

Combining spectral and molecular approaches to capture leaf pigment dynamics

Do measurement uncertainties permit the detection leaf pigment dynamics associated with species traits?

Uncertainties associated with field spectral measurements

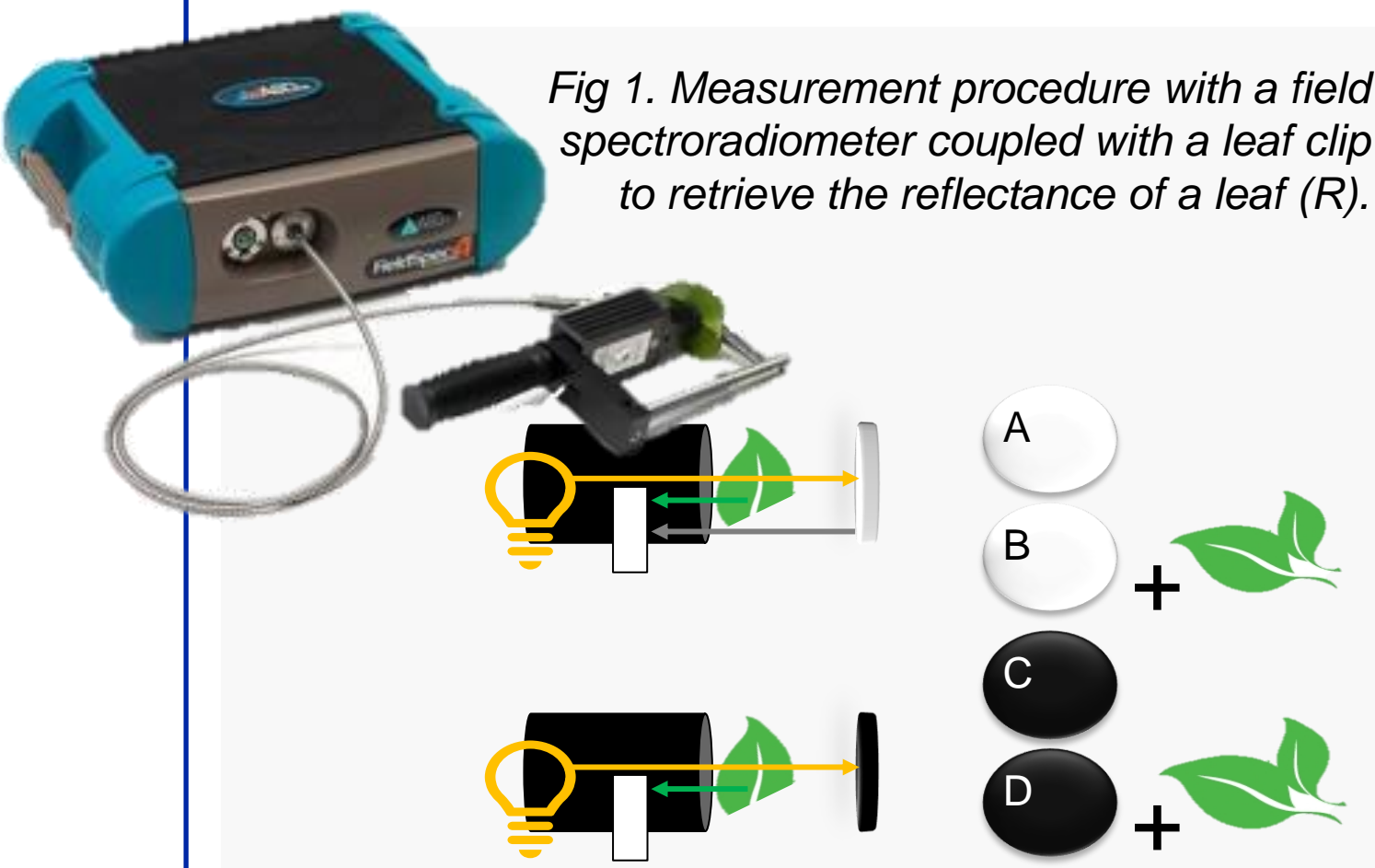
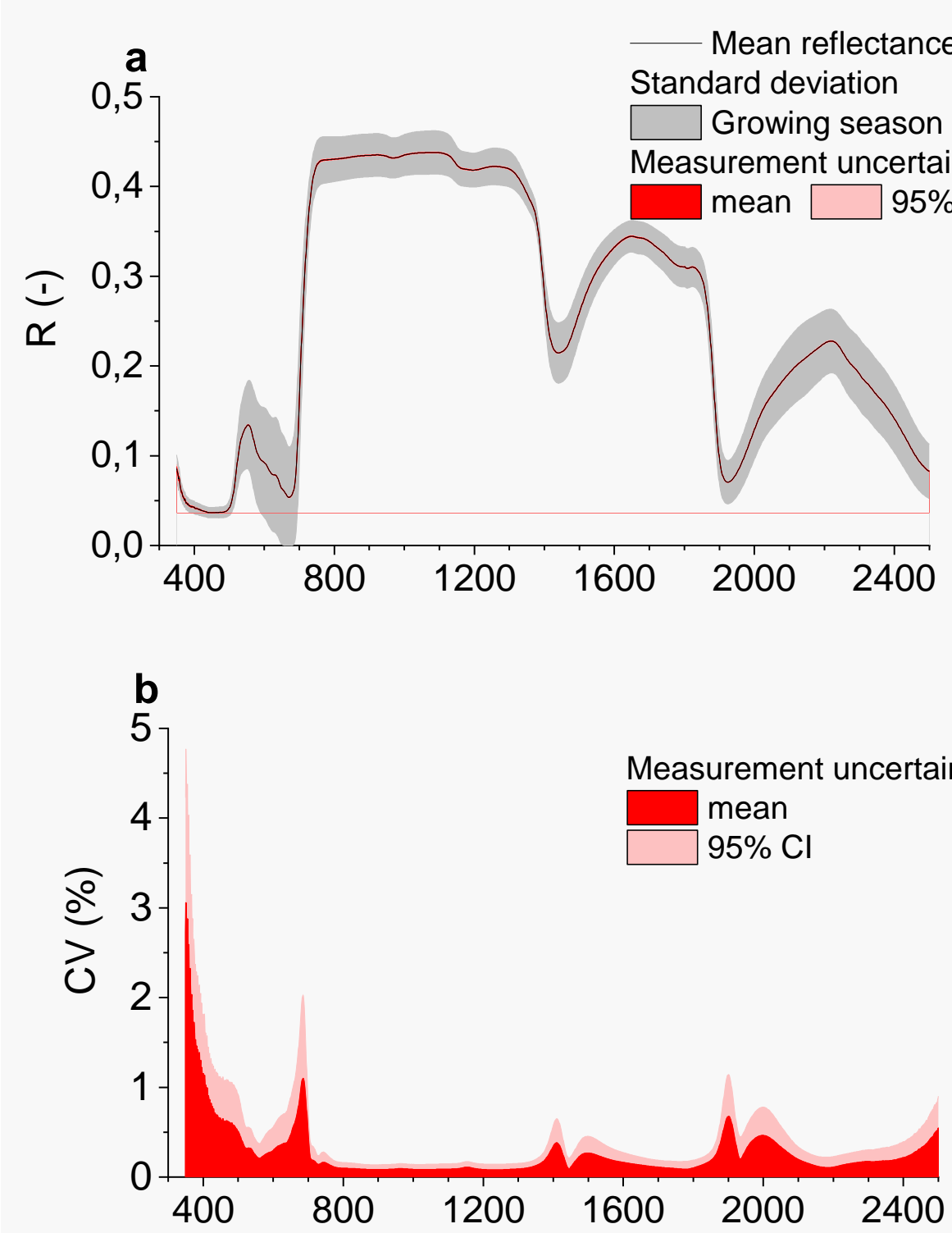


