidermis is more than 10 (Miedema and Prins, 1992; Gaberščik and Mazej, 1995). *C. placentula* can tolerate a broad range of pH conditions (Gasse and Tekaia, 1983; Pither and Aarssen, 2006; DeNicola, 2000), and therefore its presence at the adaxial and abaxial leaf surfaces of both of these pondweeds was not surprising. The alkaline microenvironment at the upper epidermis also results in the precipitation of calcium carbonate from the water (Miedema and Prins, 1992), which might contribute to the total mass of the epiphyton in our study.

The effects of the epiphyton on the light regime in leaves depends on the epiphyton properties and its biomass (Brandt and Koch, 2003). The communities with abundant diatoms have outstanding optical properties, as diatom silica frustules absorb mainly in the UV-A, violet and blue parts of the spectra (Yamanaka et al., 2008). Diatom frustules have highly porous silica shells that encapsulate the protoplasm and are joined together by a girdle (Cox, 2011). Light entering the pore structures is scattered, which increases the probability of photon absorption (Yamanaka et al., 2008). With *Coscinodiscus wailesii*, frustules have been shown to even the light focusing ability (De Stefano and Moretti, 2007); however, simulation studies have shown that this effect does not occur in the UV range (De Tommasi et al., 2010). Due to the high light absorption capacity of silica frustules, the quality and quantity of the reflected light are only slightly affected by the presence of the epiphyton on the leaf surface of the macrophytes studied. The responses of all three of these macrophyte species were very similar, except in the NIR range of the reflectance spectra. The effects were the most pronounced in the UV, violet and blue ranges, where the reflection significantly increased after the removal of the epiphyton layer. This is in line with a study of sea-grass that reported stronger absorbance by the epiphyton in the UV range, in comparison to the visible range (Brandt and Koch, 2003). According to our expectations, the redundancy analysis showed that in these three species studied, the parameters related to the epiphyton have more influence on the reflectance and transmittance spectra than biochemical and anatomical properties of the leaves. In total, only 23% of the reflectance spectra variability was explained, and out of this, 15% of the reflectance variability was explained by the dry mass of the epiphyton at the adaxial leaf surface.

Light absorbance by the periphyton shows a non-linear decrease with increasing thickness of the periphytic layer (Brandt and Koch, 2003; Vermaat, 2005). The scattering of the light within a layer of the periphyton might additionally affect the amount of transmitted light (Vermaat, 2005; Yamanaka et al., 2008). Due to the high absorbance potential of the diatom frustules, the transmittance spectra were more affected by the epiphytic layer, in comparison to the reflectance spectra. Due to the epiphyton removal, the significant increase in transmittance occurred in the UV, violet and blue ranges, which additionally demonstrate the role of the epiphyton in absorbing the short-wavelength radiation. The absorbance in the UV range is an advantage for poorly protected aquatic plants, due to the potential harmful effects of the UV photons (Brandt and Koch, 2003). Effects similar to those of the epiphyton have been reported for silica structures in some terrestrial plants (Shen et al., 2010; Yao et al., 2011).

However, in grasses, the protection against harmful effects of UV photons is not based on the absorption of the UV radiation, but on its reflection, which is contributed to by different near-surface silicified leaf structures (Klančnik et al., 2014). An increase in transmittance spectra was also detected in the green-yellow range. This might be a consequence of the absorption of light by the diatom pigments fucoxantin and the carotenoids (Fujiki and Taguchi, 2001). The transmittance spectra of the leaves of the macrophytes studied varied to a greater extent than their reflectance spectra. The epiphyton layer on the abaxial side of the leaves has a more important role than the epiphyton on the adaxial side, with the explanation of 18% and 3% of the spectra variability, respectively. An additional 21% of the spectra variability was explained by the leaf carotenoid content. Carotenoids have fundamental roles in light harvesting and photoprotection (Demmig-Adams and Adams, 1996) and are crucial for the function of macrophytes that live under unpredictable light conditions in aquatic environments (Van Duin et al., 2001).

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3.2 HETEROFILIJA SE ODRAŽA NA RAZLIČNIH »SPEKTRALNIH PODPISIH« VODNIH RASTLIN

Katja Klančnik, Mojca Mlinar, Alenka Gaberščik. 2012. Heterophyll results in a variety of "spectral signatures" in aquatic plant species. Aquatic Botany, 98: 20-26

Na podlagi odbojnih spektrov rastlin je mogoče razlikovati rastlinske vrste (Knapp in Carter, 1998; Castro-Esau in sod., 2006). Zanesljivost klasifikacije vrst z meritvami odbojnih spektrov pa je odvisna od lastnosti in fenotipske prožnosti rastlin, poleg teh pa lahko na spektralne podpise rastlin vplivajo tudi okoljske razmere (Baldini in sod., 1997). Heterofilne vodne rastline predstavljajo dober modelni sistem, ki omogoča preučevanje spreminjanja anatomskih in biokemijskih lastnosti listov v različnih okoljih ter odražanje teh sprememb na optičnih lastnostih listov znotraj posamezne rastlinske vrste.

V naši raziskavi smo primerjali anatomske, biokemijske in optične lastnosti (odbojnost) različnih tipov listov pri vodni dresni (*Polygonum amphibium*) in rumenem blatniku (*Nuphar luteum*). Obe vrsti sta razširjeni heterofilni vrsti na območju Cerkniškega jezera. Pri vodni dresni smo primerjali plavajoče in kopenske liste ter dve prehodni obliki listov, ki se razvijejo vzdolž gradienta med vodo in kopnim. Pri rumenem blatniku pa smo primerjali potopljene in plavajoče liste. Nato smo primerjali še lastnosti plavajočih listov obeh rastlinskih vrst, ki se razvijejo v podobnem okolju.

Pri vodni dresni so si bili različni tipi listov bolj različni po njihovih morfoloških kot biokemijskih lastnostih. Pri rumenem blatniku pa sta se različna tipa listov razlikovala prav v vseh merjenih parametrih. Z RDA smo dokazali, da na odboj sevanja pri vodni dresni značilno vpliva dolžina trihomov na zgornji povrhnjici lista, ki negativno korelira z vsemi deli odbojnega spektra in razloži 72 % njegove variabilnosti. Prav tako značilno vpliva na variabilnost odbojnih spektrov tudi masa na površino lista, ki pa je v pozitivni korelaciji z odbojem sevanja in razloži 6 % njegove variabilnosti. Na oboj sevanja pri rumenem blatniku je imela navečji vpliv vsebnost antocianov, ki je razložila 57 % variabilnosti odbojnih spektrov.

Medvrstna primerjava plavajočih listov je pokazala, da se isti tip lista dveh rastlinskih vrst v določenih biokemijskih parametrih ne razlikujejo, tudi odbojni spektri listov, razvitih pod vplivom podobnih okoljskih dejavnikov so si bili bolj podobni, kot odbojni spektri različnih tipov listov pri določeni rastlinski vrsti.

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Heterophylly results in a variety of "spectral signatures" in aquatic plant species

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Leaf properties

ABSTRACT

The leaf reflectance spectra (280–887 nm) of two heterophyllous aquatic plant species *Polygonum amphibium* (L.) and *Nuphar luteum* (L.) were compared and their relation to physical properties of the leaves examined. In *P. amphibium* contrasting environmental conditions along water–land gradient affected the majority of anatomical and morphological properties of leaves, but less differences were observed in photosynthetic pigment and total flavonoid contents. Leaf mass per area (LMA), palisade mesophyll, leaf thickness, trichome length and anthocyanin content per dry mass were correlated to the different parts of spectra. In *N. luteum* natant and submerged leaves differed significantly in all measured parameters. Chlorophyll *a*, anthocyanin and carotenoid contents per dry mass were related to reflectance in the red region, while leaf thickness, anthocyanin and total flavonoid contents per leaf area were related to reflectance in the near infrared region. Redundancy Analysis (*RDA*) indicated that in *P. amphibium* the average length of trichomes and LMA explained 72% and 6% variability of the spectra, whereas in *N. luteum* anthocyanin content per dry mass, explained 57% variability of the spectra. The comparison of natant leaves of both species showed that they were more similar than different leaf types within the single species.

1. Introduction

Aquatic habitats vary in time and space. Water regime change is the major driving factor affecting physical, chemical and biological environments. Plants, as sessile organisms, must be well "equipped" to cope with all the limitations of their habitat (Schurr et al., 2006). Some heterophyllous aquatic plant species colonize in a wide range of habitats from open water to dry land, while others develop assimilatory organs in contrasting media, air and water. The evolution of these species has resulted in outstanding phenotypic plasticity that enables undisturbed function under variable environmental conditions (Gaberščik, 1993; Germ and Gaberščik, 2003). Some of them develop differently shaped leaves (heterophylly) or growth forms (Hutchinson, 1975). In this way they can avoid stress and have a competitive advantage over completely submersed or emergent species (Braendle and Crawford, 1999; Cronk and Fennessy, 2001; Enríquez and Sand-Jensen, 2003).

Leaves have multiple tasks, the most important are collecting of solar energy, uptaking carbon dioxide and reducing water losses. Fine tuning of these functions assure optimised photosynthetic performance. This is enabled by acclimation of assimilation areas to current environmental conditions at morphological, anatomical, biochemical and functional levels, as shown for many heterophyllous aquatic plants (Gaberščik and Martinčič, 1992; Rascio et al., 1999; Robe and Griffiths, 2000; Boeger and Poulson, 2003; Šraj-Kržič and Gaberščik, 2005).

The information in reflectance spectra of leaves forms the basis for understanding photosynthetic performance and energy balance in plants (Vogelmann, 1993). It enables species classification (Knapp and Carter, 1998; Castro-Esau et al., 2006), determination of leaf biochemistry (Gitelson et al., 2002; Levizou et al., 2005; Castro and Sanchez-Azofeifa, 2008) and nutrient and water contents (Baltzer and Thomas, 2005; Baldini et al., 1997; Asner and Martin, 2008), and provides a tool for detecting stress (Gitelson et al., 2002). The reliability of these estimates depends on species traits and their phenotypic plasticity, which can strongly affect plant spectral signatures.

Leaves show great heterogeneity in optical properties that are not only species specific but also depend on environmental conditions (Baldini et al., 1997). From that point of view, heterophyllous aquatic plants constitute a perfect model system, since they occupy contrasting habitats. However, the reflectance spectra in this outstanding group of plants have not been studied, even though they are an important component in different aquatic habitats (Sand-Jensen and Frost-Christensen, 1999).

Two heterophyllous aquatic plant species, *Polygonum amphibium* (L.) and *Nuphar luteum* (L.), growing in the changing environment of intermittent Lake Cerknica were studied. We hypothesised that (1) the variability in properties of different leaf types within examined species is greater than in natant leaves of

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both species and that (2) the main determinants affecting spectra are different for *P. amphibium* and *N. luteum*. We also assumed that different epidermal structures (if present) exert more effect on the reflectance spectra than biochemical properties. To test these hypotheses we measured reflectance spectra (280–887 nm), anatomical and morphological (LMA, leaf and palisade mesophyll thickness, number and length of stomata and trichomes on both leaf surfaces) as well as biochemical properties (chlorophyll *a* and *b*, carotenoid, anthocyanin and total flavonoid contents) in different leaf types in both species.

2. Materials and methods

2.1. Site description

Lake Cerknica in the area of Rešeto was the study area. Lake Cerknica appears at the bottom of the karst field Cerkniško polje and is considered as *locus typicus* for intermittent lakes. The depression of Cerkniško polje is filled with water twice a year; in spring and late autumn or early winter, while the dry period usually starts in late spring (Kranjc, 2002). Great fluctuations of water level occur throughout the year and, due to the variable water regime, plant species with amphibious characteristics prevail (Gaberščik et al., 2003).

2.2. Plant material

P. amphibium (*Polygonaceae*) is an amphibious species with an outstanding phenotypic plasticity. It occurs over a wide environmental gradient, from open water about 2 m deep to dry land (Gaberščik and Martinčič, 1992; Gaberščik, 1993). It can develop two distinctive growth forms, namely *fusarium natans* and *fusarium terrestris*, and a variety of transitional forms (Hutchinson, 1975). *N. luteum* (*Nymphaeaceae*) is an aquatic plant with natant and submersed leaves, usually found in up to 3 m deep water.

Fully developed vital leaves were collected in August 2010. Leaves of natant and terrestrial specimens of *P. amphibium*, as well as of two transitional forms, thriving over a water-dry land gradient, were collected. E1 indicates a transient leaf type more similar to natant than terrestrial leaves, while E2 indicates a leaf type more similar to terrestrial leaves. In *N. luteum* we collected natant and submerged leaves. Measurements were carried out on five replicates of each leaf type.

All leaf types were exposed to full sunlight (cca. 2000 μmol m^{-2} s^{-1} at noon in sunny day), except submersed leaves of *N. luteum* where radiation in the intermittent water body ranged from about 25% of radiation at water surface when the water level was low and 2% at high water level (and less than one 1% following heavy rain fall).

2.3. Measurements of spectral reflectance

The reflectance of leaves was measured on the day of sampling. Measurements of reflectance were conducted using a portable spectrometer Jaz Modular Optical Sensing Suite (Ocean Optics, Inc., Dunedin, FL, USA). Individual leaves were installed under an integrating sphere ISP-30-6-R (Ocean Optics, Inc., FL, USA) connected by optical fibre QP600-1-SR-BX (Ocean Optics, Inc., Dunedin, FL, USA) with the spectrometer. Total adaxial reflectance spectra of leaves were recorded between 280 and 887 nm, with resolution approximately 0.3 nm, during illumination of leaf with a UV–VIS-NIR light source (DH-2000, Ocean Optics, Inc., FL, USA). A Spectrolon white reference panel was used to calibrate the instrument to 100% reflectance prior to leaf reflectance measurements. Reflectance spectra were calculated as the ratio of sample data to white reference under the same illumination.

2.4. Morphological and anatomical analysis

Leaf discs with known area (cm^2) were cut and oven dried at 105 °C for 24 h to determine leaf mass per area (LMA), which was derived as dry mass per unit area (mg cm^{-2}). Leaf histological analysis was performed on five transverse sections of each leaf type. Leaf sections were analyzed with an Olympus CX41 light microscope at 100× magnification to determine thickness of the leaf, epidermis and palisade mesophyll. The density and length of leaf stomata and trichomes were examined under a light microscope (Olympus CX41) at 400× magnification.

2.5. Biochemical analysis

Biochemical analyses were performed on five replicates for each leaf type. Contents of chlorophyll *a*, chlorophyll *b* and carotenoids were determined as described by Lichtenthaler and Buschmann (2001a,b). Absorbances of samples were measured with a UV/VIS spectrometer (Lambda 25, Perkin–Elmer, Norwalk, CT, USA) at 470, 645 and 662 nm. Contents of chlorophylls (Chl *a* and *b*) and carotenoids were expressed per dry mass (mg g^{-1}) and area (mg dm^{-2}) of sample. Content of anthocyanins was determined in accordance to Khare and Guruprasad (1993). Absorbances of extracts were measured at 530 nm, and pigment content was calculated per sample of dry mass and area. Content of anthocyanins was expressed in relative units.

UV-B (280–320 nm) and UV-A (320–400 nm) absorbing compounds (total methanol soluble flavonoid contents) were extracted from weighed fresh plant material, by homogenizing in a mortar and extracting with methanol: distilled water: HCl = 79:20:1 (v/v/v) (Caldwell, 1968). Absorbances of extracts were measured over the ranges 280–320 and 320–400 nm. The extinction values were integrated for each region and expressed per sample area and dry mass. Contents were expressed in relative units.

2.6. Statistical analysis

Differences in morphological, anatomical and biochemical parameters were tested, applying Student's *t*-test or non-parametric Mann–Whitney test as well as ANOVA or non-parametric Kruskal–Wallis test. Normal distribution was tested using the Shapiro–Wilk test. SPSS Statistics 19.0 was used for calculations. Data points of reflectance spectra were averaged within 5 nm intervals. Significance of differences between reflectance spectra were assessed by Kruskal–Wallis test (R 2.12.0) with a Bonferroni's correction. To find possible relationships between measured parameters Spearman's rank correlation analysis was performed (IBM SPSS Statistics 19). Redundancy analysis (RDA) was used to determine whether variations in response variable (reflectance spectra) were related to explanatory variables (morphological, anatomical and biochemical parameters) using CANOCO for Windows 4.0. Monte Carlo permutation tests were carried out to test the significance of the relationships. The level of significance was accepted at $p \leq 0.05$.

3. Results

3.1. P. amphibium

Comparison of different types of leaves of *P. amphibium* showed that morphological and anatomical characteristics differ more markedly along the gradient water–dry land than biochemical ones (Table 1). In general, different leaf types differ most significantly in

Table 1
Leaf morphological, anatomical and biochemical parameters in *P. amphibium*. Values are means ± SD. Letters indicate the significance of comparisons between different leaf types ($p \leq 0.05$).

Properties	Natant (n)	Emersed 1 (E1)	Emersed 2 (E2)	Terrestrial (t)
LMA (mg/cm^2)	$5.05 \pm 0.4^{E1,t}$	$5.83 \pm 0.11^{E2,t}$	$4.85 \pm 0.45^{E1,t}$	$3.22 \pm 0.3^{n,E1,E2}$
Leaf thickness (μm)	243 ± 31^{E1}	$274 \pm 26^{n,E2,t}$	241 ± 29^{E1}	235 ± 22^{E1}
Palisade mesophyll (μm)	$116.6 \pm 14.6^{E1,t}$	$133.7 \pm 15.5^{n,E2,t}$	105.1 ± 17.1^{E1}	$96.1 \pm 16.9^{n,E1,E2}$
Upper surface				
Trichome density (mm^{-2})	$0^{E1,E2,t}$	4.6 ± 2^{n}	2.6 ± 1.3^{n}	2.8 ± 0.6^{n}
Trichome length (μm)	$0^{E1,E2,t}$	$142 \pm 31^{n,E2,t}$	$228 \pm 63^{n,E1,t}$	$367 \pm 77^{n,E1,E2}$
Stomata density (mm^{-2})	$146 \pm 13^{E1,E2}$	$118 \pm 31^{n,E2,t}$	$20 \pm 11^{n,E1}$	$29 \pm 13^{n,E1}$
Stomata length (μm)	34.6 ± 2.4^{t}	35 ± 4^{t}	33.8 ± 2.8^{t}	$41.3 \pm 2.8^{n,E1,E2}$
Lower surface				
Trichome density (mm^{-2})	$0^{E1,E2,t}$	$2.3 \pm 0.4^{n,t}$	$2.3 \pm 1.3^{n,t}$	$4 \pm 1.3^{n,E1,E2}$
Trichome length (μm)	$0^{E1,E2,t}$	$184 \pm 27^{n,t}$	$161 \pm 48^{n,t}$	$323 \pm 67^{n,E1,E2}$
Stomata density (mm^{-2})	$0^{E1,E2,t}$	$135 \pm 30^{n,E2}$	$192 \pm 19^{n,E1,E2}$	$136 \pm 26^{n,E2}$
Stomata length (μm)	$0^{E1,E2,t}$	32.7 ± 3.6^{n}	$30.8 \pm 2.5^{n,t}$	$36.1 \pm 2.1^{n,t}$
Biochemical parameters				
Chlorophyll *a* (mg/g)	6.19 ± 1.62^{t}	6.79 ± 1.02	6.41 ± 0.67	8.2 ± 0.72^{n}
Chlorophyll *a* (mg/dm^2)	2.91 ± 0.8	3.96 ± 0.59	3.11 ± 0.33^{t}	2.85 ± 0.25^{E2}
Chlorophyll *b* (mg/g)	2.83 ± 0.44	3.05 ± 0.45	3.15 ± 0.48	2.85 ± 0.3
Chlorophyll *b* (mg/dm^2)	1.33 ± 0.21^{E1}	$1.78 \pm 0.26^{n,t}$	1.53 ± 0.23^{t}	$1.0 \pm 0.1^{E1,E2}$
Carotenoids (mg/g)	1.76 ± 0.51^{t}	1.746 ± 0.29^{t}	1.73 ± 0.06^{t}	$2.48 \pm 0.12^{n,E1,E2}$
Carotenoids (mg/dm^2)	0.83 ± 0.24	1.02 ± 0.17	0.84 ± 0.03	0.86 ± 0.04
Anthocyanins (relative units per dry mass)	$120 \pm 15^{E2,t}$	131 ± 8^{t}	148 ± 7^{n}	$160 \pm 17^{n,E1}$
Anthocyanins (relative units per area)	$56.2 \pm 7.1^{E1,E2}$	$76.3 \pm 4.9^{n,t}$	$72.0 \pm 3.6^{n,t}$	$55.5 \pm 5.8^{E1,E2}$
UVB-absorbing compounds (relative units per dry mass)	1905 ± 321	1767 ± 596	2225 ± 567	1838 ± 222
UVB-absorbing compounds (relative units per area)	894 ± 150	1029 ± 347	1080 ± 275^{t}	639 ± 77^{E2}
UVA-absorbing compounds (relative units per dry mass)	2704 ± 234^{t}	2494 ± 342^{t}	3066 ± 370	$3720 \pm 537^{n,E1}$
UVA-absorbing compounds (relative units per area)	1270 ± 110	1450 ± 200	1490 ± 180	1290 ± 190

length of trichomes ($p \leq 0.01$) (Fig. 1) and stomata on the adaxial leaf surface and in palisade mesophyll thickness. Of the biochemical parameters anthocyanin contents (per area) varied significantly. On the other hand, contents of chlorophyll *b* and UV-B absorbing compounds expressed per dry mass, did not differ between leaf types, as well as carotenoids and UV-A absorbing compounds expressed per unit area. The analysis showed that the transitional forms of leaves (E1 and E2) differed least, while natant and terrestrial leaves differed most.

Comparison of reflectance spectra from different leaf types in *P. amphibium* showed that the transitional forms of leaves (E1 and E2) function similarly (Fig. 4a), with some exceptions observed in the region of the visible spectrum and in some narrow regions in the UV spectra. Spectra with the greatest qualitative differences were obtained for E1 and terrestrial leaf type. Differences occurred along the whole reflectance spectrum, except for some regions in the visible and NIR regions (Fig. 4a). However, reflectance spectra of natant and terrestrial leaves exhibited the greatest quantitative differences, the exceptions being in some narrow regions in the visible spectrum (Fig. 4a). E2 and terrestrial leaves had similar reflectance in some narrow regions in the UV spectrum, in the some regions of the visible spectrum, and in the NIR region. Reflectances of natant and transitional forms of leaves were similar in the NIR region.

Fig. 1. The replica of multicellular trichomes on adaxial epidermis in *P. amphibium*.

In correlation analysis of the spectra and leaf properties only relevant parameters (adaxial leaf surface, anatomical and biochemical properties) were taken into account. Most regions of spectra were strongly related to the average and total length of trichomes (Table 2). LMA, leaf and palisade mesophyll thickness correlated with selected parts of the reflectance spectra. The only biochemical parameter related to spectra was the content of anthocyanins expressed per dry mass (Table 2; only parameters that correlated significantly are shown).

Redundancy Analysis (RDA) revealed that the average length of trichomes and LMA correlated significantly with spectra, explaining 72% and 6% of their variability (Fig. 2). Length of trichomes on adaxial epidermis correlated negatively with the reflectance spectrum, while LMA correlated positively. RDA analysis confirmed the results obtained in correlation analysis. The distribution of different leaf types in the tri-plot shows four distinct groups, forming the gradient from high reflection at the left part of the tri-plot via transitional in the middle to low reflection at the right hand side of the plot.

3.2. *N. luteum*

Comparison of natant and submersed leaves showed that all measured anatomical, morphological and biochemical properties differed significantly ($p \leq 0.05$) (Table 3). The same holds true for the reflectance spectra of submersed and natant leaves, which differed significantly at the majority of wavelengths ($p < 0.05$). Submersed leaves tend to have higher relative reflectance in the region of the visible spectrum, but in the UV and NIR regions the reflectance of submersed leaves was lower than that of natant leaves (Fig. 4c).

Table 2
Spearman's correlation coefficient (*r*) for relationships between spectral reflectance and leaf properties of *P. amphibium*.

	UVB	UVA	Violet	Blue	Green	Yellow	Red	NIR
LMA	0.64^{**}	0.60^{**}	0.61^{**}	0.34	0.14	0.42	0.58^{**}	0.39
Average trichome length	-0.91^{**}	-0.93^{**}	-0.93^{**}	-0.80^{**}	-0.47^{*}	-0.67^{**}	-0.81^{**}	-0.75^{**}
Total trichome length	-0.76^{**}	-0.81^{**}	-0.80^{**}	-0.73^{**}	−0.36	-0.52^{*}	-0.60^{**}	-0.59^{**}
Palisade mesophlyll	0.76^{**}	0.70^{**}	0.70^{**}	0.33	0.13	0.35	0.64^{**}	0.39
Leaf thickness	0.45^{*}	0.43	0.40	0.05	0.22	0.36	0.55^{*}	0.10
Anthocyanins (per dry mass)	-0.71^{**}	-0.75^{**}	-0.76^{**}	-0.58^{**}	−0.32	-0.57^{**}	-0.64^{**}	-0.59^{**}

$N = 20$.
* $p \leq 0.05$.
** $p \leq 0.01$.

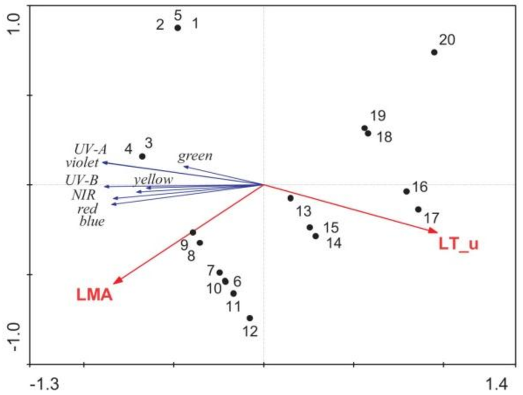

Fig. 2. RDA plot examining the strength of associations between morphological, anatomical biochemical, parameters and different regions of reflectance spectra in *P. amphibium*. Variables are represented by arrows, numbers indicate different leaf types: 1–5 natant leaves, 6–10 E1 leaves, 11–15 E2 leaves and 16–20 terrestrial leaves. LMA – leaf mass per area, LT_u–trichome length on the upper leaf surface.

Correlation analyses showed that differences in the red region were related significantly to the content of chlorophyll *a* ($r = 0.71$, $p < 0.05$), anthocyanins ($r = -0.69$, $p < 0.05$) and carotenoids ($r = 0.67$, $p < 0.05$) expressed per dry mass. The NIR region was significantly related to leaf thickness ($r = 0.82$, $p < 0.01$), UV-B absorbing compounds ($r = 0.77$, $p < 0.01$) and anthocyanins ($r = -0.66$, $p < 0.05$) expressed per dry mass.

RDA indicated that the most influential parameter affecting spectra was the content of anthocyanins per dry mass explaining 57% of the reflectance spectrum variability (Fig. 3). When RDA was run, taking into account pigments per area, anthocyanins explained 35% of the reflectance spectrum variability. Anthocyanin content per dry mass correlated positively with the regions of spectra over 500 nm, except that, in the NIR region, the correlation was negative, as in correlation analysis. Grouping of samples in the RDA plot also indicates the existence of two groups formed by the samples of two different leaf types.

3.3. *Interspecies comparison of natant leaves*

Comparison analysis of the natant leaves of the two plant species was performed. All morphological characteristics were significantly different ($p < 0.05$) while, at the biochemical level, the differences were not so consistent. The contents of chlorophyll *a*, anthocyanins and UV-A absorbing compounds expressed per area were very similar in the natant leaves of the two species (Table 3). Reflectance spectra of natant leaves in *P. amphibium* and *N. luteum* differed, with exceptions in the visible and NIR regions (Fig. 4b).

Table 3
Morphological, anatomical and biochemical parameters of *N. luteum* leaves. Values are means ± SD. Asterisks indicate the significance of comparisons of the two leaf types in *N. luteum* ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$). Dots indicate the significance of comparisons of natant leaves in both plant species (●$p \leq 0.05$, ●●$p \leq 0.01$).

Properties	Natant	Submersed
LMA (mg/cm^2)	7.58 ± 1.66●	$2.77 \pm 0.39^{**}$
Leaf thickness (μm)	643 ± 274●●	$345 \pm 27^{**}$
Palisade mesophyll (μm)	203.4 ± 36●●	Not differentiated
Upper surface		
Stomata density (mm^{-2})	595 ± 80●	–
Stomata length (μm)	21.4 ± 1.8●	–
Lower surface		
Trichome density (mm^{-2})	0	–
Trichome length (μm)	0	–
Stomata density (mm^{-2})	0	–
Stomata length (μm)	0	–
Biochemical parameters		
Chlorophyll *a* (mg/g)	4.49 ± 0.36●	$7.34 \pm 0.37^{*}$
Chlorophyll *a* (mg/dm^2)	3.41 ± 0.27	$2.03 \pm 0.1^{**}$
Chlorophyll *b* (mg/g)	1.33 ± 0.12●●	$2.94 \pm 0.23^{**}$
Chlorophyll *b* (mg/dm^2)	1.00 ± 0.09●	$0.81 \pm 0.06^{**}$
Carotenoids (mg/g)	1.62 ± 0.13	$2.06 \pm 0.23^{**}$
Carotenoids (mg/dm^2)	1.23 ± 0.09●●	$0.57 \pm 0.06^{**}$
Anthocyanins (relative units per dry mass)	81 ± 4●●	$120 \pm 40^{*}$
Anthocyanins (relative units per area)	6160 ± 350	$3320 \pm 880^{**}$
UVB-absorbing compounds (relative units per dry mass)	1905 ± 65●●	$2932 \pm 1008^{**}$
UVB-absorbing compounds (relative units per area)	14.45 ± 0.5●●	$8.12 \pm 2.79^{**}$
UVA-absorbing compounds (relative units per dry mass)	1752 ± 44●●	$1291 \pm 204^{**}$
UVA-absorbing compounds (relative units per area)	1330 ± 30	$360 \pm 60^{**}$

4. Discussion

4.1. *P. amphibium*

The outstanding phenotypic plasticity, detected in water bistort, was partly shown in the studies of Gaberščik and Martinčič (1992) and Gaberščik (1993) and, to some extent, reported for some other heterophyllous aquatic species (Nielsen, 1993; Nielsen and Sand-Jensen, 1993; Braendle and Crawford, 1999). Anyhow we observed limited biochemical response that is probably a consequence of the fact that structures on the leaf surface, such as waxy cuticule and trichomes, reduce the penetration of radiation into the mesophyll (Holmes and Keiller, 2002). The growth of trichomes also demands low energy investment and they are cost effective due to their multiple functions i.e. preventing water loss and protecting against excessive radiation (Ehleringer, 1980; Woodman and Fernandes, 1991). However, in *P. amphibium* the presence of trichomes was certainly one of the most marked differences. Along the

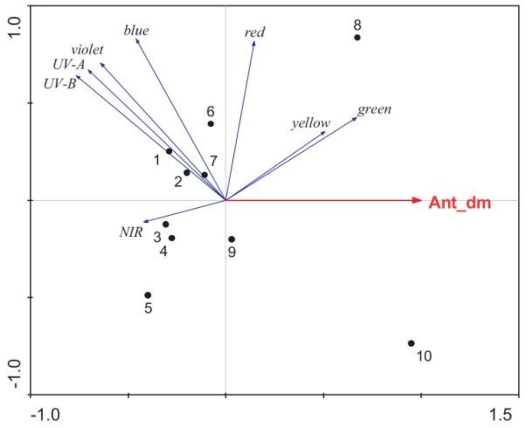

Fig. 3. RDA plot examining the strength of associations between morphological, anatomical, biochemical parameters and different regions of reflectance spectra in *N. luteum*. Variables are represented by arrows, numbers represents different leaf types: 1–5 natant leaves and 6–10 submersed leaves. Ant_dm–anthocyanins per dry mass.

gradient water–dry land the aridity increased, resulting in increased pubescence, as also reported by Filella and Peñuelas (1999).

The most pronounced quantitative differences were found between natant and terrestrial leaves that also differed at the morphological, anatomical and biochemical levels. Interestingly, the most qualitative differences were observed between E1 and terrestrial leaves. The possible reasons are the differences in LMA, which is the greatest in E1 leaves, and significantly affected spectra as revealed from correlation analyses and RDA.

It is generally accepted that leaf reflectance in the NIR region is primarily affected by leaf structures, in the visible region by photosynthetic pigments and in the mid-IR region by water content (Slaton et al., 2001). In water bistort, the thickness of palisade mesophyll and LMA were positively related to reflectance in UV, violet and red regions. Overall correlation analysis indicated that spectral reflectance correlates highly with average length and total length of trichomes on adaxial epidermis. Even though the majority of studies support the outcomes of our research, this was not the case in the study of Grant et al. (2003). Anthocyanins were significantly negatively correlated with different regions along the entire reflectance spectra, except for the green region. Anthocyanins are believed to be beneficial for plant survival under biotic and abiotic stresses i.e.

