

## Assessing PROSPECT performance on aquatic plant leaves

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### ABSTRACT

PROSPECT is the most widely used optical leaf model for a wide range of remote sensing applications on vegetation and has been developed and parameterised based on empirical data measured almost exclusively on terrestrial plant leaves. As aquatic plants differ substantially from terrestrial plants in leaf morphology and physiology, the validity of the relationships underlying PROSPECT in aquatic plants needs to be verified empirically. To this end, we compiled a comprehensive dataset of leaf spectra and biochemical-structural parameters sampled along a water affinity gradient, including floating and emergent hydrophytes, helophytes and riparian species, and terrestrial plants. In parallel, we designed a multidimensional experiment to explore the performance of PROSPECT across different groups and to characterise sources of modelling error, focusing on aquatic plants. Our results showed that estimates of most leaf parameters from PROSPECT inversions diverged increasingly from measured traits when moving from terrestrial to aquatic species. The suboptimal performance of PROSPECT on aquatic plants appears to be driven by three main factors: difficulties in disentangling leaf dry matter components (particularly proteins), unresolved issues related to the overlap of primary and secondary pigment mixtures and absorption, and the peculiarities of internal leaf structure (i.e. the presence of 'aerenchyma'). These findings highlight the need for careful preliminary evaluation of the applicability and limitations of PROSPECT when applied to vegetation types that differ significantly from the typical terrestrial trees and grasses used for model calibration, including aquatic plants. Such evaluation should be preferably based on empirical data covering natural heterogeneity, so that future applications of remote sensing for mapping aquatic and wetland vegetation characteristics can be improved in terms of robustness and transferability.

### 1. Introduction

Aquatic plants, or macrophytes – defined as large phototrophic organisms (i.e., not phytoplankton) adapted to live and grow with organs completely or partially covered by water, either permanently or periodically (Chambers et al., 2008) – make up a small proportion of the total species pool: different estimates have quantified that they cover between 0.2% (Govaerts et al., 2021) and 1% (Murphy et al., 2019) of all vascular plant species at global scale. Despite this, aquatic plants play a key role in water bodies, wetlands, and coastal areas: they act on ecosystem functioning (e.g. nutrient and carbon cycles), regulate the quality and balance of water and sediments, provide food and habitat for fish and birds, protect shorelines and riparian areas, and overall sustain biodiversity in freshwater and marine ecosystems (Himes-Cornell et al., 2018; Thomaz, 2021). To emphasise the difference with terrestrial

plants, we will henceforth refer to macrophytes simply as aquatic plants.

The foundation of remote sensing of vegetation in the visible to shortwave infrared domain (400–2500 nm) is the relation between leaf biophysical parameters – or traits – and their reflectance features (Jacquemoud and Ustin, 2019). This link has been exploited to retrieve foliar traits starting from reflectance spectra measured with different sensors (from proximal spectroradiometers to spaceborne imagers), using either statistical, empirical or physically based approaches (Verrelst et al., 2015). Among the latter, PROSPECT, a radiative transfer model that simulates leaf reflectance and transmittance spectra from a limited set of traits (pigments, water and dry matter content, mesophyll structure; Féret et al., 2021; Jacquemoud and Baret, 1990), is by far the most widely used (Jacquemoud and Ustin, 2019). Although the physical theory behind the PROSPECT is intended to be generalisable across plant functional groups and species, the model components (e.g. specific

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absorption coefficients, refractive index) have been parameterised based on empirical datasets consisting almost exclusively of terrestrial plants, e.g. LOPEX (Hosgood et al., 1993) and ANGERS (Jacquemoud et al., 2003).

Villa et al. (2021) recently investigated the association between leaf spectra and functional traits in aquatic plant species based on a systematic collection of empirical data covering environmental heterogeneity, highlighting that structural, biochemical, and physiological traits can be predicted from reflectance via PLSR models with variable reliability. Indeed, the currently available literature on spectroscopic applications for leaf trait retrieval is largely based on terrestrial plants - with a few exceptions focusing on specific groups (Proctor and He, 2013; Penuelas et al., 1993; Villa et al., 2021). Therefore, the relationships between optical properties and reflectance based on leaf optical models such as PROSPECT cannot be taken for granted in aquatic plants, which have significant differences from terrestrial plants in terms of morphology and physiology (Maberly and Gontero, 2018).

Compared to terrestrial plants, species with submerged and/or floating leaves tend to have lower dry matter content per unit leaf weight (Pierce et al., 2012; Klančnik et al., 2014a) and invest fewer resources in structural components such as lignin (Grasset et al., 2015), sometimes with mechanisms for building hard tissues based on biogenic silica (Schoelynck et al., 2010). Large lacunal space within the parenchyma tissue (also called 'aerenchyma') is common in both leaves and stems of aquatic plants (Große, 1996; Jung et al., 2008), aiming to enhance gas transport between plant organs when oxygen availability in water or sediments is low (Marzocchi et al., 2019). This feature is reflected in the arrangement of mesophylls, which is different from that of most terrestrial species, and promotes the relocation of chloroplasts close to the adaxial side of the leaf (Ronzhina, and P'yankov, V. I., 2001). Living in an environment with extremely variable light availability - very low for submerged species due to water absorption or very high for floating and emergent species with limited leaf shading possibilities - aquatic plants tend to be more plastic in pigment balance and pool composition compared to terrestrial species (Ronzhina et al., 2004), which are characterised by a major role of different types of carotenoids as well as other accessory pigments (Horppila et al., 2022; Klančnik et al., 2012).

In this context, a fundamental question arises: is the PROSPECT model capable of predicting structural and biochemical parameters of aquatic plant leaves with similar reliability as demonstrated for terrestrial plant leaves (see: Féret et al., 2019; Jacquemoud et al., 2009; Verrelst et al., 2015)? To address this question, we designed the collection and analysis of new empirical data to test the capabilities of the PROSPECT model inversion on plant species distributed along a gradient of water affinity, i.e. sampling leaves from more aquatic to terrestrial species, taking into account both the plant life form (growth habit) and the degree of leaf contact with water. Target species include floating and emergent hydrophytes, helophytes and riparian species, as well as terrestrial plants.

The objectives of this work are: i) to evaluate the effect of varying settings (spectral input, model version, mesophyll structure parameter estimation) on PROSPECT inversion results; ii) to quantify the accuracy of leaf parameters estimated via PROSPECT across species grouped by water affinity; and iii) to characterise the source of bias in PROSPECT performance over aquatic plant leaves, focusing on their peculiarities with respect to terrestrial plants.

## 2. Materials and methods

### 2.1. Data collection

We measured multiple spectral and biophysical parameters on leaves systematically sampled along a gradient of water affinity within plant communities of the Mantua lakes system, northern Italy, in the summer of 2021. This freshwater system (45°10' N, 10°47' E) is composed of

three shallow, turbid lakes (mean depth of 3.5 m) and two connected wetlands (upstream and downstream of the lakes), which host a substantial diversity of aquatic plant species, especially floating and emergent hydrophytes and helophytes (Villa et al., 2017).

The final dataset includes measured parameters for a total of 190 leaves, covering 13 species and incorporating natural spatial and temporal variability, i.e. representing samples from two different spatially separated populations (5 leaves from different individuals/branches per population) for each species, measured at two moments of the growing season, at early vegetative (09 June 2021) and mature (20–22 July 2021) stages. As anticipated, the sampled species cover a gradient of water affinity ranging from aquatic to terrestrial growth forms, and with leaves in (partial) contact with water to exclusively aerial leaves, which are divided into five groups: i) floating-leaved hydrophytes (group FL), represented by *Nelumbo nucifera* (juvenile, floating leaves), *Nuphar lutea*, *Trapa natans*; ii) hydrophytes with emergent leaves (group ER), represented by *Ludwigia hexapetala*, *N. nucifera* (adult leaves emergent above water); iii) helophytes (group H), represented by *Carex riparia*, *Phragmites australis*; iv) riparian species (group RR), represented by *Humulus lupulus*, *Salix alba*, *Salix cinerea*; v) terrestrial species (group TV), represented by *Carpinus betulus*, *Quercus robur*, *Taraxacum officinale*, *Trifolium pratense* (see sample locations and photos in Suppl. Fig. S1-S2).

Leaf spectral response, biophysical and biochemical parameters (or traits) were measured on all sampled leaves. Radiance reflected from leaves was measured using a contact probe with a halogen light source attached to a full range (350–2500 nm) spectroradiometer (SR-3500, Spectral Evolution). Within seconds of detachment, the leaf surface was gently wiped with a paper tissue and spectra readings were then taken with the same leaf placed against two different backgrounds: dark (black neoprene, ~4% absolute reflectance) and bright (Spectralon, nominal absolute reflectance >95%). The reflected radiance measured with dark and bright backgrounds was used to derive leaf reflectance (BRF) for each sample, adapting the simplified approach proposed by Lillesaeter (1982) – see Supplementary Materials for equations used. This approach to spectral measurements was adopted to ensure a better match between leaf spectra and parameters, especially for pigments and physiological parameters, and to avoid spectral changes due to leaf cutting (Richardson and Berlyn, 2002; Rascher et al., 2007). In fact, in this setup, i.e. measuring spectra on a boat for in vivo work, it is not logistically feasible to use an integrating sphere to derive hemispherical reflectance (DHRF). Indeed, the directional effects introduced by our setup in the leaf reflectance measurements are not a major distortion (Potůčková et al., 2016), and the BRF has been shown in previous work not to significantly bias the model-estimated leaf parameters (e.g. Stuckens et al., 2009; Shiklomanov et al., 2016). Based on extensive data on terrestrial plant leaves from temperate to tropical sites in China and the United States, Wang et al. (2023) recently demonstrated that PROSPECT inversions from bidirectional reflectance can estimate foliar traits with good accuracy, similar to or better than PROCOSINE, which was developed specifically for BRF (Jay et al., 2016); this performance was attributed by the authors to a possible overestimation of the mesophyll structure parameter in PROSPECT inversions from BRF, counterbalancing the effects of specular reflectance. In addition, Li et al. (2023) found that for small viewing angles (as is the case for contact probe reflectance measurements), the inversion accuracy of PROSPECT is similar to that of a modified model accounting for leaf angular reflectance anisotropy.

