



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

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



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)



- Species traits (i.e., within- \bullet 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



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 abundancy, 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*).

Uncertainty ~ 3% of spectral variation observed within Information an individual

Fig 5.. Pigment extraction and characterization procedure



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.



Species trait

Uncertainty ~ up to 20% of differences between methods

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.