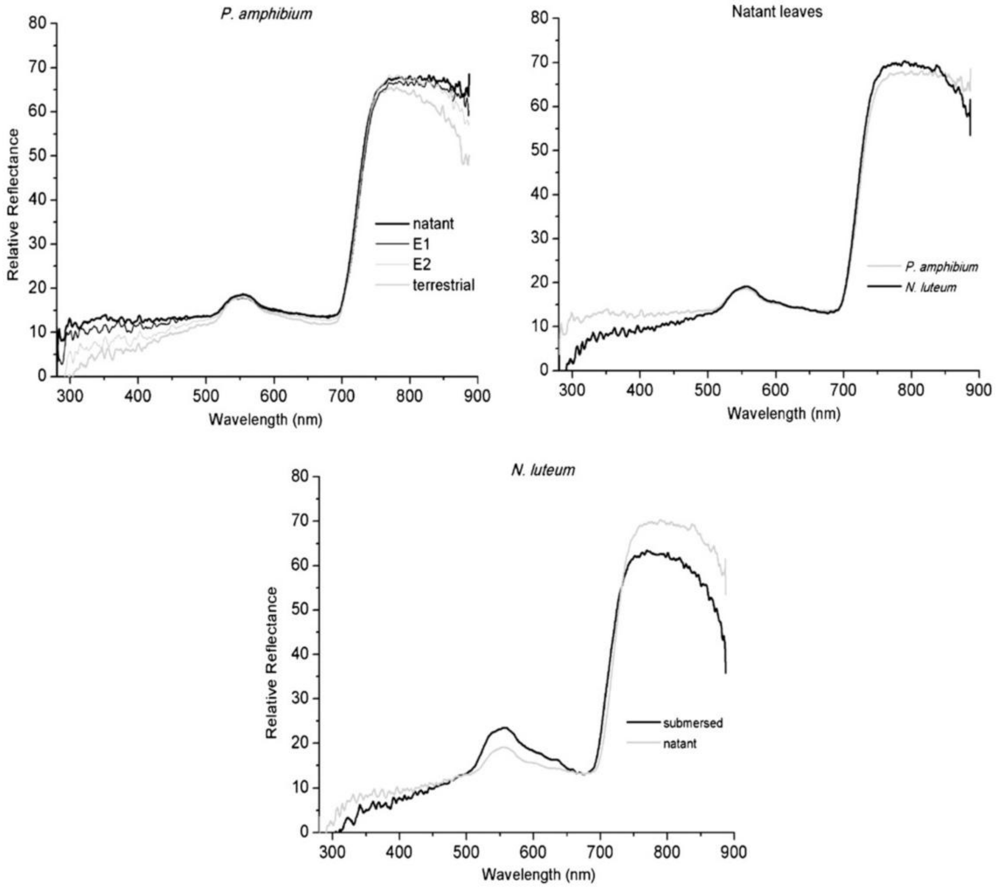

Fig. 4. Average relative reflectance spectra ($n = 5$).

high temperatures, drought, nitrogen shortage (Steyn et al., 2002; Close and Beadle, 2003) which can occur frequently in the changing environment of intermittent wetlands (Gaberščik et al., 2003). Anthocyanins also strongly absorb light and may function as a substitute for the protective role of the xanthophyll cycle (Manetas et al., 2002).

RDA confirmed the importance trichomes on adaxial leaf surface, that explainied 72% of the variability of the spectra. The role of trichomes in shaping reflectance spectra has been demonstrated in many studies (Ehleringer et al., 1976; Levizou et al., 2005), the effects being mainly positive. However, negative correlation in the case of *P. amphibium* showed that the absence of trichomes, rather than pubescence, increased the reflectance. This is in accord with the study of Holmes and Keiller (2002) who detected higher reflectance from waxy leaves than from hairy ones. The strongest negative correlation was obtained in the UV region, therefore we presumed that the trichomes of water bistort contain UV absorbing compounds, as was shown for some other species (Karabourniotis et al., 1992; Skaltsa et al., 1994).

An additional, significant effect in RDA was obtained for LMA, which explained 6% of the variability of spectra. Asner and Martin (2008) also found correlations between specific leaf area and reflectance spectrum for tropical forest canopy species.

4.2. *N. luteum*

N. luteum shows temporal (ontogenetic) segregation of leaf forms. It develops submerged leaves in the juvenile stage followed later by development of large floating leaves (Fox, 1992). Submerged leaves in heterophyllous aquatic plants are usually thinner, with higher specific leaf area, thinner cuticle and mesophyll, with no or fewer stomata, lower contents but more efficient photosynthetic pigments than emerged leaves (Rascio et al., 1999; Rascio, 2002; Enríquez and Sand-Jensen, 2003; Kuwabara and Nagata, 2002). In the case of *N. luteum*, submersed leaves produced significantly lower amounts of UV absorbing compounds expressed per area while the amounts of other pigments expressed per dry mass were higher than in natant leaves. According to Rozema et al. (2002), aquatic and amphibious plants have retained some of the biochemical characteristics of their terrestrial ancestors that protect against UV-B radiation. However the majority of emerged specimens produced higher contents of UV-B absorbing compounds than submerged, which is consistent with the higher levels of UV-B radiation on land than in water (Šraj-Kržič and Gaberščik, 2005; Šraj-Kržič et al., 2009).

The two leaf types had significantly different reflectance spectra. Interestingly, submersed leaves had higher relative reflectance in the region of the visible spectrum. The possible reason is lower content of pigments per projected area, above all anthocyanins as revealed from correlation analysis and RDA. Higher reflection is also beneficial for plants because it also prevent damage due to excess light (Ehleringer et al., 1976).

We obtained significant correlations between contents of chlorophyll *a*, anthocyanins and carotenoids and the red region of spectra. The correlation with carotenoids is possibly an artefact since carotenoids exhibit overlapping absorption of chlorophyll wavebands and it is difficult to obtain relationships between reflectance and carotenoids (Zur et al., 2000; Gitelson et al., 2002).

Carotenoids can play a role of accessory pigments under light limitation (Demmig-Adams and Adams, 1996). Further, levels of carotenoids in some species were significantly negatively related to photochemical efficiency of PSII, preventing photodamage via non-photochemical quenching of the chlorophyll *a* fluorescence of PSII (Adams and Demmig-Adams, 2004). Photoinhibition in aquatic environment could occur, not only under high light intensities, but also indirectly under nutrient shortage and limited CO_2 availability in water (Steyn et al., 2002; Nielsen and Nielsen, 2006) that may also induce the production of carotenoids, and increased carotenoid-to-chlorophyll ratio (Solovchenko, 2010). In our case the ratio was rather low therefore we presumed that the protective role of carotenoids is of lesser importance.

In the NIR region of reflectance spectra, strong correlations were obtained with leaf thickness ($r = 0.82$, $p < 0.01$), in line with the results of Ourcival et al. (1999) and Knapp and Carter (1998) who examined 26 species of various growth forms.

RDA revealed that the content of anthocyanins per dry mass explained 57% of the variability of the spectra. Anthocyanins have a strong absorption peak in the green region of the spectrum, with the "tails" reaching the yellow and the violet regions of the spectrum (Solovchenko and Merzlyak, 2008).

4.3. Interspecies comparison

Both intra-species and inter-species comparison of leaf types in general revealed that morphological differences are more pronounced than biochemical ones. In *P. amphibium* and *N. luteum* different plant traits that develop in leaf types optimise their function in the changing environment (Gaberščik and Martinčič, 1992; Gaberščik, 1993; Šraj-Kržič and Gaberščik, 2005), affecting the reflectance spectrum in different ways. In *N. luteum* leaves were not hairy, but pubescence was proved to be the most influential parameter shaping the reflectance spectrum in *P. amphibium*. This is possibly the main reason why, in *N. luteum*, the influence of pigments was more pronounced, as has been shown for many other species (Asner and Wessman, 1997; Slaton et al., 2001; Baltzer and Thomas, 2005; Gitelson et al., 2002; Castro-Esau et al., 2006; Underwood et al., 2007).

The comparison of natant leaves of both species showed that they were more similar than different leaf types within the single species. This is a consequence of similar environmental conditions; they are floating on the water surface, being fully exposed to the sun's rays.

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3.3 NA OPTIČNE LASTNOSTI LISTOV AMFIBIJSKIH RASTLIN VPLIVAJO MNOGE LASTNOSTI LISTOV

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Amfibijske rastline so sposobne razviti asimilacijske površine v povsem različnih okoljih, npr. v vodi in v zraku, kar jim daje pomembno tekmovalno prednost v spremenljivih okoljih (Enriquez in Sand-Jensen, 2003; Capon in Brock, 2006), kot je območje Cerkniškega jezera.

Namen raziskave je bil preučiti razlike optičnih lastnosti vodnih in kopenskih listov. Pri izbranih amfibijskih rastlinskih vrstah navadna streluša (*Sagittaria sagittifolia* L.) in velika zlatica (*Ranunculus lingua* L.), smo izmerili odbojne in transmisijske spektre v območju valovnih dolžin med 280 nm in 880 nm. Spektre smo primerjali in poiskali dele, kjer se spektralne krivulje listov, razvitih v vodi in listov, razvitih na kopnem, med seboj najbolj razlikujejo. Določili smo anatomske in biokemijske parametre, ki največ prispevajo k različnosti spektralnih krivulj med vrstama.

Primerjava listov znotraj posamezne rastlinske vrste je pokazala razlike v anatomskih in biokemijskih lastnostih, ki so se odražale tudi v različnih optičnih lastnostih listov, razvitih v vodi in na kopnem. Različni tipi listov znotraj vrste so se bolj razlikovali v količini in kakovosti prepuščenega sevanja, kot pri odbojnosti. Medvrstna primerjava listov pa je pokazala večje razlike med listi, razvitimi na kopnem, kot med listi, razvitimi v vodi. Analiza odbojnih spektrov je pokazala, da se potopljeni listi med vrstama bolj razlikujejo v prepustnosti sevanja, med tem ko se kopenski listi bolj razlikujejo v odbojnosti. V splošnem je bilo pri odbojnih spektrih potopljenih listov opaziti manj izrazit vrh v zelenem delu spektra. Listi, razviti v vodi so, prepuščali sevanje vzdolž celotnega merjenega spektra, listi, razviti na kopnem, pa so prepuščali sevanje le v območju zelenega in NIR dela spektra.

Na razlike pri odbojnih spektrih so imele večji vpliv biokemijske lastnosti listov (klorofil *a* in *b*, UV-B in UV-A absorbirajoče snovi), pri transmisijskih spektrih pa so bile bolj vplivne anatomske lastnosti (SLA, debelina stebričastega tkiva in debelina povrhnjic).

Raziskava je poleg vpliva okoljskih razmer na optične lastnosti rastlin pokazala tudi vpliv njihove evolucijske starosti v povezavi z izgradnjo UV-absorbirajočih snovi, katerih vsebnost je vplivala na odbojnost sevanja pri preučevanih rastlinskih vrstah.

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PLANTS IN HYDROSYSTEMS

Leaf optical properties in amphibious plant species are affected by multiple leaf traits

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Abstract The amphibious plant species *Sagittaria sagittifolia* and *Ranunculus lingua* here serve as model systems to study differences in leaf optical properties of different leaf types that develop in aquatic and terrestrial environments. We aimed to determine leaf traits that explain most of the variability in the reflectance and transmittance spectra in the range from 280 to 880 nm. Comparisons of leaves of the same form revealed marked differences in their structures and particularly in the content of total methanol-soluble UV-absorbing compounds. Submerged leaves transmit radiation over the whole range measured, but emerged leaves transmit only at wavelengths from 500 to 650 nm, and above 690 nm. Redundancy analysis shows that biochemical leaf traits, namely the UV-absorbing compounds chlorophyll *a* and *b*, together with the specific leaf area (SLA), significantly affect the reflectance spectra, explaining 60% of the spectra variability. Pigment levels negatively affect reflectance, while the effect of SLA is positive. Physical traits like thickness of the palisade mesophyll, SLA, and thickness of the lower and upper epidermis, along with anthocyanin content, explain 62% of the transmittance spectra variability. This study provides new insight into the understanding of data collected for aquatic and semi-aquatic plants based on spectral analyses.

Keywords *Sagittaria sagittifolia* L. · *Ranunculus lingua* L. · Leaf optical properties · Leaf traits

Introduction

Amphibious plants can successfully withstand variable water regimes (Braendle & Crawford, 1999; Warwick & Brock, 2003; Šraj-Kržič & Gaberščik, 2005) and thrive in environments distinctly unfavourable for other plants. They have evolved adaptations that enable their survival in water and/or dry land. The most important adaptations are related to their assimilation areas, which are highly plastic. Many of them are heterophyllous since they develop different leaf forms, submerged and/or floating leaves in water and aerial/terrestrial leaves in air, as well as numerous transitional leaf forms that enable undisturbed functioning (CO_2 uptake and light absorption) in contrasting environments (Gaberščik, 1993; Klančnik et al., 2012). The growth of different leaf types is induced by different environmental factors, the most important being the quality of incoming radiation, partial

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pressure of CO_2 and relative humidity (Kuwabara & Nagata, 2002). The investment of energy into the growth of properly adapted assimilation areas in amphibious plant species represents an important competitive advantage against fully aquatic and terrestrial plants in changing environments (Enríquez & Sand-Jensen, 2003; Capon & Brock, 2006).

Leaves are complex structures that regulate photon harvesting at multiple levels, from the biophysical structure to the orientation of leaves (Ustin et al., 2001). Different leaf forms have specific optical properties (Klančnik et al., 2012) that could be estimated by measurements of light reflected, absorbed and/or transmitted through the leaf (Woolley, 1971). The information about leaf reflectance spectra provides a widely used tool for determination of leaf biochemistry (Gitelson et al., 2002; Levizou et al., 2005), detecting stress (Gitelson et al., 2002) and enables species classification (Knapp & Carter, 1998). Submerged leaves are usually thinner, with higher specific leaf areas (SLA), thinner cuticles and mesophyll tissue, without or with lesser stomata and lower contents of photosynthetic pigments compared to natant and emerged leaves (Frost-Christensen & Sand-Jensen, 1995; Rascio et al., 1999; Klančnik et al., 2012). The absorbance of light in submerged leaves is lower in comparison to that in natant leaves (Frost-Christensen & Sand-Jensen, 1995). Special features of the leaves that develop in plants growing in water-saturated soil are extended air spaces in the tissue (Hroudová et al., 1988; Hostrup & Wiegleb, 1991; Robe & Griffiths, 1998), which could also contribute to the differences in optical properties among submerged, floating and terrestrial leaves (Zimmerman, 2006).

Different leaf forms that develop in a water/air gradient are not only exposed to different media but also to different radiation environments. Because of their plastic response and specific, variable radiation environment, they offer a challenging system to study responses and adaptations related to the absorption of light (Germ et al., 2002). Submerged leaves usually grow in dense stands in water, where light availability is modest; natant leaves are directly exposed to full sunlight, while emergent leaves avoid direct sunrays during high noon. In this study, we aimed to determine differences in optical properties of different leaves developed in water and air in two heterophyllous aquatic plant species *Sagittaria sagittifolia* L. and *Ranunculus lingua* L. We also wanted to point out biochemical and morphological traits of leaves that explain the most of the variability of reflectance and transmittance spectra. As the intensity of radiation passing through the leaf decreases exponentially when reaching the successive cell layer (Larcher, 2003), we assumed that the presence of aerenchyma will also play an important role in radiation balance in leaves.

Materials and methods

Study sites

Plants were sampled in two surface flows of the intermittent Ljubljanica River *s. lat.* The Ljubljanica River *s. lat.* is formed by the karst streams Trbuhovica, Obrh, Stržen, Rak, Unica and Pivka and the lowland river Ljubljanica *s. str. S. sagittifolia* was collected in the lower flow of the Rak River, while *R. lingua* was collected in the Stržen River. The growth conditions at both sampling locations are similar regarding sediment, while they might differ in flow velocity. Both rivers have intermittent water regimes, influenced by specific karst geology. Due to extreme water level fluctuations and occasional intermittence, amphibious plant species prevail (Gaberščik et al., 2003).

Studied species

Sagittaria sagittifolia L. is a stoloniferous aquatic perennial found in a variety of wetland habitats throughout Europe. Due to its great phenotypic plasticity, *S. sagittifolia* is able to produce a variety of submerged, floating and emergent leaves in accordance with the ontogenetic stage of the plant and environmental conditions (Hroudová et al., 1988; Dorken & Barrett, 2003).

Ranunculus lingua L. is an emergent aquatic plant found throughout Eurasia, with its core distribution in central and eastern Europe (Meusel, 1965). *R. lingua* occurs in shallow water at the edges of lakes, rivers, ditches and swamps. It often grows in dense stands of other emergent plants (Johansson & Nilsson, 1993), which are subjected to water level fluctuations during the growth season (Rybka & Duchoslav, 2007). *R. lingua* develops narrow lanceolate-shaped leaves in water and air.

At the growth location, natant and emerged leaves of both plant species were exposed to full sunlight with an intensity of about 2000 μmol m^{-2} s^{-1} at noon on a sunny day. Submerged leaves of *S. sagittifolia* that developed in monospecific stands were exposed from 10 to 50% full sunlight, depending on the water level. Submerged leaves of *R. lingua* were developed in water where the intensity of radiation ranged from 50 to 80% radiation measured at the water surface.

We examined fully developed submerged, floating and emergent leaves in *S. sagittifolia* and submerged and emergent leaves in *R. lingua*. Measurements were carried out on ten replicates of each leaf type.

Measurements of spectral reflectance and transmittance

Leaf optical properties of vital leaves were measured on the day of sampling. Leaf reflectance and transmittance were measured throughout the 280–880 nm spectrum using a Jaz Modular Optical Sensing Suite portable spectrometer (Ocean Optics, Inc., Dunedin, Florida, USA), which was fitted with an ISP-30-6-R integrating sphere (Ocean Optics, Inc., FL, USA) and QP600-1-SR-BX optical fibre (Ocean Optics, Inc., Dunedin, Florida, USA).

Measurements of reflectance followed the procedure by Klančnik et al. (2012). Total adaxial reflectance spectra were recorded during illumination of leaves with a UV–VIS–NIR light source (DH-2000, Ocean Optics, Inc., FL, USA). Radiance reflected from the surface was transmitted from the integrating sphere to the spectrometer. The spectrometer was calibrated to 100% reflectance using a white reference panel (Spectrolon, Labsphere, North Sutton, New Hampshire, USA) prior to sample measurement. Reflectance spectra were calculated as the ratio of sample data to a white reference panel under the same illumination.

For measurements of transmittance spectra, the samples were installed under an integrating sphere in a way that the lower leaf side was exposed to the integrating sphere. The UV–VIS–NIR light source (DH-2000, Ocean Optics, Inc., FL, USA) illuminated the adaxial leaf surface, and light passed through the sample into the integrating sphere. The spectrometer was calibrated to 100% transmittance with a light beam passing directly to the integrating sphere interior. Transmittance spectra were calculated as the ratio of reduction of light beam intensity after passing the leaf tissue.

Morphological and anatomical analysis

SLA was determined as leaf area per unit of dry mass (cm^2 mg^{-1}). To estimate the extent of aerenchyma, leaf tissue density was calculated as unit dry mass per leaf volume (mg cm^{-3}). Leaf histological analysis was conducted on ten transverse sections of each leaf type. The leaf thicknesses of cuticle, epidermis, palisade and spongy mesophyll were measured at ×100 magnification, using an Olympus CX41 microscope equipped with an Olympus XC30 digital camera and CellSens software (Olympus, Japan). The density and length of the leaf stomata and trichomes were also examined.

Biochemical analysis

Contents of chlorophyll *a*, chlorophyll *b* and carotenoids were determined according to Lichtenthaler & Buschmann (2001a, b). The absorbance levels of samples were measured at wavelengths 470, 645 and 662 nm using a UV/VIS spectrometer (Lambda 25, Perkin-Elmer, Norwalk, CT, USA). The contents of chlorophylls and carotenoids were expressed per area of sample (mg dm^{-2}).

The content of anthocyanins was determined as previously described by Drumm & Mohr (1978). The absorbance levels of extracts were measured at 530 nm; the content of the pigment was calculated per sample area (relative units per dm^{-2}).

Total methanol-soluble UV-B- and UV-A-absorbing compounds were extracted from weighed fresh plant material according to Caldwell (1968). The absorbance levels of extracts were measured in the spectral ranges of 280–319 and 320–400 nm. The extinction values were integrated for each UV region and expressed in relative units per sample area.

Statistical analysis

The normal distribution of data was tested using the Shapiro–Wilk test. Differences in anatomical and biochemical parameters were tested, applying Student's *t* test or non-parametric Mann–Whitney test as well as ANOVA or the non-parametric Kruskal–Wallis test. Measurements of reflectance and transmittance spectra were averaged within 5-nm intervals. The significance of differences between reflectance and transmittance spectra was assessed with a

Kruskal–Wallis test with Bonferroni's correction. To find possible relationships between spectra and measured leaf traits, Spearman's rank correlation analysis was performed. SPSS Statistics 19.0 was used for the aforementioned calculations. The level of significance was accepted at $P \leq 0.05$. Detrended correspondence analysis was used for exploratory data analysis applying the CANOCO for Windows 4.5 programme package. Due to the obtained gradient lengths (<3 SD) (ter Braak & Šmilauer, 2002), redundancy analysis (RDA) was used to determine whether variations in response variable (reflectance and transmittance spectra) were related to explanatory variables (anatomical and biochemical parameters). The significance of effects of variables was assessed using a Monte Carlo test with 999 permutations. Forward selection of explanatory variables was used to avoid co-linearity between variables. Non-significant variables ($P > 0.05$) were excluded from further RDA. All variables used in analysis were standardized.

Results

Anatomical and biochemical traits of leaves

A comparison of anatomical and biochemical parameters of different leaf types of *S. sagittifolia* and *R. lingua* revealed differences between leaves developed in contrasting environmental conditions. The changes in measured parameters reflected the conditions of the water/air gradient. SLA decreased with increasing aridity of the environment (Table 1). In contrast, tissue density, leaf thickness, palisade mesophyll thickness and contents of pigments were increasing (Table 1). The thickness of the epidermis was the only parameter that did not vary significantly between different leaf types within a species, while it differed significantly between species. However, in submerged leaves of *S. sagittifolia*, we observed an outstanding structure of the upper epidermis that consisted of bubble-shaped cells without chloroplasts. The leaves developed in water and those developed in air also differed in leaf surfaces, such as cuticle, stomata and trichomes (Table 1).

An interspecies comparison of anatomical and biochemical traits of different leaf types revealed that submerged leaves of both species were more alike than emerged leaves (Table 1).

Leaves' spectral properties

A comparison of optical properties showed that different leaf types varied in their reflectance and transmittance spectra (Fig. 1). In general, the reflection spectra of different leaf types differed less than the transmission spectra. In the case of *S. sagittifolia*, the reflection of submerged leaves was significantly greater in comparison to natant and emerged leaves (Fig. 1a). Those two leaf types exhibited smaller differences in their reflectance spectra; in the green, yellow and NIR regions of the spectra, curves did not differ significantly. In contrast, different leaf types of *R. lingua* differed significantly in their reflectance spectra throughout the measured range. Emerged leaves reflected significantly more incoming radiation than submerged leaves (Fig. 1b). However, submerged leaves of both plant species transmitted significantly more light than other leaf types (Fig. 1c, d). For all leaf types, relative transmittance was the most pronounced in the green and NIR regions of the spectra.

Interspecies comparisons of the spectral properties of submerged leaves showed that reflectance and transmittance spectra differed significantly between species. However, the differences of reflectance spectra were not so consistent, compared to those of the transmittance spectra (Fig. 1). Submerged leaves of *S. sagittifolia* reflected and transmitted more incoming radiation than the leaves of *R. lingua*. In general, the relative transmittance of both studied species was greater than the relative reflectance (Fig. 1). Furthermore, emerged leaves of both species differed markedly in their spectral properties. In the case of emerged leaves, however, differences of transmittance spectra were not so consistent, compared to those of the reflectance spectra. Transmittance spectra of both plant species differed significantly, except in the range from 565 to 615 nm. The emerged leaves of *R. lingua* reflected significantly more light than leaves of *S. sagittifolia*, while leaves of *S. sagittifolia* transmitted more incoming radiation (Fig. 1).

In general, the results of relative reflectance showed that the green peak in reflectance curves was less pronounced in aquatic leaf forms (Fig. 1a, b). The results of relative transmittance revealed that submerged leaves transmitted more incoming radiation along the whole spectrum, while emerged leaves transmitted only the wavelengths from 500 to 650 nm and above 690 nm (Fig. 1c, d).

Table 1 Leaf anatomical and biochemical parameters in *S. sagittifolia* and *R. lingua*

	S. sagittifolia			*R. lingua*	
	Submerged (s)	Natant (n)	Emerged (e)	Submerged (s)	Emerged (e)
Traits					
SLA (cm^2/mg)	$0.91 \pm 0.17^{n,e}$	0.40 ± 0.12^{s}	0.35 ± 0.07^{s}	$0.42 \pm 0.1^{e,**}$	$0.19 \pm 0.0^{s,*}$
Leaf thickness (μm)	$186 \pm 54^{n,e}$	242 ± 40^{s}	253 ± 31^{s}	219 ± 37^{e}	258 ± 33^{s}
Tissue density (mg/mm^3)	$0.066 \pm 0.02^{n,e}$	0.108 ± 0.03^{s}	0.116 ± 0.02^{s}	$0.114 \pm 0.03^{e,**}$	$0.219 \pm 0.05^{s,**}$
Palisade mesophyll (μm)	$0^{n,e}$	$46.3 \pm 11.4^{s,e}$	$66.2 \pm 17.4^{n,s}$	0^{e}	$87.35 \pm 21.2^{s,*}$
Spongy mesophyll (μm)	131 ± 33	136 ± 33	130 ± 15	$166 \pm 47.7^{e,*}$	121.3 ± 21^{s}
Upper surface					
Cuticle thickness (μm)	$0^{n,e}$	3.9 ± 1^{s}	3.6 ± 1^{s}	0^{e}	$2.5 \pm 0^{s,**}$
Epidermis thickness (μm)	30.8 ± 8.2	28.1 ± 5	28.9 ± 2.7	15.5 ± 3.5*	16.6 ± 3.7*
Stomata density (mm^{-2})	$0^{n,e}$	$47 \pm 18^{s,e}$	$66 \pm 14^{s,n}$	0	$33 \pm 7^{s,**}$
Stomata length (μm)	$0^{n,e}$	54.8 ± 3.6^{s}	49.9 ± 4.2^{s}	0^{e}	46.9 ± 14.5^{s}
Lower surface					
Epidermis thickness (μm)	26.8 ± 4.2	30.2 ± 5.9	27.7 ± 5.0	17.1 ± 2.9*	16.7 ± 4.2*
Cuticle thickness (μm)	$0^{n,e}$	$1.9 \pm 0.25^{s,e}$	$2.1 \pm 0.21^{s,n}$	0^{e}	$2.5 \pm 0^{s,*}$
Stomata density (mm^{-2})	$0^{n,e}$	$34 \pm 13^{s,n}$	$55 \pm 11^{s,e}$	0^{e}	$34 \pm 7^{s,*}$
Stomata length (μm)	$0^{n,e}$	53.9 ± 5.3^{s}	46.5 ± 3.6^{s}	0^{e}	52.9 ± 9.4^{s}
Trichome density (cm^2)	0	0	0	0^{e}	$59 \pm 27^{s,**}$
Biochemical parameters					
Chlorophyll *a* (mg/dm^2)	$0.85 \pm 0.14^{n,e}$	$1.78 \pm 0.6^{s,e}$	$3.42 \pm 0.87^{s,n}$	$1.28 \pm 0.39^{e,**}$	$2.5 \pm 0.38^{s,*}$
Chlorophyll *b* (mg/dm^2)	0.30 ± 0.03^{e}	0.55 ± 0.03^{e}	$1.96 \pm 0.66^{s,n}$	0.76 ± 0.36**	0.81 ± 0.13**
Carotenoids (mg/dm^2)	$0.30 \pm 0.06^{n,e}$	$0.56 \pm 0.15^{s,e}$	$0.93 \pm 0.18^{s,n}$	0.41 ± 0.12^{e}	0.89 ± 0.11^{s}
Anthocyanins (relative units per dm^2)	$25.9 \pm 10.4^{n,e}$	$143.9 \pm 34.2^{s,e}$	$93.9 \pm 22^{s,n}$	26.8 ± 5.6	42.6 ± 16.6**
UVB-absorbing compounds (relative units per dm^2)	$224 \pm 64^{n,e}$	594 ± 217^{s}	821 ± 191^{s}	$89 \pm 31^{e,**}$	$241 \pm 61^{s,**}$
UVA-absorbing compounds (relative units per dm^2)	$352 \pm 87^{n,e}$	$1125 \pm 404^{s,e}$	$1569 \pm 329^{s,n}$	$92.6 \pm 28^{e,**}$	$239 \pm 55^{s,**}$

Values are mean ± SD. Letters indicate significant differences between different leaf types in *S. sagittifolia* and *R. lingua* ($P \leq 0.05$). Asterisks indicate significant differences between the same leaf type of two plant species (* $P \leq 0.05$, ** $P \leq 0.01$)

Relation between leaf traits and spectra

RDA indicated that the most influential parameters shaping reflectance spectra were SLA and the contents of pigments, i.e. chlorophyll *a* and chlorophyll *b*, UV-A and UV-B absorbing compounds (Fig. 2). All aforementioned parameters explained together 60% of the variability of the reflectance spectra; most of this was explained by the content of UV-A-absorbing compounds (33%). The contents of UV-B absorbing compounds, chlorophyll *b*, chlorophyll *a* and SLA, accounted for 11, 8, 3 and 5% variability of the reflectance spectra, respectively. All pigments were negatively correlated with different regions of the spectra, while in the case of SLA, the correlation was positive. The tri-plot also showed the formation of five distinct groups, representing five groups of different leaf types of both species (Fig. 2).

The parameters that affected transmittance spectra were SLA, the thickness of palisade mesophyll and the thickness of lower and upper epidermis, as well as the anthocyanin content (Fig. 3). All parameters together explained 62% of the variability of the transmittance spectra. Palisade mesophyll thickness accounted for 36% of the variability, the thickness of lower epidermis 13% of the variability of the transmittance spectra, while anthocyanin content, SLA and thickness of upper epidermis accounted for 6, 4 and 3% of the of variability, respectively.

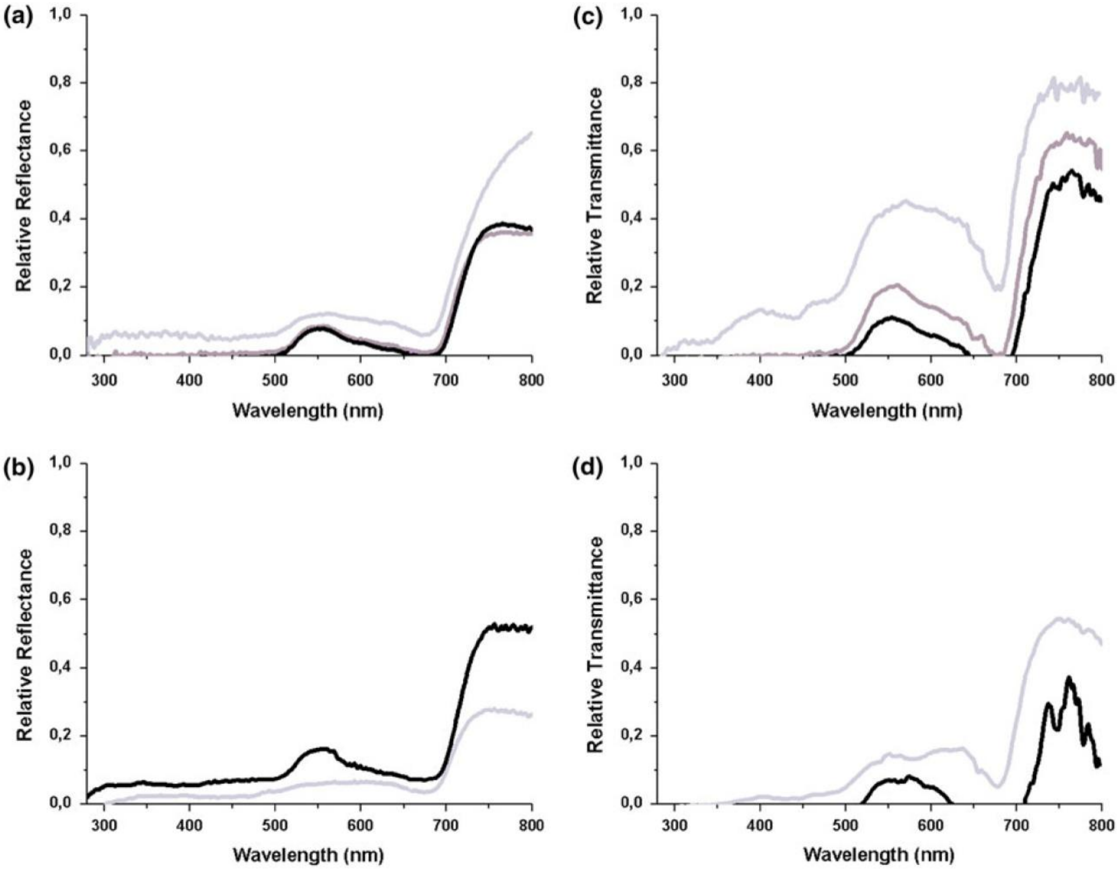

Fig. 1 Average relative reflectance (**a**) and transmittance (**c**) in *S. sagittifolia*, and average relative reflectance (**b**) and transmittance (**d**) in *R. lingua*. Spectral curves are averaged within 5-nm intervals, $n = 10$. *Black line* emergent leaves; *Dark grey line* natant leaves; and *Light grey line* submerged leaves

Discussion

Anatomical and biochemical traits of leaves

The amphibious species *S. sagittifolia* and *R. lingua* have the ability to develop different leaf forms that enable survival in extreme water level fluctuations in the intermittent aquatic ecosystem. Despite many characteristics common for submerged plants (Frost-Christensen & Sand-Jensen, 1995; Rascio et al., 1999), the submerged leaves of two species differed in their structure because they thrive in different habitats and they differ significantly in their evolutionary age, which is related to different plant biochemistry above all the content of UV-absorbing compounds (Rozema et al., 2002). The most important environmental factor that affects leaf traits is light intensity, which decreases quickly within the macrophyte stand (Titus & Adams, 1979). Ribbon-like submerged leaves of *S. sagittifolia* developed in deep water under low light, influenced by moderate flow velocity, while elongated leaves of *R. lingua* developed in shallow still water near the bank, where light intensity was high. As a result, submerged leaves of *S. sagittifolia* were thinner, with denser leaf tissue, higher SLA and lower chlorophyll contents per projected area (Table 1). The epidermis of *S. sagittifolia* consisted of enlarged bubble-shaped cells, which may contribute to buoyancy and orientation of the leaf lamina in the water column. The differences in anatomical and biochemical characteristics of emerged leaves of both species were even more pronounced in comparison to submerged leaves (Table 1). The mesophyll tissue of emerged leaves of

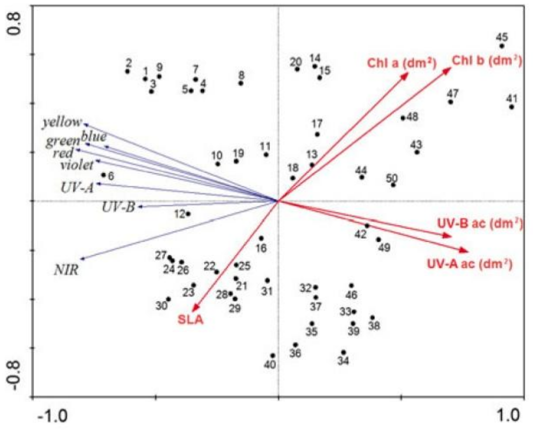

Fig. 2 RDA plot presenting the strength of associations between anatomical and biochemical parameters and different regions of the reflectance spectra. Eigenvalues for the first two axes were 0.553 and 0.037. *Numbers* represent analysed leaf types: *1–10* terrestrial leaves of *R. lingua*, *11–20* submerged leaves of *R. lingua*, *21–30* submerged leaves of *S. sagittifolia*, *31–40* natant leaves of *S. sagittifolia* and *41–50* terrestrial leaves of *S. sagittifolia*. Abbreviations for plant traits are the following: *Chl a* contents of chlorophyll *a*, *Chl b* contents of chlorophyll *b*, *UV-A ac* UV-A absorbing compounds, *UV-B ac* UV-B absorbing compounds

R. lingua was denser with higher SLA and a thicker palisade layer in comparison to emerged leaves of *S. sagittifolia*. The aerenchyma in leaves of *S. sagittifolia* was more developed than in *R. lingua*, which was related to the more terrestrial character of the latter. There were also pronounced differences in aerenchyma between different leaf types within a species. The aerenchyma ratio usually increases with inundation and is therefore higher in submerged specimens of amphibious plants (Braendle & Crawford, 1999; Boeger & Poulson, 2003). Important differences were also observed in structures on the leaf surface. In *R. lingua*, numerous long trichomes were found on the abaxial leaf surface (Table 1). On one hand, trichomes on the lower leaf surface of emerged leaves may protect leaf tissue against excessive radiation of light reflected from the water surface when plants grow in water and, on the other, hand they could also prevent water loss during dry period (Ehleringer, 1980; Woodman & Fernandes, 1991).