Nevertheless, to compensate surface effects on leaf reflectance, mainly due to directional anisotropy with near-specular properties, a correction factor based on Li et al. (2019) was applied to the derived reflectance spectra to enhance comparability with DHRF measurements. Average and standard deviation of measured leaf reflectance for the five groups sampled across the Mantua water affinity gradient are shown in Fig. 1 (further details on leaf spectra measured are provided in Suppl. Fig. S3-S8).

A number of functional traits were determined on the same leaves

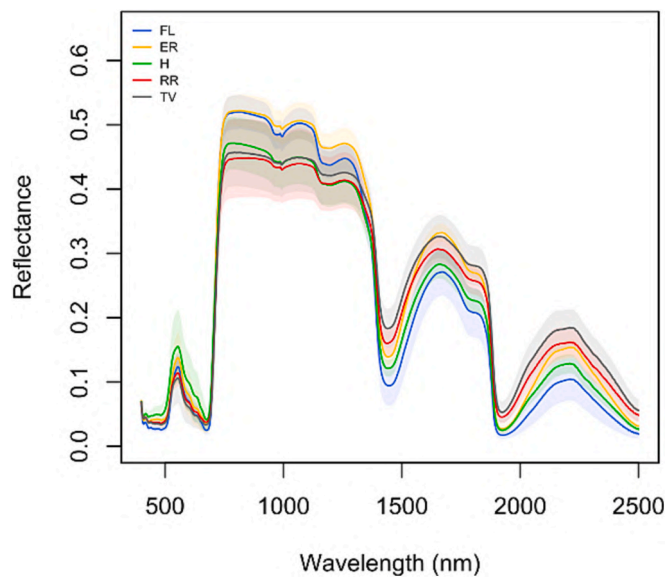


Fig. 1. Leaf reflectance measured across the Mantua water affinity gradient, as mean and standard deviation by plant group.

used for the spectral response measurements. Leaf disks of known surface area ( $24.8 \text{ mm}^2$ ) were used for the determination of pigment content (chlorophyll-*a*, chlorophyll-*b*, carotenoids), leaf dry matter content (LDMC, as dry mass to fresh mass ratio), equivalent water content (EWT, as water content per area) and leaf mass per area (LMA, as dry mass per area). Pigments were extracted from leaf discs in 80% acetone and the extracts were stored in the dark at  $4 \text{ }^\circ\text{C}$  for 24 h. After centrifugation of the samples, the supernatant was read spectrophotometrically at wavelengths of 470, 646, 663 and 750 nm for pigment calculation according to Wellburn (1994). Fresh weight and dry weight (after drying in an oven at  $50 \text{ }^\circ\text{C}$  until a constant weight was reached) of leaf discs were measured with a precision balance (0.1 mg) and used for the determination of LDMC, EWT and LMA (Perez-Harguindeguy et al., 2013). Leaf carbon content (LCC) and leaf nitrogen content (LNC) were determined from an aliquot of ground dry material by combustion analysis using an elemental analyser (Thermo FlashEA 1112). Total protein content was calculated from the LNC content using a generalised conversion factor of 4.43 (Yeoh and Wee, 1994), and carbon-based compounds (CBC) content - including lignin, cellulose, hemicellulose, and other carbohydrates - was calculated as the difference between LMA and total protein content (Féret et al., 2021). For the determination of leaf thickness, thin sections were cut and placed on a microscope slide; the slides were observed with an optical microscope (ZEISS Primostar) and thickness was determined as the mean of three measurements per section, avoiding the main veins.

## 2.2. Experiment setup

Starting from this dataset, we thoroughly assessed the performance of PROSPECT across species with different water affinity features by performing a series of PROSPECT inversion tests based on leaf reflectance via the `prospect` package in R (Féret and de Boissieu, 2022), varying with: i) spectral range covered - the full spectrum (FS: 400–2500 nm) or optimised subsets depending on the parameter (OPT: Spafford et al., 2021); ii) model version - PROSPECT-D (D: Féret et al., 2017) or PROSPECT-PRO (PRO: Féret et al., 2021); and iii) computation of the mesophyll structure parameter  $N$  ( $N_{\text{struct}}$ ) - estimated as an inversion output (Nest) or prior setting from near-infrared spectral features (Nprior: Spafford et al., 2021). We also compared the performance of PROSPECT inversions on our dataset with those obtained on other leaf datasets, and the accuracy of leaf parameters estimated from

PROSPECT inversions with those estimated from other methods. Finally, we examined the differences between measured leaf spectra and those modelled with PROSPECT to investigate the specific leaf features that affect the reliability of PROSPECT in aquatic plant species. A summary of the experiment setup is shown in Fig. 2.

### 2.2.1. Comparison of PROSPECT inversion settings

We first compared the inversion results, in terms of retrieved Chl-*ab*, Car, EWT, LMA, and  $N$ , of different PROSPECT versions (D vs. PRO) testing different combinations of settings, i.e. spectral range (FS or OPT), and  $N_{\text{struct}}$  computation (Nest or Nprior). In addition, we compared the inversion results over the same parameters starting from different spectral ranges (FS vs. OPT) and using as input estimated  $N_{\text{struct}}$  (Nest) or prior  $N_{\text{struct}}$  (Nprior).

### 2.2.2. Leaf parameter estimation along a water affinity gradient

We then assessed the differences between selected leaf parameters (Chl-*ab*, Car, EWT, LMA) estimated from the PROSPECT inversion or derived from their ratios ( $\text{Chl}/\text{Car} = \frac{\text{Chl-}ab}{\text{Car}}$ ,  $\text{LDMC} = \frac{\text{LMA}}{\text{LMA}+\text{EWT}}$ ) with those measured in our reference dataset in terms of precision (Bias), root mean square error (RMSE), normalised root mean square error (nRMSE), and coefficient of determination ( $R^2$ ). Such comparisons were made based on the outputs from the best setting for PROSPECT-PRO (FS-Nest) to those from the best setting for PROSPECT-D (OPT-Nprior). We performed this evaluation separately for the five plant groups distributed along the water affinity gradient (FL, ER, H, RR, TV) and further aggregated them into three macro-groups: hydrophytes (FL and ER groups), riparian plants (H and RR groups), and terrestrial plants (TV group). In addition, the comparison was extended to leaf proteins and CBC content, as well as estimated C/N ratio (Féret et al., 2021) by considering inversions of PROSPECT-PRO with two different settings (FS- Nest vs. OPT-Nprior).

### 2.2.3. PROSPECT inversion performance on different datasets

To check the consistency of results derived from our reference dataset, we compared the estimation performance (modelled vs. measured leaf parameters) of PROSPECT inversions on our dataset with that derived using homologous leaf datasets publicly available online: i) LOPEX (Hosgood et al., 1993), excluding dry and needle leaf samples; ii) NEON (Wang, 2020), excluding needle leaf samples; and iii) IFGG/KIT (Kattenborn et al., 2017), excluding pigment content data as these were estimated from the PROSPECT inversion based on leaf spectra. Comparisons were based on the same model version and settings for all datasets, i.e. PROSPECT-D (OPT-Nprior). Although doubts have been raised about the reliability of pigment measurements in LOPEX (Féret et al., 2017), we did not exclude these parameters from our comparison in order to fully exploit the richness of this dataset, which is still the most comprehensive among the freely available datasets. Other leaf datasets were not considered because they either contained few leaf parameters or were biased towards some species (e.g., the ANGERS dataset, predominantly composed of *Acer platanoides* samples).

### 2.2.4. Leaf parameter estimation with different methods

After evaluating performance on different datasets, we compared the performance of leaf parameter estimation using PROSPECT inversions (version D, settings OPT-Nprior) with that of other semi-empirical methods previously documented in the literature: i) linear regression based on optimised spectral indices (LRM\_Git, Gitelson et al., 2006), ii) partial least squares regression based on leaf reflectance across a range of species (PLSR\_Sch, Schweiger et al., 2018), and iii) partial least squares regression based on leaf reflectance of floating and emergent hydrophytes (PLSR\_Vil, Villa et al., 2021). As in section 2.2.2, we perform this evaluation separately for three macro-groups along the water affinity gradient (hydrophytes, riparian plants, and terrestrial plants).

