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ADJUSTMENT OF OPTICALLY MEASURED LEAF TRAITS TO PATTERNS OF SOLAR SPECTRAL IRRADIANCE IN PLANT TAXA FROM HIGH ELEVATIONS AND FROM FOREST UNDERSTOREYS

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DOCTORAL DISSERTATION

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ABSTRACT

The attenuation of radiation in forest canopies has been studied in depth within the photosynthetically active radiation (PAR, 400-700 nm), but we are still lacking knowledge on how the spectral composition of ultraviolet radiation (UV-B 280-315 nm, UV-A 315-400 nm) varies. Advances in knowledge on the effects of UV radiation has led to growing interest in its study as a trigger of regulatory responses in plants, rather than as a stressor, which is now considered to be rare in plants growing under natural conditions. Furthermore, a growing number of studies suggest that there are complex interactions in perception, signalling and responses of plants to solar spectral irradiance. My dissertation research is focussed on the acclimation and adaptation of leaf flavonoids to solar radiation in plant taxa from contrasting environments assessed through the study of optically measured leaf traits. These responses were studied in forest understorey taxa growing in a seasonally dynamic, but mostly low UV radiation environment, and in taxa growing at high elevation experiencing a high UV radiation environment in a "common garden" setting. More precisely, we examined: 1) how understorey spectral irradiance changes across shade, leaf-shade and sunflecks through the spring and among different forest stands, 2) how optically measured leaf traits and leaf flavonoids in understorey plants change through the growing season, 3) how these patterns relate to seasonal changes in spectral irradiance especially those in UV region, 4) do the patterns of optically measured leaf traits from a large set of taxa, mainly growing at high elevation, follow their patterns of phylogenetic relatedness and 5) how do their leaf traits relate to climatic conditions at their original collection sites. To study these questions, we adopted relatively new monitoring approaches, enabling us both to measure simultaneously the *in situ* spectral irradiance from the UV to near infra-red (NIR) regions, and leaf flavonols/flavones *in vivo* repeatedly with a leaf-clip in the field. Our analysis revealed a hierarchy among those factors affecting spectral composition of solar radiation in forest understoreys; most importantly understorey position (sunflecks, shade or radiation transmitted through the canopy of leaves), then stand composition, and date during spring. We found the optically measured leaf flavonol/flavone index (I_{flav}) in forest understorey species to be plastic and to adjust to changes in climatic conditions. Furthermore, species' leaf retention strategy (e.g., summer green, overwintering leaves) and new leaf production were found to affect the I_{flav} of plants. All these factors are reflected in the seasonal trends we describe in leaf flavonoids, measured optically (I_{flav}) and via leaf extracts, across understorey plant communities. For mountain environments, our objectives were to determine factors that underpin leaf flavonoid accumulation of high elevation taxa and whether patterns in optically measured leaf traits followed their phylogenetic relatedness or climatic conditions at their origin. Both these patterns could potentially constrain plant responses. To see if either pattern was present, we tested for a phylogenetic signal particularly in I_{flav} from a large set of taxa growing in a high-elevation environment and the relationship of mean I_{flav} of plants to climatic variables. The tests for a phylogenetic signal (Pagel's λ, range from 0 to 1) gave intermediate fitted λ values with significant results for I_{flav} and anthocyanin index (I_{ant}), while for the smaller set of taxa growing in Kumpula Botanical Garden (southern Finland) only chlorophyll index (I_{chl}) showed significant results. Despite the relatively low signal for I_{flav}, we identified certain genera with mainly positive local autocorrelations (local Moran's *I*) meaning they contained species showing either with mostly high or mostly low leaf trait values. This suggests potential limitations in their leaf flavonol/flavone accumulation responses. Hence, some of these genera may be less well prepared against higher maximum UV radiation and may encounter constraints in migrating

upwards, if other compensatory photoprotection mechanisms fail. We did not find a relationship between I_{flav} and climate at the plants' origin, while our results suggested UV irradiance in the plants' current microhabitat to be important, albeit not the only driver for flavonoid accumulation. In most taxa, we did not find a clear indication of constraints on leaf flavonoid accumulation, thus no evidence that high UV radiation is a detrimental factor in their environment. The values of these optically measured leaf traits represent the outcome of complex interactions between the evolutionary and biogeographical history, and acclimation to the current growing conditions of the plants. In general across the three studies, these results provide evidence that optically measured leaf traits related to flavonoid accumulation are largely flexible and acclimate to local changes in the environment, as well as adjusting over the growing season.