Fig 1. Measurement procedure with a field spectroradiometer coupled with a leaf clip to retrieve the reflectance of a leaf (R).

Leaf reflectance was retrieved from field spectral measurements (Fig. 1) The measurement uncertainty represents 0.3-4% of the leaf reflectance, which corresponds to 2-25% of the spectral variation observed within a tree (*F. sylvatica*) sampled over one growing season (2018) at different sampling heights and light exposition (shade and sun leaves) (Fig. 7a). Maxima of uncertainty occur at the detector limits (low signal-to-noise ratio) and at low reflectance (Fig. 2).

The biological variation capturing pigment dynamics (400-800nm) increased over the growing season, indicating an increasing diversity of spectral features within a tree as leaf mature and senesce (Fig. 3). The biological variation between trees was greater than within a tree, but did not allow for differentiating between sampling sites (Fig. 4). (Petibon et al., 2021)

Fig 2. (a) Mean leaf reflectance, measurement uncertainty, and spectral variation within a tree; (b) relative measurement uncertainty.



Reflectance $R = \frac{A+D-C+B}{A-C}$

Measurement uncertainty ~ propagated uncertainty

$$U_{R,abs}^2 = 100 * \sqrt{\left(\frac{\partial R}{\partial A} \frac{SD_A}{\sqrt{N}}\right)^2 + \left(\frac{\partial R}{\partial B} \frac{SD_B}{\sqrt{N}}\right)^2 + \left(\frac{\partial R}{\partial C} \frac{SD_C}{\sqrt{N}}\right)^2 + \left(\frac{\partial R}{\partial D} \frac{SD_D}{\sqrt{N}}\right)^2}$$