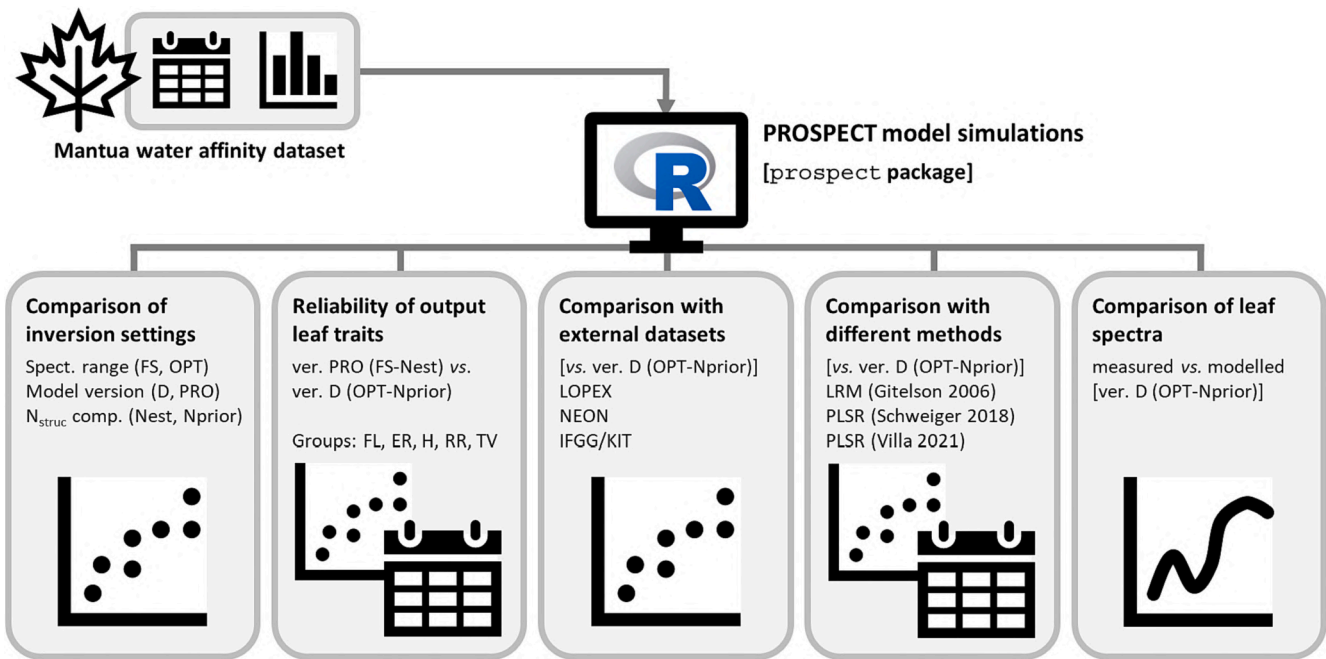


Fig. 2. Summary scheme of the experimental setup for the evaluation of PROSPECT simulations on the Mantua water affinity dataset.

2.2.5. Differences in leaf spectra measured and modelled by PROSPECT

As a final step, we compared the leaf reflectance modelled by forward runs of PROSPECT-D, fed with leaf parameters measured on our samples - where anthocyanin content was derived following Féret et al. (2017) and prior  $N_{struct}$  from Spafford et al. (2021) – to reflectance measured on corresponding leaves. To do this, we calculated the RMSE (absolute and relative) of modelled reflectance to measured values, aggregated by group (along the water affinity gradient); we also calculated the relative reflectance difference (modelled measured/measured) at the detailed scale of the sampled populations (species/growth stage) to investigate relationships between spectral modelling errors and leaf characteristics of different aquatic plant species.

3. Results and discussion

3.1. Comparison of PROSPECT inversion settings

Fig. 3 (row 1) shows that, when inverted from the full spectral range (400–2500 nm), PROSPECT-PRO tends to predict slightly lower Chl-ab,

Car and EWT values compared to version D. The discrepancy is substantial for LMA, where the underestimation by the PRO version peaks at 40% for most of the samples from FL group (especially *T. natans* samples) and riparian *Salix* spp. At the root of these inconsistencies, there appear to be systematic differences in the estimation of  $N_{struct}$  between the D and PRO versions when the spectral inversion input is limited to reflectance. This pattern is highlighted for all groups (although the effect is minimal for terrestrial plants) and may have a cascading effect on all other leaf parameters. Féret et al. (2021) showed that for PROSPECT-PRO the effect of  $N_{struct}$  variability on leaf absorbance is null, while it is evident for leaf reflectance, but the issue of different results for D and PRO versions in this respect needs further investigation.

Using optimised spectral ranges specific to each leaf parameter (Spafford et al., 2021), PROSPECT-PRO and -D inversions differ slightly in predicted Chl-ab (2–3% higher for the PRO version), while predicted Car is similar. As PROSPECT-D and -PRO do not differ with respect to leaf pigments (Féret et al., 2021), this Chl-ab inversion bias is difficult to explain, unless some unreported differences in the prospect package code between the two model versions play a role.

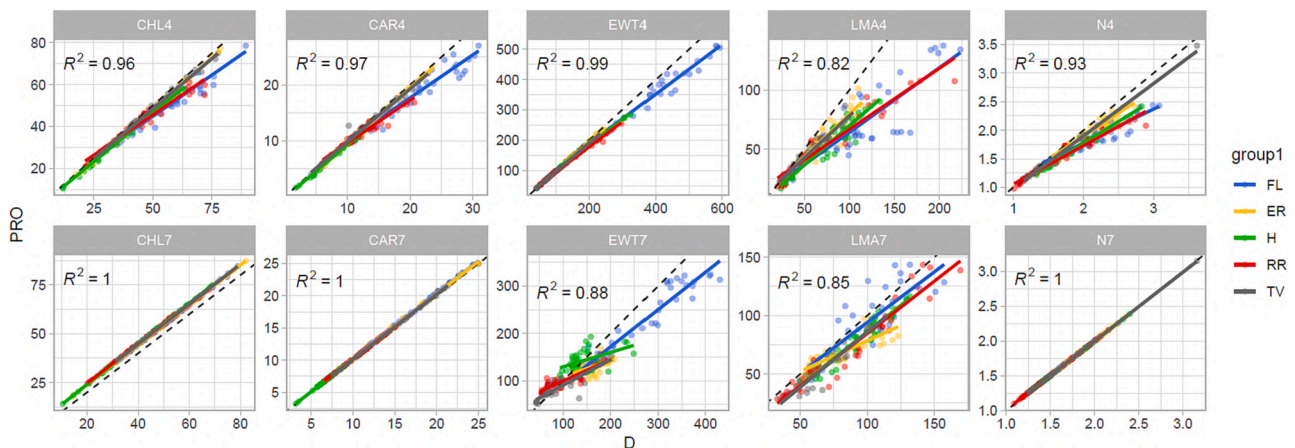


Fig. 3. Comparison of leaf parameters estimated from the inversion of PROSPECT-D and -PRO under variable settings: row 1 (suffix 4): R-FS-Nest; row 2 (suffix 7): R-OPT-Nprior.

Estimates of structural parameters are generally consistent between PROSPECT versions ( $R^2 = 0.88$  and  $0.85$  respectively; Fig. 3, row 2), with a tendency to lower estimated values for PRO versions, more evident for EWT and across samples from FL group (showing EWT that can surpass  $200 \text{ g m}^{-2}$ ).

Fig. 4 shows the differences between leaf parameters estimated based on the full spectrum or optimised spectral ranges, using either PROSPECT-D or PRO model versions. Chl-ab predicted values are slightly higher when using optimised ranges, compared to inversions based on full spectrum, especially over riparian and terrestrial plants and except for *N. lutea* samples. For Car estimation, the situation is generally inverted, i.e. the use of optimised ranges as input tends to produce lower values compared to the full spectrum, with a few samples (mainly the FL group and some *N. nucifera* leaves) overcoming  $20 \mu\text{g cm}^{-2}$ . The differences in inversion performance between the two input strategies, are more marked for structural parameters, and in particular for EWT - which is consistently estimated to be higher with full spectrum input, especially for leaves in the FL group due to the patterns shown for  $N_{\text{struct}}$  (Fig. 4, fifth column).

Compared to the limited output variability shown in Fig. 3, the notable discrepancy in PROSPECT inversion results shown in Fig. 4 shows that inversion performance is generally more influenced by the choice of input spectral range than by the model version used, which is expected based on the relative similarity of PROSPECT-D and -PRO over most leaf parameters (Féret et al., 2017; Féret et al., 2021).

Looking at individual leaf parameters, PROSPECT results appear to be more sensitive to inversion settings over LMA (especially for samples in the FL and RR groups) and carotenoids, which have a minor impact on model results compared to EWT and chlorophylls in the SWIR and VNIR regions, respectively (Féret et al., 2019; Sun et al., 2019). The estimation of chlorophylls is instead quite consistent with changing settings.

### 3.2. Performance of leaf parameter estimation along a water affinity gradient

Comparing the performance of PROSPECT inversions in estimated parameters against the actual leaf measurements in the Mantua water affinity dataset (Table 1), using the best settings for the PRO (FS-Nest) and D (OPT-Nprior) versions, we observed that PROSPECT-PRO inverted over the full spectrum generally provides better results for leaf pigments, on average - i.e. lower RMSE ( $12.8\text{--}15.8 \mu\text{g cm}^{-2}$  for Chl-ab,  $3.8\text{--}8.3 \mu\text{g cm}^{-2}$  for Car, and  $1.35\text{--}1.43$  for Chl/Car) and nRMSE (22–41%, 36–105%, 45–58%, respectively), but the overall level of precision is subpar ( $R^2$  never exceeds 0.5). Inversions of PROSPECT-D based on optimised spectral ranges score more precise estimates for

Chl-ab ( $R^2 > 0.6$ ) for both hydrophytes and terrestrial species, with a tendency towards overestimation (Bias in the range  $14.6\text{--}16.7 \mu\text{g cm}^{-2}$ ). For leaf structural parameters the situation is mixed: EWT and LDMC are better predicted on average by inverting PROSPECT-D from optimised spectral ranges, with RMSE of  $32.6\text{--}78.5 \text{ g m}^{-2}$  for EWT (nRMSE = 19–24%), and  $0.09\text{--}0.12 \text{ g g}^{-1}$  for LDMC (nRMSE = 22–56%), while LMA estimates are more accurate using PROSPECT-PRO based on the full spectrum, with RMSE of  $13.9\text{--}22.7 \text{ g m}^{-2}$  (nRMSE = 22–29%).

Accuracy figures in general, i.e. pooling all groups in the Mantua water affinity dataset, are in line or slightly lower than those recently reported by Spafford et al. (2021) and Wang et al. (2023), remarking that pigments estimation tend to be less sensitive to input spectral range, while structural trait estimates are improved by using specifically selected spectral domains, especially for EWT.