Leaves' optical properties

Differences in anatomical and biochemical characteristics of different leaf types affected their optical properties. RDA indicated that the biochemical characteristics of leaves exerted more effect on the reflectance spectra, anatomical characteristics of leaves exerted more effect on transmittance spectra (Figs. 2, 3), while leaf density, which is the measure of the extent of aerenchyma, exerted no significant effect on variability of spectra. The former is in accordance with other studies, which also confirmed correlations between reflectance spectra and biochemical traits of leaves (Baltzer & Thomas, 2005; Levizou et al., 2005; Castro & Sanchez-Azofeifa, 2008). Knapp & Carter (1998), who evaluated leaf optical properties of various plant species from different habitats, report that aquatic plants *Typha latifolia* and *Eichhornia crassipes* with abundant air spaces did not differ significantly from other plant species. Most of the variability of the reflectance spectra was explained by contents of UV-A- (33%) and UV-B-absorbing compounds (11%). The amount of UV-absorbing compounds significantly differed among species. Rozema et al. (2002) report that the amount and complexity of UV-absorbing substances in plants

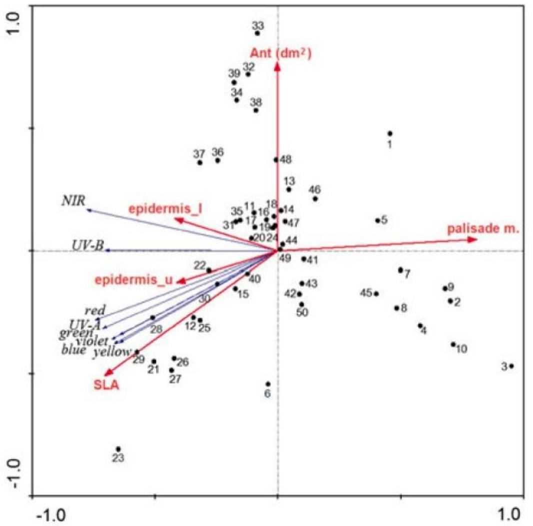

Fig. 3 RDA plot presenting the strength of associations between anatomical and biochemical parameters and different regions of the transmittance spectra. Eigenvalues for the first two axes were 0.552 and 0.059. *Numbers* represent analysed leaf types: *1–10* terrestrial leaves of *R. lingua*, *11–20* submerged leaves of *R. lingua*, *21–30* submerged leaves of *S. sagittifolia*, *31–40* natant leaves of *S. sagittifolia* and *41–50* terrestrial leaves of *S. sagittifolia*. Abbreviations for plant traits are the following: *Ant* content of anthocyanins, *palisade m.* palisade mesophyll thickness, *epidermis_l* thickness of lower epidermis, *epidermis_u* thickness of upper epidermis, *SLA* specific leaf area

were increasing during plant evolution, which was also shown in some other studies (Germ et al., 2002, 2006; Germ & Gaberščik, 2003). Since the genus *Ranunculus* is evolutionarily much older in comparison to the genus *Sagittaria*, the difference in the amount of UV-absorbing substances comparing the same leaf form seems to be more evolutionary than environmentally based. However, significantly higher amounts of UV-absorbing substances were obtained when comparing areal and aquatic leaf forms within the species. This is in accordance with the research of Les & Sheridan (1990), which revealed reduced complexity of UV-B absorption in submerged leaves of *Potamogeton natans* compared to natant leaves. Moreover, Germ et al. (2002) estimated significantly lower amounts of UV-absorbing compounds in submerged leaves of *Potamogeton alpinus* in comparison to natant leaves.

Submerged leaves of *S. sagittifolia* reflected and transmitted more incoming radiation than submerged leaves of *R. lingua*. A possible reason was high SLA, which was one of the most influential parameters shaping reflectance and transmittance spectra as shown by RDA (Figs. 2, 3). Lower SLA might increase the backscatter effect, which consequently increases total light absorbance, reduces sieving effects and increases path-lengthening effects (Lee et al., 2000). Correlations between SLA and reflectance spectra were also observed by Asner & Martin (2008), who studied optical properties of tropical forest canopy species. RDA also revealed the importance of chlorophyll contents, which were negatively correlated with reflectance spectra (Fig. 2). Due to lower chlorophyll contents, the reflection of light of submerged leaves of *S. sagittifolia* was greater than that of *R. lingua*. The role of pigments in shaping reflectance spectra has already been confirmed in many other studies (Slaton et al., 2001; Baltzer & Thomas, 2005; Levizou et al., 2005; Castro & Sanchez-Azofeifa, 2008). In addition to the SLA, RDA also revealed significant effects of palisade mesophyll thickness, the thickness of epidermis and anthocyanin content on the transmittance spectra (Fig. 3). In contrast to submerged leaves, emerged leaves of *R. lingua* reflected significantly more incoming radiation (Fig. 1b), which was possibly related to thick cuticles. RDA showed that contents of chlorophylls and contents of UV-absorbing compounds correlated negatively with reflectance spectra (Fig. 2). Emerged leaves of *S. sagittifolia* contain more UV-A- and UV-B-absorbing compounds per projected area (Table 1), which could explain its lower reflectance spectra, especially in the regions below 500 nm (Fig. 1a, b). In any case, emerged leaves of *S. sagittifolia* were less shiny in comparison to emerged leaves of *R. lingua*. The structure of the cuticle probably differed between species, which could also significantly shape reflectance spectra (Holmes & Keiller, 2002). Emerged leaves of *S. sagittifolia* transmitted more incoming radiation than emerged leaves of *R. lingua*. Emerged leaves of *R. lingua* had lower SLA, which positively correlated with transmittance spectra; the epidermis and cuticle were thinner, but the lower leaf surface was covered with trichomes. The presence of trichomes may significantly affect leaf optical properties (Klančnik et al., 2012) and decrease leaf transmittance (Baldini et al., 1997). Despite the fact that interspecies comparisons revealed significant differences in leaf traits, which had developed either in water or in air, the obtained results showed that the leaves function similarly. The green peak in reflectance curves was less pronounced when leaves were developed in water (Fig. 1a, b). Those leaves also transmitted more incoming radiation along the whole measured range (Fig. 1c, d), possibly because of lower amounts of all pigments per volume of leaf tissue.

We conclude that reflectance and transmittance spectra of both submerged and emerged leaves differed significantly between species. We found that not only the variability of habitat characteristics that affected plant traits but also evolutionary age which is related to the production of secondary substances (UV-absorbing compounds) significantly affected the optical properties in the leaves of two species. The results showed that the biochemical traits exerted more effect on the reflectance spectra, while transmittance spectra were mainly influenced by anatomical traits; however, the presence of aerenchyma did not statistically significantly affect light absorbance of leaves. The information gained in this study may contribute to the interpretation of data collected on aquatic and semi-aquatic plants by remote-sensing techniques, based on reflectance spectra.

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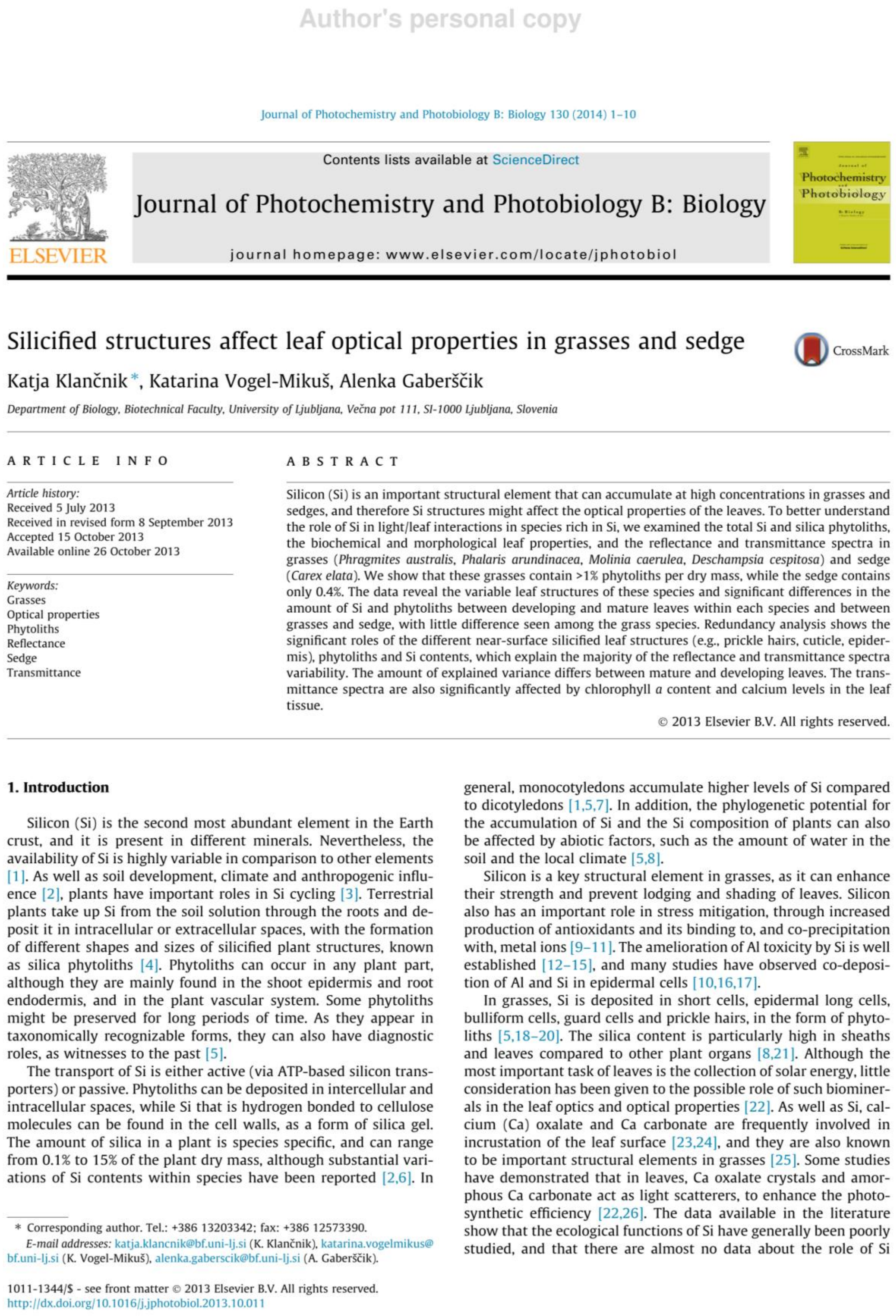
3.4 SILICIJEVE STRUKTURE VPLIVAJO NA OPTIČNE LASTNOSTI LISTOV TRAV IN ŠAŠA

Katja Klančnik, Katarina Vogel-Mikuš, Alenka Gaberščik. 2014. Silicified structures affect leaf optical properties in grasses and sedge. Journal of Photochemistry and Photobiology B: Biology 130: 1-10

Predstavniki trav in šašev so znani kot akumulatorji Si, njegove vsebnosti pa so navadno največje v listih (Piperno, 2006). Si se pogosto odlaga v epidermalnih celicah in strukturah na ali blizu površini lista (Kaufman in sod., 1985; Cauhan in sod., 2011). Znano je, da strukturiranost listne površine močno vpliva na usodo sevanja, ki pade na list (Ehleringer in Björkman, 1978; Barker in sod., 1997; Holmes in Keiller, 2002; Klančnik in sod., 2012), vendar pa je o vplivih prisotnosti Si, silicijevih struktur in drugih mineralov na optične lastnosti listov malo znanega.

Pri preučevanju vpliva Si na interakcije lista s sevanjem, smo izbrali štiri vrste trav; navadni trst (*Phragmites australis*), pisanko (*Phalaris arundinacea*), modro stožko (*Molinia caerulea*), rušnato masnico (*Deschampsia cespitosa*) in togi šaš (*Carex elata*). Na območju Cerkniškega jezera smo nabrali mlade in zrele liste izbranih rastlinskih vrst. Na listih smo izmerili odbojne in transmisijske spektre v območju od valovnih dolžin od 280 do 880 nm. Sledila je anatomska in biokemijska analiza listov. S pomočjo XRF smo analizirali vsebnosti Si, Ca in Al v mladih in zrelih listih. Z gravimetrično metodo pa smo določili tudi vsebnost silicijevih fitolitov v različno starih listih izbranih rastlinskih vrst.

Analiza rastlinskega materiala je pokazala, da listi trav vsebujejo >1 % fitolitov na suho maso lista, medtem ko vsebnost fitolitov v listih šaša ne presega 0,43 %. Vsebnosti fitolitov in Si so se razlikovale med različno starimi listi znotraj vrste. Pri rušnati masnici in togem šašu je vsebnost Ca presegala vsebnost Si. Z RDA smo dokazali vpliv različnih silificiranih struktur blizu ali na površini lista, vsebnosti Si in silicijevih fitolitov na optične lastnosti listov. Vpliv teh parametrov je bolj opazen v primeru zrelih listov. Na prepustnost sevanja je pri zrelih listih poleg Si vplivala tudi vsebnost Ca v listnem tkivu.



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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 (1359g, 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.

Clean and dry leaves were weighed (300 mg) in 18-ml glass tubes. These leaf samples were soaked in 10 ml 65% nitric acid, and placed in a thermostat at 80 °C for 3 h. After this digestion at 80 °C, the temperature was increased to 100 °C for 1 h. When the digestion was complete, and all of the remaining material had sedimented to the bottom of the tubes, the nitric acid was carefully removed. These extracted phytoliths were washed in distilled water and in 10% HCl, to remove any Ca oxalate crystals, with the phytoliths in the samples sedimented by centrifugation between washes (339g, 10 min, room temperature). Then these phytolith suspensions were filtered using a vacuum filter system (IsoLab, Wertheim, Germany) with polytetrafluorethylene membrane filters of pore size 0.45 μm (Sartorius Stedim Biotech, Goettingen, Germany). The filters with the extracted phytoliths were rinsed three times with distilled water and placed in an oven at 75 °C for 24 h. Due to the large amounts of extracted phytoliths, some encrusted organic material might not have oxidised completely, and therefore the dry-ashing method was used next. The extracted phytoliths were removed from the filters and placed into porcelain crucibles. The crucibles were heated in a muffle furnace at 500 °C for 24 h. The phytolith contents were expressed as percentages of the leaf dry mass and as mass per volume of tissue. These phytolith extractions were carried out as three replicates for each plant species.

2.7. Leaf bulk element analysis

The concentrations of Si, Al and Ca in the leaves were determined by X-ray fluorescence (XRF) spectrometry. The dried plant material was pulverised and homogenised with a pestle and mortar. Depending on the available material, 100–500 mg of the powdered leaves were pressed into pellets using a pellet die and hydraulic press. ^{55}Fe (25 mCi; Isotope Products Laboratories, Valencia, USA) was used as the primary excitation source for the analysis. The fluorescence radiation emitted was collected using an energy dispersive X-ray spectrometer, equipped with a Si(Li) detector (Canberra, Meriden, USA), with a 25-μm-thick Be window. The energy resolution of the spectrometer at count rates below 1000 counts s^{-1} was 175 eV at 5.9 keV. The XRF analysis was performed under vacuum, and the samples were irradiated for 5000 s to obtain spectra with sufficient statistics [38]. The analysis of the X-ray spectra was performed using an iterative least-squares programme, as included in the quantitative X-ray analysis system software package. Element quantification from the measured spectra was performed using the quantitative analysis of environmental samples based on fundamental parameters [39]. Quality assurance for the element analysis was performed using standard reference materials: NIST SRM 1573a (tomato leaves as a homogenised powder) and OU-10 (geological sample of longmyndian greywacke, GeoPT24, certified through an international proficiency test of the International Association of Geoanalysts); these were all analysed in the form of pressed pellets.

2.8. Statistical analysis

The normal distribution of the data was tested using Shapiro-Wilk tests. Differences in morphological, anatomical and biochemical parameters were tested by applying one-way analysis of variance followed by Tukey's *post-hoc* multiple comparison tests or non-parametric Kruskal–Wallis tests. Measurements of reflectance and transmittance spectra were averaged within 5-nm intervals. The significances of the differences between reflectance and transmittance spectra of the different plant species were assessed using Kruskal–Wallis tests with Bonferroni's correction. Differences in phytolith contents between the leaves were tested by applying the Student's *t*-test. One-way analysis of variance followed by Tukey's *post-hoc* multiple comparison tests were used to assess differences between the concentrations of the elements in the leaves. SPSS Statistics 19.0 was used for the calculations. The level of significance was accepted at $p \leqslant 0.05$.

Redundancy analysis (RDA) was used to determine the effects of the explanatory variables (i.e., anatomical and biochemical parameters, phytolith content, concentrations of elements) on the response variables (i.e., reflectance and transmittance spectra), by applying the CANOCO for Windows 4.5 programme package [40]. Monte Carlo permutation tests with 999 permutations were carried out to test the significance of the effects. Forward selection of explanatory variables was used to avoid co-linearity. The level of significance was accepted at $p \leqslant 0.05$. Non-significant explanatory variables were excluded from the further analysis. A further RDA was carried out with a subset of only the selected variables. All of the variables in the RDA analysis were standardised to remove the influence of magnitude differences between scales and units.

3. Results

3.1. Morphological and biochemical parameters

The morphological and biochemical characteristics of the mature and developing leaves of the plant species studied were very variable (Tables 1 and 2). The majority of the measured morphological parameters of the mature leaves differed significantly ($p < 0.05$); namely, as thicknesses of different leaf layers, prickle hair densities and lengths, and leaf stomatal parameters. The biochemical parameters were more uniform, with fewer significant differences detected among the species. The single species that differed the most from the other species was *P. arundinacea* (Table 1). The differences among the species were also pronounced in the developing leaves (Table 2). The *C. elata* (the sedge) leaf morphology and biochemistry differed a lot from the other species (the grasses). There were fewer significant differences when the mature and developing leaves of the same species were compared. Most of the significant differences were obtained for the thickness of the cuticle, the prickle hair properties, the stomata densities in the upper and lower surfaces, and the carotenoid contents (Tables 1 and 2).

3.2. Phytolith contents and leaf bulk element analysis

In the mature leaves of the grasses, the phytoliths accounted for >1% of the dry weight, while the leaves of the sedge (*C. elata*) contained lower amounts (0.4% dry weight) (Table 3). The phytolith content per dry mass in the *C. elata* leaves was similar for the developing and mature leaves, while for the grasses, the differences between the developing and mature leaves were pronounced (Table 3). The phytolith content per dry mass in the mature leaves of *P. australis* (July), *P. arundinacea* and *M. caerulea* was significantly greater than in their respective developing leaves ($p < 0.01$). The *P. australis* leaves of the same phenological phases were sampled twice in the season, with the mature leaves showing increased phytolith content in July comparing to June ($p < 0.01$). The differences between leaves were observed also when comparing the mass of phytoliths per volume (Table 3). The greatest amounts were determined for *P. australis* and *D. cespitosa*. In *P. australis*, there were significant differences when leaves sampled in June and July were compared ($p < 0.01$). Significant differences between developing and mature leaves were obtained in *P. australis*, *M. caerulea* and *D. cespitosa* ($p < 0.01$).

The XRF spectroscopy revealed that in the leaves of the grasses, Si generally occurred at the highest concentrations, with the exception of *D. cespitosa*, where the Ca concentration was

Table 1
Morphological and biochemical parameters in the mature leaves of the four grasses and the sedge. Data are means ± standard errors ($n = 10$).

Properties	Grasses					Sedge
	Phragmites australis		*Phalaris arundinacea*	*Molinia caerulea*	*Deschampsia cespitosa**	*Carex elata*
	June	July	June	July	July	July
Specific leaf area (cm^2 g^{-1})	92.1 ± 3.3	93.8 ± 5.4	238.9 ± 9.4	164.9 ± 9.3	151.5 ± 11.6	130.1 ± 4.8
Leaf thickness (μm)	272 ± 5.8	219 ± 9.3	253 ± 6.5	124 ± 5.7	624 ± 22	250 ± 6.8
Mesophyll thickness (μm)	210.1 ± 5.5	180.2 ± 8.8	194.8 ± 5.9	99 ± 4.6	501.9 ± 20.6	94.5 ± 2.5
Upper surface						
Cuticle thickness (μm)	1.4 ± 0.06	1.2 ± 0.03	2.8 ± 0.13	4.5 ± 0.18	4.7 ± 0.16	2.3 ± 0.09
Epidermis thickness (μm)	14.1 ± 0.55	17.7 ± 0.51	27.4 ± 1.02	8.7 ± 0.60	15.4 ± 0.94	11.2 ± 0.39
Prickle hair density (mm^{-2})	62 ± 8	104 ± 22	1 ± 0	238 ± 11	132 ± 28	32 ± 17
Prickle hair length (μm)	16 ± 0.27	16 ± 0.4	9 ± 1.7	38 ± 1.7	26 ± 1.7	53 ± 2.1
Stomata density (mm^{-2})	332 ± 10	376 ± 18	61 ± 4	38 ± 22	146 ± 30	2 ± 1
Stomata length (μm)	20 ± 0.2	20 ± 0.19	45 ± 1	30 ± 0.9	31 ± 2.7	17 ± 3
Lower surface						
Cuticle thickness (μm)	1.4 ± 0.10	1.3 ± 0.10	2.5 ± 0.09	4.7 ± 0.27	5.6 ± 0.33	4.1 ± 0.41
Epidermis thickness (μm)	17.4 ± 0.41	13.7 ± 0.53	28 ± 0.95	8 ± 0.51	17.4 ± 0.58	22.5 ± 1.13
Prickle hair density (mm^{-2})	278 ± 22	291 ± 28	51 ± 8	12 ± 9	12 ± 7	2161 ± 81
Prickle hair length (μm)	20 ± 0.7	19 ± 0.5	39 ± 1.3	35 ± 3.9	40 ± 13	35 ± 2.1
Stomata density (mm^{-2})	560 ± 23	459 ± 32	71 ± 6	25 ± 11	55 ± 9	1142 ± 34
Stomata length (μm)	20 ± 0.3	20 ± 0.2	44 ± 0.9	32 ± 0.5	34 ± 1.8	17 ± 0.25
Biochemical parameters						
Chlorophyll *a* (g m^{-2})	0.273 ± 0.009	0.405 ± 0.020	0.36 ± 0.002	0.234 ± 0.030	0.218 ± 0.010	0.365 ± 0.016
Chlorophyll *b* (g m^{-2})	0.098 ± 0.005	0.203 ± 0.010	0.065 ± 0.004	0.186 ± 0.029	0.185 ± 0.030	0.194 ± 0.009
Carotenoids (g m^{-2})	0.065 ± 0.003	0.106 ± 0.005	0.039 ± 0.001	0.214 ± 0.046	0.127 ± 0.015	0.113 ± 0.006
Anthocyanins (relative units per cm^{-2})	0.964 ± 0.083	1.259 ± 0.075	0.685 ± 0.039	0.564 ± 0.069	0.504 ± 0.079	0.811 ± 0.060
UVB-absorbing comp. (relative units per cm^{-2})	14.86 ± 0.50	24.01 ± 1.25	13.81 ± 0.61	10.11 ± 1.52	23.84 ± 3.72	14.94 ± 2.08
UVA-absorbing comp. (relative units per cm^{-2})	25.01 ± 1.09	32.95 ± 1.69	24.22 ± 0.93	16.83 ± 2.42	36.31 ± 5.58	21.02 ± 2.42

* Thickness of individual leaf layers were measured at the thickest parts of the leaf.

Table 2
Morphological and biochemical parameters in the developing leaves of the four grasses and the sedge. Data are means ± standard errors ($n = 10$).

Properties	Grasses					Sedge
	Phragmites australis		*Phalaris arundinacea*	*Molinia caerulea*	*Deschampsia cespitosa**	*Carex elata*
	June	July	June	July	July	July
Specific leaf area (cm^2 g^{-1})	102.8 ± 3.6	90.2 ± 10.3	225.7 ± 9.0	182.2 ± 4.6	114.5 ± 6.9	122.8 ± 6.3
Leaf thickness (μm)	239 ± 6.9	142 ± 4.6	246 ± 7.1	128 ± 2.0	427 ± 19.0	228 ± 11.8
Total mesophyll (μm)	163.4 ± 5.4	113.7 ± 4.2	56.0 ± 3.2	104.5 ± 2.1	343.6 ± 16.4	101.8 ± 5.2
Upper surface						
Cuticle thickness (μm)	1.4 ± 0.06	1.2 ± 0.06	3.0 ± 0.13	4.8 ± 0.08	5.6 ± 0.27	2.0 ± 0.08
Epidermis thickness (μm)	12.9 ± 0.45	15.1 ± 0.39	26.6 ± 0.98	8.09 ± 0.29	11.8 ± 0.53	11.3 ± 0.40
Prickle hair density (mm^{-2})	92 ± 14	123 ± 11	1 ± 1	228 ± 15	158 ± 20	13 ± 4
Prickle hair length (μm)	15 ± 0.34	13 ± 0.40	33 ± 3.40	35 ± 0.95	25.3 ± 3.10	54 ± 13.30
Stomata density (mm^{-2})	398 ± 12	460 ± 26	133 ± 9	244 ± 11	90 ± 21	1 ± 1
Stomata length (μm)	20 ± 0.3	18 ± 0.2	34 ± 0.8	31 ± 0.8	39 ± 0.5	17 ± 17
Lower surface						
Cuticle thickness (μm)	1.3 ± 0.04	1.3 ± 0.04	2.9 ± 0.07	4.8 ± 0.12	5.7 ± 0.20	2.7 ± 0.11
Epidermis thickness (μm)	14.1 ± 0.56	11.7 ± 0.40	27.9 ± 0.93	7.9 ± 0.46	11.6 ± 0.52	20.3 ± 0.88
Prickle hair density (mm^{-2})	254 ± 28	333 ± 38	182 ± 16	43 ± 20	42 ± 15	2371 ± 88
Prickle hair length (μm)	16 ± 0.6	15 ± 0.4	31 ± 1.1	19 ± 4.5	26.9 ± 3.3	35 ± 35
Stomata density (mm^{-2})	605 ± 27	723 ± 51	163 ± 9	94 ± 9	14 ± 6	1225 ± 50
Stomata length (μm)	20 ± 0.3	18 ± 0.2	34 ± 0.8	32 ± 1.1	34 ± 1.9	16 ± 0.3
Biochemical parameters						
Chlorophyll *a* (g m^{-2})	0.271 ± 0.013	0.273 ± 0.012	0.205 ± 0.020	0.320 ± 0.021	0.217 ± 0.020	0.458 ± 0.021
Chlorophyll *b* (g m^{-2})	0.113 ± 0.008	0.122 ± 0.060	0.089 ± 0.009	0.184 ± 0.014	0.132 ± 0.012	0.198 ± 0.001
Carotenoids (g m^{-2})	0.058 ± 0.004	0.077 ± 0.003	0.058 ± 0.006	0.175 ± 0.033	0.209 ± 0.024	0.115 ± 0.005
Anthocyanins (relative units per cm^{-2})	0.857 ± 0.017	0.897 ± 0.039	0.427 ± 0.030	1.053 ± 0.097	0.796 ± 0.063	0.881 ± 0.081
UVB-absorbing comp. (relative units per cm^{-2})	15.25 ± 0.42	18.66 ± 1.02	29.97 ± 1.65	15.85 ± 1.37	14.41 ± 0.83	20.50 ± 2.14
UVA-absorbing comp. (relative units per cm^{-2})	24.32 ± 0.81	34.21 ± 2.22	48.23 ± 2.78	33.92 ± 2.38	23.42 ± 1.32	31.11 ± 3.08

* Thickness of individual leaf layers were measured at the thickest parts of the leaf.

several-fold higher than the concentration of Si ($p < 0.01$) (Table 3). *P. arundinacea* was also exceptional, with concentrations of Ca similar to those of Si. The *C. elata* leaves contained lower concentrations of Si compared to the leaves of the grasses ($p < 0.01$). Furthermore, the ratio between the elements was different in the leaves of the sedge, where the Ca concentrations were higher than those of Si ($p < 0.01$) (Table 3).

Comparisons of the developing and mature leaves showed that the mature leaves contained higher concentrations of Si ($p < 0.01$); the exceptions here were *P. australis* and *C. elata*, where the Si

Table 3
Concentrations of silicon (Si), calcium (Ca) and aluminium (Al) and phytolith contents in the leaves of the different plant species. Data are means ± standard deviations ($n = 3$). Limit of detection <0.1%.

Grass/sedge	Stage-month	Element concentration (mg g^{-1} dry mass)			Phytoliths (% per dry mass)	Phytoliths (mg cm^{-3})
		Si	Ca	Al		
P. australis	D-June	9.89 ± 2.18	1.98 ± 0.17	<LOD	1.23 ± 0.11	5.13 ± 1.42
	M-June	8.96 ± 0.45	6.39 ± 1.01	4.95*	1.02 ± 0.14	3.97 ± 0.56
	D-July	10.8 ± 1.84	2.9 ± 0.32	5.84 ± 1.11	1.36 ± 0.16	10.92 ± 2.15
	M-July	10.33 ± 1.1	9.15 ± 0.67	3.83 ± 0.51	2.11 ± 0.17	9.26 ± 0.73
P. arundinacea	D-June	4.41 ± 1.02	5.08 ± 0.41	<LOD	1.09 ± 0.01	2.05 ± 0.36
	M-June	10.14 ± 0.67	12.03 ± 1.53	<LOD	2.14 ± 0.07	3.28 ± 0.24
M. caerulea	D-June	6.14 ± 0.91	2.36 ± 0.17	<LOD	1.63 ± 0.1	6.05 ± 0.46
	M-July	11.57 ± 0.25	5.35 ± 0.95	<LOD	1.88 ± 0.12	9.36 ± 1.92
D. cespitosa	D-June	6.68 ± 1.72	43.87 ± 9.95	6.37 ± 1.7	1.76 ± 0.18	10.85 ± 1.09
	M-July	10.91 ± 0.94	34.33 ± 4.58	6.52*	2.23 ± 0.33	14.73 ± 2.07
C. elata	D-June	1.83*	2.82 ± 0.19	<LOD	0.40 ± 0.05	1.73 ± 0.70
	M-July	2.09 ± 0.54	4.02 ± 0.24	<LOD	0.43 ± 0.07	1.41 ± 0.33

D, developing leaves; M, mature leaves.
LOD, limit of detection.
* Only two samples were relevant.

contents of the different phenologic stages of the leaves did not differ significantly (Table 3). The trend of higher concentrations of these elements in the mature leaves was observed also in the case of Ca, with the exception of *D. cespitosa* (Table 3). Comparison of the same phenological phases of the leaves sampled at different times of the season showed an inverse relationship between the concentrations of Si and Ca. In the *P. australis* leaves, the Si concentrations did not increase later in the growing season, as was observed for Ca ($p < 0.01$). In most of the samples, the Al concentrations were below the limit of detection (0.1%), with the exception of *P. australis* and *D. cespitosa* (Table 3).