TIIVISTELMÄ

Metsien kenttäkerroksen valo-oloja ja niiden muutoksia keväällä latvuston sulkeutumisen myötä on tutkittu paljon erityisesti niillä aallonpituuksilla, joita kasvit hyödyntävät fotosynteesissä (400-700 nm). Ultraviolettisäteilyn (UV) muutoksista metsien kenttäkerroksessa tiedetään sitä vastoin verraten vähän. UV-säteily (UV-B 280-315 nm, UV-A 315-400 nm) muodostaa pienen osan maanpäällisestä auringon kokonaissäteilystä, mutta sen tiedetään olevan tärkeä kasvien vasteiden stimuloija. Auringon säteilyn eri aallonpituuksien vaikutukset kasveihin eivät ole suoraviivaisia, vaan niihin liittyy monimutkaisia vuorovaikutussuhteita aina valon havainnoinnista tuotettuun vasteeseen asti, esimerkiksi fotoreseptorien signalointireittien komponenttien välillä. Eräs usein havaittu kasvien vaste UV-säteilylle on tuottaa ja kerryttää sekundaarisia aineenvaihduntatuotteita (kuten flavonoideja), jotka osaltaan suojaavat kasveja säteilyn haitallisilta vaikutuksilta (engl. photoprotection). Tässä väitöskirjassa tutkin kasvien lehtien flavonoidien akklimaatiota ja adaptaatiota auringon säteilyyn kahdessa hyvin erilaisissa ympäristöissä. Metsän kenttäkerrosta luonnehtii tyypillisesti alhainen auringon säteilytaso ja lyhytaikainen korkea säteilytaso valoaukoissa. Vuoristoissa UV-säteilyarvot sitä vastoin ovat usein korkeita, ja siellä tutkimme eri puolilta maailmaa tuotuja kasveja samassa ympäristössä. Tutkimme 1) miten metsän kenttäkerroksen auringon säteilyn määrä ja säteilyn suhteellinen määrä spektrin eri aallonpituuksilla muuttuvat keväällä eri latvustoisissa metsiköissä, 2) miten aluskasvuston lehtien flavonoidien määrä muuttuu kasvukauden aikana, mitattuna optisesti sekä määrittäen lehtiuutteista, 3) miten nämä muutokset vertautuvat auringon säteilyn ja erityisesti UV-alueen säteilyn muutoksiin keväällä, 4) noudattavatko vuoristossa kasvavien kasvilajien optisesti mitatut lehtien ominaisuudet taksonien evolutiivisia sukulaisuussuhteita ja 5) korreloivatko nämä mitatut lehtien ominaisuudet kasvien alkuperäisen keräyspaikan ilmasto-olojen kanssa. Käytimme näiden kysymysten tutkimiseen tekniikoita, jotka mahdollistivat laajan (UV-säteilystä lyheen infrapunasäteilyyn) auringon säteilyspektrin mittauksen samanaikaisesti. Lehtien flavonoidipitoisuuden mittaamiseen käytimme optista laitetta (Dualex Scientific+), joka mahdollisti toistuvan seurannan kasvia vahingoittamatta. Havaitsimme, että auringon säteilyspektriin metsien kenttäkerroksessa vaikuttivat eniten varjostuksen määrä (umbra, penumbra, valolaikku) sekä laskevassa järjestyksessä metsikkö (eroavat puulajit, tiheys) ja ajankohta kevään aikana. Kasvien flavonoidi-indeksissä havaittiin plastisuutta ja muutoksia vallitsevien ympäristömuuttujien mukaisesti. Lisäksi kasvien lehtistrategiat, sekä uusien lehtien tuotanto vaikuttivat kasvien lehtien flavonoidi-indeksiin. Vuoristossa kasvavien lajien lehtien ominaisuuksien mahdollisia rajoitteita tutkittiin testaamalla niiden fylogeneettinen signaali (Pagel's λ), sekä korrelaatio kasvien alkuperäisen ympäristön ilmastomuuttujien ja lehtien ominaisuuksien välillä. Emme havainneet korrelaatiota kasvien alkuperäisten ilmasto-olojen sekä flavonoidi-indeksin välillä, mikä viittaa kasvien akklimaatioon niiden nykyiseen ympäristöönsä. Lisäksi havaitsimme, että lehtien ominaisuuksien (flavonoidi- ja antosyaani-indeksi) fylogeneettinen signaali oli heikko mutta merkitsevä, ja pienemmästä lajiryhmästä, mitkä kasvoivat Kumpulan kasvitieteellisessä puutarhassa (Etelä-Suomi) ainoastaan klorofylli-indeksi antoi merkitsevän tuloksen. Lähemmässä tarkastelussa (local Moran's *I*) erotimme sukuja, joiden lajit osoittivat samankaltaisuutta (paikallinen positiivinen autokorrelaatio) lehtien ominaisuuksissa, pääasiassa joko korkeita tai alhaisia indeksiarvoja. Tämä viittaa siihen, että näissä suvuissa flavonoidien kerääntyminen voi olla evolutiivisten sukulaisuussuhteiden mukaisesti rajoittunutta. Tämä voi mahdollisesti vaikeuttaa joidenkin lajien kohdalla migraatiota vuoristossa -varsinkin suvuissa, joissa havaitsimme pääasiassa