Along the water affinity gradient, the error of Chl-ab and Car estimates from PROSPECT inversions tends to be slightly lower for terrestrial species than for hydrophytes. Both EWT and LMA estimates are less biased for terrestrial species (TV group) than for true hydrophytes (FL and ER groups), with riparian plants (H and RR groups) occupying the middle ground between the extremes. This is consistent with the ecological setting of the latter group, which typically occupies the transitional zone in wetland ecotones (Lamb and Mallik, 2003; Klančnik and Gaberšček, 2016), as reflected in the water affinity gradient we investigated.

The suboptimal performance in terms of precision of PROSPECT-PRO in estimating LMA, particularly when using the full spectrum ( $R^2 = 0.36$ ; Fig. 5), is mainly due to the mis-estimation of protein content ( $R^2 < 0.4$ ). This is particularly evident for hydrophytes, but it also affects other species groups, while CBC estimation is relatively reliable ( $R^2 = 0.51$ ) when using optimised ranges. Indeed, Wang et al. (2015) have already highlighted that proteins are estimated with lower accuracy compared to other LMA components (lignin and cellulose) in fresh leaf samples of terrestrial species. Reinforcing what Wang et al. (2023) recently highlighted, the feasibility of assessing nitrogen content and C/N balance based on PROSPECT-PRO inversions - demonstrated over a limited sample of terrestrial species (Féret et al., 2021) - need to be further investigated for their transferability to a wider range of plant species, especially hydrophytes. Within our dataset, hydrophytes do indeed have a significantly different C:N balance ( $p < 0.05$ ) compared to riparian and terrestrial plants, linked to a higher LNC ( $p < 0.05$ ), which may be due to the peculiar composition of proteins and amino acids in aquatic plants (Boyd, 1970). A possible explanation for this pattern is that for terrestrial vegetation (with leaves developing in the air), the reflectance-transmittance relationship is mediated by the structure of the mesophylls (Jacquemoud and Ustin, 2019), whereas this may not be

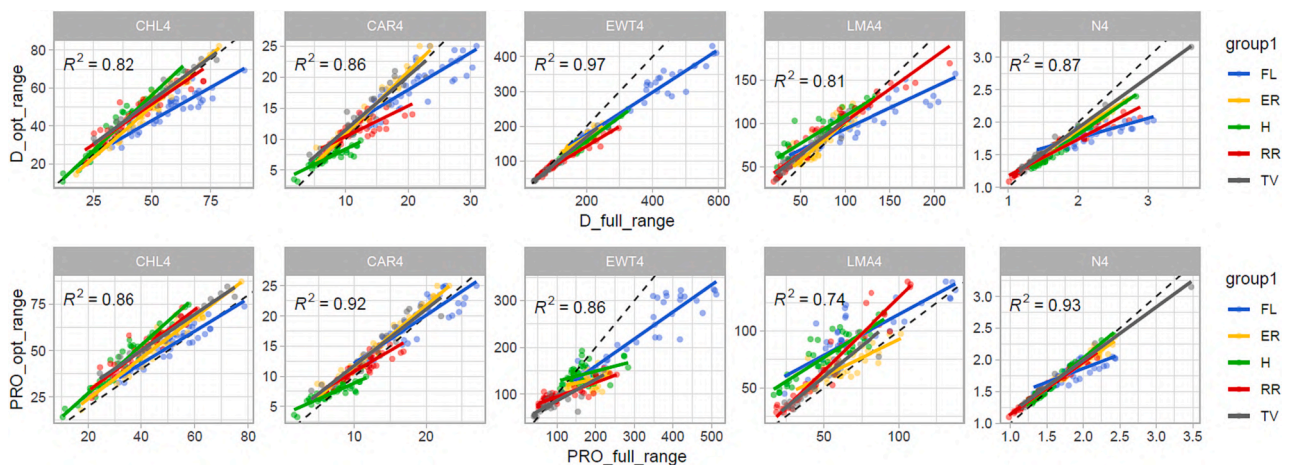
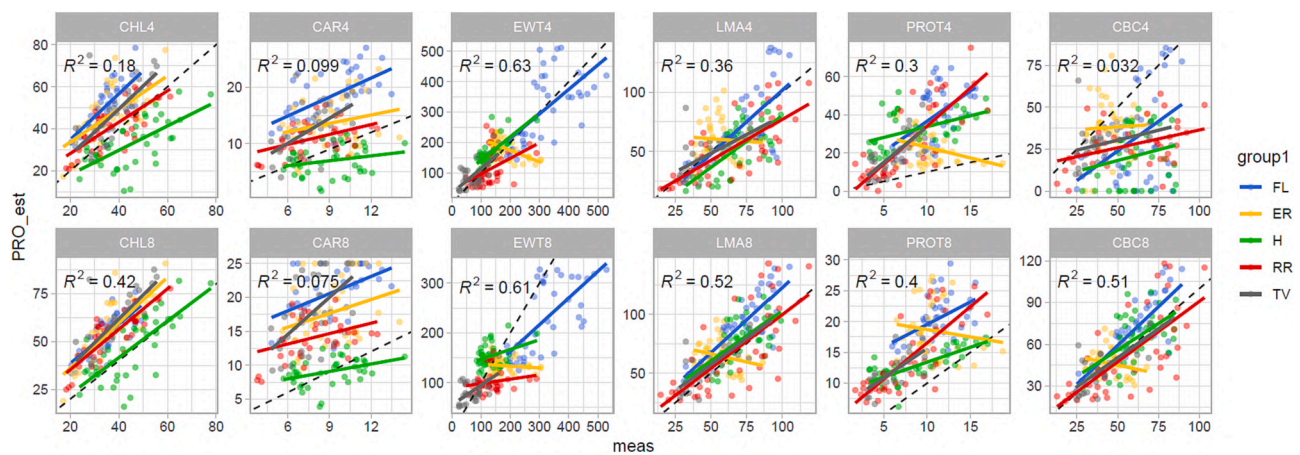


Fig. 4. Comparison of leaf parameters estimated from the inversion of two PROSPECT versions with the full spectrum or optimised spectral ranges as input: row 1: version D (R-FS-Nest vs. R-OPT-Nprior); row 2: version PRO (R-FS-Nest vs. R-OPT-Nprior).

**Table 1**

Performance (precision and accuracy metrics) of PROSPECT inversion in modelling selected leaf parameters for different macro-groups in the Mantua water affinity dataset, expressed as Bias, RMSE, nRMSE,  $R^2$ . Results from PROSPECT-PRO (FS-Nest) and from PROSPECT-D (OPT-Nprior) are compared (best performing in bold)

		PROSPECT-PRO (FS-Nest)				PROSPECT-D (OPT-Nprior)			
		Bias	RMSE	nRMSE	$R^2$	Bias	RMSE	nRMSE	$R^2$
Hydrophytes (FL + ER)	Chl-ab ( $\mu\text{g cm}^{-2}$ )	13.1	<b>15.8</b>	<b>0.38</b>	0.44	14.6	16.4	0.39	<b>0.65</b>
	Car ( $\mu\text{g cm}^{-2}$ )	6.9	<b>8.3</b>	<b>0.90</b>	0.13	9.8	10.5	1.14	<b>0.15</b>
	Chl/Car	-0.98	<b>1.43</b>	<b>0.45</b>	0.14	-1.41	1.68	0.52	<b>0.24</b>
	EWT ( $\text{g m}^{-2}$ )	-5.5	81.7	0.20	0.47	-30.0	<b>78.5</b>	<b>0.19</b>	<b>0.49</b>
	LMA ( $\text{g m}^{-2}$ )	2.4	<b>21.5</b>	<b>0.29</b>	<b>0.28</b>	21.0	30.0	0.41	<b>0.28</b>
	LDMC ( $\text{g g}^{-1}$ )	0.01	<b>0.04</b>	<b>0.23</b>	<b>0.45</b>	0.07	0.09	0.56	0.13
Riparian plants (H + RR)	Chl-ab ( $\mu\text{g cm}^{-2}$ )	-4.3	12.8	0.22	0.25	4.2	<b>12.3</b>	<b>0.21</b>	<b>0.43</b>
	Car ( $\mu\text{g cm}^{-2}$ )	0.5	<b>3.8</b>	<b>0.36</b>	<b>0.01</b>	3.0	5.0	0.47	0.01
	Chl/Car	-0.81	<b>1.39</b>	<b>0.52</b>	0.13	-1.06	1.54	0.58	<b>0.30</b>
	EWT ( $\text{g m}^{-2}$ )	8.3	52.3	0.22	<b>0.24</b>	-5.0	<b>47.3</b>	<b>0.19</b>	0.22
	LMA ( $\text{g m}^{-2}$ )	-13.8	<b>22.7</b>	<b>0.22</b>	0.49	15.8	23.5	0.23	<b>0.65</b>
	LDMC ( $\text{g g}^{-1}$ )	-0.06	<b>0.10</b>	<b>0.22</b>	0.21	0.06	<b>0.10</b>	<b>0.22</b>	<b>0.28</b>
Terrestrial plants (TV)	Chl-ab ( $\mu\text{g cm}^{-2}$ )	9.3	<b>13.6</b>	<b>0.41</b>	0.49	16.7	18.9	0.57	<b>0.61</b>
	Car ( $\mu\text{g cm}^{-2}$ )	4.9	<b>6.2</b>	<b>1.05</b>	0.27	9.7	10.8	1.84	<b>0.29</b>
	Chl/Car	-1.05	<b>1.35</b>	<b>0.58</b>	0.01	-1.76	1.94	0.83	<b>0.03</b>
	EWT ( $\text{g m}^{-2}$ )	19.0	38.3	0.28	0.45	13.6	<b>32.6</b>	<b>0.24</b>	<b>0.48</b>
	LMA ( $\text{g m}^{-2}$ )	-0.7	<b>13.9</b>	<b>0.22</b>	0.43	19.4	23.0	0.36	<b>0.57</b>
	LDMC ( $\text{g g}^{-1}$ )	-0.07	0.14	0.25	0.34	0.03	<b>0.12</b>	<b>0.22</b>	<b>0.44</b>



**Fig. 5.** Comparison of leaf parameters measured over the Mantua water affinity dataset with those estimated from the inversion of PROSPECT-PRO with two different settings: row 1 (suffix 4): FS-Nest; row 2 (suffix 8): OPT-Nprior.

the case for aquatic plants, i.e. when leaves develop in water or at the air-water interface. This affects the validity of the Beer-Lambert law assumptions underlying PROSPECT and significantly biases the model inversion performance.