Spectral variation ~ coefficient of variation

$$CV = 100 * \frac{R_{SD}}{R_{MEAN}} (\%)$$

Biological variation = $CV - U_{R,rel}$

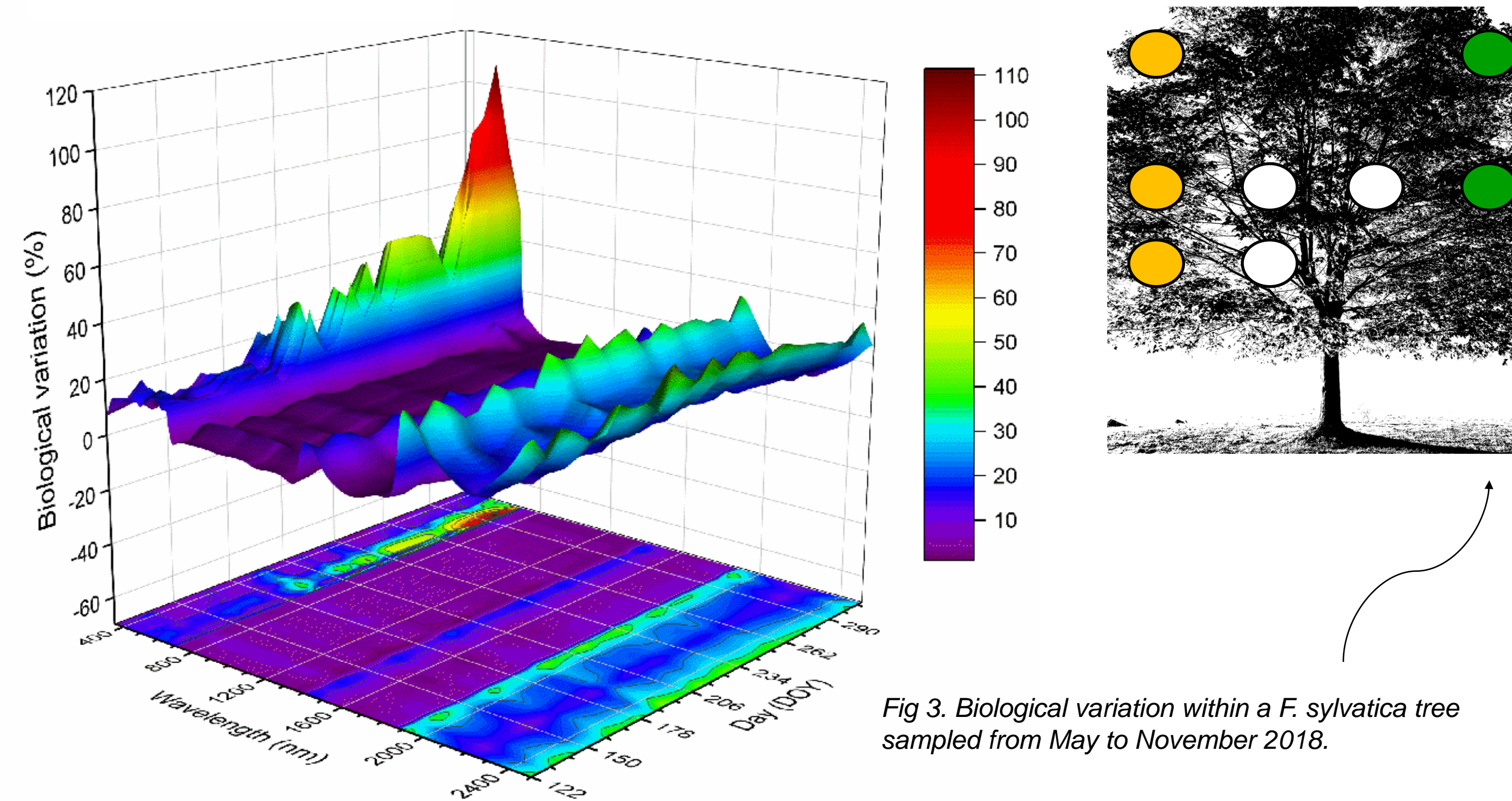


Fig 3. Biological variation within a *F. sylvatica* tree sampled from May to November 2018.

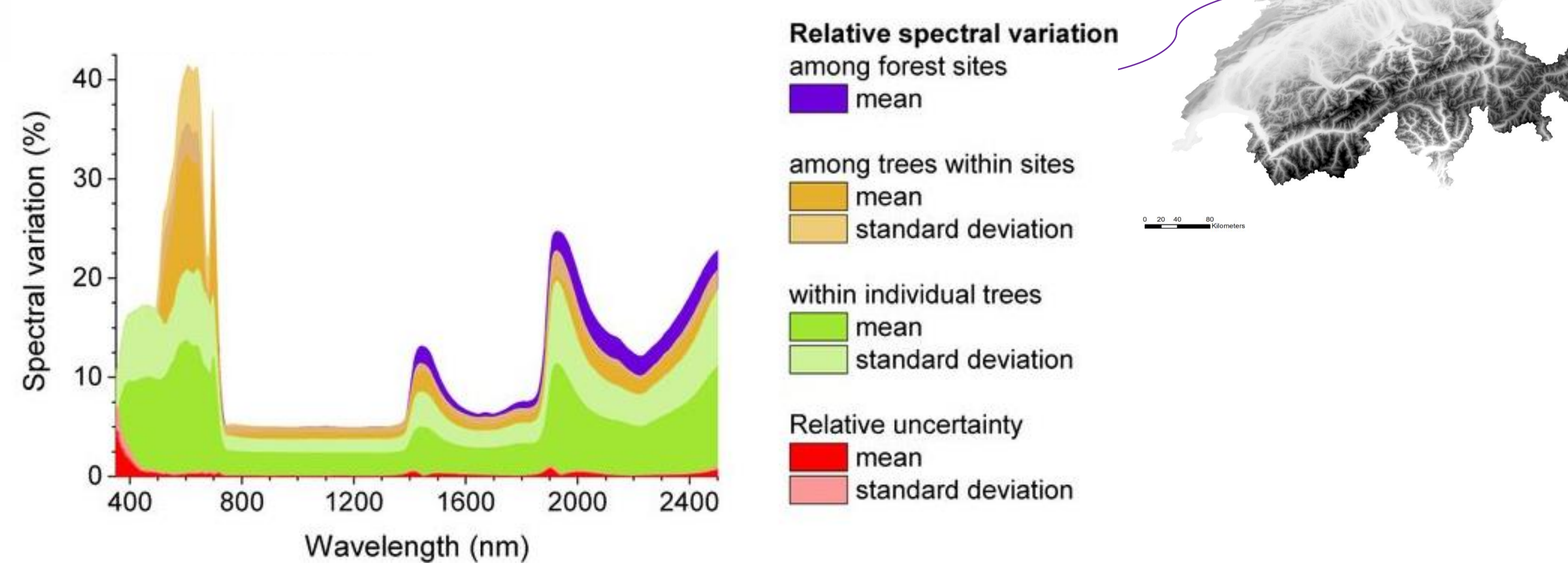


Fig 4. Variation within and between *F. sylvatica* trees sampled in 5 Swiss forest sites on 25-26 of July 2019.