matalia flavonoidi-indeksin arvoja. Emme kuitenkaan löytäneet selkeää merkkiä rajoitteista vuoristoissa kasvavien lajien lehtien flavonoidien akkumuloitumisessa, joten todisteita korkean UV-säteilyn haitoista niiden nykyisessä ympäristössä ei ollut. Kaiken kaikkiaan tuloksemme osoittavat, että optisesti mitatut lehtien ominaisuudet, liittyen lehtien flavonoidien kertymiseen, ovat joustavia ja akklimoituvat paikallisiin ympäristön muutoksiin, mukaan lukien kasvukauden aikaiset muutokset.

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CONTENTS

ABSTRACT

TIIVISTELMÄ

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LIST OF ORIGINAL PUBLICATIONS AND MANUSCRIPTS

This thesis is based on the following publications and manuscripts:

I. **Hartikainen, S. M.**, Jach, A., Grané, A., & Robson, T. M. (2018). Assessing scale-wise similarity of curves with a thick pen: As illustrated through comparisons of spectral irradiance. *Ecology and Evolution*, 8(20), 10206–10218. https://doi.org/10.1002/ece3.4496

II. **Hartikainen, S. M**., Pieristè, M., Lassila, J., & Robson, T. M. (2020). Seasonal patterns in spectral irradiance and leaf UV-A absorbance under forest canopies. *Frontiers in Plant Science*, 10, 1762. https://doi.org/10.3389/fpls.2019.01762

III. **Hartikainen, S.M,** & Robson T. M. The roles of species' relatedness and climate of origin in determining optical leaf traits over large set of taxa from high elevation and latitude. Manuscript.

The publications and manuscripts are referred to in the text by their roman numerals.

AUTHORS' CONTRIBUTIONS TO THE PUBLICATIONS

The doctoral candidate's independent contribution in the publications or article manuscripts is as follows:

I   SMH, AJ, AG, and TMR conceived the ideas for the manuscript. SMH and TMR designed the field methodology. SMH collected the data. AJ, AG, and TMR analysed the data. SMH and TMR led the writing of the manuscript.