In terms of group-specific characteristics, the plots in Fig. 5 highlight that samples from the ER group are the most challenging for the estimation of structural characteristics (EWT, LMA) from the PROSPECT inversion. Intra-group differences in emergent hydrophytes result in contrasting patterns for the inversion performance of leaf structural parameters, i.e. LMA and its components, which are mainly driven by the negative correlation between leaf thickness and  $N_{\text{struct}}$  in *N. nucifera* samples, possibly related to the relevant effect of leaf age and turnover - not assessed in our dataset - on lotus traits (Tsuchiya, 1991).

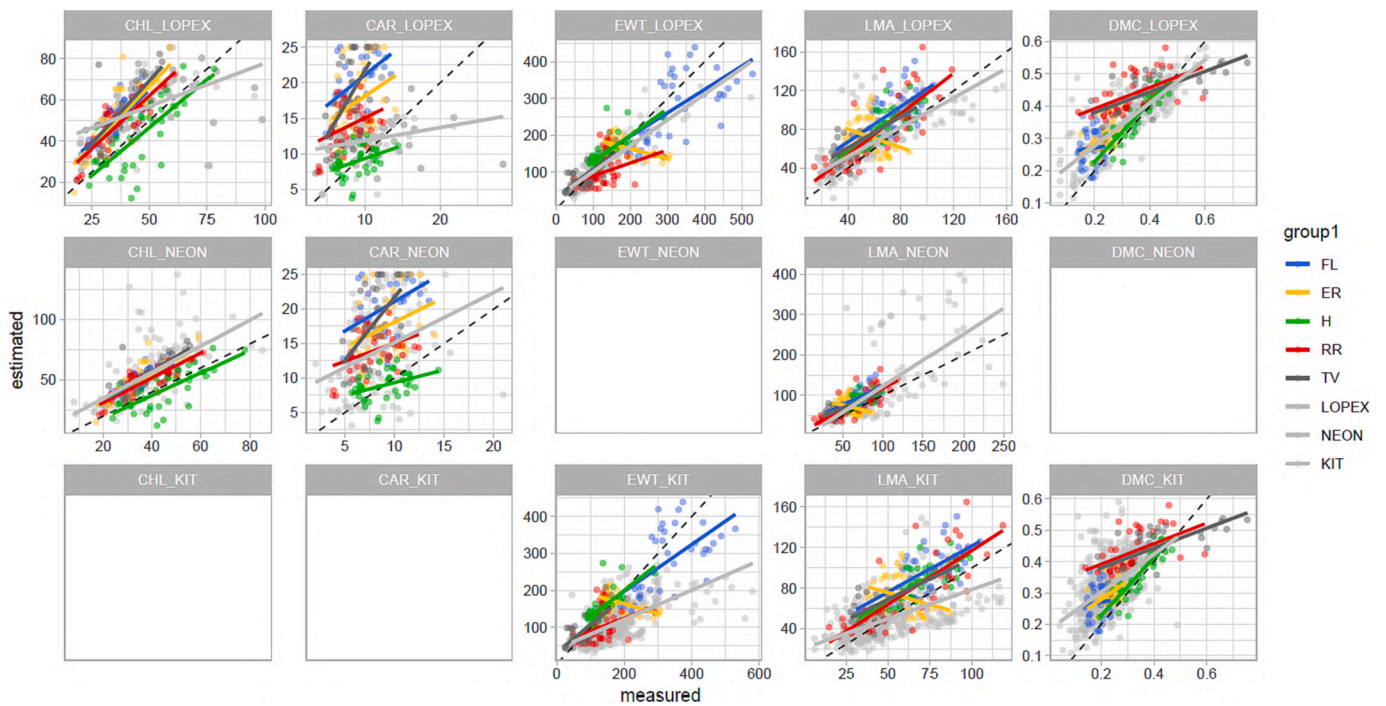
### 3.3. Comparison of PROSPECT inversion performance on different datasets

The scatter plots in Fig. 6 show that performance of PROSPECT-D inversions, highlighted for the Mantua water affinity dataset, is generally in line with other homologous datasets - covering different plant groups and consisting almost entirely of terrestrial species (light grey dots in Fig. 6 plots).

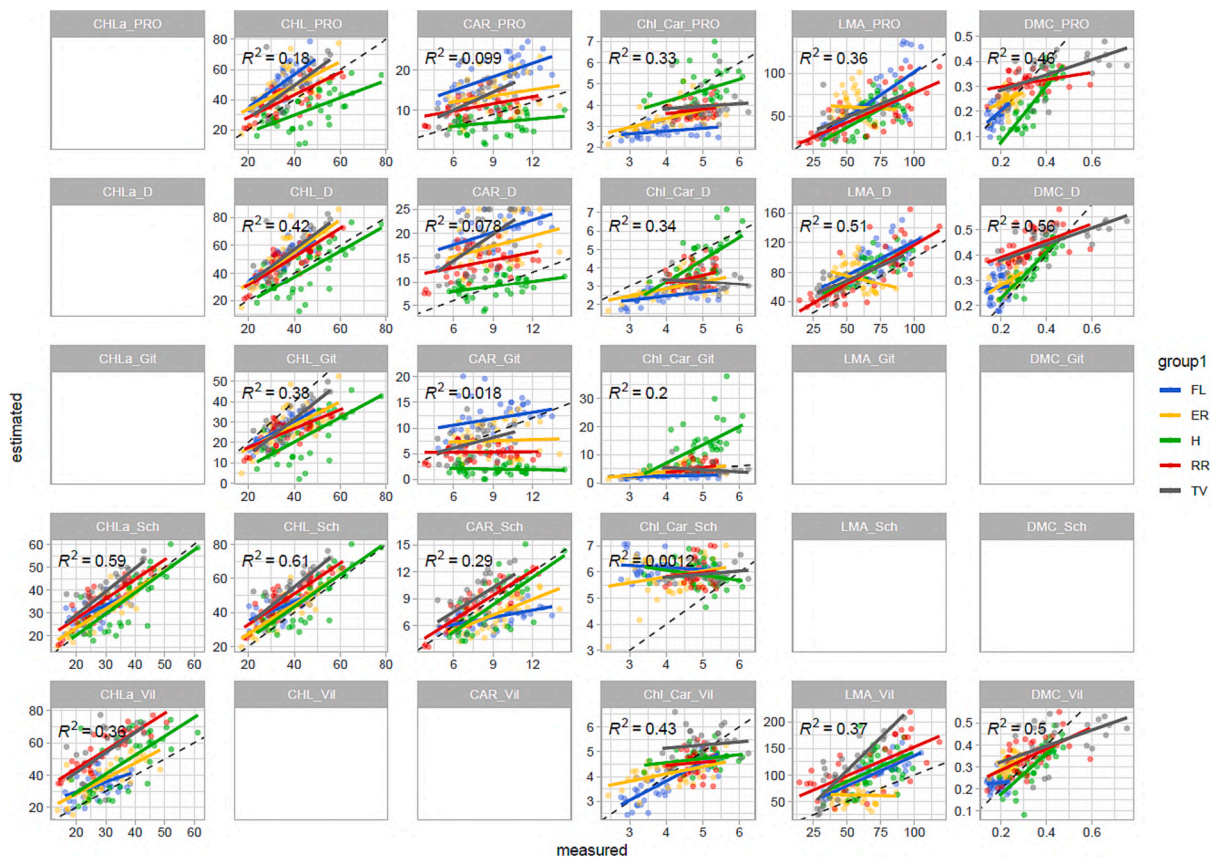
Notable differences with the LOPEX dataset are shown for

carotenoids, which appear to be sensibly overestimated in the Mantua water affinity dataset, especially for most hydrophyte samples (FL and ER groups). PROSPECT-D estimates of pigments over the NEON dataset appear to be similarly biased, with a systematic overestimation of measured chlorophylls, even more pronounced for carotenoids; this pattern is noteworthy because pigment content in the NEON dataset was measured by high performance liquid chromatography, which should provide more reliable results than spectrophotometric measurements of leaf extracts used for the Mantua water affinity dataset and LOPEX. The weak performance of PROSPECT in estimating carotenoids across different datasets, where such pigments were derived by different methods, seems to indicate unresolved challenges in separating absorption features of different pigments, even in the latest versions of the model (Wang et al., 2023; Zhang et al., 2022), which are more evident for aquatic plant samples - characterised by higher total carotenoid content than terrestrial species in our dataset ( $p < 0.05$ ).

PROSPECT-D inversion comparisons for structural parameters tend to diverge from measured data in the IFGG/KIT dataset, which consists exclusively of grassland species, showing a trend towards underestimation of both EWT and LMA at higher values. The overall consistency of PROSPECT-based estimates compared to the three external datasets tested - which include different methods for deriving leaf parameters and spectra and have already been recognised in previous literature



**Fig. 6.** Comparison of leaf parameters estimated from the inversion of PROSPECT-D (setting: OPT-Nprior) and those measured on leaf samples in our dataset, showing the same comparison (measured vs. modelled parameters) over other leaf datasets available online, shown as light grey dots on the background: row 1 LOPEX; row 2 NEON; row 3 IFGG/KIT.