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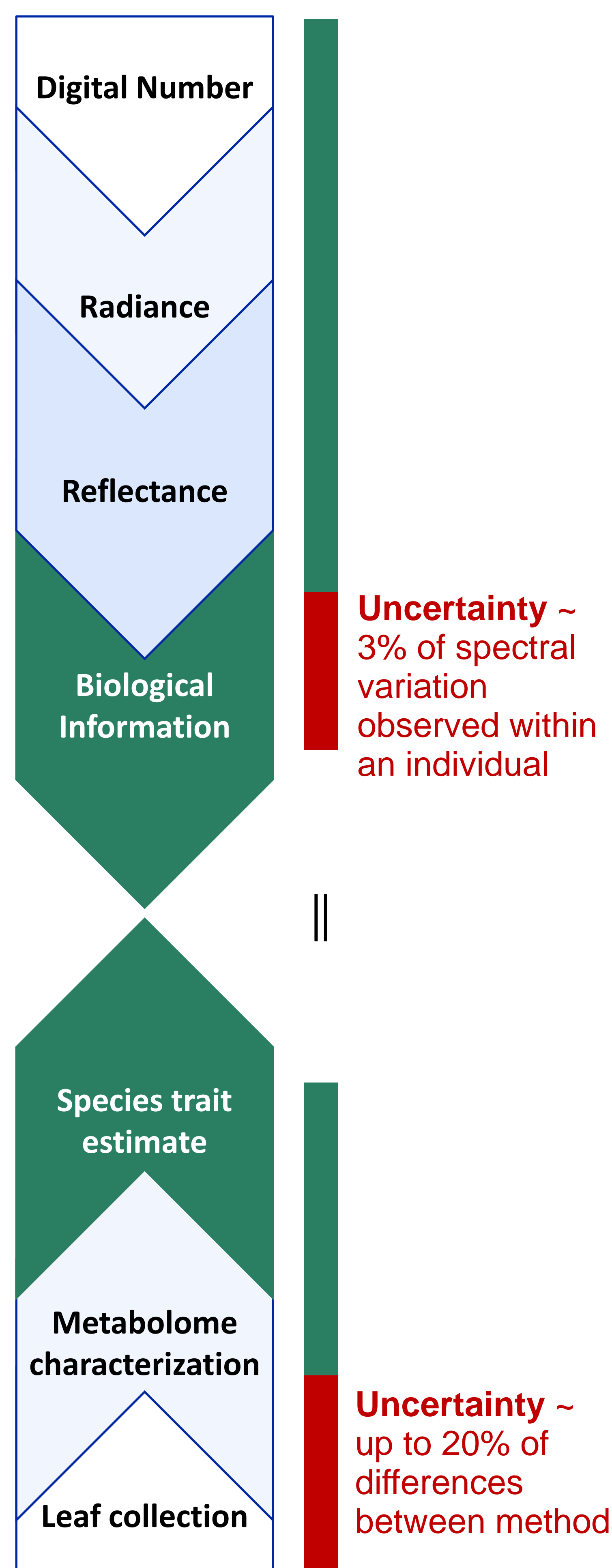
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- Species traits (i.e., within-species variation) are important indicators of biodiversity.
- Field spectral measurements still permit species traits detection despite uncertainties
- Leaf pigments are important indicators of plant performance and acclimation.
- Pigment derivatives better capture species traits than chlorophyll a & b.