II   SMH and TMR designed the study, and SMH collected the data in 2015 and leaf extract data in 2016. JL collected the optically measured leaf trait data from 2016. TMR processed the spectral irradiance data, and SMH analysed all data. SMH wrote the publication under supervision of TMR, with editorial input from JL and MP.

III   SMH and TMR conceived the ideas for the manuscript and SMH designed the field methodology. TMR collected and processed the spectroradiometer data, but SMH collected and analysed all other data. SMH wrote the manuscript and TMR supervised all stages.

ABBREVIATIONS

ΦPS II	Operating efficiency of PS II photochemistry from light-adapted leaves under ambient conditions
BSWF	Biological spectral weighting function
CI	Confidence interval
CIE	UV action spectrum for erythema induced on human skin, McKinlay and Diffey (1987)
CWM	Community weighted mean
DHP	Digital hemispherical photography
DNA(N)	UV action spectrum for damage to naked DNA, Setlow (1974)
DOY	Day of the year
FLAV	UV action spectrum for accumulation of flavonoids, Ibdah et al. (2002)
F_v/F_m	Maximum quantum efficiency of PS II photochemistry
GEN(G)	Mathematical formulation of Caldwell's (1971) generalized plant action spectrum by Green et al. (1974)
GEN(T)	Mathematical formulation of Caldwell's (1971) generalized plant action spectrum by Thimijan et al. (1978)
HCAs	Hydroxycinnamic acids
I_{ant}	Anthocyanin index measured with an optical leaf clip Dualex Scientific[+] with excitation wavelength of 515 nm
I_{chl}	Chlorophyll index measured with an optical leaf clip Dualex Scientific[+]
I_{flav}	Flavonol/flavone index measured with an optical leaf clip Dualex Scientific[+] with excitation wavelength of 375 nm
NIR	Near infra-red radiation, often determined as 770-3000 nm, when referring to our solar spectral irradiance dataset 750-900 nm
NPQ	Non-photochemical quenching, calculated as $(F_m-F_m')/F_m'$
Y(NPQ)	Equivalent to quantum yield of regulated non-photochemical energy loss in PS II i.e. describing the fraction of energy dissipated in form of heat through the regulated photoprotective NPQ mechanism, Klughammer and Schreiber (2008)
Y(NO)	Equivalent to quantum yield of non-regulated non-photochemical energy loss in PS II i.e. describing the fraction of energy passively dissipated in form of heat and fluorescence, Klughammer and Schreiber (2008)

PAI	Plant area index, $m^2\ m^{-2}$
PAR	Photosynthetically active radiation, 400-700 nm
PG	UV action spectrum for plant growth, Flint and Caldwell (2003)
TPMA	Thick pen measure of association
TPT	Thick pen transform
UV	Ultraviolet radiation, 280-400 nm
UV-A	Ultraviolet-A radiation, 315-400 nm
UV-B	Ultraviolet-B radiation, 280-315 nm