**Fig. 7.** Comparison of leaf parameters measured on the Mantua water affinity dataset with those estimated using different approaches: inversion of PROSPECT-PRO (settings: FS-Nest) in row 1, and of PROSPECT-D (settings: OPT-Nprior) in row 2, LRM models from Gitelson et al. (2006) in row 3, PLSR models of Schweiger et al. (2018) in row 4, and Villa et al. (2021) in row 5.

(Féret et al., 2021; Kattenborn et al., 2019; Wang et al., 2022) - demonstrates the substantial reliability of Mantua water affinity gradient data for quantitative assessment of model performance (especially for terrestrial plant leaves).

### 3.4. Performance of leaf parameter estimation with different methods

Among the compared methods presented in Section 2.2.4, the best performance on the Mantua water affinity dataset for estimating leaf pigment content (Fig. 7, Table 2) is achieved by the PLSR models calibrated by Schweiger et al. (2018) for Chl-a (nRMSE = 15%), Chl-ab (nRMSE = 19%) and Car (nRMSE = 21%), although a clear underestimation of carotenoids is highlighted over hydrophyte samples (FL and ER groups, Bias =  $-2.0 \mu\text{g cm}^{-2}$ ). Pigments balance is instead better estimated by the PLSR model developed by Villa et al. (2021), with nRMSE = 17% for Chl/Car. Concerning structural parameters, the best performance is achieved by PROSPECT for LMA (nRMSE = 20% and  $R^2 = 0.36$  for PRO version based on full range, nRMSE = 25% and  $R^2 = 0.51$  for D version based on optimised ranges) and by the PLSR model from Villa et al. (2021) for LDMC (nRMSE = 14%).

Looking at the results disaggregated by the three water affinity macro-groups, the best estimates of chlorophyll content are given by PLSR of Schweiger et al. (2018) for hydrophytes (nRMSE = 22%) and riparian plants (nRMSE = 18%), and by LRM of Gitelson et al. (2006) for terrestrial species (nRMSE = 29%), with PLSR of Villa et al. (2021) (nRMSE = 30% on hydrophytes) or PROSPECT-PRO (nRMSE = 22–41% on riparian and terrestrial species) as the second best methods. On the other hand, the PLSR model calibrated by Villa et al. (2021), although based only on floating and emergent aquatic species, is the best option for direct estimation of the Chl/Car ratio for all the groups covered in our data (nRMSE < 35%), with other methods having more than twice the error (nRMSE > 45%). In any case, the error of Chl/Car estimation with PLSR from Villa et al. (2021) increases when moving from hydrophytes (nRMSE = 18%) to terrestrial plants (nRMSE = 35%).

Among the structural parameters, the best results in estimating LMA and LDMC in our dataset are overall achieved by PROSPECT, although when restricted to hydrophytes and riparian plants (i.e. excluding the TV group), the performance of Villa et al. (2021) PLSR for LDMC is comparable or slightly better. Although LMA is better estimated by PROSPECT-PRO across all groups (nRMSE = 20%), errors tend to increase from terrestrial (RMSE =  $13.9 \text{ g m}^{-2}$  or 22%) to hydrophyte species (RMSE =  $21.5 \text{ g m}^{-2}$  or 29%).

In the latter group, the performance of the PLSR model specifically calibrated for floating and emergent aquatic plants (Villa et al., 2021) is comparably accurate to that of PROSPECT-D (RMSE =  $30.3 \text{ g m}^{-2}$ ) and tend to be more precise than PROSPECT-PRO ( $R^2 = 0.48$ ), mainly due to a systematic overestimation bias of  $19.8 \text{ g m}^{-2}$ .

These results confirm the superior flexibility of PLSR models in estimating leaf traits from reflectance, provided they are trained on representative, high-quality data (Kothari et al., 2023). PLSR models implemented by Schweiger et al. (2018) and Villa et al. (2021) achieve lower estimation errors for pigment content compared to PROSPECT, even more so for aquatic plant leaves (FL and ER groups). In particular, PLSR achieves good results for the direct estimation of the Chl/Car ratio (Song and Wang, 2022), possibly because the mutual correlation of both pigment groups and overlapping light absorption bands are handled more effectively in this way than in the simplified PROSPECT model formulation (Shiklomanov et al., 2016; Zhang et al., 2022). On the other hand, the good reliability of the PROSPECT inversion for the estimation of structural parameters (LMA and LDMC) generally confirms the recent findings of Féret et al. (2021), Spafford et al. (2021) and Wang et al. (2023) on LMA, although it should be noted that the PLSR model calibrated by Villa et al. (2021) can provide a slight performance advantage in terms of precision on FL and ER groups. As improved LMA estimation has been achieved by some authors by including in PROSPECT additional terms that account for surface and internal leaf structure (Li et al.,

2023; Qiu et al., 2018; Wan et al., 2021), testing these refined versions would be an interesting direction for further investigation of model performance on aquatic plants.

### 3.5. Differences between measured and modelled leaf spectra from PROSPECT

The RMSE between measured leaf reflectance and that modelled from PROSPECT-D (settings: OPT-Nprior) differs among plant groups, and oscillates within 1–6% range in the VNIR and 1–5% range in the SWIR (Fig. 8, row 1).

With the exclusion of helophytes (group H) in the VNIR and a localised peak for hydrophytes (groups FL, ER) in the red edge region (690–720 nm), the modelled reflectance with PROSPECT-D has an absolute RMSE < 0.05 over the whole spectrum. The high RMSE of the H group in VNIR reflectance (Fig. 8, row 1, left panel) is caused by the anomalous underestimation of modelled leaf reflectance in *C. riparia* samples, which could be due to biomineral encrustations (silica and calcium) on the leaf of this graminoid (Klančnik et al., 2014b), increasing surface light scattering (Li et al., 2023). Averaged across the Mantua water affinity dataset, the RMSE of leaf reflectance is approximately 1–4% for wavelengths below 800 nm and 1–3% for wavelengths above 800 nm.

Apart from the localised peaks mentioned, such modelling errors are in line with previous work with similar comparisons, i.e. 1–5% and 1–3% for wavelengths below and above 800 nm respectively, using different datasets and older versions of PROSPECT (Féret et al., 2008); 1–2% using the ANGERS dataset and other data for wavelengths below 800 nm (Féret et al., 2017); 1–3% using the LOPEX dataset for wavelengths above 800 nm (Wang et al., 2015), 1–3% using various datasets and wavelengths above 1000 nm (Féret et al., 2021). This indicates that our measurement setup, including a specularly correction factor (Li et al., 2019) applied to BRF measurements, resulted in leaf reflectance spectra that were not substantially biased compared to what is expected from DHRF measurements, which are typically assumed in PROSPECT models. The reliability of the leaf reflectance data in the Mantua water affinity dataset is further supported by the evaluation of differences (in absolute and relative terms) between measured and modelled spectra compared to homologous differences derived from the CABO dataset (Kothari et al., 2022) - the largest and most comprehensive (over 1900 samples from 105 species, including *P. australis*) publicly available dataset of leaf optical properties to date. The results of this evaluation showed that the reflectance modelling bias for our dataset is not substantially different from that of the CABO dataset (using DHRF measurements) averaged over all samples (Suppl. Fig. S9, row 1). Considering only *P. australis* samples, a helophyte species common to both datasets, the reflectance measured and modelled by PROSPECT tends to differ more in CABO (as DHRF) than Mantua (as corrected BRF) data in the visible region (below 750 nm) and vice versa in the NIR region (750–1100 nm) - with the bias in our data averaging +2–3% and –1–2% for wavelengths below and above 1000 nm, respectively (Suppl. Fig. S9, row 2). The relative RMSE of the modelled leaf reflectance (Fig. 8, row 2) clearly shows that the PROSPECT modelling error in the VNIR tends to decrease along the water affinity gradient, i.e. from floating hydrophytes to riparian species (relative RMSE decreases in this sequence: FL-ER-H-RR), with localised peaks (relative RMSE > 50%) particularly visible for hydrophytes (FL, ER groups). The positions of these peaks - i.e. around 510–520 nm (related to carotenoids and/or anthocyanins), 690–710 nm (related to chlorophyll-a) and 620–630 nm (possibly related to accessory pigments such as chlorophyll-b or anthocyanins) - again point to possible inconsistencies not fully resolved in PROSPECT regarding pigment mixture and absorption overlap (Shiklomanov et al., 2016; Zhang et al., 2022), which become deeper with increasing water affinity. On the other hand, hydrophytes in our dataset do not show significant differences in terms of RMSE of PROSPECT-modelled reflectance compared to riparian plants (H, RR groups) in