3.3. Optical properties of the leaves

The reflectance spectra of the mature leaves of the grasses and the sedge showed the greatest differences in the UV (below 400 nm) and NIR (above 700 nm) regions of the spectra (Fig. 1). In the visible region, *P. arundinacea*, *M. caerulea* and *D. cespitosa* had higher relative reflectance, while *P. australis* and *C. elata* reflected less incoming radiation (Fig. 1). In general, only the leaves of *C. elata* reflected more radiation in the UV region in comparison with the grasses, while in the visible parts of the spectra, the leaves of *C. elata* reflected radiation to the lowest extent. Comparisons of mature leaves of *P. australis*, which were harvested in June and July, showed that they reflect light to very similar extents, except in the NIR region (Fig. 1).

In general, the transmittance spectra of the mature leaves of the plant species studied varied to a greater extent between plant species than the reflectance spectra (Fig. 2). In the UV region, *C. elata* transmitted significantly more incoming radiation compared to the leaves of the grasses, where the UV light transmittance was negligible (Fig. 2). The green peak in the transmittance spectra was most pronounced in *D. cespitosa* and *M. caerulea*. The leaves of *P. australis* transmitted incoming radiation to the lowest extent in the visible part of the spectra (Fig. 2).

Redundancy analysis was performed to determine which parameters explained most of the variability of the reflectance and transmittance spectra. As some of the parameters examined might overlap (e.g., Si structures, thickness of cuticle, Si contents), we ran RDA with three different datasets. In the first run, we

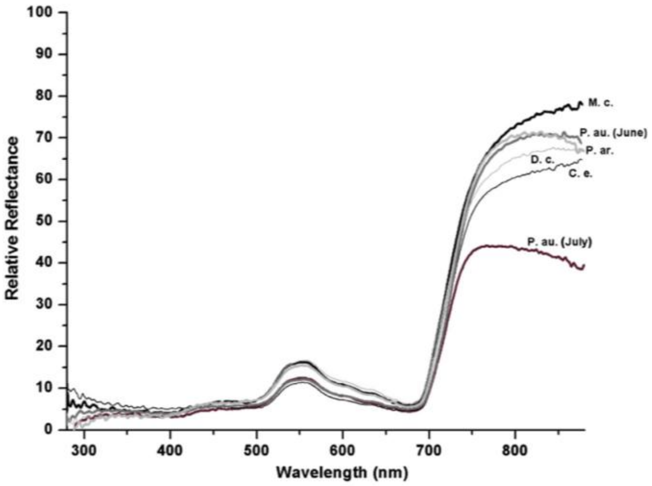

Fig. 1. Mean relative reflectance spectra of the mature leaves. Data are means over 5-nm intervals ($n = 10$). P. au., *Phragmites australis*; P. ar., *Phalaris arundinacea*; M. c., *Molinia caerulea*; D. c., *Deschampsia cespitosa*; C. e., *Carex elata*.

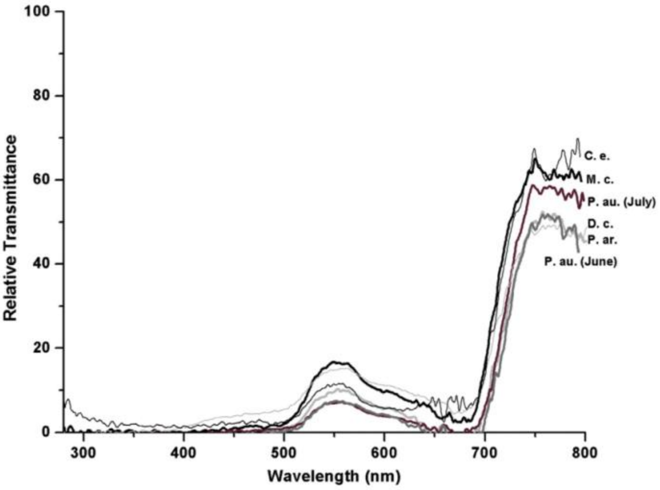

Fig. 2. Mean relative transmittance spectra of the mature leaves. Data are means over 5-nm intervals ($n = 10$). P. au., *Phragmites australis*; P. ar., *Phalaris arundinacea*; M. c., *Molinia caerulea*; D. c., *Deschampsia cespitosa*; C. e., *Carex elata*.

looked at the role of the phytoliths, and therefore we included all of the biochemical and morphological parameters, including the phytolith mass per volume. As we analysed only three parallel samples for the phytoliths, in this analysis we included just the data of the corresponding biochemical and morphological analyses. In the mature leaves, the most influential parameter that affected the reflectance spectra was the average length of prickle hairs at the adaxial leaf surface, which explained 54% of the spectra variability. The phytolith mass per volume explained 21% of the variance of the reflectance spectra (Table 4). The phytolith mass per volume correlated positively with the visible parts of the spectra. When analysing the transmittance spectra, the RDA showed that phytolith mass per volume explained 11% of the spectra variability. However, the cuticle thickness and density of prickle hairs at the abaxial surface explained a greater portion of the transmittance spectra variability, 47% and 19%, respectively (Table 4). In the case of developing leaves, the phytolith mass per volume did not demonstrate any significant role in the reflectance spectra (Table 4); however, the phytolith content significantly affected the transmittance spectra, explaining 31% of its variability.

In the second RDA run, we excluded the phytoliths, and included only the data from the biochemical and morphological analyses performed on 10 parallel samples. In the mature leaves, most of the variability of the reflectance spectra was explained by the thickness of the cuticle at the adaxial leaf surface (19%), followed by the average length of the prickle hairs at the adaxial surface (16%), the adaxial epidermis thickness and the chlorophyll *a* content per area (4%) (Fig. 3). The thickness of the cuticle positively correlated with the visible range, as did the length of the prickle hairs with the UV range (Fig. 3). When RDA was run to explain the variability of the transmittance spectra, the abaxial cuticle thickness explained 32%, the density of prickle hairs at the abaxial leaf surface explained 15%, the mesophyll thickness explained 3%, and the biochemical parameter of chlorophyll *a* content explained 3% (Fig. 4).

Different results were obtained when RDA was performed with the data collected for the developing leaves. All of the significant

Table 4
The RDA run with the different datasets for plant traits (explanatory variables) that significantly affected the variability of the reflectance spectra, and the % of explained variance and p-values.

Dataset	*n*	Stage	Explanatory variable	Explained variance (%)	*p*
Phytoliths, morphological and biochemical traits	3	Developing	Prickle hairs length (ad)	22	0.031
	3	Mature	Prickle hairs length (ad)	54	0.001
			Phytoliths (mg cm^{-3})	21	0.002
			Chl *a*	10	0.001
			Mesophyll thickness	6	0.001
			Chl *b*	2	0.033
Morphological and biochemical traits	10	Developing	Anthocyanins	22	0.001
			Total prickle hairs length (ad)	13	0.001
			Chl *a*	13	0.001
	10	Mature	Cuticle thickness (ad)	19	0.001
			Prickle hairs length (ad)	16	0.001
			Epidermis thickness (ad)	4	0.01
			Chl *a*	4	0.043
Element content	3	Developing	Si	42	0.002
	3	Mature	Si	18	0.041

ad, adaxial leaf surface.

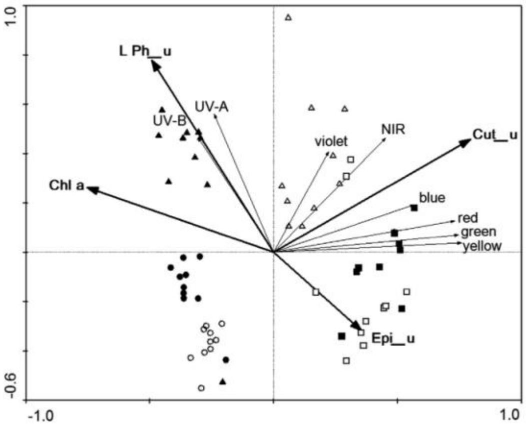

Fig. 3. Redundancy analysis ordination diagram showing the strength of the associations between the significant morphological and biochemical parameters ($p < 0.05$) and the regions of the reflectance spectra in mature leaves. The eigenvalues for the first two axes are 0.246 and 0.165. Symbols: open circles, *Phragmites australis* (June); filled circles, *Phragmites australis* (July); open squares, *Phalaris arundinacea*; filled squares, *Deschampsia cespitosa*; open triangles, *Molinia caerulea*; filled triangles, *Carex elata*. L Ph_u, mean length of prickle hairs on the adaxial leaf surface; Cut_u, cuticle thickness on the adaxial leaf surface; Epi_u, epidermis thickness on the adaxial leaf surface; Chl a, chlorophyll *a* content per area.

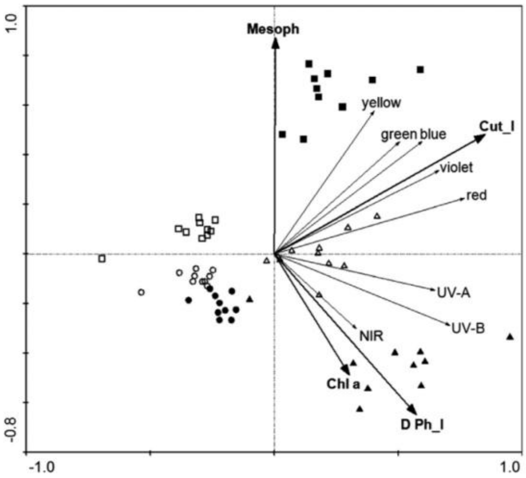

Fig. 4. Redundancy analysis ordination diagram showing the strength of the associations between the significant morphological and biochemical parameters ($p < 0.05$) and the regions of the transmittance spectra in mature leaves. The eigenvalues for the first two axes are 0.41 and 0.097. Symbols: open circles, *Phragmites australis* (June); filled circles, *Phragmites australis* (July); open squares, *Phalaris arundinacea*; filled squares, *Deschampsia cespitosa*; open triangles, *Molinia caerulea*; filled triangles, *Carex elata*. Mesoph, mesophyll thickness; Cut_l, cuticle thickness on the abaxial leaf surface; D Ph_l, average density of prickle hairs on the abaxial leaf surface; Chl a, chlorophyll *a* content per area.

biochemical and morphological variables explained 50% of the total variance of the reflectance spectra. The most influential parameter was anthocyanins content, followed by the total length of the prickle hairs at the adaxial leaf surface, and the chlorophyll *a* content (Table 4). For the transmittance spectra, RDA revealed that the most influential parameter was chlorophyll *a* content, which explained 26% of the variability of the spectra, while the average length of the prickle hairs at the adaxial leaf surface explained 7%, the abaxial cuticle thickness 4%, and the density of the prickle hairs at the abaxial leaf surface 3% (Table 5). Among these parameters, the chlorophyll *a* content and the density of prickle hairs at the abaxial leaf surface correlated negatively with the regions of the transmittance spectra.

The third run was performed using the leaf contents of Si, Ca and Al only. The data showed the importance of Si in the optical properties of the mature leaves of the species examined. The Si content explained 18% of the variability of the reflectance spectra (Fig. 5) and 39% of the variability of the transmittance spectra (Fig. 6). The variability of the transmittance spectra was additionally explained by the Ca content (18%). In both cases, Si showed a negative correlation with the UV range, while the transmittance in the visible part of the spectrum was positively correlated with Ca (Fig. 6).

In the developing leaves, Si was the only significant variable, and it explained 42% of the variability of the reflection spectra (Table 4), while the variability of the transmittance spectra was significantly explained only by Ca (31%) (Table 5).

4. Discussion

The presence of silicified leaf structures located in different parts of the leaf tissue [4,5,41] can affect the optical properties of mature and developing leaves of these grasses (*P. australis*, *P. arundinacea*, *M. caerulea*, *D. cespitosa*) and the sedge (*C. elata*). The effects on the reflectance spectra were less pronounced than those on the transmittance spectra.

The phytolith extraction showed that the mature leaves of *P. australis* (July), *P. arundinacea* and *M. caerulea* contain higher amounts of phytoliths per leaf dry mass than their developing leaves. In general, grasses and sedges are known to be efficient Si accumulators [2,6], and to have a high production of phytoliths [5]. In the leaves of the sedge here, the phytoliths accounted for only 0.4% of the dry mass, which would classify it as among the intermediate silicon accumulators, according to Takahashi et al. [25]. On the other hand, the mature leaves of grasses, which contained more than 1% phytoliths per dry mass, are considered to be high accumulators. Mature leaves of *P. australis* were sampled twice in the season, and this showed increased phytolith content in July comparing to June. This is in agreement with studies that have reported that the majority of phytoliths in grasses are deposited later in the growing season [8,42]. For explanation of the leaf optical properties, phytolith mass expressed per volume is more relevant because of the backscattering and transmittance of light by the phytoliths, and their effects on the leaf tissue properties [22,23].

The RDA showed that 21% of the variation of the reflectance spectra in the mature leaves was explained by phytolith content per volume of tissue, while for the variability of the transmittance spectra, the phytoliths explained 11% of the spectra variability. The phytolith content correlated positively with the visible regions of the reflectance spectra. However, when RDA was run as phytolith content per dry mass, the explained variance of the reflectance spectra was only 6%, while for the variability of the transmittance spectra, the phytoliths per dry mass did not have any significant role. The differences in the explained variance is possibly because phytoliths do not occur on the leaf surface only, but can also be found in the different leaf layers [4,41], and therefore their effects might be adverse.

However, when the phytoliths were omitted from the RDA, we could explain a large portion of the reflectance spectra variability

Table 5
The RDA run with the different datasets for plant traits (explanatory variables) that significantly affected the variability of transmittance spectra, and the % of explained variance and p-values.

Dataset	*n*	Stage	Explanatory variable	Explained variance (%)	*p*
Phytoliths, morphological and biochemical traits	3	Developing	Phytoliths (mg cm^{-3})	31	0.020
			Total prickle hairs length (ad)	18	0.022
	3	Mature	Cuticle thickness (ab)	47	0.001
			Prickle hairs density (ab)	19	0.005
			Phytoliths (mg cm^{-3})	11	0.019
			Chl *a*	9	0.006
			Prickle hairs length (ab)	2	0.045
Morphological and biochemical traits	10	Developing	Chl *a*	26	0.001
			Prickle hairs length (ad)	7	0.001
			Cuticle thickness (ab)	4	0.030
			Prickle hairs density (ab)	3	0.045
	10	Mature	Cuticle thickness (ab)	32	0.001
			Prickle hairs density (ab)	15	0.001
			Mesophyll thickness	3	0.013
			Chl *a*	3	0.047
Element content	3	Developing	Ca	31	0.012
	3	Mature	Si	39	0.006
			Ca	18	0.020

ad, adaxial leaf surface; ab, abaxial leaf surface.

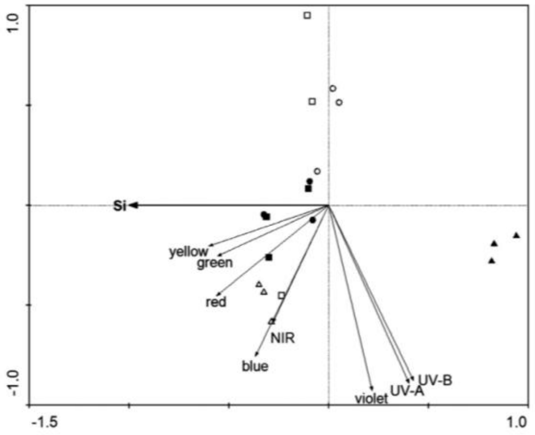

Fig. 5. Redundancy analysis ordination diagram showing the strength of the associations between the significant element contents in the leaves ($p < 0.05$) and the regions of the reflectance spectra in mature leaves. The eigenvalues for the first two axes are 0.200 and 0.016. Symbols: open circles, *Phragmites australis* (June); filled circles, *Phragmites australis* (July); open squares, *Phalaris arundinacea*; filled squares, *Deschampsia cespitosa*; open triangles, *Molinia caerulea*; filled triangles, *Carex elata*.

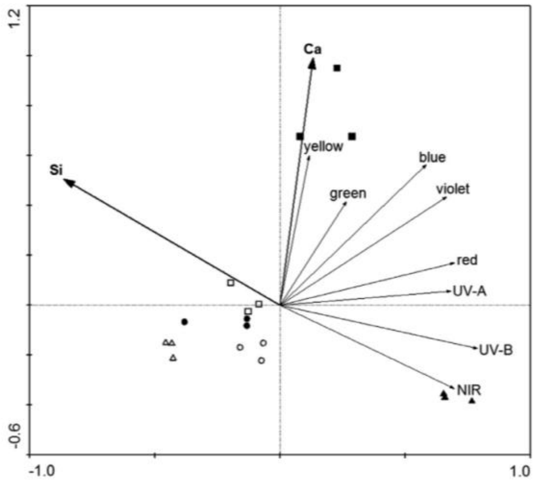

Fig. 6. Redundancy analysis ordination diagram showing the strength of the associations between the significant element contents in the leaves ($p < 0.05$) and the regions of the transmittance spectra in mature leaves. The eigenvalues for the first two axes are 0.500 and 0.069. Symbols: open circles, *Phragmites australis* (June); filled circles, *Phragmites australis* (July); open squares, *Phalaris arundinacea*; filled squares, *Deschampsia cespitosa*; open triangles, *Molinia caerulea*; filled triangles, *Carex elata*.

according to the upper leaf layers; namely, with the cuticle, the length of the prickle hairs, and the epidermis. This is in agreement with previous studies that have shown that the leaf surface that directly interacts with incoming light has the most important role in leaf reflectance [33,43,44]. All of these structures are known to be subjected to Si incrustation [5,19,42,45]. For *P. australis* leaves, it has been shown that the Si supply in the soil promotes Si deposition as a double silica layer in the epidermis, beneath the cuticle [46].

The RDA where the transmittance spectra in grasses and sedge were related to the leaf morphological and biochemical traits revealed that the abaxial cuticle thickness was the most influential parameter. In addition, the density of prickle hairs at the lower leaf surface also has important roles in explaining the transmittance spectra variability. A role of the abaxial leaf surface in the leaf transmittance was also shown in another study [47].

The analysis in which the element contents were related to the reflectance and transmittance spectra confirmed the role of silicified leaf structures in the explanation of the spectra variance. The variability of the transmittance spectra was additionally explained by the Ca content. Only a few studies have shown that different biominerals in plants can affect their light absorption. Agrarie and co-workers [27] compared the optical properties of leaves in Si-treated and non-treated rice plants. The higher content of silicified structures did not increase the amount of light transmitted and reflected to the mesophyll, and the light-use efficiency and quantum yield of Si-treated plants was even slightly lower. Nevertheless, they reported that the absorption in the visible part of the spectra was lower in plants treated with Si, while in the NIR significant differences were obtained. However, in our case the differences were the most pronounced in the regions below 400 nm and above 700 nm. A similar study to that of Agrarie et al. [27] was conducted by Kaufman et al. [18], who also rejected the hypothesis that Si structures can facilitate the transmission of light to the mesophyll. However, our data show that near-surface Si structures (i.e., prickle hairs, cuticle, epidermis) [19,42,45,48] correlate positively with the reflectance spectra, and indicate that they might scatter incoming visible radiation and thus possibly reduce its penetration deeper into the leaf tissue. As reported by Wang et al. [49], biosilicified epidermal tissue enhanced the emission of mid-infrared radiation, and therefore reduced the leaf temperature. On the contrary, Björn and Li [50] did not confirm the theory that near-surface silica increased radiative heat dissipation over the mid-infrared range. In the present study, the surface structures also affected the reflection of the UV radiation. The significant positive correlations obtained between the lengths of prickle hairs and the reflectance in UV regions in this study demonstrate their importance in scattering short-wave radiation. The study of Goto et al. [28] showed lower UV absorbance in the epidermis, schlerenchyma and metaxylem vessel walls in rice plants treated with Si, in comparison to non-treated plants. Furthermore, it has been shown that leaves containing more Si produced lower concentrations of phenolic compounds [51]. It was suspected that the near-surface silica layer might supplement the UV screening effect of the phenolic substances, which protects tissues against harmful UV radiation [21]. Therefore the availability of Si for plants and the production of Si structures might reduce the stress due to UV radiation [29,52].

As well as Si, Ca carbonate is also often found as an incrustation on the leaf surface, and might be present in the cell walls of hairs [24,53,54]. XRF spectroscopy revealed that the *D. cespitosa* and *C. elata* leaves contained higher concentrations of Ca compared to Si, which might also contribute to the light reflectance from the leaf surface. Recently, Gal et al. [22] confirmed that cystoliths (Ca structures) and Ca oxalate druses have light-scattering functions, as they help to distribute the light inside the leaf more evenly. The dispersion of light by druse Ca oxalate crystals might also be beneficial for the photosynthesis of undergrowth plants [26].

Biogenic silicon in plants might also contain inclusions of other elements (e.g., Al, Na, Mg, K, Ca, Mn, Fe, Cu, Ti, P, N, organic C), which usually represent up to 5% of the total phytolith weight [55,56]. These inclusions can disperse and or reflect, and thereby these can reduce the internal intensity of incoming radiation. Inclusions might also be present in the prickle hairs, and contribute to the scattering of the UV radiation. Studies have also shown that Si has an important role in stress mitigation, by binding and coprecipitating metal ions, as seen by the co-deposition of Si and Al [10]. Co-deposition has been shown in tea leaves, where the high Al contents in the epidermal cell walls were associated with increased levels of Si and Mg [16]. Al and Si also co-deposit in the epidermis of the needles of four conifer species, where the Al content increases towards of the tips of needles [10]. It is likely that Al can also co-deposit with Si in *P. australis* and *D. cespitosa*, where we measured significant amounts of Al.

Phytoliths affected the reflectance and transmittance spectra also in the case of the developing leaves. As expected, the variability of the reflectance spectra was at most affected by the prickle-hair length. In addition, the anthocyanin content affected the reflectance spectra. Anthocyanins can occur in the upper leaf layers [44,57,58], and they are beneficial for plant survival under different stresses. They also have photoprotective features that decrease the risk of photoinhibition, which can occur especially in young, developing leaves [59,60].

According to previous studies that have indicated the importance of the biochemical structure for light transmittance [61,62], the RDA where the transmittance spectra in grasses and sedge were related to the leaf plant traits revealed that beside phytolith per volume, the chlorophyll *a* content is also an influential parameter. Chlorophyll *a* correlated negatively with all of the measured parts of the spectra. This was expected, as the key role of the pigments is the interception of the light that passes through the leaf, and consequently a reduction in the transmittance [44,63,64].

The analysis of the optical properties of the developing leaves was aimed at investigating the role of the elements, and it revealed that the effect of Si was even more pronounced than in mature leaves. Silicon significantly affected the variability of the reflection spectra, while for the transmittance spectra, Ca was shown to be a highly significant variable. This is probably due to the variable amounts of Si that were deposited in the form of phytoliths, and the great variability of the Ca content in the developing leaves. A study by Motomura and co-workers [19] showed that the leaves of grasses deposit Si during the leaf expansion only in the silica cells, while silicification of epidermal cells occurs after the full differentiation of the leaves [65]. At the same time, the accumulation of silica in developing leaves is important for the reduction of the leaf consumption by herbivores [66]. The outcomes of the spectra analysis in the developing leaves show additionally the role of biominerals in the optical properties of these grasses and the sedge.

The data from the present study have thus shown the important role of silicified leaf structures and different leaf traits in the leaf optical properties of five monocotyledons. At the same time, the differences between the reflected and transmitted light indicate that silicified leaf structures influence internal light gradients that might also affect photosynthesis [44,58]. However, localisation studies of Si in plant tissue are needed to define the incidence of Si distribution within the different leaf layers and in the different structures, and to obtain more detailed information on the contents of silica in prickle hairs and the epidermis with cuticle, which are shown as the most influential parameters in the present study.

Acknowledgments

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3.5 NA OPTIČNE LASTNOSTI LISTOV VPLIVAJO MESTO ODLAGANJA IN VRSTE BIOMINERALOV V LISTIH

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Biominerali v rastlinah imajo številne vloge, ki so odvisne od njihove oblike, številčnosti, velikosti, lokacije in kemijske sestave (He in sod., 2012). Vrste iz družine trav in ostričevk so znane po kopičenju velikih količin različnih biomineralov (Motomura in sod., 2000; Hodson in sod., 2005; Piperno in sod., 2006), vendar je o njihovem prispevku na optične lastnosti listov malo znanega (Gal in sod., 2012; Klančnik in sod., 2014).

V raziskavi smo preučili vpliv potencialno inkrustiranih struktur, na ali blizu listne površine, na optične lastnosti listov pri navadnem trstu (*Phragmites australis*), pisanki (*Phalaris arundinacea*), modri stožki (*Molinia caerulea*), rušnati masnici (*Deschampsia cespitosa*) in togem šašu (*Carex elata*). Z RDA smo dokazali vplive gostote in dolžine trihomov ter debeline kutikule in povrhnjice na odboj in prepustnost sevanja pri izbranih rastlinskih vrstah. Z metodo mikro-PIXE smo določili razporeditev in koncentracije Si, Ca, Mg in Al v listnem tkivu. Pri navadnem trstu, pisanki in modri stožki je bila listna površina inkrustirana s Si, Ca pa se je kopičil v mezofilu listov. Pri rušnati masnici je bila listna površina inkrustirana s Si in Ca, ta se je kot pri ostalih vrstah trav pojavljal tudi v mezofilu. Za razliko od trav, je bila zgornja listna površina togega šaša inkrustirana s Ca, na spodnji strani lista pa se je na površini pojavljal tudi Si. Mg se je pri izbranih rastlinskih vrstah kopičil predvsem v predelu mezofila, pri travah *M. caerulea* in *D. cespitosa* pa je bil opazen tudi v predelu povrhnjic. Al se je pri večini vrst pojavljal v majhnih koncentracijah z izjemo navadnega trsta. Z metodo LEXRF smo natančneje določili elementno sestavo in koncentracije elementov v plasteh v bližini listne površine in v bodičkah na površini. Izkazalo se je, da se v pri travah nalaga Si tudi v bodičkah, medtem ko pri togem šašu tega nismo opazili. Natančnejša analiza listnega tkiva je pokazala kopičenje Mg tudi v vakuolah in mehurčastih celicah zgornje povrhnjice. Velike koncentracije Mg lahko vplivajo na turgor v celicah epidermisa (Pei in sod., 1999) in preprečujejo zvijanje listov. S korelacijsko analizo smo preverili še soodvisnost pojavljanja posameznih elementov v različnih plasteh in strukturah listov. Hkratno pojavljanje Si in Al smo opazili v bližini listne površine ter v bodičkah in fitolitih. Si in Ca sta se izključevala in pojavljala ločeno v različnih plasteh listov, medtem ko sta se Si in Mg pojavljala neodvisno drug od drugega.

Leaf optical properties are affected by the location and type of deposited biominerals

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Abstract

This study aimed to relate the properties of incrusted plant tissues and structures as well as biomineral concentrations and localisation with leaf reflectance and transmittance spectra from 280 nm to 880 nm in the grasses *Phragmites australis*, *Phalaris arundinacea*, *Molinia caerulea* and *Deschampsia cespitosa*, and the sedge *Carex elata*. The following leaf properties exerted significant effect on optical properties of a single species: prickle-hair length on adaxial surface and thickness of lower epidermis in *P. australis*; prickle-hair density at both surfaces and thickness of epidermis on adaxial leaf surface in *P. arundinacea*; thickness of epidermis on adaxial leaf in *D. cespitosa*; prickle-hair density on adaxial leaf surface and thickness of cuticle in *M. caerulea*; and prickle-hair length on adaxial leaf surface and cuticle thickness of the lower side in *C. elata*. Micro-PIXE and LEXRF elemental localization analysis shows that all of these structures and tissues are encrusted by Si in three of the grasses, *P. australis*, *P. arundinacea* and *M. caerulea*, and/or by Ca, in *D. cespitosa* and *C. elata*. Reflectance spectra variability were significantly affected by the concentrations of Ca in the adaxial epidermis, while Si and Mg concentrations in the adaxial epidermis and the Ca concentrations in the mesophyll, significantly affected transmittance spectra. High concentrations of Mg were detected in epidermal vacuoles of *P. arundinacea*, *M. caerulea* and *D. cespitosa*. Al co-localises with Si in the cuticle, epidermis and/or prickle hairs. The Si and Ca contents and their distribution patterns differ among the species and affected spectral properties.

Keywords

Optical properties; Element distribution; Silica; Calcium; Aluminium

Introduction

In plant leaves, biominerals have multiple functions that depend on their structure, abundance, location and chemistry [1]. Biominerals can affect the water and energy balance of a plant [2, 3], as well as increase the plant resistance to pathogens and herbivores [4, 5, 6, 7]. Plant species of the families Poaceae and Cyperaceae are known to accumulate large amounts of different biominerals in their organs [8, 9, 10].

Amorphous silicon (Si) dioxide (silica), amorphous calcium (Ca) carbonate and Ca oxalate are the most common biominerals in plants [11, 12]. Silica and Ca carbonate are important structural elements that increase the rigidity of the plant tissue, thus enhancing the strength [13, 14]. Deposition of silica in plants occurs in intercellular and intracellular spaces and in the cell walls. Silica is not accumulated uniformly among various cell types and tissues, and it is found in high concentrations especially in the cell walls of epidermal cells, guard cells, bulliform cells and prickle hairs [8, 10, 15, 16, 17].

The depositions of biominerals in the upper layers of plant organs are known also as encrustations, the levels of which depend on leaf age and the side of the leaf (upper or lower leaf surface) [16, 18, 19]. Silica can also be found in the mesophyll tissue, although usually at lower concentrations than in the epidermal layers [18]. Silicon is deemed to be non-essential for plants, but it is well known for its beneficial effects [20, 21, 22, 23]. Silicon can substitute carbon (C) as a structural element [24, 25] because the energy costs for Si structures are 10 to 20 times lower than those of C. It has been suggested that Si accumulation increased in the plants that developed in the Miocene (e.g., Poales), when the concentration of CO_2 in atmosphere decreased [26]. Silicon also has an important role in stress mitigation, as it promotes an increase in the production of antioxidants and binds and co-precipitates metal ions [27, 28, 29]. The amelioration of aluminium (Al) toxicity by Si is well established [23, 30, 31, 32], and many studies have observed co-deposition of Al and Si in epidermal cells [28, 33, 34].

Calcium is an essential nutrient that has a crucial role in several processes in plant cells, especially signalling and signal transduction [35]. Therefore intracellular Ca^{2+}

concentrations need to be carefully regulated in order to maintain cellular homeostasis. For this reason, excess Ca^{2+} is first compartmented in the vacuole and/or endoplasmic reticulum, and can be also deposited in an inactive form as amorphous Ca carbonate and/or as crystal Ca oxalate [12]. Calcium carbonate bodies are known as cystoliths, and these are regularly distributed in the epidermis, and their deposition can also extend into the mesophyll [17]. Cystolith bodies are attached to the peripheral cell wall by a silicate stalk that has an important role in C and Ca storage and pH regulation [36]. As indicated above, Ca oxalate can be abundant and frequently occur, and it has also been observed in plants from most taxonomic groups [37]; however, Ca oxalate is rarely present in grasses and sedges [38]. Calcium oxalate crystals can develop in epidermal and vascular tissues of different plant parts; namely: leaves, stems, flowers, fruit, seeds and roots [39]. These Ca oxalate crystals are usually formed within intravacuolar membrane chambers of specialised cells known as idioblasts [40]. Once Ca is deposited in the vacuoles, it is rarely redistributed [41]. However, in the mature leaves of *Morus australis* (Chinese mulberry), both Ca oxalate and Ca carbonate served as forms of Ca storage that can be reused during the growth of new leaves [42]. As well as Ca oxalate, magnesium (Mg) oxalate has also been reported in plants [1]. Magnesium is a central atom in the chlorophyll molecule and it is essential for the function of different enzymes in plant cells [43]. High Mg^{2+} contents in leaves can disturb the metabolic processes, as has been shown in sunflower during drought stress [44]. Excessive Mg^{2+} can be bound in the cell wall or sequestered in vacuoles, where it occurs in this ionic form as Mg^{2+} or it is bound to anionic ligands, like sulphate and phosphate [43]. Magnesium crystals can also form a Mg sink that regulates the Mg^{2+} levels in the cell cytoplasm [12].

There is a lot of literature on different biominerals, although the studies on their ecological functions are rare, and there are almost no data about the role of biomineral encrustations in UV and photosynthetically active radiation reflection and transmission in plants [17, 19]. Biominerals in the leaves can distribute the light flux more evenly inside the leaf tissue, which increases the energy availability for the lower tissues and prevents photoinhibition in the upper mesophyll [36]. Biominerals that appear at or close to the leaf surface can significantly affect the leaf optical properties [19]. Among such biominerals, Si and Ca appear to have the most important roles, as they usually reach high concentrations in the epidermal tissue, particularly in different grasses and sedges [3, 15, 17, 18]. Our previous study showed that biomineralised structures at or close to the leaf surface affects the reflection and transmittance of light in the leaves of four grasses and a sedge [19]. That study lacked any analysis of the responses of the single grass or sedge species, and did not provide the localisation and amounts of the different biominerals in the different tissues. This information is crucial to define the importance of certain biominerals in terms of the effects that they might have on the leaf optical properties in certain species.