1. INTRODUCTION

1.1. Dynamic solar radiation in the forest understorey

1.1.1. How understorey spectral irradiance relates to solar elevation angle and atmospheric properties

Solar elevation angle changes seasonally, diurnally and with latitude affecting both the amount and spectral composition of solar radiation at the Earth's surface. Any individual photon may interact with particles or molecules within the atmosphere and the longer the pathlength travelled -increasing with decreasing solar elevations above the horizon-, the more likely absorption and other physical interactions (scattering) are to occur (Monteith and Unsworth, 2013) (Figure 1). Global radiation consists of two components: direct and diffuse radiation (Monteith and Unsworth, 2013). Diffuse radiation is typically scattered multiple times within the atmosphere before reaching Earth's surface and therefore has changed its original direction of propagation (Blumthaler, 2012). The size of molecules or particles influence their scattering properties (Monteith and Unsworth, 2013). Rayleigh scattering efficiency of molecules is proportional to the inverse fourth power of the wavelength, λ^{-4} (illustrated in Lindfors and Ylianttila, 2016), while the dependency between scattering efficiency and wavelength is usually weaker for larger particles (Mie scattering) (Ångström, 1964). As a result of these complex interactions, UV-B radiation is proportionally more diffuse compared to longer wavelengths (such as PAR), although the proportion of diffuse radiation increases with decreasing solar elevation angle and with the amount of scattering molecules and particles in the atmosphere (Grant, 1997, Seckmeyer et al., 2008). Furthermore, diffuse UV-B radiance (directional) is more homogenously distributed across the sky hemisphere than UV-A or PAR, although not isotropic (Blumthaler et al., 1996, Grant et al., 1997), while relative variation in UV-B irradiance seasonally and diurnally is more pronounced than in UV-A irradiance, with more pronounced UV-B irradiance maxima around midsummer and noon (Seckmeyer et al., 2008). Although the main proportion of incident UV radiation at the ground level is UV-A radiation, the higher energy of photons within the UV-B region may produce particular effects in plants (Robson et al., 2015b, Jenkins, 2017).

Absorption of specific wavelengths of radiation by elements, mainly in the outer layers of the sun, create typical valleys in the spectrum of extra-terrestrial solar radiation called Fraunhofer lines (for the extra-terrestrial solar spectrum see Wehrli, 1985). In addition, absorption by constituents of the Earth's atmosphere (e.g., H_2O and O_2) creates characteristic drops in the terrestrial solar spectrum (Gueymard, 2004). Photochemical reactions in the stratosphere result in the attenuation of all UV-C radiation and most of the UV-B radiation from terrestrial radiation (WMO, 2018). Changes in stratospheric ozone concentration seasonally, with latitude, or historically due to emissions of ozone depleting substances (chlorofluorocarbons and similar compounds) affect terrestrial UV-B radiation (WMO, 2018). Especially in polar regions, cold temperatures in the polar vortex are among a combination of factors that promote photochemical reactions, which may result in the formation of stratospheric ozone holes (WMO, 2018). In general, weather conditions may significantly alter the spectrum of radiation at ground level and in this context clouds are particularly important (Bais et al., 2019). Effects of clouds on terrestrial radiation are variable yet in general clouds reduce irradiance and increase the proportion of diffuse to direct radiation (Bais et al., 2019). However, under a partially clouded sky, radiation reaching ground level can sometimes increase beyond that received under a clear-sky conditions, both in the PAR and UV regions,