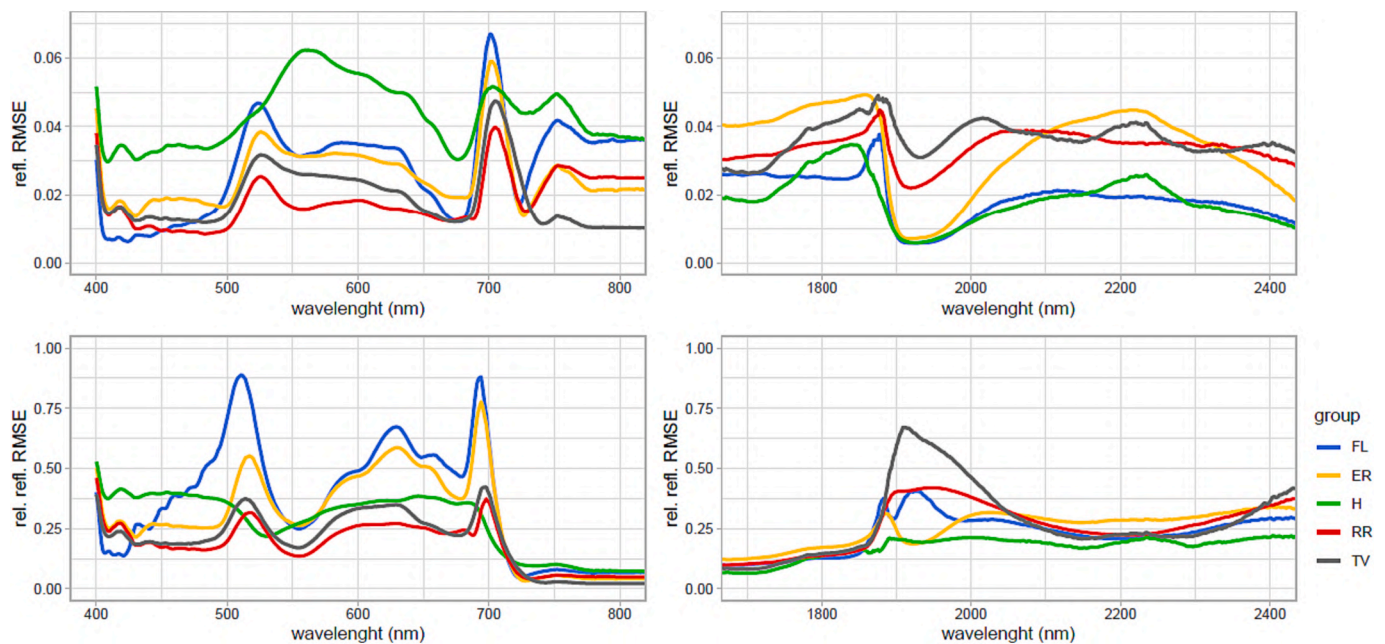


**Table 2**

Performance (precision and accuracy metrics) of different methods in modelling a subset of leaf parameters (LMA, LDMC, Chl-a/ab, Chl/Car) for different macro-groups in the Mantua water affinity dataset, expressed as Bias, RMSE, nRMSE, R<sup>2</sup>. The results of five methods are compared: PROSPECT-PRO (FS-Nest), PROSPECT-D (OPT-Nprior), LRM models of Gitelson et al. (2006), PLSR models of Schweiger et al. (2018), and PLSR models of Villa et al. (2021) (best performing in bold).

		PROSPECT-PRO (FS-Nest)				PROSPECT-D (OPT-Nprior)				LRM_Git (Gitelson et al., 2006)				PLSR_Sch (Schweiger et al., 2018)				PLSR_Vil (Villa et al., 2021)			
		Bias	RMSE	nRMSE	R <sup>2</sup>	Bias	RMSE	nRMSE	R <sup>2</sup>	Bias	RMSE	nRMSE	R <sup>2</sup>	Bias	RMSE	nRMSE	R <sup>2</sup>	Bias	RMSE	nRMSE	R <sup>2</sup>
Hydrophytes (FL+ER)	LMA (g m <sup>-2</sup> )	2.4	<b>21.5</b>	<b>0.29</b>	0.28	21.0	30.0	0.41	0.28									19.8	30.3	0.41	<b>0.48</b>
	LDMC (g g <sup>-1</sup> )	0.01	<b>0.04</b>	<b>0.23</b>	<b>0.45</b>	0.07	0.09	0.56	0.13									0.06	0.07	0.45	0.39
	Chl (µg cm <sup>-2</sup> )*	13.1	15.8	0.38	0.44	14.6	16.4	0.39	<b>0.65</b>	-8.8	11.2	0.27	0.47	7.0	<b>9.2</b>	<b>0.22</b>	0.61	7.3	10.4	0.30	0.44
	Chl/Car	-0.98	1.43	0.45	0.14	-1.41	1.68	0.52	0.24	-0.72	1.61	0.50	0.18	2.05	2.25	0.70	0.01	-0.04	<b>0.58</b>	<b>0.18</b>	<b>0.45</b>
Riparian pl. (H+RR)	LMA (g m <sup>-2</sup> )	-13.8	<b>22.7</b>	<b>0.22</b>	0.49	15.8	23.5	0.23	<b>0.65</b>								45.0	55.3	0.53	0.37	
	LDMC (g g <sup>-1</sup> )	-0.06	0.10	0.22	0.21	0.06	0.10	0.22	0.28									-0.01	<b>0.07</b>	<b>0.16</b>	<b>0.38</b>
	Chl (µg cm <sup>-2</sup> )*	-4.3	12.8	0.22	0.25	4.2	12.3	0.21	0.43	-16.7	19.2	0.32	0.40	7.1	<b>10.8</b>	<b>0.18</b>	<b>0.63</b>	18.4	21.9	0.46	0.39
	Chl/Car	-0.81	1.39	0.52	0.13	-1.06	1.54	0.58	<b>0.30</b>	4.26	7.57	2.83	0.24	1.04	1.25	0.47	0.01	-0.24	<b>0.61</b>	<b>0.23</b>	0.04
Terrestrial plants (TV)	LMA (g m <sup>-2</sup> )	-0.7	<b>13.9</b>	<b>0.22</b>	0.43	19.4	23.0	0.36	0.57								54.9	65.2	1.01	<b>0.75</b>	
	LDMC (g g <sup>-1</sup> )	-0.07	0.14	0.25	0.34	0.03	<b>0.12</b>	<b>0.22</b>	<b>0.44</b>									-0.02	0.13	0.23	0.29
	Chl (µg cm <sup>-2</sup> )*	9.3	13.6	0.41	0.49	16.7	18.9	0.57	0.61	-8.2	<b>9.7</b>	<b>0.29</b>	<b>0.72</b>	14.8	16.3	0.49	0.68	24.0	27.1	1.03	0.33
	Chl/Car	-1.05	1.35	0.58	0.01	-1.76	1.94	0.83	0.03	-0.36	1.90	0.81	<b>0.08</b>	0.91	1.08	0.46	0.07	0.26	<b>0.82</b>	<b>0.35</b>	0.02

\* Total chlorophylls (Chl-ab) for PROSPECT, LRM\_Git, PLSR\_Sch; Chl-a for PLSR\_Vil.



**Fig. 8.** Plant group-specific mean differences in leaf reflectance modelled from PROSPECT-D and measured with a light-equipped contact probe attached to the SR-3500 spectroradiometer over the Mantua water affinity dataset, in terms of RMSE (row 1), and relative RMSE (row 2).

the SWIR region, with relative reflectance difference values generally lower than in the VNIR region, i.e. between 10 and 40% (Fig. 8, row 2, right panel).

At the species level, the differences between PROSPECT-modelled and measured leaf reflectance, shown in Fig. 9, highlight some peculiarities of aquatic plant species. First, the highest overestimation peak in the 510–520 nm region for *T. natans* samples is associated with low carotenoid content and a high Chl/Car ratio. Second, the peak of reflectance overestimation around 600–650 nm could be related to anthocyanin content in floating and emergent plants, especially for species characterised by a reddish abaxial side during some growth stages, such as juvenile *N. nucifera* and mature *T. natans*. Thirdly, the small peak of reflectance difference around 650–660 nm pairs with the different chlorophyll pool balance (high Chl-a/Chl-b ratio) of the floating hydrophyte *T. natans*. Fourth, the spectral variability of the relative reflectance difference in the 1900–2000 nm range - where water absorption is the main driver - for many hydrophytes (*N. nucifera*, *N. lutea*, *T. natans*) suggests that the patterns linking leaf water content and spectra in aquatic plants may be different from those considered in the PROSPECT formulation, which is mainly shaped by the biochemical and structural arrangements of terrestrial plant leaves (Féret et al., 2017). Furthermore, the generally low leaf C/N ratio (10–15) of hydrophyte species (up to the helophyte *P. australis*) - reflecting the typical absence of nitrogen-limiting conditions in mostly eutrophic wetland systems - indicates a different balance of N- and C-based compounds as dry matter components.

This might pose a challenge for PROSPECT to reliably model aquatic plant LMA, especially the PRO version, as it is based on stoichiometric ranges calibrated on terrestrial species (Féret et al., 2021).

Finally, the scatter plots of Fig. 10 comparing two proxies for leaf structural complexity - one measured (thickness) and the other modelled (N mesophyll parameter from PROSPECT-D inversions) - show a strong decoupling of leaf thickness from  $N_{\text{struct}}$  for more water-affine species (*N. lutea*, *N. nucifera*, *T. natans*) characterised by thicker leaves, which is not seen in terrestrial species (in the LOPEX dataset: Fig. 10 right panel). In the wake of observations by other authors on the volume scattering modelling in PROSPECT, which showed different degrees of reliability across species and functional types (Qiu et al., 2018; Féret et al., 2019), such decoupling suggests that the internal leaf structure for hydrophytes

- related to the presence of large void spaces that form the aerenchyma typical of aquatic plants (Große, 1996; Jung et al., 2008) - and peculiarities in cuticle morphology (Maberly and Gontero, 2018) may strongly influence the differentiation of their leaf optical properties from those of most terrestrial plants. Therefore, the simplified modelling of volumetric scattering implemented so far in PROSPECT, which relies on  $N_{\text{struct}}$  as the sole descriptor of internal leaf structure, may lead to significant biases in the estimation of leaf spectral parameters for aquatic plant species.