The spatial distribution of Mg, Al, Si and Ca within biological tissues can be studied using micro-analytical techniques that are based on the emission of characteristic X-rays, which are excited either by charged particles (e.g., protons) or by photons (synchrotron radiation) [45, 46]. In the present study, we assessed the spatial resolution of Si, Mg and Ca at the tissue level using micro-proton induced X-ray emission (micro-PIXE) spectroscopy. In addition, a method with high sensitivity for Al and higher spatial resolution, known as synchrotron low energy X-ray fluorescence (LEXRF) micro-spectroscopy [46, 47, 48] was used to probe the localisation of Si, Mg and Al in the upper leaf epidermis and in prickle hairs.

The aims of the present study were therefore to define the role of the encrusted near-surface leaf tissues and structures for the leaf reflectance and transmittance spectra in the grasses *Phragmites australis* (common reed), *Phalaris arundinacea* (reed canarygrass), *Molinia caerulea* (purple moor grass) and *Deschampsia cespitosa* (tufted hairgrass), and in the sedge *Carex elata* (tufted sedge); and to localise and estimate the concentrations of the biominerals Si, Ca and Mg in the different layers of the leaf tissue, to determine their relation with leaf optical properties, as well as to investigate the possible co-localisation of other elements with Si and Ca in near-surface leaf structures. We hypothesised (1) that the importance of near-surface encrustations for leaf optical properties differ among these species; (2) that the presence and amount of biominerals in different leaf tissues and structures is species specific; (3) that the presence of biominerals affect reflectance and transmittance spectra and (4) that possible co-localisation of biominerals with other elements might occur in selected species.

Materials and methods

Plant material

The plants were sampled in the area of an intermittent lake, Lake Cerknica (45° 46’ 15” N, 14° 21’ 20” E). The majority of the shallower parts of the lake are colonised by the cosmopolite common reed species *P. 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 [49].

The leaves of the grasses *P. australis* (Cav.), *P. arundinacea* L., *M. caerulea* L., *D. cespitosa* L. and the sedge *C. elata* L. were collected in the summer of 2012 and 2013. Fully developed vital leaves were sampled. The measurements of morphological parameters were carried out on 10 replicates of leaves for each plant species. The micro-PIXE analysis was performed on three sub-samples for each plant species, and the mapping of the elemental distributions using LEXRF spectroscopy was performed on three or four samples of each plant species.

Measurements of spectral reflectance and transmittance

The optical properties of the leaves were measured on the day of sampling. The leaf reflectance and transmittance spectra were measured in a range from 280 nm to 880 nm, at a resolution of approximately 0.3 nm, using a portable spectrometer (Jaz Modular Optical Sensing Suite; Ocean Optics, Inc., Dunedin, USA) fitted with an integrating sphere (ISP-30-6-R; Ocean Optics, Inc.) and an optical fibre (QP600-1-SR-BX; Ocean Optics, Inc.). The reflectance and transmittance spectra were measured for the same part of the leaf.

The reflectance measurements followed the procedures in Klančnik et al. (2012) [50]. Adaxial leaf surfaces were illuminated with a UV-VIS-near infrared light source (DH-2000, Ocean Optics, Inc.). The spectrometer was calibrated to 100% reflectance using a white reference panel (Spectralon; Labsphere, North Sutton, USA).

For the transmittance spectra measurements, the integrating sphere was positioned at the abaxial leaf surface, while the adaxial surface was illuminated by the above-mentioned light source. Prior to the sample measurement, the spectrometer was calibrated to 100% transmittance with a light beam that passed directly into the interior of the integrating sphere.

Anatomical analysis

The thickness of the total mesophyll, cuticles and epidermis for the upper and lower leaf surfaces were measured on leaf transects at 100× magnification, using a CX41 microscope equipped with a XC30 digital camera and CellSens software (Olympus, Hamburg, Germany). The density and length of the prickle hairs on the upper and lower leaf surfaces were also determined using this microscopy system.

Sample preparation for mapping the element distributions

The leaves of each plant species were cut into small pieces (2 mm × 5 mm) with a razor blade, and these were immediately put inside 2-mm-wide stainless steel needles, embedded with a droplet of tissue freezing medium (Jung) to provide support during the cutting, and rapidly frozen in liquid propane cooled with liquid nitrogen [45, 46, 51]. The leaf pieces were then sectioned with a CM3050 cryo-microtome (Leica, Bensheim, Germany) with head and chamber temperatures in the range of -30 °C to -25 °C, and a section thickness of 50 µm for micro-PIXE analysis, and 20 µm for LEXRF analysis. The sections were placed in pre-cooled Al holders, and freeze-dried at -30 °C and 0.4 mbar pressure, for 3 days in an Alpha 2-4 Christ freeze dryer, using a cryo-transfer assembly that was cooled by liquid nitrogen [45, 46, 51]. Dry leaf cross-sections were then mounted between two layers of pioloform foil [45] for the micro-PIXE and LEXRF analyses. The images of the cross-sections were obtained with an Axioskop 2 MOT microscope (Carl Zeiss, Goettingen, Germany), using a visible and blue-light excitation source, an Axiocam MRc colour digital camera, and the AxioVision 3.1 software.

Micro-PIXE spectroscopy analysis

The spatial distributions of the mineral elements at the tissue level were determined in leaf cross-sections using micro-PIXE spectroscopy, with the nuclear microprobe of the Jožef Stefan Institute, Slovenia, as previously described [45, 52].

The proton beam used was at 3 MeV energy, with a diameter varying from 1.2 µm to 2.0 µm at ion currents ranging from 100 pA to 500 pA. The X-rays with energies from 0.9 keV up to 40 keV were detected by a pair of X-ray detectors. The first comprised a high-purity Ge X-ray detector with an active area of 95 mm^2, a 25-µm-thick Be window, and a 100-µm-thick polyimide absorber, positioned at an angle of 135° with respect to the beam direction. The second X-ray detector for low energy X-rays comprised a Si(Li) detector with an area of 10 mm^2 and an 8-µm-thick Be window, at an angle of 125° with respect to the beam direction; this covered the detection energy range from 0.9 keV to 4 keV. The samples were sprayed with low-energy electrons that originated from a hot tungsten filament, to prevent sample charging during proton-beam irradiation. The proton dose was determined by an in-beam chopping device that was positioned in the beam line after the last collimation of the beam, and before the beam was focussed with a magnetic quadrupole lens. The rotating chopper was gold-plated graphite that periodically intersected the beam, with a frequency of approximately 10 Hz, which made the method insensitive to beam intensity fluctuations [53]. The spectrum of the back-scattered protons from the chopper was recorded in parallel with the PIXE spectra, in the list mode. The high-energy part of the spectrum consisted of protons scattered from the gold layer and appeared as a separate peak. The area of the gold peak is proportional to the proton flux, and this was used for dose normalisation.

From the micro-PIXE measurements, the quantitative mineral element distribution maps were generated using the GEOPIXE II software package [54]. The corresponding concentrations of the mineral elements at the tissue level were extracted from the numerical matrices obtained with the GEOPIXEII software, using the ImageJ programme [51, 55].

LEXRF micro-spectroscopy analysis

The localisation of Si, Al and Mg at the cellular level was performed at the TwinMic X-ray spectro-microscopy beamline (BL 1.1L), Elettra Sincrotrone Trieste, Italy [56]. The X-ray microprobe for the TwinMic is formed using diffractive focusing optics, which achieves a lateral resolution between 0.03 μm to 1.00 μm, depending on the imaging mode. This is operated in the 400 eV to 2,200 eV photon energy range, and as such, the TwinMic accesses the main low-Z elements (from C to P, via K X-ray fluorescence lines, and from Cr to Rb, via L X-ray fluorescence lines) [47, 48]. In the present study, the X-ray microprobe with lateral resolution of 1.2 μm was formed by a tungsten zone plate (Zoneplates.com, UK) with a diameter of 320 μm, and an outermost zone width of 50 nm. The transmission signal was acquired using a fast readout, electron-multiplying, CCD camera (Andor Ixon) coupled to a phosphor-screen-based X-ray to visible-light converting system, which allowed simultaneous detection of brightfield or absorption, differential absorption, and differential phase-contrast signals [57, 58]. The morphological analysis of the specimens at the cellular and sub-cellular levels was complemented by a LEXRF spectrometry set-up consisting of eight Si-drift detectors (PNSensor, Germany) (seven with an active area of 30 mm^2 and one of 20 mm^2), in an annular back-scattering configuration positioned around the specimen [59], and a customised preamplifier and bias electronics established in collaboration with the Politecnico Milano/ National Institute of Nuclear Physics, Italy [60]. The maps were acquired at 1.9 keV over an area of 80 μm by 80 μm with a spatial resolution of 1.2 μm.

The data processing and image analysis were performed using the PyMCA software [61], by fitting the peaks of X-ray fluorescence lines to a Gaussian function after background stripping The quantification procedure was an extension of the quantification used in conventional X-ray fluorescence analysis [51] and was based on the fundamental parameters. These parameters were taken from the database of Elam et al. (2002) [62], and the system of equations for the measured elements was based on the relationship initially developed by Sherman (1955) [63]. The areal density of the freeze-dried cuttings (ρd) was calculated from the absorption of X-rays at an energy of 1900 eV in the sample ($I/I^0 = -\ln \mu\rho d$, where μ is the absorption coefficient in cellulose at 1900 eV). The calibration of the X-ray fluorescence system was performed with a set of thin (few 10 μg cm^{-2} thick) standard samples (MgF_2, Al, Fe; from Micromatter Co.) he respective geometry and/or instrumental constants were calculated [64]. Additionally, the geometric constant of the approximately same value was obtained from the measured incident photon beam by the photodiode placed behind the sample, and considering the solid angles extended from the sample to the detector. Using the fundamental parameters and the obtained geometry constant the respective element sensitivities were then calculated. The uncertainties introduced by the fundamental parameters and the calibration procedure were partially compensated because the experimental sensitivities were used in further quantifications. The measurements were validated by scanning multi-element conventional standard reference materials obtained by the proficiency test: OPY-1 and OU-10 (International Association of Geoanalysts). The certified and measured values are presented in ESM (S1).

Co-localisation analysis

The co-localisation analysis of Si concentrations with other elements measured using micro-PIXE or LEXRF spectroscopy was performed using the ImageJ programme and the ‘Intensity correlation analysis’ plug-in, which generated the Pearson’s correlation coefficients (*r*) [46, 51]. The Pearson’s correlation coefficients range from 1 to -1, where a value of 1 represents perfect correlation/ co-localisation; a value of -1 represents perfect exclusion; and a value of 0 represents random localisation. The number of comparisons (pixels) in the micro-PIXE maps was 256×256, and in the LEXRF maps, 66×66.

Statistical analysis

Differences between spectra were assessed by Kruskal-Wallis test with a Bonferroni’s correction, using the IBM SPSS Statistics 19.0 programme. Redundancy analysis (RDA) was used to determine whether variations in response variables (spectra) were related to explanatory variables (anatomical parameters). Monte Carlo permutation tests with 999 permutations were used to test the significance of the effects. Forward selection of anatomical parameter variables was used to avoid co-linearity of variables. The level of significance was accepted at $p \leq 0.05$. Non-significant anatomical parameters were excluded from further analysis. Another RDA was run with a subset of only the significant parameters. All of the variables in the RDA analysis were standardized. The concentrations of elements in different plant tissues were z-transformed. The complete set of RDA analyses was performed using CANOCO for Windows 4.5 programme package [65].

Results

Influence of leaf traits on leaf optical properties

In the first step we examined the properties of plant tissues and structures subjected to biomineral encrustations in all species (see S2 in ESM) and measured their reflectance and transmittance spectra. Relative cumulative values of different spectral ranges of reflected and transmitted light in studied plant species are presented in Table 1. The differences in reflectance were found along the whole range, with the most pronounced differences in UV range. The most pronounced differences in light transmittance were detected in green and NIR ranges. Redundancy analysis was run for each species to examine the relationships between leaf traits i.e. the thickness of the total mesophyll, cuticle, and epidermis, and the density and length of the prickle hairs on the adaxial and abaxial leaf surfaces and the leaf optical properties. The leaf traits that significantly affect the variability of the reflectance and transmittance spectra in each species are summarised in Table 2. The traits that had significant influences on the reflectance spectra variability differed among the species: in *P. australis*, the prickle-hair length on the adaxial surface explained 28%; in *P. arundinacea* and *D. cespitosa*, the thickness of epidermis on the adaxial leaf surface explained 35% and 31%, respectively; and in *M. caerulea*, the thickness of the cuticle on the adaxial leaf surface explained 14%. In the leaves of the sedge *C. elata*, none of the measured parameters had any significant role in explaining the variance of the leaf reflectance spectra.

Table 1

The transmittance spectra variability was also significantly affected by the leaf traits in studied species (Table 2): in *P. australis*, the thickness of epidermis on the abaxial leaf surface explained 32%; in *P. arundinacea*, the density of the prickle hairs on the adaxial and abaxial leaf surfaces explained 38% and 12%, respectively; and in *M. caerulea*, the length of the prickle hairs on the adaxial surface explained 22%. The transmittance spectra in the leaves of *D. cespitosa* were not significantly affected by the parameters measured. In *C. elata*, the cuticle thickness on the abaxial surface and the prickle-hair density on the adaxial surface had significant effects on the transmittance spectra variability, explaining 40% and 11%, respectively.

Table 2

Mapping the element distributions and element concentrations using the micro-PIXE spectroscopy method

In the next step, we examined the presence and type of encrustations for the different tissues, through micro-PIXE spectrometry analysis of the elemental compositions and localisations in the different leaf layers in these four grasses and one sedge (Fig. 1).

Figure 1

For the leaves of *P. australis*, *P. arundinacea* and *M. caerulea*, the analysis of the transverse leaf sections showed very similar Si and Ca distribution patterns (Fig. 1a-c). Silicon was deposited in the adaxial and abaxial epidermal layers. The quantitative element distribution maps showed higher concentrations of Si in the adaxial epidermis in *P. australis* and *P. arundinacea,* while in *M. caerulea*, Si was equally abundant in both epidermal layers (Table 3). The highest concentrations of Si in the epidermal layers were in the *P. australis* leaves. In contrast to the Si distribution pattern in these three grasses, Ca was accumulated in the mesophyll, and particularly in the areas of the vascular bundles (Fig. 1a-c). However, this pattern was less pronounced in the leaves of *P. australis*, where there was Ca throughout the mesophyll tissue (Fig. 1a). Among these grass species studied, *D. cespitosa* was exceptional, as in addition to Si, Ca was also deposited in the epidermal layers (Fig. 1d). Both elements, Si and Ca were homogenously deposited in the abaxial and adaxial epidermis (Table 3). As in the other grass species, in the *D. cespitosa* leaves, Ca was also deposited in the areas of the vascular bundles (Fig. 1d). In the leaves of the sedge *C. elata,* Ca was deposited in the upper epidermal layer, where in contrast to the grass species, Ca was the most abundant element (Table 3). Ca was homogenously encrusted in the adaxial epidermis in *C. elata*,

while Si appeared only in selected spots (Fig. 1e). In the abaxial epidermis of *C. elata*, both Si and Ca were deposited in selected spots only (Fig. 1e).
Table 3

In all of the leaves studied, Mg was distributed in the mesophyll tissue. However, in the *D. cespitosa* and *M. caerulea* leaves, there was Mg also in the epidermal layers (Fig. 1c). *M. caerulea* accumulated Mg only in the abaxial epidermis, while in *D. cespitosa*, there was accumulation of Mg in the adaxial and abaxial leaf epidermis (Fig. 1d). Out of all of the species examined, *D. cespitosa* showed the highest concentrations of Mg in leaf tissue (Table 3).

The concentrations of Al were low in most of the samples, so using micro-PIXE spectroscopy the number of counts for the Al-K line was not sufficient to obtain distribution maps with sufficient statistics. Al was therefore analysed by complementary synchrotron LEXRF.

The relation between element concentrations and optical properties

The redundancy analysis revealed that element concentrations in different leaf tissues affected the light reflectance and transmittance in different way (Table 4). The reflectance spectra variability was significantly affected by the concentrations of Ca in the adaxial epidermis that explained 28% of the variability of spectra. Ca concentrations in the upper epidermis were positively related to short wave lengths (UV-B, UV-A and violet ranges), while they were negatively related to other colour bands of visible light (blue, green, yellow, orange, red) and NIR band. On the other hand the Si and Mg concentrations in the adaxial epidermis and the Ca concentrations in the mesophyll, significantly affected transmittance spectra, explaining 32%, 18% and 16% of their variability, respectively. In the case of transmittance spectra Si concentrations in the upper epidermis and Ca concentrations in mesophyll negatively correlated with all colour bands, while Mg concentrations in the upper epidermis were negatively related to UV and NIR bands, and positively to the others.
Table 4

Mapping the element distributions and element concentrations using the synchrotron LEXRF micro-spectroscopy method

In the third step we performed the detailed study of the element presence and distribution in the near-leaf-surface structures. These data show the deposition of Si in the upper epidermal layer of the leaves of *P. australis, P. arundinacea* and *M. caerulea* (Fig. 2a-c), which is in line with the micro-PIXE analysis. The mapping of the elements also showed Si deposition in the prickle hairs of these three grasses (Fig. 2a-c). Analysis of the leaves of the fourth grass, *D. cespitosa*, showed the highest concentrations of Si for the top of the leaf ribs with the prickle hairs (Fig. 2d). In general, in all of these grass species, there were significantly higher Si concentrations in the adaxial epidermal layer than the mesophyll layer (Table 5). The same holds true for the Al concentrations, which were significantly higher in the adaxial epidermis than in the mesophyll layer (Table 5).

Figure 2

In the *C. elata* leaves, the concentrations of Si in adaxial epidermis were significantly lower in comparison to those for the grasses (Table 5). There was Si in the phytoliths only, which is in line with the micro-PIXE analysis. The mapping of the element distribution in *C. elata* showed high concentrations of Mg (Fig. 2e), with no Si detected in the *C. elata* prickle hairs.

Table 5

In all of the plant species studied, Mg was preferentially localised in the mesophyll cells, although it was also detected in large vacuoles of epidermal cells, including bulliform cells (Fig. 2). The concentrations of Mg in the epidermal layer were relatively high (Table 5).

Co-localisation analysis of elements in leaves

Co-localisation analysis at the tissue level using the micro-PIXE spectroscopy element distribution maps revealed negative correlations in the distributions between Si and Ca. The *r* values for the leaves of *P. australis, P. arundinacea* and *M. caerulea* were -0.28, -

0.28 and -0.25, respectively. Interestingly, the mapping of the elements using the micro-PIXE method also revealed Mg-Cl co-localisation, although only in the bulliform cells of *P. arundinacea* and *M. caerulea* (maps not shown).

Detailed co-localisation analysis at the cellular and sub-cellular levels from the LEXRF spectroscopy maps showed positive correlations between the Si and Al concentrations in certain cells and tissues. The strongest correlations in the epidermal tissue were obtained for *P. arundinacea*, *M. caerulea* and *P. australis*, with *r* values of 0.96, 0.95 and 0.93, respectively. In *P. australis* and *M. caerulea*, Si and Al were encrusted together for the whole leaf surface, including the prickle hairs (Fig. 2a, c), while in *P. arundinacea*, there was co-localisation of Si and Al in the prickle hairs only (Fig. 2b). In *D. cespitosa*, we calculated a significant correlation also between Si and Al (r = 0.69). Si and Al co-localised in the prickle hairs at the tops of the leaf ribs and in the phytoliths, which were formed in the epidermal and mesophyll tissues (Fig. 2d). Co-localisation of Si and Al in the phytoliths was also seen for *C. elata*, where the correlation coefficient was 0.77 (Fig. 2e).

There were no significant correlations between the Si-Mg and Al-Mg distributions in these plant species studied, as these did not co-localise in any of the tissues or cells examined.

Discussion

Leaf traits in relation to leaf optical properties

The most important task of the leaves is collecting of solar energy that depends on the leaf optical properties. As the leaf surface properties greatly influence the fate of the incoming radiation, the encrustations at or close to the leaf surface can exert great effect on collecting solar energy [17]. The RDA, that was run to assess the effects of the structures on the leaf surface and the near-surface leaf layers, reveal the importance of the thickness of the cuticle and epidermis, as well as the density and length of the prickle hairs in explaining significant portion of the reflectance and transmittance spectra variability. The traits that affect optical properties differed among studied plant species. For example the RDA with the *C. elata* dataset showed that the prickle-hair density at the adaxial leaf surface, along with the cuticle thickness at the lower leaf surface, could have significant effects on the light transmittance, while in the case of *P. australis*, the thickness of epidermis on the abaxial leaf surface was the most important. The leaf optical properties of studied species are a result of specific leaf morphology [19] and the nature of incrustations, since the accumulation and compartmentation of a certain element in plants appear to be species and cell specific [1, 15].

The nature of encrustations and their location

Si and Ca might contribute significantly to the encrustation of the leaf surface [8, 66, 67]. However, Si and Ca deposits also occur in the structural and vascular tissues, as has also been shown in other studies [14, 15]. Large deposits of Si are very common in the leaf blades of grasses [8, 9, 18, 68]. The mapping of the element distributions and the determination of the element concentrations in the different leaf layers of grasses and sedge using micro-PIXE and LEXRF spectrometry methods in studied species show that the different near-surface leaf layers and the structures on the leaf surface are encrusted with either Si and/or Ca; however, the level of the encrustation of a single structure differed among these species. The micro-PIXE spectrometry maps revealed that in *P. australis*, *P. arundinacea* and *M. caerulea,* Si is deposited in the upper and lower epidermal layers. Motomura and co-workers (2004) [18] showed similar patterns of Si deposition in bamboo (*Sasa veitchii*), where the deposition of Si in the epidermal tissue in particular takes place after leaf development, in the mature phase. In *P. australis* and *P. arundinacea*, the adaxial leaf surface is more encrusted than the abaxial leaf surface. This is in line with other studies, which have also reported higher Si deposition for the adaxial leaf surface [16, 18]. In the leaves of *P. australis* and *P. arundinacea*, the upper and lower epidermis was evenly encrusted with silica, as has been seen for the leaves of maize [3], which differed in terms of the pattern in the grass *Brachiaria brizantha* [16].

The accumulation and compartmentation of the different elements may also vary within a species [1, 15]. The rate of leaf tissue silicification is usually positively related to the transpiration rates of the leaves, which appears to be due to passive movement of silicic acid with water, and the polymerisation of silicic acid monomers as the water evaporates [16, 69]. Polymerisation of silicic acid results in deposits of amorphous silica in the cell walls and lumens, and in intercellular cavities, or as sheets under the leaf cuticle [34]. As silica polymerises in the plant tissue, it cannot dissolve and it appears to be chemically inactive [69]. In the species studied here, the transpiration process is usually not disturbed and reaches high rates, because these species thrive in permanently flooded and/or wet habitats [70, 71], and therefore the silification rate might also be high.

The mapping using the LEXRF spectroscopy analysis showed the detailed elemental compositions of the prickle hairs in the grasses, which are mainly encrusted with Si, while in the prickle hairs of the sedge, Si was not detected. In *P. australis* and *M. caerulea*, Si was deposited mainly in the outer cell walls of the epidermal cells, which affects the thickness of the cuticle. In *P. australis*, silica can form a double layer beneath the cuticle [72]. In the upper cuticle of *M. caerulea*, the thickness significantly affects the light reflectance, while in *P. australis*, there is a significant effect of the prickle-hair density on the upper leaf surface. In the prickle hairs, Si is deposited over their entire surface. This is also the case in bamboo, where in bulliform cells and prickle hairs, Si is deposited continuously after leaf maturation [18]. The study of maize leaf encrustations has shown that Si deposits in the cell walls of the leaf epidermis where it is mostly in the form of polymerised silica, which occurs as longitudinal strips along the veins of the leaves [3]. A similar pattern was found for *D. cespitosa* in the present study, where the thickest deposits of Si were detected at the top of the leaf ribs with the prickle hairs. The trichomes can also accumulate large quantities of Ca^{2+} into crystal-containing idioblasts and/or into Ca oxalate crystals [66, 73], even though the presence of Ca in the prickle hairs was not observed in the present study.

In the upper leaf surface of the sedge *C. elata*, there are Si deposits in selected spots, while Ca is evenly distributed. In the lower epidermis, the distribution of both of these elements is uneven. In the plant species studied here, the majority of the Ca appears to be in an inactive form, as amorphous Ca carbonate, as Ca oxalate crystals are rare or absent in Cyperaceae, while they are normally not found in Poaceae, although they are occasionally present in the *Panicum* species [38].

Plant vacuoles have a major role in elemental storage and in the compartmentation of toxic substances [74]. The results of the present study show high concentrations of Mg in vacuoles of the cells of the upper epidermis, including the bulliform cells as it was previously shown in a case of *P. australis* [75].

Tissue elemental concentrations and optical properties

It is believed that Si and Ca encrustations both provide similar benefits for plants [15]. According to Setoguchi and co-workers (1989) [36], the presence of Ca body cystoliths reduces the steep light gradient into the leaf. The present study shows that in the sedge *C. elata*, the light reflectance is not significantly affected by the encrusted layers and structures; however, the Si concentrations are significantly lower in comparison to those of Ca, which is mostly encrusted at the adaxial leaf surface. RDA taking into account the concentrations of elements in different leaf tissues of studied species showed that Ca concentrations in the upper epidermis are important in explaining light reflectance, while Si and Mg concentrations in the adaxial epidermis and the Ca concentrations in the mesophyll, significantly affected transmittance spectra. The importance of Ca may be also related to the inverse relation between Ca and Si in certain tissue. For example in *P. australis*, the increase in the Si content in the leaf tissue coincided with a decrease in the Ca content in the leaves, although the presence of Ca at the leaf surface was not consistent in all of the samples. It has been shown that the Ca content negatively correlates with the Si availability [76]. In the case of *C. elata*, much greater amounts of Ca were deposited in the upper epidermal layers in comparison to Si, while the lower epidermis was less encrusted. Some studies have claimed that different taxa have evolved different ways of biomineralisation, and that biomineralisation with either Si or Ca has occurred [15]. However, some plant species do not fit into this model, as for *D. cespitosa*, where we detected both elements at relatively high levels.

The impact of the encrusted structures and tissues on the interactions of the leaves with the radiation are mainly related to the radiation in the UV range [19], as also shown from present data analysis. On the one hand, it has been suggested that an amorphous Si double layer close to the epidermis can reduce the transmission of UV radiation [72], while on the other hand, it has been shown that prickle hairs can efficiently reflect the radiation in the UV range, depending on their length [19]. As silica is transparent for UV and visible light, this might be due to the co-localisation of Al with Si. In the studied plant species, the locations of the Al deposits significantly corresponded to those of Si, which was also the situation for the prickle hairs of *P. arundinacea, P. australis* and *M. caerulea*. Co-deposition of silica with Al prevents potential toxic effects of Al and allows better growth of the plants [28, 34, 77], as encrustations with silica are irreversible. In conifers, increased amounts of Al were found at the very tips of the needles [34]. The same distribution pattern was observed in prickle hairs at the tops of the leaf ribs in *D. cespitosa*. Silicon can often co-localise with potentially harmful elements in the cell walls of metabolically less active tissues [34, 78].

High concentrations of Mg in vacuoles of the cells of the upper epidermis, including the bulliform cells appear to affect turgor of the epidermal cells [79], and may take part in maintainance of the leaves of the grasses unrolled or unfolded. Loss of turgor in the bulliform cells and/or in other epidermal cells on the adaxial surface is a mechanism that induces the rolling and/or folding of the grass leaves [80] and prevents stress during drought [81]. The rolling and unrolling of the leaves affect the size and shape of the area exposed to the rays of the sun, and therefore these increased concentrations of Mg also indirectly affect the optical properties and the energy balance in the leaves. Different studies have reported that the cell walls of bulliform cells can be silicified [8, 16, 82]. This is because in the part of the leaves with the bulliform cells, the transpiration rate is high [83] which might increase the encrustation of the walls of those cells. A study of Motomura et al. (2004) [18] showed that even the lumina of bulliform cells in bamboo (*Sacha veitchii*) are filled with granulate silica, which is potentially a mechanism that prevents silicification of the cell walls. In the present study, the elemental distribution analysis at the cellular level revealed that the walls of the bulliform cells in *P. arundinacea* and *M. caerulea* also included Mg, which occurs together with Ca.

Conclusions

The present study has shown that the prickle hairs, cuticle and epidermis, which significantly affect the light reflectance and transmittance of these four grasses and one sedge, are mainly encrusted with amorphous silica, as for *P. australis, P. arundinacea* and *M. caerulea*, or with presumably amorphous Ca carbonate, as for *C. elata*, or with both, as for *D. cespitosa*. There is co-localisation of Al with Si in *P. australis* and *M. caerulea* in the cuticle, epidermis and prickle hairs, which potentially changes the optical properties of the light-transparent amorphous silica and increases the light reflectance. In the other species, if there is co-localisation of Al with Si, this is in the prickle hairs or in spots only. The concentrations of elements in different tissues of studied species showed that Ca concentrations in the upper epidermis are important in explaining light reflectance, while Si and Mg concentrations in the adaxial epidermis and the Ca concentrations in the mesophyll, significantly affected transmittance spectra. High concentrations of Mg possibly affect turgor of the epidermal cells, to maintain the leaves of the grasses unrolled or unfolded in *P. australis*, *P. arundinacea* and *D. cespitosa*. This affects the area of the leaves that is exposed to the rays of the sun, and through this also, indirectly, the optical properties and the energy balance in the leaves.

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Table 1 Relative cumulative values (mean ±SE, *n* = 10) of different spectral ranges of reflected and transmitted light in studied plant species

Species	**Optical property**	**Spectral ranges**							
		UV-B	**UV-A**	**violet**	**blue**	**green**	**yellow**	**red**	**NIR**
P. australis	Reflectance	4.8 ±0.6	5.0 ±0.3	5.3 ±0.2	6.3 ±0.2	11.3 ±0.3	9.1 ±0.3	6.5 ±0.2	49.3 ±1.9
	Transmittance	0	0	0	0.6 ±0.1	6.8 ±0.5	5.6 ±0.4	1.6 ±0.4	49.0 ±3.1
P. arundinacea	Reflectance	1.5 ±1.2	3.6 ±0.5	5.3 ±0.3	6.5 ±0.3	12.9 ±0.2	10.6 ±0.2	7.1 ±0.2	60.7 ±0.5
	Transmittance	0	0	0	0.3 ±0.2	9.0 ±0.7	7.8 ±0.6	2.1 ±0.6	36.6 ±2.1
M. caerulea	Reflectance	9.9 ±1.1	5.8 ±0.4	6.1 ±0.2	7.1 ±0.2	13.9 ±0.3	11.1 ±0.3	7.9 ±0.2	54.6 ±2.4
	Transmittance	0	0	0.2 ±0.1	1.4 ±0.2	12.8 ±0.4	10.9 ±0.4	5.4 ±0.3	48.1 ±3.3
D. cespitosa	Reflectance	2.7 ±0.4	3.2 ±0.1	5.1 ±0.1	6.5 ±0.2	13.7 ±0.5	11.8 ±0.5	8.2 ±0.3	58.7 ±0.4
	Transmittance	0	0	3.0 ±0.4	5.1 ±0.6	13.0 ±1.1	12.2 ±1.1	7.9 ±0.8	41.5 ±3.7
C. elata	Reflectance	1.9 ±1.7	3.1 ±0.7	4.0 ±0.4	4.5 ±0.3	10.1 ±0.5	8.0 ±0.4	5.3 ±0.2	51.7 ±1.0
	Transmittance	0	0	0.8 ±0.7	1.9 ±0.8	9.3 ±1.2	7.9 ±1.2	3.6 ±1.5	12.1 ±14.3

Table 2 Redundancy analysis for the plant traits that significantly affected the variabilities of the reflectance and transmittance spectra in the range from 280 nm to 880 nm

Species	***n***	**Optical property**	**Explanatory variable**	**Explained variance** (%)	**p**
P. australis	40	Reflectance	Prickle-hair length (ad)	28	0.001
		Transmittance	Epidermis thickness (ab)	32	0.001
P. arundinacea	20	Reflectance	Epidermis thickness (ad)	35	0.005
		Transmittance	Prickle-hair density (ad)	38	0.004
		Transmittance	Prickle-hair density (ab)	12	0.046
M. caerulea	20	Reflectance	Cuticle thickness (ad)	14	0.044
		Transmittance	Prickle-hair length (ad)	22	0.032
D. cespitosa	20	Reflectance	Epidermis thickness (ad)	31	0.006
		Transmittance	/	/	/
C. elata	20	Reflectance	/	/	/
		Transmittance	Cuticle thickness (ab)	40	0.003
		Transmittance	Prickle-hair density (ad)	11	0.047

ad, adaxial leaf surface; ab, abaxial leaf surface.

/, none explanatory variable significantly affected the variabilities of the spectra

Table 3 Element concentrations in the adaxial and abaxial epidermis and the mesophyll, as calculated from the micro-PIXE spectrometry element distribution maps

Element	**Leaf tissue**	**Element concentration in each species** (mg g^{-1})				
		P. australis	***P. arundinacea***	***M. caerulea***	***D. cespitosa***	***C. elata***
Si	Adaxial epidermis	189 ±24.7	85.5 ±15.1	24.4 ±3.4	25.1 ±8.0	5.94 ±3.53
	Mesophyll	6.81 ±2.03	1.77 ±0.48	0.90 ±0.18	7.63 ±2.82	3.68 ±1.56
	Abaxial epidermis	66.8 ±10.40	47.3 ±12.40	29.0 ±10.30	43.5 ±12.00	6.81 ±2.69
Ca	Adaxial epidermis	4.22 ±0.28	3.96 ±0.75	2.51 ±0.33	11.9 ±2.20	19.6 ±1.97
	Mesophyll	12.3 ±1.36	13.7 ±1.86	8.67 ±0.77	12.2 ±2.54	2.75 ±0.19
	Abaxial epidermis	4.25 ±0.54	1.93 ±0.61	2.30 ±0.92	9.79 ±1.55	5.21 ±1.10
Mg	Adaxial epidermis	6.46 ±0.89	4.14 ±0.86	2.48 ±0.80	7.28 ±1.09	2.50 ±0.31
	Mesophyll	7.10 ±0.63	2.30 ±0.42	2.37 ±0.50	13.7 ±1.47	2.19 ±0.18
	Abaxial epidermis	4.51 ±0.60	0.62 ±0.14	2.12 ±0.37	4.74 ±0.56	2.15 ±0.32

Data are means calculated in particular tissues of subsamples (*n* = 3) ±SE.