Uncertainties associated with leaf metabolome characterization

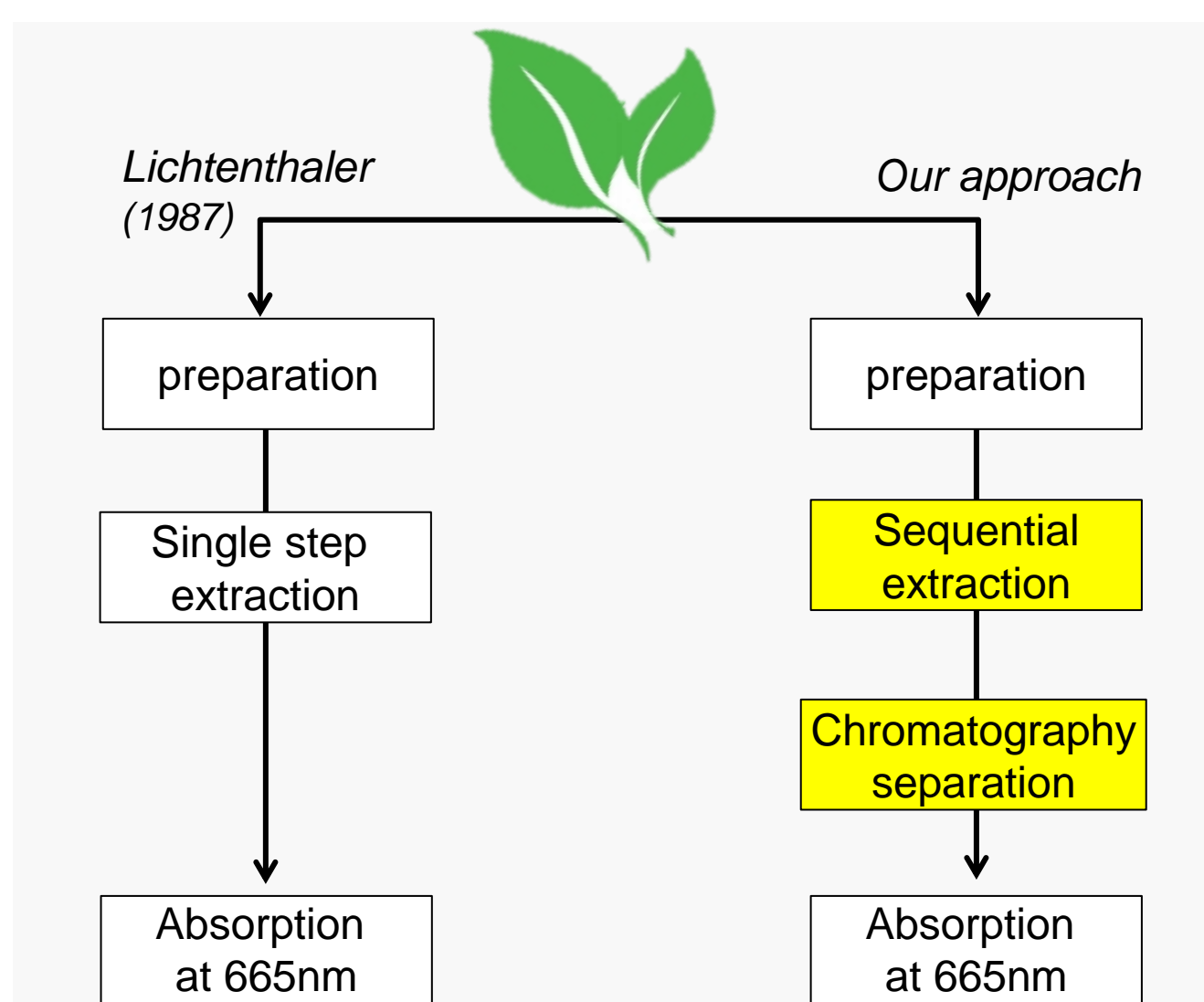


Fig 5. Pigment extraction and characterization procedure

Sequential extraction allows for the extraction of ca. 3 times more pigments than a single step extraction (Fig. 5). Solvent polarity gradient improves the recovery of low abundant apolar pigments, resulting in a better characterization of the entire leaf pigment profile (Fig. 6).