#### 4. Conclusions

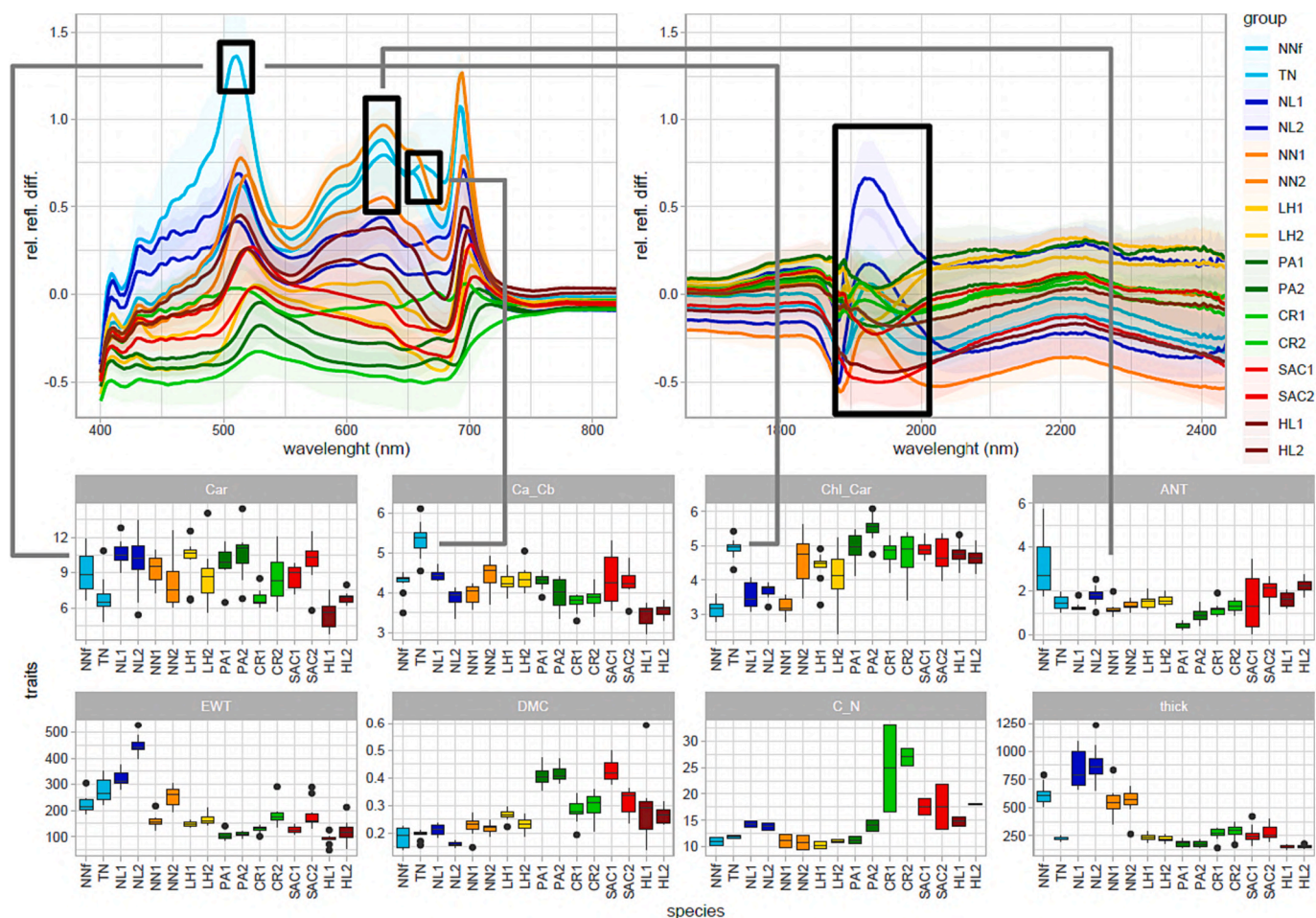
Based on empirical data of leaf reflectance and measured parameters (biochemical and structural), a multidimensional assessment of PROSPECT model capabilities was performed over a water affinity gradient from floating and emergent hydrophytes to terrestrial plants, through helophytes and riparian species.

Overall, PROSPECT results for the estimation of LMA and carotenoids appear to be more sensitive to changing inversion settings compared to chlorophyll estimation, with the choice of input spectral range playing a prominent role in the model version used. Nevertheless, the PRO version showed some inconsistencies in LMA estimation in  $N_{\text{struct}}$  retrieval than the D version.

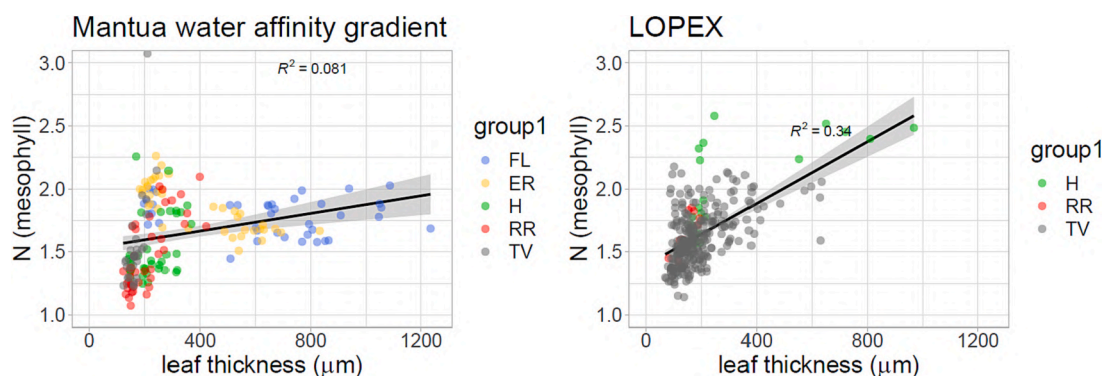
Along the water affinity gradient, PROSPECT-based estimates of most parameters (carotenoids, EWT and LMA) diverge increasingly from measured values from hydrophytes to riparian plants to terrestrial species, while the situation is more uniform across groups for chlorophylls.

As the suboptimal performance of PROSPECT-PRO for LMA ( $R^2 < 0.4$ ) is primarily driven by errors in the estimation of protein content, the capabilities of this latter version for the assessment of nitrogen-based compounds and the carbon-nitrogen balance in leaves need to be further evaluated for transferability to aquatic plants - and possibly to other plant groups that show significant differences from common terrestrial species.

In parallel with the degradation of model inversion performance with water affinity, the error in leaf reflectance simulated with PROSPECT tends to increase from terrestrial to aquatic leaf samples, with localised error peaks for hydrophyte species in the visible spectrum. This pattern points to unresolved modelling issues dealing with pigment



**Fig. 9.** Relative differences in leaf reflectance modelled from PROSPECT-D (settings: R-OPT-Nprior) and measured with a light-equipped contact probe attached to the SR-3500 spectroradiometer over the Mantua water affinity dataset (excluding terrestrial plants), grouped by species and time of sampling (odd entries in the species list are from samples measured in early June, even entries from samples measured in late July). The last row shows the box plots of the main leaf characteristics measured for each species. *Nuphar lutea* (NL), *Trapa natans* (TN), *Nelumbo nucifera* (Nnf, NN); *Ludwigia hexapetala* (LH), *Phragmites australis* (PA), *Carex riparia* (CR); *Salix* spp. (SAC), *Humulus lupulus* (HL).



**Fig. 10.** Comparison between measured leaf thickness and  $N_{struct}$  estimated from the PROSPECT-D inversion (settings: OPT-Nprior) extracted from the Mantua water affinity and LOPEX datasets.

mixture and absorption overlap, which become more apparent with increasing water affinity (and water content). Indeed, more flexible PLSR models can effectively deal with multicollinearity and avoid absorption overlap issues when trained on high quality reference data, outperforming PROSPECT in leaf pigment estimation.

Another source of relevant bias in the model results is the strong decoupling of estimated  $N_{struct}$  from leaf thickness for more water-affine

species, suggesting that peculiarities in the internal leaf structure of hydrophytes strongly deviate from the basic assumptions of PROSPECT.

The reliability of our results is supported by the consistency of the PROSPECT inversion performance across different external datasets and the modelling error of leaf spectra, which is in line with previous work, although based almost exclusively on terrestrial plants.

The overall picture of our findings underscores the need for a

quantitative assessment of the PROSPECT capabilities over leaves with significant structural and biochemical differences (e.g. aquatic plants) compared to those of the terrestrial species that make up the calibration data used to develop the model. Such an assessment is a prerequisite for characterising and improving the performance of remote sensing applications on aquatic and wetland vegetation. In fact, these applications often rely on the upscaling of leaf spectral characteristics to canopy and ecosystem scales using airborne and spaceborne hyperspectral data, based on models that incorporate PROSPECT as a cornerstone, although its assumptions have only been empirically tested for terrestrial vegetation, including crops.

Potential improvements in PROSPECT performance for vegetation other than temperate broadleaved trees and crops, which have been the main source of model calibration to date, e.g. aquatic plants, can be achieved by recalibrating the specific absorption coefficients (especially for secondary pigments) and the refractive index on the basis of extensive and comprehensive leaf datasets that take into account natural heterogeneity in terms of functional groups, growth stages and environmental conditions, as well as by including additional model parameters that account for leaf structural complexity (e.g. mesophyll stratification or presence of aerenchyma).

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## CRediT authorship contribution statement

**Paolo Villa:** Conceptualization, Methodology, Investigation, Formal analysis, Supervision, Writing – original draft. **Alice Dalla Vecchia:** Data curation, Resources, Methodology, Writing – review & editing. **Erika Piaser:** Data curation, Investigation, Writing – review & editing. **Rossano Bolpagni:** Data curation, Resources, Methodology, Supervision, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no competing interests.

## Data availability

The datasets generated and analysed during the current study will be made available on EcoSIS (ecosis.org) upon publication.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.rse.2023.113926>.

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