Table 4 Redundancy analysis for the concentrations of elements in different plant tissues that significantly affected the variabilities of the reflectance and transmittance spectra in the range from 280 nm to 880 nm. Redundancy analysis was performed on z-transformed values

Optical property	**Explanatory variable**	**Explained variance** (%)	**p**
Reflectance	Ca concentrations in the adaxial epidermis	28	0.012
Transmittance	Si concentrations in the adaxial epidermis	32	0.007
	Mg concentrations in the adaxial epidermis	18	0.022
	Ca concentrations in the mesophyll	16	0.012

Table 5 Element concentrations in the adaxial epidermal and adaxial mesophyll layers, as calculated from the quantitative LEXRF spectrometry element distribution maps

Element	**Leaf tissue**	**Element concentration in each species** (mg g^{-1})				
		P. australis	***P. arundinacea***	***M. caerulea***	***D. cespitosa***	***C. elata***
Si	Adaxial epidermis	62.06 ±19.95	10.3 ±5.10	20.7 ±7.32	14.9 ±3.78	0.63 ±0.46
	Mesophyll	4.57 ±1.96	1.06 ±0.54	0.97 ±0.24	1.77 ±0.62	1.02 ±0.81
Mg	Adaxial epidermis	1.38 ±0.55	4.99 ±1.81	3.20 ±2.07	0.60 ±0.13	2.19 ±0.93
	Mesophyll	2.27 ±0.57	6.16 ±2.83	2.52 ±1.24	0.17 ±0.02	1.42 ±0.22
Al	Adaxial epidermis	0.554 ±0.117	0.410 ±0.222	0.213 ±0.075	0.206 ±0.033	0.146 ±0.09
	Mesophyll	0.075 ±0.021	0.076 ±0.007	0.042 ±0.013	0.061 ±0.008	0.070 ±0.003

Data are means calculated in particular tissues (*n* = 3-4) ±SE.

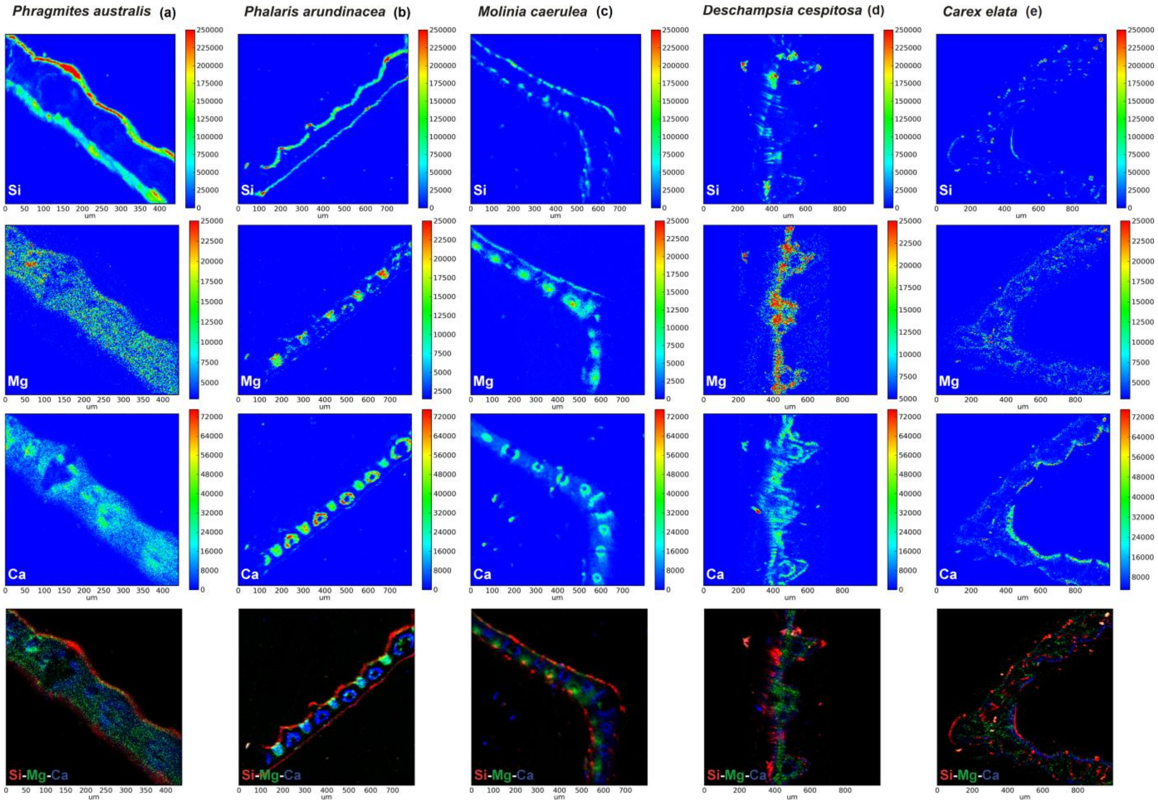

Fig. 1 Silicon (Si), magnesium (Mg) and calcium (Ca) distributions and concentrations (right: colour coding; µg g^{-1}) in the leaves of the four grasses and one sedge (as indicated), using the micro-PIXE spectroscopy method. The size of all images is indicated in microns

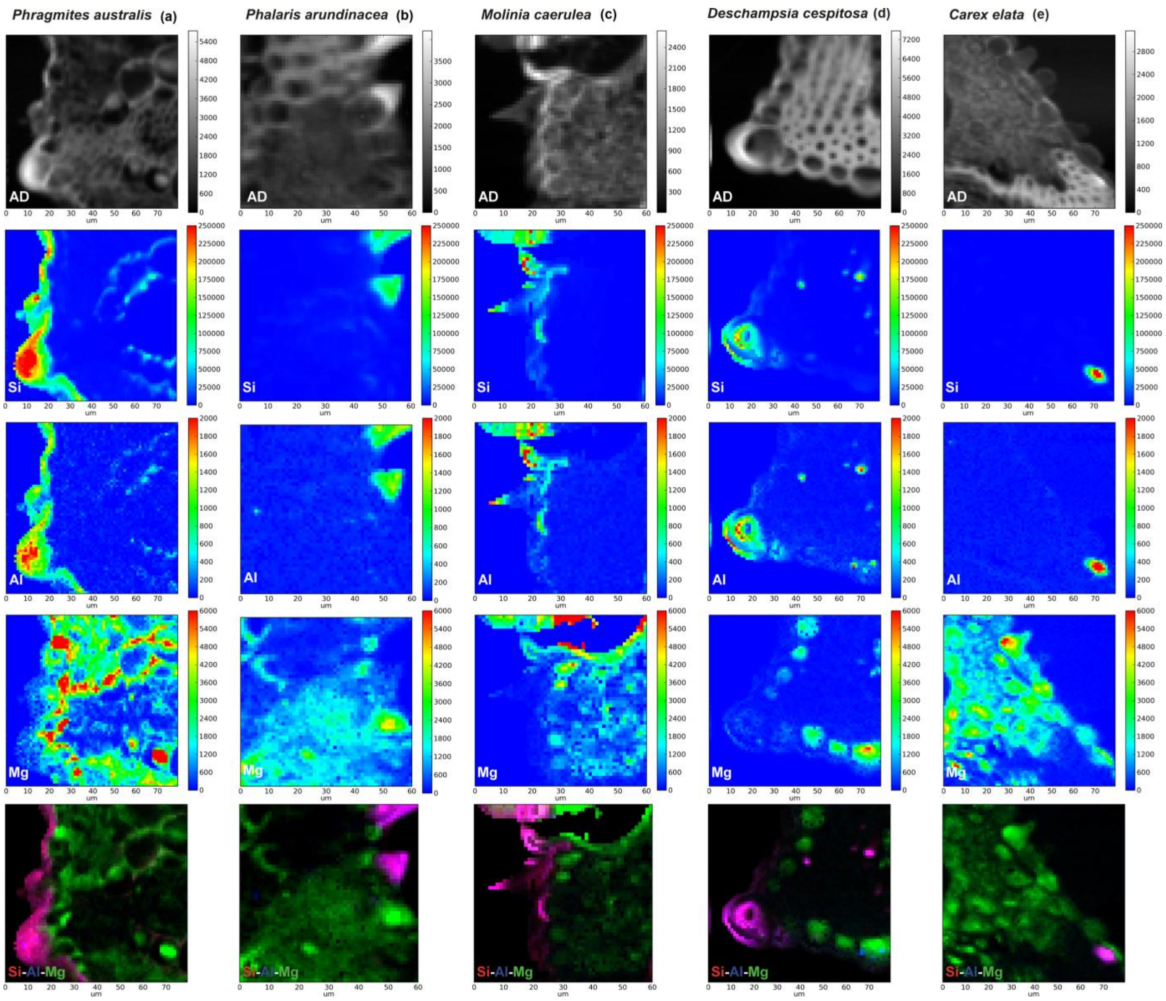

Fig. 2 Silicon (Si), aluminium (Al) and magnesium (Mg) distributions and concentrations (right: colour coding; µg g^{-1}) in the adaxial epidermal and mesophyll regions of the leaves of the four grasses and one sedge (as indicated), using the LEXRF spectroscopy method and respective absorption images (AD) of the analysed areas. The size of all images is indicated in microns

Supplementary Information

Table S1 Element concentrations in the OPY-1 and OU-10, as analysed by quantitative synchrotron radiation micro-x-ray fluorescence at the Twinmic beamline, Synchrotron Elettra, Trieste. The uncertainties of the measurement are given in parentheses.

Element	**Standard reference material analysis**					
	OPY-1			**OU-10**		
	Measured (ppm)	**Certified** (ppm)	**Discrepancy** (%)	**Measured** (ppm)	**Certified** (ppm)	**Discrepancy** (%)
Fe	86060 (12000)	82800	3.8	30900 (3780)	34400	10.2
Na	8670 (880)	8200	5.4	20630 (1380)	18000	12.7
Mg	138000 (14900)	129200	6.4	11600 (2300)	10700	7.8
Al	44600 (3600)	42200	5.4	57150 (9500)	58300	2.0
Si	218000 (17000)	206000	5.5	308400 (38000)	341900	9.8

OPY-1, Ultramafic rock (GeoPT20), analysed in the form of a pressed pellet; certified through an international proficiency test of the International Association of Geoanalysts

OU-10, Longmyndian greywacke (GeoPT24), analysed in the form of a pressed pellet; certified through an international proficiency test of the International Association of Geoanalysts

Uncertainties of the measurements in parentheses.

Table S2 Anatomical parameters of plant tissues and structures subjected to biomineral encrustations of the four grasses and the sedge

Properties	**Grasses**				**Sedge**
	Phragmites australis	***Phalaris arundinacea***	***Molinia caerulea***	***Deschampsia cespitosa***	***Carex elata***
Mesophyll thickness (µm)	166 ±6	125 ±16	102 ±3	423 ±22	98 ±3
Upper leaf surface					
Cuticle thickness (µm)	1.2 ±0.05	2.9 ±0.09	4.7 ±0.10	5.3 ±0.20	2.2 ±0.07
Epidermis thickness (µm)	14.9 ±0.36	27.0 ±0.69	8.4 ±0.33	13.6 ±0.67	11.3 ±0.27
Prickle hair density (mm^{-2})	95 ±8	0 ±0	233 ±8	142 ±17	32 ±9
Prickle hair length (µm)	15 ±0.24	6 ±2.9	37 ±1.1	/	53 ±6.2
Lower leaf surface					
Cuticle thickness (µm)	1.3 ±0.02	2.7 ±0.07	4.8 ±0.14	5.7 ±0.19	3.4 ±0.26
Epidermis thickness (µm)	14.2 ±0.40	27.9 ±0.65	7.9 ±0.33	14.5 ±0.77	21.4 ±0.74
Prickle hair density (mm^{-2})	289 ±15	116 ±17	28 ±11	27 ±9	2266 ±63
Prickle hair length (µm)	18 ±0.41	35 ±1.26	22 ±4.01	40 ±12.97	34.6 ±2.1

Data are means ±SE (*n* = 20-40).

3.6 ALI ODBOJNI SPEKTRI RAZLIČNIH RASTLINSKIH SESTOJEV V MOKRIŠČIH ODRAŽAJO LASTNOSTI VRSTE?

Katja Klančnik, Igor Zelnik, Primož Gnezda, Alenka Gaberščik. Do reflectance spectra of different plant stands in wetland indicate species properties? Poslano v objavo kot poglavje v monografiji The Role of Natural and Constructed Wetlands in Nutrient Cycling and Retention on the Landscape. Vymazal J. (ur.), Springer

Daljinsko merjenje optičnih lastnosti rastlin nam omogoča spremljanje večjih površin in primerjave podatkov v času in prostoru (Ollinger, 2010). Kljub velikim obetom o uporabnosti daljinskega merjenja optičnih lastnosti rastlin, so meritve v bližini sestoja in na ravni lista še vedno nepogrešljive za ustrezno tolmačenje podatkov pridobljenih z daljinskim merjenjem.

S terensko spektroskopijo smo izmerili odbojne spektre (280-887 nm) različnih sestojev na območju Cerkniškega jezera. Tekom vegetacijske sezone smo izvajali meritve na 23 izbranih lokacijah z različno vrstno sestavo in zastopanostjo različnih vrst, ki naseljujejo habitate vzdolž hidrološkega gradienta presihajočega jezera. Na posamezni lokaciji smo poleg meritev odbojnih spektrov izvedli še popise strukturnih lastnosti sestojev: število prisotnih rastlinskih vrst, pogostost posamezne vrste, višina sesestoja, vitalnost in prevladujoča fenološka faza rastlin v sestoju ter prevladujoč naklon listov. Nato smo odbojne spektre, izmerjene na ravni sestojev, primerjali z odbojnimi spektri, izmerjenimi na ravni lista prevladujoče rastlinske vrste v sestoju.

Primerjava odbojnih spektrov je pokazala značilne razlike spektralnih krivulj, izmerjenih na ravni lista in na ravni sestoja. V splošnem so bili izmerjeni relativni odboji sevanja na ravni sestojev manjši od tistih, izmerjenih na ravni lista. V monospecifičnih sestojih lahko variabilnost odbojnih spektrov razložimo z lastnostmi listov prevladujoče vrste v sestoju. Vendar pa lastnosti, ki vplivajo na odbojni spekter niso enake za različne rastlinske vrste. Pri mešanih sestojih, kjer je prevladujoča vrsta pokrivala 50 % merjene površine, se je vpliv lastnosti izmerjenih na ravni lista prevladujoče vrste zmanjšal. Na odbojni spekter sestoja je vplivala tudi njegova struktura. Med naborom parametrov, merjenih na ravni sestojev, sta bila homogenost sestoja in naklon listov najbolj vplivna za odboj sevanja. Omenjena parametra sta značilno sooblikovala odbojni spekter sestoja in skupaj razložila 14 % njegove variabilnosti. Rezultati kažejo, da je uporabnost informacij o sestojih, pridobljenih z meritvami njihovih odbojnih spektrov, odvisna od poznavanja strukturnih in biokemijskih lastnosti ne le na ravni sestoja, temveč tudi na ravni lista.

Do Reflectance Spectra of Different Plant Stands in Wetland Indicate Species Properties?

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Abstract

This contribution discusses the relationships between the reflectance spectra obtained by field spectroscopy and the properties of the leaves of the species that form a stand, and the relation between the reflectance spectra and the stand characteristics. We thus investigate the reliability of conclusions made at the species levels on the basis of the reflectance spectra of the stands. We studied monospecific and mixed stands that thrive in the habitats along a hydrological gradient in the intermittent Lake Cerknica. The reflectance spectra differed significantly at the stand and leaf levels; however, although the shape of the reflectance spectra of a monospecific stand with *Phalaris arundinacea* was similar to the shape of the leaf spectra, this was not the case for mixed stands. The leaf morphological and biochemical properties that explain most of the variability of the spectra differed for graminoids and different dicotyledons. This study shows that based on the reflectance spectra, the species properties for monospecific stands can be deduced, while for mixed stands, such deductions can be misleading.

1 Introduction

Ecosystem structure and function depends on multiple environmental factors that affect habitats, species properties and their distribution (Ustin 2010). The key factor is the amount of incoming radiation and its fate in the plant community. The majority of light is absorbed by different plant organs while some of the light can either penetrate through the stand or is reflected from the plant surface. Thus, only a small proportion of the incoming solar radiation reaches the stand floor. The interactions between the radiation and the plant communities are very complex, due to stand diversity, species architecture, and leaf structural properties (Larcher 2003). Leaves that thrive in specific environments have specific traits that optimise their capture of the solar energy, and prevent the damage due to excessive and/or harmful photons (Gurevitch et al. 2002). This fine tuning is made possible through special adaptations of the leaves at the morphological, anatomical, biochemical and functional levels (Robe and Griffiths 2000, Boeger and Poulson 2003, Šraj-Kržič and Gaberščik 2005, Klančnik et al. 2012).

The light that is reflected from plant leaves can provide a basis for an understanding of the photosynthetic performance and energy balance of the plants (Vogelmann 1993). It also provides information on the leaf biochemistry (Levizou et al. 2005, Castro and Sanchez-Azofeilfa 2008) and nutrient and water status (Baltzer and Thomas 2005, Asner and Martin 2008), and can serve as a tool for stress detection (Gitelson et al. 2002); in some cases, this also allows species classification (i.e., through their spectral signatures) (Castro-Esau et al. 2006). Similarly, light that is reflected from plants can indicate the condition of a stand (Asner 1998, Ullah 2012).

Different indices that are based on species and/or stand reflectance spectra have been developed to determine the properties of different plant species and plant functional groups (Levizou et al. 2005). However, without detailed knowledge of the basic parameters that define the spectral signatures at the species level, reflectance spectra might not provide reliable information (Milton et al. 2009).

In comparison to the measurements of leaf optical properties, which are time consuming, remote sensing allows for surveying and monitoring of relatively large areas, as well as comparisons of data across time and space (Ollinger 2010). Therefore, one of the main reasons for detailed research and a need to understand leaf optical properties is the establishment of libraries of species spectral signatures, along with species leaf properties (Chandrasekharan 2005).

Remote sensing includes two types of spectroscopy: ‘field spectroscopy’, which is based on measurements within or close to a stand; and ‘imaging spectroscopy’, which is the detection of the spectra from a distance (e.g., from aircrafts or satellites). In comparison to remote sensing, field spectroscopy is technically less demanding and less influenced by atmospheric conditions (Gao et al. 2009).

In the present study, we aimed to define the properties of stands and leaves in the intermittent Lake Cerknica affecting the reflectance spectra that can be obtained by field spectroscopy, and to compare the reflectance spectra at the stand level to that at the leaf level. We also examined how reliable conclusions at the species level can be on the basis of the reflectance spectra of a stand.

2 Materials and Methods

2.1 Site Description

The intermittent Cerknica Lake appears at the bottom of the karst Cerknica Polje (38 km2). Due to abundant precipitation in spring and autumn, the polje changes into a shallow lake of 20 km2 to 25 km2 in size. On average, the floods last for 260 days a year, and the dry period usually starts in late spring (Kranjc 2003). The result of this intermittence of Cerknica Lake is the zonation of the plant communities along a hydrological gradient that depends on the duration and extent of the flooding.

2.2 Field Spectroscopy and Stand Properties

For the purpose of the present study, we selected plant stands at 23 locations along the hydrological gradient (Table 1). The selected stands were homogenous, as either monospecific or mixed species. We performed two to four sets of 20 scans/stand in the vegetative period. These measurements of reflectance between 280 nm and 887 nm were carried out using a portable spectrometer (Jaz Modular Optical Sensing Suite; Ocean Optics, Inc., Dunedin, FL, USA). Prior to the leaf reflectance measurements, a white reference panel (Spectralon®, Labsphere, North Sutton, USA) was used to calibrate the spectrometer to 100% reflectance. The reflectance spectra were then calculated as the ratios of the sample data to the white reference under the same illumination. The scans were recorded between 10:00 hours and 14:00 hours. The detector was positioned 90 cm above the stands, at a constant angle that was adjusted according to the position of the sun. At each sampling plot, the properties of plant stands were determined as: number of species; species abundance; total plant and specific species cover (%); height of the stand; and species properties (i.e., plant phenological phases, vitality, leaf angle). The species abundance was estimated according to the Braun-Blanquet method (Braun-Blanquet 1964). The amount of photosynthetically active radiation, and the air temperature and relative humidity were also measured.

Table 1 Plant species composition and abundance (in brackets) at selected locations during the growing season

Location	Month of measurement	RDA code[a]	Plant species composition (abundance[b])
1	May	1	*Euphorbia lucida* (3), *Phalaris arundinacea* (3), *Carex elata* (3)
	June	2	*E. lucida* (4), *P. arundinacea* (2), *C. elata* (2)
	Aug	3	*E. lucida* (5), *C. elata* (2), *P. arundinacea* (2)
	Sept	4	*P. arundinacea* (4), *E. lucida* (3), *C. elata* (2)
2	May, June,	5-6	*P. arundinacea* (5)
	Aug, Sept	7-8	*P. arundinacea* (5)
3	Aug	9	*Myosotis scorpioides agg.* (5), *Mentha aquatica* (3), *Teucrium scordium* (2)
4	Sept	10	*T. scordium* (4), *M. aquatica* (3), *M. scorpioides agg.* (2), *Agrostis sp.* (2)
5	May	11	*Gratiola officinalis* (5), *Plantago altissima* (2)
	Aug	12	*G. officinalis* (4), *P. altissima* (3)
	Sept	13	*G. officinalis* (4), *P. altissima* (2), *C. elata* (2)
6	May	14	*Senecio paludosus* (4), *Polygonum amphibium* (3)
	Aug,4	15-16	*S. paludosus* (5), *P. amphibium* (2)
7	June	17	*Phragmites australis* (5)
8	June	18	*Molinia caerulea* (5), *P. altissima* (2)
9	June	19	*Deschampsia cespitosa* (5), *P. altissima* (2)
10	June	20	*C. elata* (5)
11	May	21	*Apiaceae* (5), *M. scorpioides agg.* (2), *M. aquatica* (2)
	Aug	22	*M. scorpioides agg.* (3), *Apiaceae* (2), *M. aquatica* (2), *T. scordium* (2)
	Sept	23	*Apiaceae* (3), *M. aquatica* (2), *T. scordium* (2), *M. scorpioides agg.* (2)
12	May	24	*P. altissima* (4), *Carex panicea* (3), *Molinia caerulea* (2)
	Aug	25	*P. altissima* (4), *C. panicea* (3), *M. caerulea* (2)
	Sept	26	*P. altissima* (3), *M. caerulea* (2), *C. panicea* (2), *M. aquatica* (2)
13	May	27	*M. aquatica* (3), *Rorippa amphibia* (3), *P. arundinacea* (2)
	Aug, Sept	28-29	*M. aquatica* (4), *R. amphibia* (2)
14	May	30	*R. amphibia* (3), *P. amphibium* (2), *M. aquatica* (2)
	Aug, Sept	31-32	*P. amphibium* (4), *R. amphibia* (3), *M. scorpioides agg.* (2), *M. aquatica* (2)
15	May	33	*C. elata* (5), *P. altissima* (2), *G. officinalis* (2)
	Aug	34	*C. elata* (4), *G. officinalis* (3), *P. altissima* (2), *L. salicaria* (2)
	Sept	35	*C. elata* (4), *G. officinalis* (3), *P. altissima* (2)
16	May	36	*E. lucida* (5), *P. altissima* (3)
	June	37	*E. lucida* (4), *P. altissima* (3)
	Aug, Sept	38-39	*E. lucida* (5)
17	May	40	*C. panicea* (4), *P. altissima* (3), *M. caerulea* (2), *Succisa pratensis* (2)
	June	41	*C. panicea* (3), *P. altissima* (2), *M. caerulea* (2)
	Aug	42	*P. altissima* (4), *C. panicea* (3), *M. caerulea* (3)
	Sept	43	*P. altissima* (4), *C. panicea* (2), *M. caerulea* (2)
18	May, Sept	44-45	*P. amphibium* (5)
	Aug	46	*P. amphibium* (5), *R. amphibia* (2)
19	May, Aug,	47-48	*P. amphibium* (5)
	Sept	49	*P. amphibium* (5)
20	May, Aug,	50-51	*P. amphibium* (5)
	Sept	52	*P. amphibium* (5)
21	May	53	*G. officinalis* (4), *P. altissima* (3)
	Aug	54	*P. altissima* (4), *G. officinalis* (3), *C. panicea* (2)
	Sept	55	*G. officinalis* (4), *P. altissima* (3)
22	May	56	*Schoenus nigricans* (5), *Centaurea jacea agg.* (2)
	June	57	*S. nigricans* (4), *P. altissima* (3), *C. jacea agg.* (2)

	Aug, Sept	58-59	*S. nigricans* (4), *P. altissima* (3), *C. jacea agg.* (2), *M. caerulea* (2), *C. panicea* (2)
23	May, Aug	60-54	*Salix rosmarinifolia* (5)
	Sept	55	*S. rosmarinifolia* (5), *M. caerulea* (2)

[a], RDA code in Figure 5

[b], abundance according Braun-Blanquet (1964)

2.3 Measurements at the Leaf Level

The reflectance spectra of the leaves were measured on the day of sampling with the above-mentioned portable spectrometer. The individual leaves were positioned under an integrating sphere (ISP-30-6-R; Ocean Optics, Inc., FL, USA) connected to the spectrometer via an optical fibre (QP600-1-SR-BX; Ocean Optics, Inc., Dunedin, FL, USA). During the illumination of the leaf with an ultraviolet-visual-near infrared (UV-VIS-NIR) light source (DH-2000, Ocean Optics, Inc., FL, USA), the total adaxial reflectance spectra of the leaves were recorded between 280 nm and 887 nm, with a resolution of approximately 0.3 nm.

For the same leaves, the following morphological, anatomical and biochemical properties were determined: specific leaf area; thickness of the leaf, cuticle, epidermis and mesophyll; density and length of the leaf stomata, trichoma and prickle hairs (silicified trichoma in graminoids); contents of chlorophyll *a*, chlorophyll *b*, carotenoids and anthocyanins; and amount of UV-B (280-320 nm) and UV-A (320-400 nm) absorbing compounds. These analyses followed the procedures and methods as described and cited previously (Klančnik et al. 2012, 2013a).

2.4 Statistical Analysis

The measurements of the reflectance spectra are given as the means across 5-nm intervals. The significances of the differences between the reflectance spectra were assessed with Kruskal-Wallis tests with Bonferroni’s correction. Detrended correspondence analysis was used for the exploratory data analysis, using the CANOCO 4.5 programme package. The gradient length was <3 S.D., and therefore redundancy analysis (RDA) was used to determine the possible effects of the explanatory variables (i.e., leaf traits, stand properties) on the reflectance spectra variability (ter Braak and Šmilauer 2002). Each variable was entered separately into the analysis, and the significance of its gross effects was assessed using Monte Carlo tests with 999 permutations. To avoid possible co-linearity between explanatory variables, forward selection was used. Non-significant variables ($p > 0.05$) were excluded from the further analysis.

3 Results

3.1 Reflectance Spectra at Leaf and Stand Levels

The comparisons of the reflectance spectra differed among the stands and leaves. We compared the reflectance spectra of monospecific and mixed stands of Phalaris arundinacea and the reflectance measurements on the leaves (Figure 1). Three main differences were observed: (1) the leaves reflected significantly more light than the stands; (2) the variability of the reflectance in the different colour bands was more pronounced for the leaves, with the least variability observed for the mixed stands; and (3) the greatest differences were obtained in the UV, green and NIR ranges.

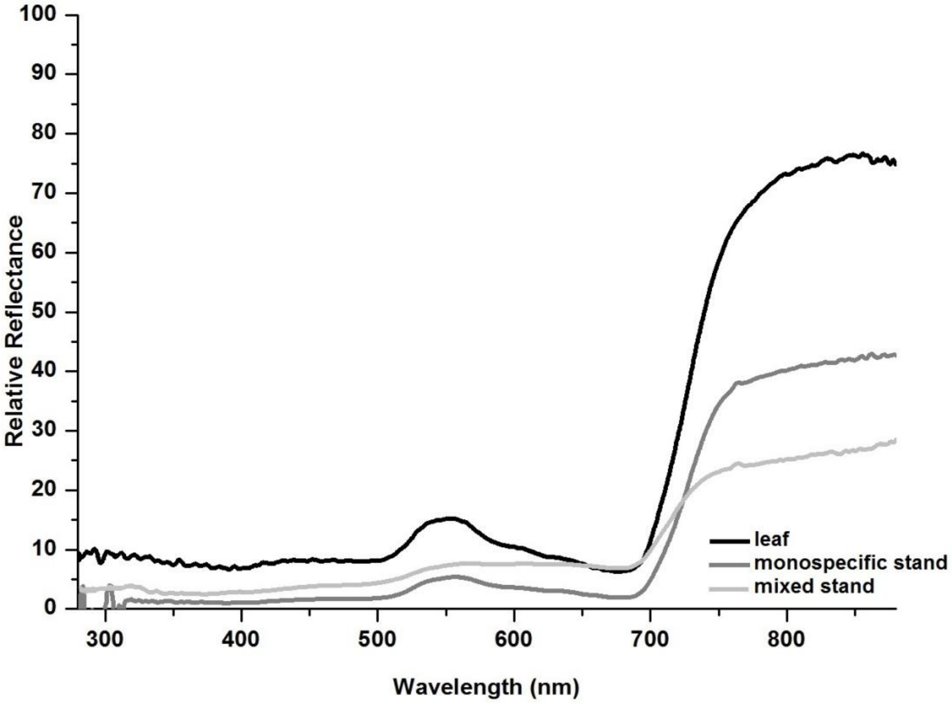

Fig. 1. Mean relative reflectance spectra of a *P. arundinacea* leaf, a *P. arundinacea* monospecific stand, and a mixed stand where *P. arundinacea* covered 25% of the sampling plot. Data are means over 5-nm intervals (n = 10)

3.2 Leaf Reflectance Spectra and Leaf Traits

RDA was performed to define the parameters that explained most of the variability of the reflectance spectra, taking into account the different datasets. In the first run, the data on the biochemical and anatomical leaf traits and the corresponding leaf reflectance spectra were used. In this case, the thickness of the upper epidermis explained as much as 17% of the variability of the reflectance spectra, the trichome density, 16%, the amount of carotenoids and the length of the prickle hairs, 8% each, and the specific leaf area, an additional 7% (Fig. 2). The length of the prickle hairs was negatively related to the reflectance, while the density of the trichoma showed a positive relationship. The species studied were distributed along the full gradient of the visible wavelengths, which showed differences in reflectance and formed optical groups, with the exception of the specimens of *Myosotis scorpioides* agg., which were scattered throughout the whole plot. The graminoids *Carex elata*, *Molinia caerulea* and *Phragmites australis* formed a single group, while the single dicotyledon species were located distinctly apart (Fig. 2).

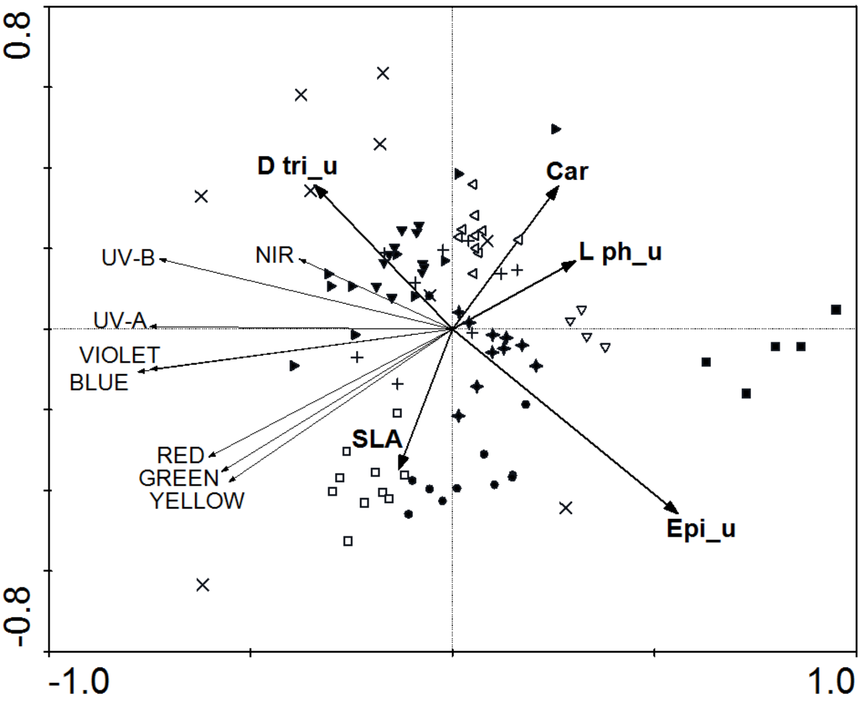

Fig. 2. Redundancy analysis ordination diagram showing the strength of the associations between the significant leaf traits ($p < 0.05$) and the regions of the leaf reflectance spectra. Plant species: filled circles, samples of *P. arundinacea*; open squares, *Gratiola officinalis*; filled squares, *Polygonum amphibium*; filled upside-down triangles, *C. elata*; open upside-down triangles, *Euphorbia lucida*; filled right-pointing triangles, *M. caerulea*; open left-pointing triangles, *P. australis*; pluses (+), *Deschampsia cespitosa*; crosses (×), *M. scorpioides*; thick pluses (+), *Senecio paludosus*. D tri_u, mean trichome density on the adaxial leaf surface; L ph_u, mean prickle-hair length on the adaxial leaf surface; Epi_u, epidermis thickness on the adaxial leaf surface; SLA, specific leaf area; Car, carotenoids content per leaf area

3.3 Monospecific Stand Reflectance and Leaf Traits

In the second RDA, we examined the relationships between the reflectance spectra of the monospecific stands and the biochemical and anatomical leaf traits of the species that formed these stands. The amount of total explained variance was 76%, which was even higher than in the first RDA. The length of the prickle hairs of the upper epidermis and the density of the trichoma explained 32% and 18% of the spectra variability, respectively, the UV-A absorbing compounds and chlorophyll a, 6% each, and the other significant parameters, 1% to 3% each. As shown by Figure 3, the graminoids reflected more in the UV range, while the reflectance in the visible range was very variable.