because of additional light scattered from clouds adding to unattenuated direct radiation (McKenzie et al., 2017, Bais et al., 2019). This enhancement effect is higher for the UV-A and PAR regions than for the UV-B region, and typically results in a less than 20 % increase in radiation compared to clear-sky conditions for UV-B radiation, while for the PAR region an increase of up to 50 % may occur (McKenzie et al., 2017, Bais et al., 2019).
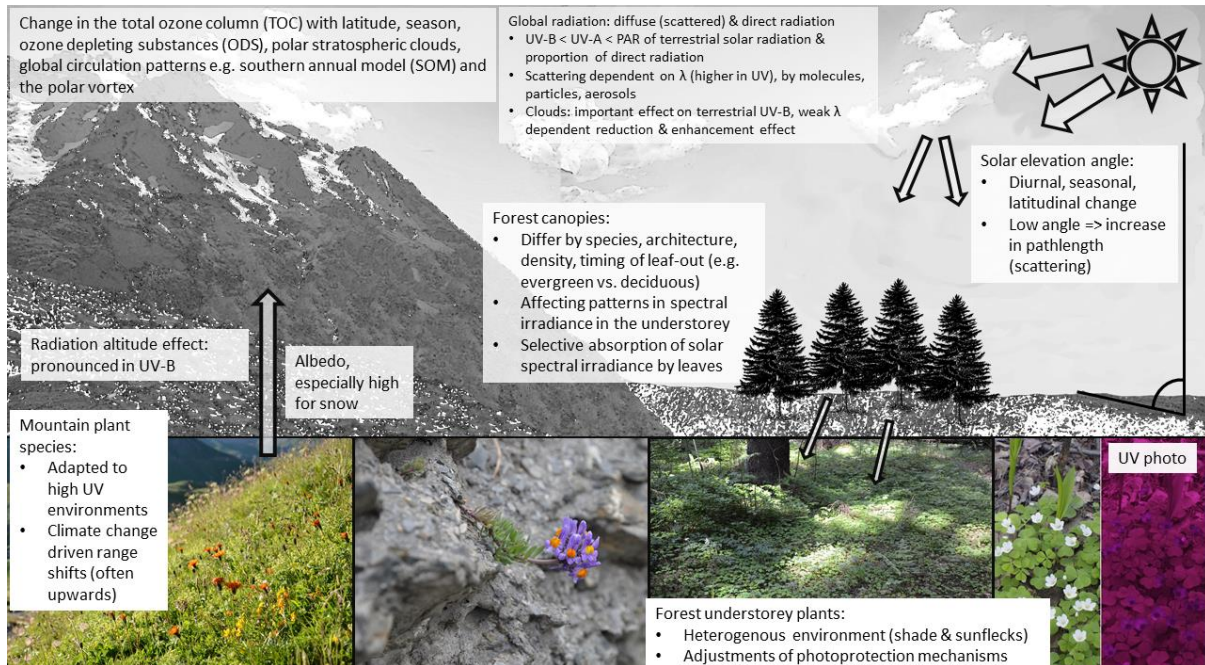
Figure 1. Summarising general environmental factors affecting terrestrial solar spectral radiation, especially UV radiation, and respective plant responses in contrasting mountain and forest understorey plant communities relevant to this study.

1.1.2. How understorey spectral irradiance relates to forest stand architecture and canopy foliage

Radiation within forest understoreys is typically enriched in far-red and NIR that are less efficiently absorbed across plant canopies than blue, red and UV regions of solar radiation (Grant, 1997). Correspondingly, individual leaves reflect and transmit more of the green, far-red and NIR regions relative to other spectral regions (Grant, 1997, Qi et al., 2010). Additionally, woody material increasingly reflects radiation at longer wavelengths towards the NIR (e.g., Rautiainen et al., 2018 and references therein), which can influence the composition of spectral irradiance measured in forest understoreys. Trees optimise their light use efficiency by adjusting their leaf traits to suit the light conditions they experience at various levels within the canopy (Niinemets, 2012, Raabe et al., 2015). The regulation of traits, such as those related to leaf morphology and anatomy, foliage distribution and leaf inclination angle may serve to improve intra-canopy light interception (reviewed by Niinemets, 2010). Interestingly, previous studies -although concerning a crop species- imply that differences in leaf inclination angles affect the received UV-B doses incident on the leaf (Grant, 1999), even though the UV-B region has higher diffuse component compared to PAR, and it should hence be less dependent on the direction. However, it may be that plants more commonly use their capacity to adjust leaf inclination angles to optimise their light interception rather than to avoid excess sunlight (Robson et al., 2015b).

The consequences of differences among tree species in their architecture and foliage are perhaps most strikingly demonstrated by comparison of evergreen and deciduous forests (Federer and Tanner, 1966; evergreen: Coombe, 1957, Dengel et al., 2015; deciduous: Baldocchi et al., 1984, Grant et al., 2005). For instance, changes in light penetration through a canopy of conical evergreen species is greatly affected by solar elevation angle, whereas the most apparent changes in deciduous stands follow phenological phases of leaf-out and leaf fall (Leuchner et al., 2011). Diffuse radiation, particularly blue, which penetrates deeper within the canopy (Dengel et al., 2015), partially contributes to a reported increase in net ecosystem exchange under cloudy conditions (Urban et al., 2007). Similarly, due to greater scattering within the UV region in the atmosphere, the UV-B:PAR ratio is often reported to be higher in the shaded portion of the understorey than in sunflecks or sunlit portions of gaps (Brown, 1994, Flint and Caldwell, 1998). In summary, forest density and canopy architecture are important features defining the understorey light conditions.