Despite of low abundance, pigment derivatives better capture the biological variation within a tree. The contribution of pigment derivatives to the leaf pigment profile is maximal in spring and autumn (Fig. 7).

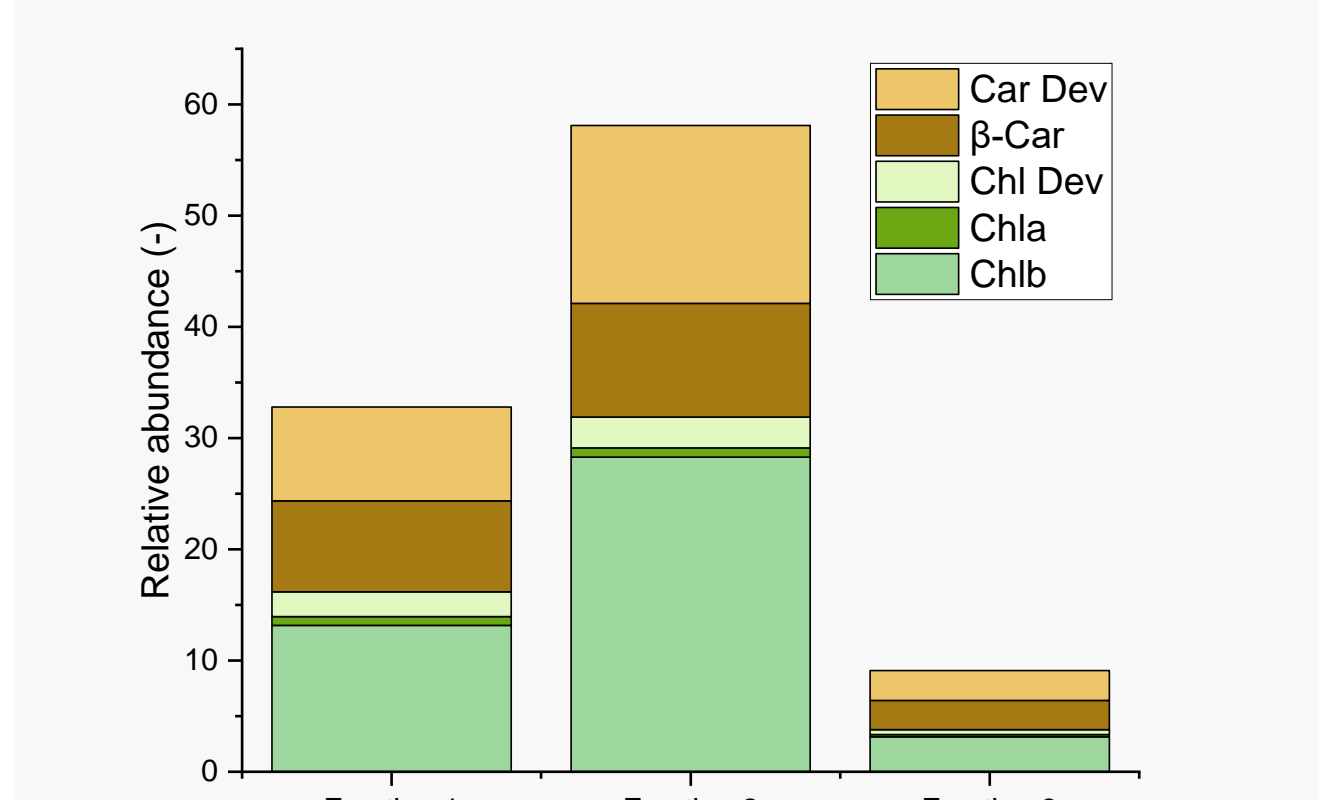


Fig 6. Relative abundance in leaf pigments at each step of the sequential extraction using acetone:water (fraction 1), acetone (fraction 2) and isopropanol:hexane (fraction 3).