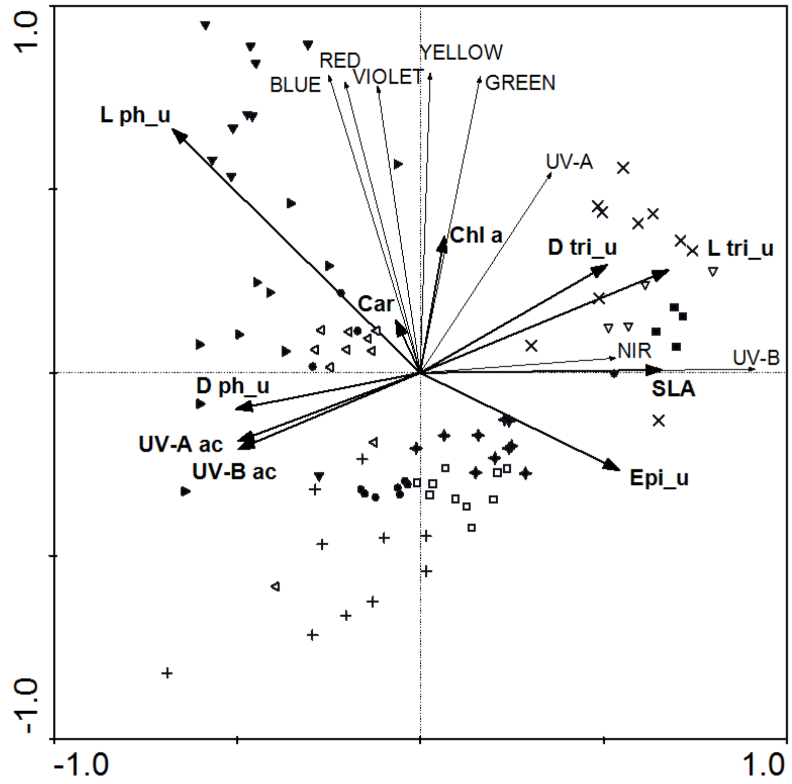

Fig. 3. Redundancy analysis ordination diagram showing the strength of the associations between the significant morphological and biochemical leaf traits ($p < 0.05$) and the regions of the monospecific stand reflectance spectra. Plant species: filled circles, samples of *P. arundinacea*; open squares, *Gratiola officinalis*; filled squares, *Polygonum amphibium*; filled upside-down triangles, *C. elata*; open upside-down triangles, *Euphorbia lucida*; filled right-pointing triangles, *M. caerulea*; open left-pointing triangles, *P. australis*; plusses (+), *Deschampsia cespitosa*; crosses (×), *M. scorpioides*; thick pluses (+), *Senecio paludosus*. D tri_u, trichome density on the adaxial leaf surface; L tri_u, mean trichome length on the adaxial leaf surface; D ph_u, prickle-hair density on the adaxial leaf surface; L ph_u, prickle-hair length on the adaxial leaf surface; Epi_u, epidermis thickness on the adaxial leaf surface; SLA, specific leaf area; Chl a, chlorophyll *a* content per leaf area; Car, carotenoids content per leaf area; UV-A ac, UV-A absorbing compounds per leaf area; UV-B ac, UV-B absorbing compounds per leaf area

3.4 Mixed Stand Reflectance and Leaf Traits

In the next step, we related the reflectance spectra of the mixed stands to the biochemical and anatomical leaf traits of the species that covered half of the sampling area of the plot. In this case, the majority of species that formed the stands were dicotyledons (except *C. elata*), and therefore the outcomes were somewhat different. With the species traits, a total of 74% of the variability of the reflectance spectra was explained. Chlorophyll *a* explained 31%, the thickness of the cuticle, 26%, and other parameters exerted little influence on the spectra variability (up to 5% each). The thickness of the cuticle was negatively related to all of the ranges of the spectra (Fig. 4).

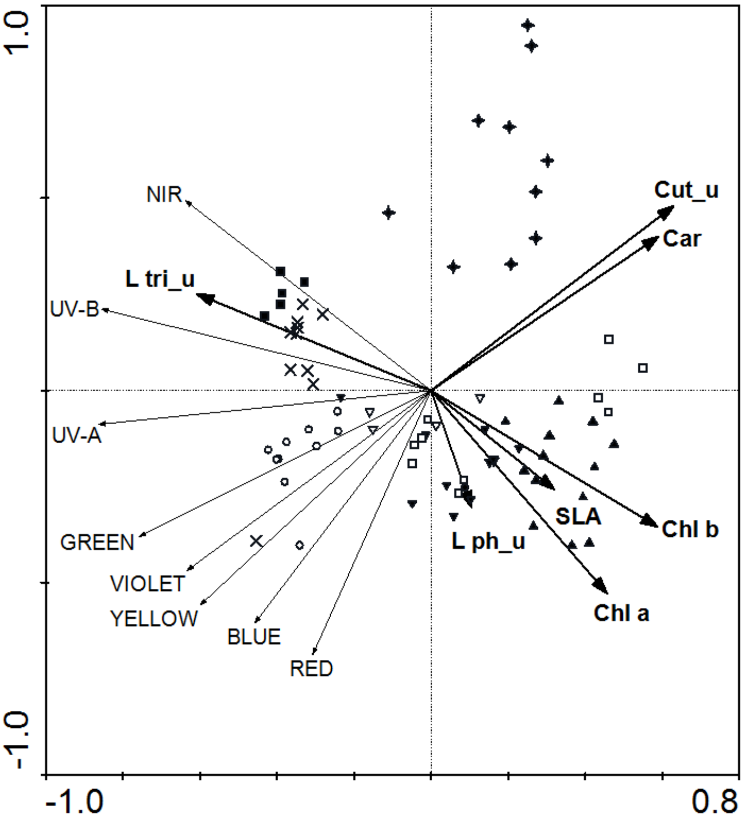

Fig. 4. Redundancy analysis ordination diagram showing the strength of the associations between the significant morphological and biochemical leaf traits ($p < 0.05$) and the regions of the mixed stands reflectance spectra (prevailing species covers 50%). Prevailing plant species: open circles, samples of *Mentha aquatica*; open squares, *Gratiola officinalis*; filled squares, *Polygonum amphibium*; filled upside-down triangles, *C. elata*; open upside-down triangles, *Euphorbia lucida*; filled triangles, *Plantago altissima*; crosses (×), *M. scorpioides*; thick pluses (+), *Senecio paludosus*. L tri_u, trichome length on the adaxial leaf surface; L ph_u, prickle-hair length on the adaxial leaf surface; Cut_u, cuticle thickness on the adaxial leaf surface; SLA, specific leaf area; Chl a, chlorophyll a content per leaf area; Chl b, chlorophyll b content per leaf area; Car, carotenoids content per leaf area

3.5 Stand Reflectance and Stand Properties

The last RDA was performed taking into account the stand reflectance spectra and the properties of the stand. Only two variables had significant effects on the stand reflectance: leaf angle and stand homogeneity. Together, these explained 14% of the variability (Fig. 5). The leaf angle was positively related to all parts of the spectra. The distribution of the stands within a plot showed that the same plots had different distributions at different times of the season.

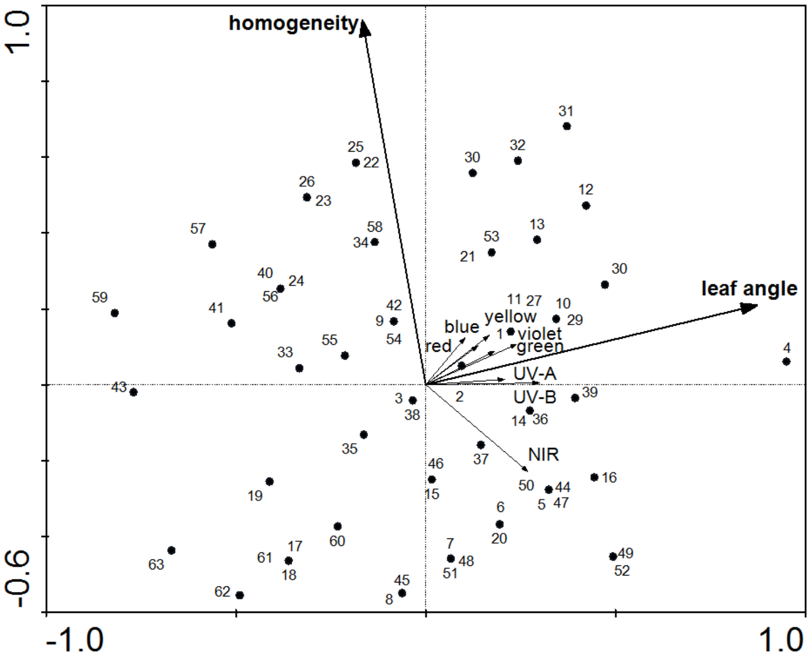

Fig. 5. Redundancy analysis ordination diagram showing the strength of the associations between the significant stand properties ($p < 0.05$) and the regions of the stand reflectance spectra. The detailed species compositions of the stands represented by the numbers are given in Table 1

4 Discussion

Field spectroscopy enables the determination of the properties of the analysed object. In the past decade, this method has significantly enhanced the understanding of the interactions between matter and energy at the levels of plant leaves and stands (Gamon, 2006). Some studies have concentrated to the reflection in the narrow wave bands, with proposals of various vegetation indices, although these have usually been tested with only a few different species (Sims in Gamon, 2002). Many field-spectroscopy studies have aimed to define the species composition and species properties as, for example, the levels of the chlorophylls, carotenoids and anthocyanins (Gamon et al. 1990, Gitelson et al. 2009). The present study has shown that such conclusions might not be always reliable.

To establish the relationships between the reflectance spectra and the species traits, we studied different stand types, with different species compositions and different species properties. The measured reflectance spectra differed significantly at the leaf and stand levels, as related to the leaf and stand properties. When the reflectance spectra of stands with different abundance of *P. arundinacea* were compared, this showed that the reflectance curves of monospecific stands of *P. arundinacea* have similar shapes to those of the *P. arundinacea* leaves, while mixed stands reflected less radiation along the whole spectra. The most pronounced differences were observed in the UV, green and NIR ranges. This was apparently related to the more complex architecture of the mixed stands, in comparison to the monospecific stands (Schulze et al. 2005).

The development of individual plant-leaf properties depends on the species genotype and site conditions, while the structure of a stand mainly depends on the species that constitute the stand, and especially on their growth forms (Larcher 2003). For Lake Cerknica, the specific water regime creates an environmental gradient (Martinčič and Leskovar 2003) that supports a variety of different communities with the different species that were included in the present study. We applied RDA to explain the variability of the spectra with the properties of the species that formed the stands. The data show that the reflectance spectra of monospecific stands can be explained by the species properties, while different properties are indicative of different species or optical groups. The majority of the significant parameters to the monospecific stand reflectance were largely expected. The exceptions were for the contents of chlorophyll *a* and the carotenoids, where the relationships with reflectance were positive, although they explained minor parts of the spectra variance (i.e., 12%). In some species and/or for some stands, the structural parameters were more important than the biochemical parameters (Klančnik et al. 2012, 2013a). It is generally accepted that the leaf surface relief greatly influences the surface reflection of light, while the structure of the mesophyll affects the light penetration. The limited role of the biochemical parameters in the reflectance spectra in some species/ stands was therefore a consequence of structures on the leaf surface, such as the waxy cuticule, trichomes or prickle hairs, which dissipate the radiation and reduce its penetration into the mesophyll (Baldini et al. 1997, Holmes and Keiller 2002). Different trichomes are present in many plant species, as they are cost-effective due to their multiple functions; i.e., the prevention of water loss and protection against excessive radiation (Ehleringer 1980, Woodman and Fernandes 1991). The reflectance in the UV range is usually very low (<10%) (Yoshimura et al. 2010, Qi et al. 2002, Holmes and Keiller 2002), due to the absorption of UV photons by phenolic substances, which usually accumulate in the upper leaf layers, and mainly in the epidermis (Pfündel et al. 2007). However, it has also been reported that in some cases, the increased reflectance is a consequence of silica structures (prickle hairs and cuticle) at the leaf surface (Klančnik et al. 2013a). Silica is a key structural element in graminoids, where it substitutes for carbon as a structural element, and enhances their strength, while preventing lodging and shading of leaves (Schoelynck et al. 2010, Schaller et al. 2012). Therefore, silica should be taken into account when studying reflectance of this plant group.

In the analyses of the mixed stands, dicotyledon species were mainly included, and their biochemical properties were revealed as more important than their structural properties, together explaining 38% of the variability of the reflectance spectra. As expected, chlorophylls a and b, which intercept the light inside the leaf, correlated negatively with the visible parts of the spectra (Klančnik et al. 2012, 2013b). Surprisingly, the cuticle thickness correlated negatively with the entire spectra, even though many studies have shown that wax on leaf surfaces effectively reflects the radiation (Holmes and Keiller 2002, Klančnik et al. 2012). This unexpected relationship potentially arose because the accompanying species in the stands contributed to the shape of the reflectance spectra and masked the role of the prevailing species in the light reflectance. The data obtained indicate that the reflectance of the monospecific stands can be explained by the species properties, while in mixed stands, the data might be misleading, even in the case of a very abundant species. In addition, the architecture of the stand can also contribute to the shape of the spectra. With the RDA where the stand reflectance was related to the stand properties, this revealed that the leaf angle and the stand homogeneity significantly affect the stand reflectance, as has also been shown in previous studies (Ganapol et al. 1999, Rautiainen et al. 2008).

Conclusions

We can conclude that: (1) the complexity of a stand negatively affects the amount of light that is reflected; (2) in monospecific stands, the reflectance can be explained by the leaf properties of the species that constitutes the stand, although the key properties differed among the various species; (3) this is not very likely for mixed stands, including those with species that occur at high abundance due to interactions in reflectance caused by specific traits of different species in the stand; (4) plant architecture might also have an important role in explaining the reflectance spectra variability; and (5) any interpretation of the results of field spectroscopy needs detailed knowledge of the structural and biochemical properties at the stand and species levels.

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4 RAZPRAVA IN SKLEPI

4.1 OPTIČNE LASTNOSTI SUBMERZNIH IN NATANTNIH LISTOV

Primerjava potopljenih in plavajočih listov rumenega blatnika (*Nuphar luteum*) je pokazala značilne razlike v odboju sevanja med obema tipoma listov. Potopljeni listi so imeli večji relativni odboj sevanja v vidnem delu spektra, kar je lahko posledica manjše vsebnosti pigmentov v submerznih listih (Ronzhina in sod., 2004; Klančnik in sod., 2012; Nedukha, 2013). Večji odboj sevanja lahko rastlino ščiti pred poškodbami zaradi prekomernega sevanja (Ehleringer in sod., 1976). Te se lahko pri potopljenih listih hitro pojavijo, saj so prilagojeni na majhne jakosti sevanja (Madsen in Adams, 1989; Sand-Jensen in Borum, 1991). Korelacijska analiza je pokazala vpliv biokemijskih parametrov (klorofila *a*, antocianov in karotenoidov) na odbojnost sevanja zlasti v rdečem delu spektra. Karotenoidi lahko imajo pomembno vlogo kot pomožna barvila pri zbiranju fotonov zlasti v slabših svetlobnih razmerah, kakršne so navadno v vodi (Demming-Adams in Adams, 1996). Prav tako lahko tudi karotenoidi preprečujejo nastajanje poškodb zaradi prekomernega sevanja in pojav fotoinhibicije pri rastlinah (Adams in Demming-Adams, 2004). Fotoinhibicija v vodnem okolju pa ni nujno povezana samo s prekomernim sevanjem, temveč se lahko pojavi tudi zaradi pomanjkanja hranil ali zmanjšane dostopnosti CO_2 za rastline (Steyn in sod., 2002; Nielsen in Nielsen, 2006). Povečano razmerje med karotenoidi in klorofili lahko kaže na pojav stresa pri rastlinah (Solovchenko in sod., 2009), kajti pri potopljenih listih je navadno večje razmerje med klorofili in karotenoidi in ne obratno (Ronzhina in sod., 2004; Klančnik in sod., 2012; Nedukha, 2013). Kordyum in Klimenko (2013) poročata, da je celoten fotosintzni aparat potopljenih listov rumenega blatnika prilagojen na šibkejše jakosti sevanja in ima lastnosti listov, ki rastejo v senčnih razmerah. To jim zagotavlja učinkovit potek fotosinteze tudi v slabših svetlobnih razmerah v primerjavi s plavajočimi listi in listi nad vodo. V naši raziskavi je RDA delno potrdila rezultate korelacijske analize, saj je vsebnost antocianov razložila kar 57 % variabilnosti odbojnih spektrov pri listih rumenega blatnika. Antociani vplivajo na absorbcijo sevanja predvsem v zelenem in rumenem delu spektra in manj v modrem delu spektra (Neill in Gould, 1999; Neill in Gould, 2003). Potopljeni listi so v zelenem in rumenem delu spektra absorbirali manj sevanja kot plavajoči listi rumenega blatnika, ki so vsebovali več antocianov na enoto površine.

Primerjava natantnih listov rumenega blatnika z natantnimi listi navadne dresni je pokazala, da se listi morfološko sicer razlikujejo, vendar pa so si bili v določenih biokemijskih lastnostih zelo podobni. Isti tip lista pri obeh rastlinskih vrstah se je razlikoval manjši meri kot pa različni tipi listov znotraj posamezne vrste (Klančnik in sod., 2012). Podobnost določenih biokemijskih parametrov je bila posledica podobnih okoljskih razmer, v katerih so se listi razvili. Relativna odbojnost sevanja natantnih listov obeh rastlinskih vrst je bila podobna v vidnem delu spektra, kjer v največji meri na optične lastnosti vpliva vsebnost barvil v listih (Slaton in sod., 2001). Odbojni spektri natantnih listov so bolj podobni odbojnim spektrom kopenskih listov kot odbojnim spektrom submerznih listov (Klančnik in sod., 2012; Klančnik in sod., 2013).

Na optične lastnosti listov lahko poleg okoljskih dejavnikov v vodnem okolju vpliva tudi prisotnost epifitona, ki se razvije na rastlinah. RDA je potrdila vpliv prisotnosti epifitona na odbojne in transmisijske spektre submerznih listov treh rastlinskih vrst (*Potamogeton perfoliatus*, *P. lucens* in *Sagittaria sagittifolia*). Vpliv epifitona na optične lastnosti listov je bil večji kot vpliv morfoloških in biokemijskih lastnosti listov. Prisotnost različnih struktur na površini lista vpliva na usodo sevanja, ki pade na list. Tudi raziskave kopenskih rastlin so pokazale, da strukture kot so trihomi in voski, vplivajo na prehajanje sevanja v globje plasti lista (Reicosky in Hanover, 1978; Robberecht in sod., 1980; Barker in sod., 1997; Holmes in Keiller, 2002), kjer doseže pigmentne molekule, zato je njihov vpliv ob prisotnosti struktur na površini manjši (Holmes in Keiller, 2002; Klančnik in sod., 2012; Klančnik in sod., 2013). V naši raziskavi je količina epifitona na površini lista vplivala na odbojne spektre. Suha masa epifitona je razložila 15 % variabilnosti odbojnih spektrov submerznih listov, 8 % variabilnosti pa je razložila še vsebnost klorofila *a* v listih makrofitov. Pri prehajanju sevanja skozi list je bil vpliv vsebnosti barvil nekoliko večji kot vpliv epifitona. Vsebnost karotenoidov je razložila 21 % variabilnosti transmisijskih spektrov, gostota diatomej na spodnji povrhnjici lista pa je razložila 18 % variabilnosti. Suha masa perifitona je v primeru prepustnosti lista razložila le 3 % variabilnosti, enak prispevek je imela še vsebnost klorofila *a* v perifitonu na spodnji strani lista. Vrstna sestava epifitona na submerznih listih preučevanih rastlin je pokazala, da epifiton pretežno tvorijo diatomeje vrste *Cocconeis placentula*. Omenjena vrsta diatomej se je pojavljala na zgornji in spodnji strani listov, njihova gostota pa je bila večja na spodnji strani listov.

Za diatomeje *C. placentula* velja, da so zelo strpne na spremenljive svetlobne razmere (Hudon in Bourget, 1981) in širok pH spekter v okolju (Gasse in Tekaia, 1983; Pither in Aarssen, 2006; DeNicola, 2000). V kolikšni meri vpliva epifiton na svetlobni režim listov, je odvisno od lastnosti epifitona in njegove biomase (Brandt in Koch, 2003). Prisotnost diatomej vpliva na optične lastnosti epifitona, saj diatomejske frustule absorbirajo sevanje v UV-A, vijoličnem in modrem delu spektra. Ker so frustule tudi strukturirane in porozne, se sevanje, ki prodira vanje, razprši, s tem pa se tudi poveča možnost njegove absorpcije (Yamanaka in sod., 2008). Zaradi zmožnosti absorpcije sevanja na frustulah, je bil vpliv epifitona na odbojne spektre manjši, odziv preučevanih vrst makrofitov pa podoben, z izjemo NIR dela spektra. Vpliv epifitona na odbojne spektre je bil najbolj opazen v UV, vijoličnem in modrem delu spektra, kjer se je relativna odbojnost listov po odstranitvi epifitona povečala. Podobno je bilo opaženo tudi v raziskavi na morski travi, kjer je perifiton absorbiral več UV sevanja, kot pa sevanja vidnega dela spektra (Brandt in Koch, 2003). V primeru transmisijskih spektrov smo v naši raziskavi prav tako opazili, da so spremembe transmisijskih spektrov po odstranitvi epifitona največje v UV, vijoličnem in modrem delu spektra. Listi brez epifitona so prepuščali več sevanja, kar dodatno potrjuje vlogo epifitona pri absorpciji kratkovalovnega sevanja. Absorpcija sevanja v UV delu spektra pa je lahko pri submerznih rastlinah prednost, saj so le-te navadno slabše zaščitene pred morebitnimi škodljivimi vplivi kratkovalovnega sevanja (Brandt in Koch, 2003).

4.2 OPTIČNE LASTNOSTI LISTOV AMFIBIJSKIH RASTLIN

Amfibijske rastline so zaradi fenotipske prožnosti in spreminjajočega se okolja, v katerem živijo, dober modelni sistem za opazovanje odzivov in prilagoditev, povezanih z absorpcijo sevanja (Germ in sod., 2002). Vodna dresen (*P. amphibium*) je rastlina z izrazitim amfibijskim značajem. Njena velika fenotipska prožnost ji omogoča razvoj različnih tipov listov vzdolž gradienta med vodo in kopnim (Gaberščik, 1993; Gaberščik in Martinčič, 1992). Raziskava optičnih lastnosti listov navadne dresni je vključevala plavajoče in kopenske liste ter dve prehodni obliki listov, ki se pojavljajo vzdolž hidrološkega gardienta.

Različni tipi listov so se med seboj bolj razlikovali v njihovih morfoloških kot biokemijskih lastnostih. Največje razlike med listi vzdolž gradienta so bile opažene pri pojavljanju struktur na površini (trihomi in reže) ter v debelini stebričastega tkiva (Klančnik in sod., 2012). Vzdolž gradienta med vodo in kopnim se povečuje možnost izgube vode iz rastlinskih tkiv, kar se kaže v povečani poraščenosti listov (Filella in Peñuelas, 1999). Korelacijska analiza je pokazala negativne korelacije povprečnih dolžin trihomov in skupnih dolžin trihomov z odbojnimi spektri. Vpliv trihomov na odboj sevanja je bil v naši raziskavi potrjen tudi z RDA, kjer je povprečna dolžina trihomov razložila kar 72 % variabilnosti odbojnih spektrov (Klančnik in sod., 2012). O vplivih trihomov na odboj sevanja z listov poročajo tudi številne druge raziskave (Ehleringer in sod., 1976; Robberecht in sod., 1980; Holmes in Keiller, 2002; Levizou in sod., 2005). Baldini in sod. (1997) poročajo, da je vpliv trihomov na odbojne spektre odvisen tudi od njihove oblike. Luskasti trihomi pri divji oljki (*Olea ouropaea*) so povečevali odboj, medtem ko pri podolgovatih trihomih navadnega smokvovca (*Ficus carica*) takšnega vpliva niso zaznali. Glede na to, da so trihomi navadne dresni v naši raziskavi negativno korelirali z odbojem sevanja tudi v UV delu spektra, pa lahko sklepamo, da so vsebovali UV-absorbirajoče snovi, kot je bilo dokazano pri nekaterih drugih rastlinskih vrstah (Karabourniotis in sod., 1992; Skaltsa in sod., 1994). Pri različnih tipih listov navadne dresni sta na odbojne spektre vplivala še parametra, povezana z debelino lista (LMA in debelina stebričastega tkiva) (Klančnik in sod., 2012). Korelacije med SLA in odbojnimi spektri sta v svoji raziskavi, ki je zajemala širok nabor različnih drevesnih vrst potrdila Asner in Martin (2008). Med biokemijskimi parametri je bil edini parameter, ki je vplival na odbojne spektre, vsebnost antocianov na suho maso lista (Klančnik in sod., 2012). Antociani ščitijo rastline pred različnimi stresnimi dejavniki, kot so visoka temperatura, pomanjkanje vode in hranil (Steyn in sod., 2002; Close in Beadle, 2003), k čemur so lahko pogosto podvržene rastline v spremenljivem okolju, kot je presihajoče jezero (Gaberščik in sod., 2003).

Podobno raziskavo o optičnih lastnostih amfibijskih rastlin smo naredili še na navadni streluši (*S. sagittifolia*) in veliki zlatici (*Ranunculus lingua*), pri katerih smo poleg odbojnih spektrov merili tudi transmisijske spektre različnih tipov listov. Želeli smo določiti morfološke in biokemijske parametre, ki najbolj vplivajo na variabilnost odbojnosti in prepustnosti sevanja pri listih. Submerzna lista obeh rastlinskih vrst sta se med sabo razlikovala po morfoloških in biokemijskih lastnostih, saj uspevata v različnih habitatih. Potopljeni listi navadne streluše so uspevali v bolj senčnih razmerah, z manjšimi intenzitetami sončevega sevanja kot potopljeni listi velike zlatice. Rezultat različnih razmer so tanjši listi navadne streluše, z večjo SLA in manjšo vsebnostjo klorofilov na površino lista (Klančnik in sod., 2013). Poleg tega se vrsti *S. sagitifolia* in *R. lingua* razlikujeta v evolucijski starosti, kar tudi vpliva na razlike v biokemičnih lastnostih lista, še posebej v vsebnosti UV-absorbirajočih snovi (Rozemain sod., 2002). Razlike med vrstama so bile večje v primeru kopenskih listov. Listi velike zlatice so imeli manj razvit aerenhim in bolj kopenski značaj v primerjavi s kopenskimi listi navadne streluše (Klančnik in sod., 2013). Razlike med različnimi tipi listov ene vrste pa so se odražale tudi v razvitosti aerenhima, saj se delež aerenhima povečuje z obsegom poplavljenosti habitata (Braendle in Crawford, 1999; Boeger in Poulson, 2003). Vendar pa vpliva gostote tkiva in razvitosti aerenhima na optične lastnosti v naši raziskavi nismo potrdili. Podobno kot pri navadni dresni pa se je vzdolž gradienta s povečevanjem suhosti povečevalo tudi število trihomov na spodnji strani listov pri veliki zlatici (Klančnik in sod., 2012, 2013). Trihomi na spodnji strani lista lahko poleg preprečevanja izgube vode (Ehleringer, 1980; Woodman in Fernandes, 1991), zaščitijo list pred prekomernim sevanjem, ki se odbija od poplavljenih tal (Klančnik in sod., 2013).

Razlike v morfoloških in biokemijskih lastnostih, ki nastanejo kot posledica različnih habitatov in evolucijske starosti rastlin, so se odražale tudi na optičnih lastnostih listov. Podobno kot so ugotovili tudi v nekaterih drugih raziskavah (Baltzer in Thomas, 2005; Castro in Sanchez-Azofeifa, 2008), so na odbojne spektre bolj vplivale biokemijske kot morfološke lastnosti listov (Klančnik in sod., 2013). Večino variabilnosti spektrov je razložila vsebnost UV-absorbirajočih snovi (44 %). Razlike v vsebnosti UV-absorbirajočih snovi lahko razložimo z evolucijsko starostjo rastlinskih vrst (Rozema in sod., 2002). Evolucijsko starejša velika zlatica je vsebovala znatno manj UV-absorbirajočih snovi, kot isti tip lista navadne streluše, kljub temu, da so bili listi velike zlatice izpostavljeni večjim jakostim sevanja (Klančnik in sod., 2013). Vsebnost UV-absorbirajočih snovi med listi znotraj posamezne vrste je bila večja v kopenskih listih. Podobno sta ugotovila tudi Les in Sheridan (1990), kjer so potopljeni listi makrofita

Potamogeton natans vsebovali manj UV-B absorbirajočih snovi, kot plavajoči listi. Podobno so tudi listi vrste *Potamgeton alpinus* vsebovali manj UV-absorbirajočih snovi v primerjavi s plavajočimi listi (Germ in sod., 2002). Med parametri, ki so še vplivali na odboj sevanja, je bila tudi SLA, ki je vplivala tudi na prepustnost (Klančnik in sod., 2013). Poleg SLA, so na prepustnost vplivali še debelina stebričastega tkiva, debelina povrhnjice in vsebnost antocianov. Večja SLA je lahko razlog, da so potopljeni listi navadne streluše odbijali več sevanja kot potopljeni listi velike zlatice. Lee in sod. (2000) namreč poročajo, da lahko manjša SLA poveča učinek sipanja sevanja znotraj lista, kar povečuje njegovo absorpcijo in podaljša pot potovanja sevanja znotraj lista. Mnogo študij tudi poroča o vplivih vsebnosti klorofilov na odboj sevanja (Slaton in sod., 2001; Baltzer in Thomas, 2005; Levizou in sod., 2005; Castro in Sanchez-Azofeifa, 2008), kar smo ugotovili tudi v primeru listov vrst *S. sagittifolia* in *R. lingua* (Klančnik in sod., 2013).

Pri kopenskih listih navadne streluše in velike zlatice smo ugotovili, da so listi slednje odbijali več sevanja. To smo pripisali lastnostim kutikule na njenih listih, katerih površina je bila bolj svetleča od površine listov navadne streluše, kar lahko nakazuje na možne strukturne razlike kutikul in vpliv na odboj sevanja na listih (Holmes in Keiller, 2002). Kopenski listi navadne streluše so presevali več sevanja kot listi velike zlatice. To je povezano s prej omenjenim dejstvom, da so imeli listi velike zlatice bolj kopenski značaj, kot listi navadne streluše. Listi velike zlatice so bili spodaj poraščeni s trihomi, ki očitno vplivajo na optične lastnosti listov (Klančnik in sod., 2012) in zmanjšujejo prehajanje sevanja skozi list (Baldini in sod., 1997).

Kljub temu, da je medvrstna primerjava istega tipa listov pokazala številne razlike med rastlinskima vrstama, pa so rezultati pokazali, da isti tipi listov kljub vsemu delujejo podobno. Odboj sevanja v zelenem delu spektra je bil manj izrazit pri listih, ki so se razvili v vodi. Prav tako so listi, razviti v vodi, prepuščali več sevanja, kar pripisujemo manjši vsebnosti pigmentov na enoto površine v primerjavi s kopenskimi listi (Klančnik in sod., 2013).

4.3 OPTIČNE LASTNOSTI MOČVIRSKIH RASTLIN IN RASTLIN MOKROTNIH TRAVNIKOV

Raziskava optičnih lastnosti rastlin je zajemala nekaj najbolj opaznih močvirskih rastlinskih vrst in rastlin mokrotnih travnikov, ki se pogosto pojavljajo na območju Cerkniškega jezera. Izbrane so bile štiri vrste trav navadni trst (*P. australis*), pisanka (*P. arundinacea*), modra stožka (*M. caerulea*) in rušnata masnica (*D. cespitosa*) ter vrsta šaša togi šaš (*C. elata*), ki jih lahko združujemo z izrazom graminoidi. Pri raziskavi optičnih lastnosti graminoidov smo se osredotočili predvsem na vpliv silicijevih struktur na interakcije lista s sevanjem. Predpostavili smo, da bodo različne silicijeve strukture v listih graminoidov poleg ostalih anatomskih in biokemijskih značilnosti listov vplivale na njihove optične lastnosti ter da se razlike v vsebnosti Si in silicijevih fitolitov v mladih in zrelih listih odražajo tudi v različnih optičnih lastnostih.