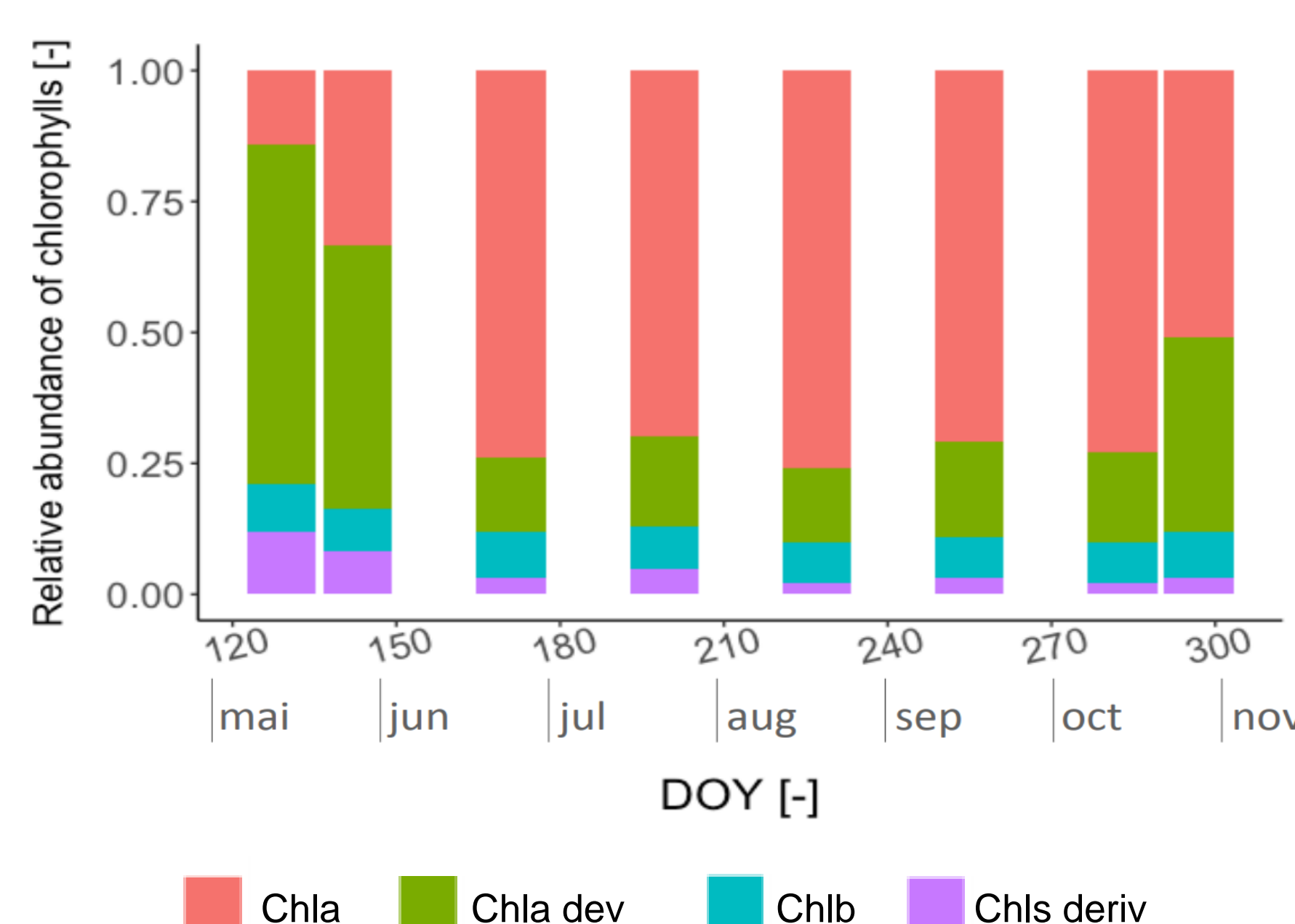


Fig 7. Relative abundance in chlorophylls of sun-exposed leaves of a *F. sylvatica* tree sampled from May to November 2018.

Reference: Petibon et al. (2021) Variation in reflectance spectroscopy of European beech leaves captures phenology and biological hierarchies despite measurement uncertainties, *bioRxiv* 2021.03.09.434578; doi: <https://doi.org/10.1101/2021.03.09.434578>

Lichtenthaler (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in enzymology*, 48, 350-382.