Pri izbranih rastlinskih vrstah smo določili vsebnosti silicijevih fitolitov, ki so splošni pokazatelj zmožnosti rastline za privzem in kopičenje Si (Piperno in sod., 2006). Ekstrakcija fitolitov je pokazala velike vsebnosti le-teh v listih trav, saj je presegala 1 % suhe mase lista, kar jih uvršča med rastline, ki zelo učinkovito kopičijo Si (Takahashi in sod., 1990). Na drugi strani so fitoliti v listih šaša predstavljali le 0,4 % njegove suhe mase, kar ga po klasifikaciji Takahasi in sod. (1990) uvršča med rastline, ki zmerno učinkovito kopičijo Si. Raziskava je potrdila, da zreli listi trav *P. australis, P. arundinacea* in *M. caerulea* vsebujejo več fitolitov na suho maso kot mladi listi. Podobno poročajo tudi nekateri drugi avtorji o povečanem kopičenju silicija v zrelih listih (de Melo in sod., 2010; Motomura in sod., 2006). Motomura in sod. (2004) so analizirali odlaganje Si v različnih tipih epidermalnih celic in ugotovili, da se Si najprej nalaga v specializiranih celicah povrhnjice, ki odmrejo in se popolnoma napolnijo s Si, ta proces pa so opazili že v mladih listih trave *Pleioblastus chino*. Nasprotno se je Si v druge celice povrhnjice (npr. mehurčaste celice, celice zapiralke) in strukture na površini (bodičke) odlagal šele, ko so bili listi že zreli. V naši raziskavi smo zrele liste navadnega trsta vzorčili dvakrat v sezoni, v juniju in juliju, in potrdili povečano kopičenje Si tekom rastne sezone, o podobnem trendu kopičenja Si pa poročajo tudi nekatere druge raziskave (Webb in Longstaffe, 2002; Motomura in sod., 2004; de Melo in sod., 2010).

Poleg vsebnosti silicijevih fitolitov smo analizirali tudi vsebnost Si, Ca in Al v izbranih rastlinskih vrstah. Si in Ca sta pogosto zastopana elementa v graminoidih, saj sta komponenti obsežnih opornih tkiv (O'Reagain in Mentis, 1989; Motomura in sod., 2004; Hodson in sod., 2005; Motomura in sod., 2006). Al pa se pogosto pojavlja v rastlini skupaj s Si, ki zmanjšuje škodljive učinke Al na rastline (Wang in sod., 2004; Liang in sod., 2007). XRF analiza je pokazala, da je v listih rušnate masnice in togega šaša vsebnost Ca presegla vsebnosti Si. Oba elementa imata v rastlinah podobno vlogo, zato lahko rastline favorizirajo kopičenje v večjih količinah le enega izmed njiju (Borrelli in sod., 2011), lahko pa se v večjih koncentracijah pojavljata tudi oba hkrati v rastlini (Setoguchi in sod., 1989). Koncentracije Al so bile pri večini analiziranih vrst pod mejo detekcije, razen pri travah *P. australis* in *D. cespitosa*, ki očitno tolerirata kopičenje Al v tkivih. Omenjeni rastlinski vrsti sta znani po prevzemanju kovin v tkiva in se uporabljata tudi v fitoremediacijskih procesih (Schat, 1999; Vymazal in sod., 2009).

Meritve optičnih lastnosti rastlin so pokazale, da se odbojni spektri izbranih rastlinskih vrst najbolj razlikujejo v UV in NIR delu spektra. Pogosto se odbojni spektri rastlin najbolj razlikujejo v NIR delu spektra, na račun strukturnih razlik med njimi (Asner in Wessman, 1997; Asner, 1998; Artigas in Yang, 2005). V splošnem so se listi izbranih rastlin bolj razlikovali pri prepustnosti kot odboju sevanja (Klančnik in sod., 2014).

Z RDA smo analizirali vpliv morfoloških in biokemijskih lastnosti listov na njihove optične lastnosti. Analiza je potrdila, da vsebnost fitolitov, izražena na prostornino tkiva, vpliva na optične lastnosti. Pri zrelih listih so fitoliti razložili 21 % variabilnosti odbojnih spektrov in 11 % variabilnosti transmisijskih spektrov. Večji delež variance spektrov je bil razložen, kadar smo upoštevali vsebnost fitolitov, izraženo na prostornino tkiva, kot pa na suho maso lista, saj se fitoliti pojavljajo v različnih plasteh lista in ne samo na njegovi površini (Motomura in sod., 2000; Bauer in sod., 2011). Nadalje je RDA potrdila tudi vpliv različnih struktur, blizu ali na površini lista, na njegove optične lastnosti. Znano je, da strukture na ali blizu listne površine pomembno vplivajo na interakcije lista s sevanjem (Klančnik in sod., 2012; Holmes in Keiller, 2002; Buschmann in sod., 2012). Debelina povrhnjice in kutikule ter dolžina bodičk na površini lista so lastnosti, ki so najbolj prispevale k odboju sevanja. Na prehajanje sevanja skozi list pa sta vplivala še debelina kutikule in gostota bodičk na spodnji strani lista. Vse omenjene strukture, ki so vplivale na odboj in prepustnost, pa so lahko tudi inkrustirane s Si (Piperno, 2006; Motomura in sod., 2006; de Melo in sod., 2010; Currie in Perry, 2007). Na prepustnost je vplivala še vsebnost klorofila *a* v listih, ki je zmanjševala prehajanje sevanja skozi list. To je pričakovano, saj je osnovna vloga pigmentov prestrezanje sevanja (Wooley in sod., 1971; Buschmann in sod., 2012). Vpliv lastnosti listov povezanih s Si na optične lastnosti, je bil potrjen še z RDA, ki je vključevala podatke o vsebnostih Si, Ca in Al v listih. Med elementi je na odboj in prepustnost sevanja vplivala količina Si v listih, poleg tega pa je na prepustnost vplivala še vsebnost Ca. Vse strukture blizu ali na površini lista so povečale odboj sevanja pri travah in šašu. Podoben vpliv Si in silicijevih struktur pa ni bil opažen pri rižu (*Oryza sativa* L.), saj se odbojni spektri rastlin z različnimi vsebnostmi Si niso razlikovale (Agarie in sod., 1996), čeprav se je pokazalo, da je absorpcija sevanja v rastlinah z več silicificiranimi strukturami manjša. Wang in sod. (2005) poročajo, da listi z več Si oddajajo več infrardečega sevanja, kar lahko pripomore k zmanjšanemu pregrevanju listov, vendar pa Björn in Li (2011) tega vpliva Si pri rastlinah nista potrdila.

Največ podatkov o vplivih Si na optične lastnosti rastlin se nanaša na UV sevanje. Različni avtorji poročajo o vplivih Si na zmanjšanje absorpcije UV sevanja pri rastlinah (Goto in sod., 2003; Schaller in sod., 2013). Podoben učinek smo opazili tudi v naši raziskavi (Klančnik in sod., 2014), saj je dolžina bodičk na površini lista, ki so pri graminoidih pogosto okremenjene (Motomura in sod., 2004; Motomura in sod., 2006), bila v pozitivni korelaciji z odbojem UV sevanja. Fine strukture na površini lista, ki so manjše od valovnih dolžin sevanja, lahko veliko bolj učinkovito odbijajo kratkovalovno sevanje kot pa sevanje daljših valovnih dolžin (Grant, 1987). Prisotnost Si v rastlinah tako lahko pripomore k zaščiti rastlinskih tkiv pred škodljivimi učinki kratkovalovnega sevanja (Yao in sod., 2011; Shen in sod., 2010). V raziskavi, ki jo je izvedel Goto s sod. (2003), so rastline riža z večjo vsebnostjo Si vsebovale manjše koncentracije fenolnih snovi, ki rastline ščitijo pred UV sevanjem. Obratno razmerje med vsebnostjo fenolnih substanc in Si lahko kaže na njihovo podobno vlogo v rastlini (Schaller in sod., 2012). Izgradnja fenolnih snovi je za rastline energetsko mnogo bolj potratna v primerjavi z odlaganjem Si v površinske plasti lista.

Aluminij, izmerjen v povečanih koncentracijah pri travah *P. australis* in *D. cespitosa,* se lahko v listih pojavlja skupaj s Si. Silicij je znan kot element, ki zmanjšuje ali preprečuje strupene učinke kovin, kot so Al, Mn, Cd, Zn idr. (Liang in sod., 2001; Liang in sod., 2005; Shi in sod., 2005; Gu in sod., 2012). Aluminij je tako lahko vključen v različne strukture poleg Si in prav tako lahko pripomore k pršenju ali odboju sevanja. Vsebnost Ca v listih trav in šašev ni vplivala na odboj sevanja, je pa vplivala na presevanje sevanja skozi liste. Ca se v rastlinah pogosto pojavlja v obliki Ca oksalata ali v obliki Ca karbonata, ki tvori t.i. cistolite (Bauer in sod., 2011). Ca strukture lahko razpršijo sevanje po tkivu in povečajo prehajanje sevanja iz zgornjih v spodnje plasti lista (Gal in sod., 2012), kar je zlasti pomembno pri rastlinah v podrasti za čim boljši izkoristek šibkega sevanja (Kuo-Huang in sod., 2007).

Pri mladih, še razvijajočih se listih graminoidov, je bil vpliv Si in silicijevih struktur na njihove optične lastnosti manjši kot pri zrelih listih. Na odboj sevanja pri mladih listih je imela največji vpliv dolžina bodičk, poleg teh pa je vplivala še vsebnost antocianov v listih. Antociani se pojavljajo v površinskih plasteh listov in ščitijo rastlino pred različnimi stresnimi dejavniki, kar je zlasti pomembno za mlade liste (Manetas in sod., 2002; Liakopoulos in sod., 2006). RDA, ki je vključevala vsebnosti Si, Ca in Al je pokazala, da vsebnost Si v mladih listih razloži večji delež variabilnosti spektra (42 %), kot pri zrelih listih (18 %). Na prepustnost sevanja pa je vplivala vsebnost Ca v mladih listih in razložila 31 % variance transmisijskih spektrov. Vsebnosti fitolitov, Si in Ca so bile v mladih listih zelo variabilne. Biomineralizacija namreč v listih poteka postopoma tekom razvoja, zato lahko povečano variabilnost podatkov pripišemo različni starosti listov (de Melo in sod., 2010; Motomura in sod., 2006). Kopičenje Si v mladih listih, pa lahko predstavlja zaščito pred objedanjem (Massey in Hartley, 2009).

Na podlagi dobljenih rezultatov o vplivih struktur na ali blizu površine lista na optične lastnosti listov graminoidov, smo natančneje raziskali, katere strukture vplivajo na optične lastnosti pri posamezni rastlinski vrsti. Najpogosteje so na optične lastnosti vplivali parametri povezani z bodičkami (gostota ali dolžina). Pomembna parametra sta bila še debelina povrhnjice in kutikule. Analizi razporejanja elementov v listih graminoidov sta pokazali, da se v omenjenih strukturah pri travah večinoma odlaga Si, z izjemo rušnate masnice, kjer je bila tudi vsebnost Ca v strukturah podobna vsebnosti Si.

V kolikšni meri rastline kopičijo in kje natančneje skladiščijo posamezne elemente, je lahko vrstno-pogojeno. Prisotnost različnih biomineralov v rastlinskih tkivih zato lahko pomaga pri prepoznavanju taksonov (Borrelli in sod., 2011). Mikro-PIXE analiza je pokazala, da se pri navadnem trstu (*P. australis*), pisanki (*P. arundinacea*) in modri stožki (*M. caerulea*) Si nalaga na zgornji in na spodnji površini lista. Podoben vzorec nalaganja Si se pojavlja tudi pri vrsti bambusa (*Sasa veitchii*) (Motomura in sod., 2004). Kljub vsemu je analiza pokazala razlike med tremi travami, saj se je pri navadnem trstu in pisanki Si bolj koncentriral na zgornji strani lista, pri modri stožki pa na spodnji strani lista. Odlaganje Si navadno pozitivno korelira s transpiracijo, saj silicijeva kislina pasivno z vodo prehaja po rastlini in se odlaga v predelih, kjer voda zapušča rastlinsko tkivo (de Melo in sod., 2009; Bauer in sod., 2011). S polimerizacijo silicijeve kisline v tkivih se amorfen Si odlaga v celičnih stenah, lumnu celic, medceličnih prostorih ali pod kutikulo (Sangster in sod., 2009). Vrste graminoidov, izbrane v naši raziskavi, uspevajo v poplavljenih oz. vlažnih habitatih, kjer je transpiracija lahko velika na račun velike dostopnosti vode (Martinčič in Leskovar, 2003; Dolinar in sod., 2010). Z večjo transpiracijo, pa je inkrustacija rastlinskih tkiv lahko večja (de Melo in sod., 2009; Bauer in sod., 2011). Mezofil listov je vseboval manjše koncentracije Si. Si se navadno odlaga v celicah mezofila, ko je njihov potencial za fotosintezo zmanjšan (Motomura in sod., 2000; Motomura in sod., 2004). V nasprotju s Si, se je v predelu mezofila odlagal Ca, predvsem okrog prevajalnih tkiv. Tudi prehajanja Ca po rastlini je vezano na prevajalna tkiva. Ker ima Ca veliko afiniteto za vezavo na lignin (Torre in sod., 1992), ga v večji količini najdemo v celicah v bližini prevajalnih tkiv, kamor se odlaga med transportom po rastlini (Pérez Cuadra in Hermann, 2013). V naši raziskavi smo pri večini vrst ugotovili negativne korelacije med Si in Ca pri odlaganju v rastlinskih tkivih. V nedavni raziskavi so Brackhage in sod. (2013) izmerili povečano vsebnost Si v listih navadnega trsta, kadar je imela rastlina na voljo več Si. Ob povečanem kopičenju Si, pa se je zmanjšalo kopičenje Ca. V naši raziskavi so listi togega šaša, za razliko od trav, v splošnem vsebovali več Ca kot Si. Prevladujoči način poteka biomineralizacije se lahko med različnimi taksoni razlikuje in ker imata Ca in Si podobno vlogo v rastlinah, lahko kopičenje enega izmed njiju zadostuje (Borrelli in sod., 2011).

Ker pa so se med strukturami, ki vplivajo na optične lastnosti graminoidov, za zelo pomembne izkazale bodičke, smo preverili prisotnost različnih biomineralov v njih in morebitne razlike med rastlinskimi vrstami. Ponovno se je vrsta *C. elata* razlikovala od trav, saj bodičke šaša niso vsebovale Si, ki pa je bil vedno prisoten v bodičkah trav. Pri šašu tudi nobena od struktur na ali blizu listne površine ni vplivala na odboj sevanja. Da kalcificirane strukture na zgornji strani lista niso vplivale na odboj je v skladu z rezultati naše prejšnje raziskave, kjer smo ugotovili, da Ca ne vpliva na odboj sevanja pri graminoidih (Klančnik in sod., 2014). V bodičkah trav se je skupaj s Si pojavljal tudi Al, ki lahko dodatno pripomore k sipanju sevanja z listne površine.

V listih graminoidov se je Mg pojavljal predvsem v mezofilu. Mg je osrednji atom v klorofilnih molekulah (Shaul, 2002), katerih največja koncentracija je v mezofilu lista. Večje koncentracije Mg so bile opažene tudi v predelu povrhnjice, natančneje v vakuolah in mehurčastih celicah, kjer se skladišči in verjetno po potrebi remobilizira v mezofil lista. Velike koncentracije Mg v vakuolah lahko vplivajo na turgor v celicah povrhnjice (Pei in sod., 1999) in preprečujejo zvijanje listov.

4.4 OPTIČNE LASTNOSTI RASTLINSKIH SESTOJEV RASTOČIH V HABITATIHVZDOLŽ HIDROLOŠKEGA GRADIENTA

Raziskava optičnih lastnosti na ravni celotnih rastlinskih sestojev je zajemala pogoste sestoje na območju Cerkniškega jezera. Spremljali smo odbojne spektre enovrstnih in mešanih sestojev tekom rastne sezone in skušali določiti lastnosti sestojev, ki najbolj vplivajo na njihove odbojne spektre. Pri nekaterih sestojih smo primerjali odbojni spekter, izmerjen na ravni sestoja z odbojnim spektrom, izmerjenim na ravni lista rastlinske vrste, ki je bila v sestoju najpogosteje zastopana. Rezultati so pokazali, da odbojni spekter enovrstnih sestojev lahko razložimo z lastnostmi vrste, vendar pa so lastnosti, ki vplivajo na spekter, med vrstami različne. Lastnosti, ki so najbolj vplivale na odboj sevanja na ravni lista (gostota trihomov, dolžina bodičk, SLA, debelina povrhnjice in vsebnost karotenoidov), so vplivale tudi na odboj sevanja v enovrstnih sestojih. Poleg omenjenih lastnosti so vplivale na odboj sevanja v sestoju še gostota bodičk, dolžina trihomov, vsebnost klorofila *a* in vsebnost UV-A in UV-B absorbirajočih snovi. Izkazalo se je, da ima na odbojne spektre listov in enovrstnih sestojev velik vpliv oblikovanost povrhnjice listov. Posledica tega je manjši vpliv biokemijskih parametrov, saj že strukture na površini listov znatno vplivajo na prodiranje sevanja v notranje plasti lista, kjer se v večini nahajajo barvila (Baldini in sod., 1997; Holmes in Keiller, 2002; Klančnik in sod., 2012). Podobno so večji vpliv morfoloških lastnosti na odbojne spektre potrdili tudi Baldini in sod. (1997), vpliv barvil je prišel do izraza samo v skrajnih primerih, kot so lisasti listi bršljana (*Hedera helix*). UV-absorbirajoče snovi se sicer navadno kopičijo v zgornjih plasteh lista, večinoma v povrhnjici, kjer prestrezajo UV sevanje (Pfündel in sod., 2007), ker so bližje površini je lahko njihov vpliv na odboj sevanja kljub strukturam na površini večji.

V mešanih sestojih so imeli biokemijski parametri nekoliko večji vpliv na odbojne spektre, kot v homogenih sestojih. To pripisujemo dejstvu, da je mešane sestoje tvorilo več rastlinskih vrst brez izrazito strukturirane povrhnjice. Pričakovano so vsebnosti pigmentov negativno korelirale z odbojnimi spektri, zaradi njihove primarne vloge absorpcije sevanja (Larcher in sod., 2003; Buschmann in sod., 2012; Klančnik in sod., 2012, 2013). Med parametri, ki so vplivali na odboj sevanja v mešanih sestojih, je bila tudi debelina kutikule, ki je negativno kolerirala z odbojem sevanja. Pričakovali bi, da kutikula povečuje odboj sevanja z lista (Holmes in Keiller, 2002; Baltzer in Thomas, 2005; Klančnik in sod., 2012), zato predvidevamo, da je dobljen rezultat najverjetneje posledica vpliva še drugih rastlinskih vrst v sestoju, ki prav tako prispevajo k odboju. Mešani sestoji imajo namreč kompleksnejšo strukturo v primerjavi z enovrstnimi sestoji (Schulze in sod., 2005), zato je težje zajeti vse dejavnike, ki prispevajo k odboju sevanja v sestoju.

Na odboj sevanja v sestoju vpliva tudi sama arhitektura sestoja (Ross, 1981; Yoder in Pettigrew-Crosby, 1995) kar smo opazili tudi v naši raziskavi. Od lastnosti sestojev na odboj sevanja poleg številčnosti vrst vpliva še naklon listov v sestoju. Naklon lista je bil tudi eden izmed pomembnejših dejavnikov, ki je vplival na odboj sevanja različnih rastlinskih sestojev v raziskavi, ki jo je izvedel Asner (1998). V isti raziskavi je bilo tudi ugotovljeno, da se optične lastnosti listov najbolj izrazito odražajo v odbojnih spektrih sestojev v predelu NIR. Kako jasna je bila povezava med spektri izmerjenimi na ravni listov in spektri izmerjenimi na ravni krošenj, pa je bilo odvisno predvsem od indeksa listne površine in naklona listov. Asner in Martin (2008) poročata, da je prenos in ohranjanje informacij z ravni lista na raven krošnje bolj uspešen pri rastlinah z večjimi indeksom listne površine.

Na podlagi dobljenih rezultatov smo zaključili, da odbojne spektre, merjene na ravni enovrstnih sestojev, lahko razložimo z lastnostmi listov rastlinske vrste, v mešanih sestojih pa je povezava med lastnostmi listov in odbojem sestoja manjša, kljub temu, da je v naših mešanih sestojih bila prevladujoča vrsta še vedno relativno pogosta (50 %). V mešanih sestojih se kompleksnost dejavnikov poveča, zato je težje spremljati vse lastnosti, ki lahko prispevajo k odboju sevanja v sestoju. Interpretacija podatkov, dobljenih s terensko spektroskopijo, pa zahteva tudi poznavanje strukturnih in biokemijskih lastnosti ne le na ravni sestoja, temveč tudi na ravni listov.

4.5 SKLEPI

Ugotovili smo, da se optične lastnosti rastlin, rastočih v habitatih, ki nastanejo vzdolž časovnih in prostorskih gradientov presihajočega jezera, razlikujejo. Pod vplivom abiotskih dejavnikov se spreminjajo morfološke in biokemijske lastnosti listov, ki vplivajo na razlike v njihovih optičnih lastnostih. Poleg posrednega vpliva okolja na optične lastnosti rastlin, lahko nanje posredno vpliva tudi njihova evolucijska starost.

Lastnosti listov, z značilnim vplivom na odbojnost in prehajanje sevanja se med vrstami in različnimi oblikami listov razlikujejo. Kadar je listna površina dodatno strukturirana je vpliv biokemijskih parametrov na optične lastnosti manjši. Na odbojnost in prepustnost sevanja pri listih graminoidov vplivajo silicificirane strukture na ali blizu listne površine, medtem ko kalcificirane strukture vplivajo na prepustnost. Pri submerznih makrofitih lahko na strukturiranost listne površine in optične lastnosti listov vpliva tudi prisotnost epifitona. Epifiton absorbira predvsem sevanje krajših valovnih dolžin in tako lahko predstavlja listom zaščito pred škodljivimi vplivi UV sevanja.

Pri amfibijskih rastlinah so potopljeni listi absorbirali manj sevanja kot plavajoči in kopenski listi. Različni tipi listov amfibijskih rastlin se znotraj rastlinske vrste lahko bolj razlikujejo, kot isti tip lista dveh različnih rastlinskih vrst. Listi, razviti v podobnem okolju, imajo podoben spektralni podpis in delujejo podobno.

Pri graminoidih vsebnost silicijevih fitolitov in Si ter z njim inkrustirane strukture neposredno vplivajo na odbojnost in prepustnost sevanja na listih. Vsebnost Ca pa vpliva na presevanje sevanja skozi liste.

V enovrstnih sestojih lahko variabilnost v odbojnosti sevanja razložimo z lastnostmi listov rastlinske vrste, ki tvori sestoj. V mešanih sestojih se vpliv lastnosti listov prevladujoče vrste na odboj sevanja zmanjša zaradi povečane kompleksnosti sestoja in raznolikosti znakov rastlinskih vrst v sestoju. Na odboj sevanja v sestojih vpliva tudi njegova struktura, natančneje zastopanost in številčnost vrst in naklon listov v sestoju. Interpretacija rezultatov, dobljenih s pomočjo terenske spektrosokopije, zahteva tudi poznavanje strukturnih lastnosti listov in sestoja.

5 POVZETEK (SUMMARY)

5.1 POVZETEK

Interakcije sevanja z rastlinskimi organi so na eni strani odvisne od valovnih dolžin sevanja, na drugi strani pa od optičnih lastnosti rastlin, ki jih opisujemo z odbojnostjo, absorptivnostjo in prepustnostjo. Optične lastnosti rastlin so odvisne od kompleksnega skupka najrazličnejših fizičnih lastnosti rastlin. Za učinkovito ugotavljanje njihovih vplivov na interakcije s sevanjem je potreben celosten pristop, ki zajema čim večji nabor možnih dejavnikov.

Namen doktorskega dela je bil podrobneje preučiti optične lastnosti rastlinskih vrst, ki rastejo v habitatih vzdolž hidrološkega gradienta presihajočega Cerkniškega jezera. Želeli smo ugotoviti, kako spremembe okolja vzdolž hidrološkega gradienta vplivajo na spremembe morfoloških in biokemijskih lastnosti listov, ki se odražajo na njihovih odbojnih in transmisijskih spektrih. Določili smo lastnosti listov, ki najbolj vplivajo na odbojnost in prepustnost na ravni lista ter na odbojnost sevanja na ravni rastlinskega sestoja. Pri rastlinskih sestojih smo ugotavljali tudi vpliv različnih strukturnih lastnosti sestoja na odbojnost sevanja v njih.

Odbojnost in prepustnost smo merili v območju valovnih dolžin od 280-880 nm. Raziskali smo morfološke in biokemijske lastnosti listov, pri vrstah graminoidov pa smo določali tudi vsebnost silicijevih fitolitov in elementov Si, Ca, Mg ter Al. Z metodami mikro-PIXE in LEXRF smo določali porazdelitve in koncentracije elementov v rastlinskih tkivih in celicah. Na ravni rastlinskih sestojev smo poleg meritev odbojnih spektrov spremljali tudi strukturne lastnosti sestoja: število rastlinskih vrst, pogostost posamezne rastlinske vrste, pokrovnost celotnega sestoja in posamezne rastlinske vrste v sestoju, višino sestoja in lastnosti posamezne rastlinske vrste (fenološko fazo, vitalnost, naklon listov).

Raziskave rastlinskih vrst, rastočih v habitatih, ki nastanejo vzdolž časovnih in prostorskih gradientov na presihajočem jezeru, so pokazale, da na optične lastnosti vplivajo različne morfološke in biokemijske lastnosti listov. Pri različnih ekoloških skupinah rastlin, ki se pojavljajo vzdolž hidrološkega gradienta se morfološke in biokemijske lastnosti, ki vplivajo na njihove optične lastnosti razlikujejo. Izkazalo se je, da imajo rastline, ki živijo v podobnih habitatih, podobne optične lastnosti listov.

Listi submerznih rastlinskih vrst odbijajo več sevanja v vidnem delu spektra kot listi natantnih in kopenskih rastlin. Potopljeni listi so bolj prepustni za sevanje, zato je absorpcija sevanja v teh listih manjša. Pri submerznih listih makrofitov na njihove optične lastnosti pomembno vpliva tudi prisotnost epifitona na listih. Epifiton, ki so ga pretežno tvorile diatomeje, je absorbiral zlasti sevanje v UV, vijoličnem in modrem delu spektra. Absorpcija kratkovalovnega sevanja v epifitonu, lahko rastlini predstavlja zaščito pred škodljivimi vplivi kratkovalovnega sevanja. Pri heterofilnih rastlinskih vrstah se spektralni podpisi različnih rastnih oblik ali različnih tipov listov znotraj vrste razlikujejo bolj kot isti tip lista različnih vrst. Na optične lastnosti imajo velik vpliv trihomi, katerih gostota in dolžina se povečuje vzdolž gradienta od vode do kopnega.

Pri močvirskih rastlinah in rastlinah mokrotnih travnikov, smo raziskali 5 pogostih predstavnikov, ki sodijo v skupino graminoidov. Največji vpliv na njihove optične lastnosti so imele silicificirane strukture na ali blizu listne površine (bodičke, kutikula in povrhnjica). Ekstrakcija silicijevih fitolitov je pokazala razlike v njihovi vsebnosti med mladimi in zrelimi listi posamezne rastlinske vrste in med vrstami. XRF analiza je pokazala razlike v vsebnostih Si, Ca in Al v različnih listih graminoidov. Med omenjenimi elementi je na odbojnost sevanja na listih vplivala vsebnost Si, na prepustnost pa je poleg Si vplivala tudi vsebnost Ca. Mikro-PIXE analiza je pokazala, da je listna površina pri travah večinoma inkrustirana s Si, medtem ko je listna površina pri šašu na zgornji strani večinoma inkrustirana s Ca, spodaj pa s Si. Korelacijska analiza porazdeljevanja elementov v listnem tkivu je pokazala negativne korelacije med Si in Ca v rastlinskem tkivu, korelacije med Si in Al pa so bile pozitivne. LEXRF analiza je pokazala, da se v bodičkah trav odlagata Si in Al, medtem ko to v bodičkah šaša ni bilo opaženo. Omenjena analiza je pokazala tudi prisotnost Mg v celicah povrhnjice, zlasti v vakuolah.

Variabilnost odbojnih spektrov homogenih rastlinskih sestojev, ki se pojavljajo vzdolž hidrološkega gradienta, je bilo mogoče razložiti s strukturnimi, morfološkimi in biokemijskimi lastnostmi rastlin, ki tvorijo sestoj. Morfološki parametri, zlasti strukture na površini listov, so imele velik vpliv na odboj sevanja v sestoju. Tudi v mešanih sestojih je mogoče razložiti odboj sevanja z lastnostmi listov prevladujoče vrste, vendar je mogoče zaznati tudi vpliv drugih rastlinskih vrst v sestoju. Na odbojnost sevanja v sestojih je pomembno vplivala tudi struktura sestoja, njegova homogenost in naklon listov na rastlinah, ki tvorijo sestoj. Za pridobivanje in interpretacijo informacij, pridobljenih na ravni sestoja, je potrebno poznavanje tudi lastnosti na ravni lista, zato je pomembno ustvarjati podatkovne knjižnice o optičnih lastnostih rastlin, ki bodo podpora številnim podatkom, ki se danes pridobivajo s satelitsko spektrosokopijo.

5.2 SUMMARY

The interactions of radiation with plant organs depend on wavelength of radiation and on leaf optical properties, which are defined by reflectance, absorbance and transmittance. Optical properties depend on complex set of different plant physical properties. The effective determination of their impact on interactions with radiation requires an integrated approach that covers a wide range of different properties/factors.

The aims of this study were to examine the optical properties of different plant species, which occur in habitats along hydrological gradient in the intermittent Lake Cerknica. We wanted to (1) examine morphological and biochemical traits of leaves of different plant species from different habitats along the hydrological gradient and to relate them with their reflectance and transmittance spectra; (2) to determine the traits of leaves that exerted significant effect on their reflectance and transmittance spectra and reflectance spectra of the plant stands and (3) to examine the potential impact of stands structural traits on their reflection of radiation.

Reflectance and transmittance were recorded in a range from 280 nm to 880 nm. In all plant species we examined morphological and biochemical traits of leaves, while in graminoids also the content of silica phytoliths and elements Si, Ca, Mg and Al were measured. Using micro-PIXE and LEXRF methods the localisation patterns and concentrations of elements were determined in plant tissues and cells. In plant stands besides reflectance spectra also structural properties of stand were determined. At each sampling plot, the following traits of plant stands were determined: number of species, species abundance, total plant and specific species cover, height of the stand, and species traits (plant phenological phases, vitality, leaf angle).

The analyses of optical properties of plants, growing in habitats along temporal and spatial gradients of intermittent lake that belonged to various ecological groups, showed that their optical properties were affected by different morphological and biochemical traits of leaves. The results revealed that the plants, thriving in habitats with similar environmental conditions, exhibited similar leaf optical properties.

In leaves of submerged species the reflectance in the visible range of the spectra was higher in comparison with leaves of natant and emergent species. Submerged leaves also transmitted more incoming radiation therefore the absorption of radiation was lower. The optical properties of submerged leaves of macrophytes were also affected by epiphyton on their surfaces. The epiphytic algal community, dominated by the diatoms, absorbed particularly radiation in the UV, violet and blue ranges of the spectra. Absorption of short-wavelength radiation in epiphyton may provide protection against its potentially harmful effects. In heterophyllous plant species the spectral signatures of different growth forms or different leaf types differed more within species than the same leaf type of different species. The presence of trichomes, which density and length increased along water/ land gradient, greatly influenced leaf optical properties.

In wetland plant species and wet-meadow plants, we examined 5 common species, which belonged to the group of graminoids. The greatest impact on their optical properties exerted silicified structures at or near-leaf surface (prickle hairs, cuticle and epidermis). Phytolith analysis showed differences in phytolith contents between developing and mature leaves within and among different plant species. XRF analyses revealed differences in Si, Ca and Al contents between different leaves of graminoid species. The Si contents affected reflectance spectra, while the transmittance spectra were affected by Si and Ca. Micro-PIXE analysis showed that the leaf surfaces of grasses were mainly encrusted by Si, while in sedge the upper leaf surface was encrusted by Ca and the lower by Si. Correlation analysis that was run to show elemental co-localisation in leaf tissue revealed negative correlations between Si and Ca, while Si and Al correlated positively. LEXRF analysis showed that Si and Al were deposited mainly in prickle hairs of grasses, while this was not the case in sedge. LEXRF analyses also revealed the presence of Mg in the epidermal cells, mainly in vacuoles.

The variability of reflectance spectra of homogenous plant stands, occurring along hydrological gradient, was explained in different extent by structural, morphological and biochemical traits of plant species forming the stand. The morphological parameters, especially structures on the leaf surface, greatly influenced the reflectance of radiation from plant stands. The variability of reflectance spectra of heterogeneous plant stands was explained by the leaf properties of prevailing plant species, but also the impact of other plant species was detected. The reflectance spectra of plant stands were also affected by their structural traits namely stand homogeneity and leaf angle of the species forming the stand. In general, the crucial role on the reflectance spectra of the stands had traits of prevailing species. For the acquisition and interpretation of the information obtained at stand level, it is important to know also traits at the leaf level, therefore it is important to create a data library of plant optical properties to support data obtained by satellite spectroscopy.

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