1.1.3. Dynamic changes in spectral irradiance in the forest understorey

In nature, plants grow under dynamic and fluctuating environmental conditions rather than under the static light and stable environment often used in controlled-environment experiments. Although these experiments have proven to be extremely valuable in revealing the physiological and molecular mechanisms that regulate plant processes, more research is needed under realistic light conditions to better understand the ecophysiology of plants in their habitat. Under closed forest canopies, solar radiation may pass through gaps in the crown, creating typically brief sunflecks on the forest floor (Smith and Berry, 2013). Depending on the height of the trees and the size of the canopy gap, a sunfleck may consist of a penumbral ring typically surrounding the direct beam, or it may be entirely penumbral (Smith et al., 1989). Although definitions vary, the most frequent sunflecks are typically short, lasting only few seconds and of relatively low irradiance, while on rare occasions values can reach close to the irradiance at the top of the canopy (Pearcy, 1990, Chazdon and Pearcy, 1991, Way and Pearcy, 2012, Smith and Berry, 2013). This short duration of sunflecks contrasts with the definition of sun-patches, which is used sometimes to describe larger patches that last at least 8 minutes (Smith and Berry, 2013). Sunflecks often represent a large portion of the daily radiation received by plants in the forest understorey (Chazdon and Pearcy, 1991, Way and Pearcy, 2012).

1.1.4. Quantifying spectral irradiance in the forest understorey

In the past and currently, quantifying PAR has been of great interest because of its significance for photosynthesis and thus in estimating e.g., potential carbon assimilation under and within forest canopies (Baldocchi et al., 1984, Grant, 1997, Leuchner and Werner, 2007). Several different measuring techniques have been used depending on the information sought. These often involve using broadband instruments that integrate irradiance over a wavelength region, such as PAR (Aphalo et al., 2012, Akitsu et al., 2017). Alternatively, scanning spectroradiometers can be used to capture spectral irradiance: they can have high performance (allow double monochromator arrangement), but are typically large and difficult to move (Aphalo, 2016). Although these two approaches are well suited for many purposes, they fail to describe dynamic changes in spectral irradiance in the understorey with the spectral or temporal resolution desired for our research. The broadband instruments lack the spectral resolution to capture subtle changes in the spectral composition of radiation, whereas scanning spectroradiometers are too slow to capture the fast dynamic changes between sunflecks and

shade that occur under forest canopies. Both these types of changes are meaningful for plant photobiology, and hence finding a way to obtain this information is potentially of value to researchers. An advantage of a portable array spectroradiometer with a wide spectral range is its ability to measure multiple spectral regions simultaneously and promptly, providing information on dynamic changes in spectral irradiance (Aphalo et al., 2012, Aphalo, 2016). However, measuring UV radiation and especially UV-B radiation is technically challenging, mainly because UV-B radiation represents a very small faction of the terrestrial solar radiation. Thus, in the UV-B region, the signal-to-noise ratio of measurements can be low, making it vital that stray light, slit function and signal noise are accounted for (Aphalo et al., 2016) to avoid obtaining erroneous and potentially misleading results (Flint et al., 2008). This entails regular calibration and proper operation of the spectroradiometer using a tailored protocol to obtain acceptable accuracy and precision for the UV-B region as well as the PAR (Ylianttila et al., 2005, Aphalo et al., 2012). Spectral data are complex to analyse, and commonly solar irradiance data are summarised as integrals of photon or energy irradiance over wavelength regions, such as photon irradiance over the UV-B region. However, using this simple approach some important information contained in high resolution (~1 nm) spectral data is ignored. In contrast, spectral analysis can extract this additional information about the structure of spectral irradiance. Statistical approaches can be used to look for differences in spectral irradiance and to identify spectral features by comparing entire spectra simultaneously, such as functional data analysis (Ramsay and Silverman, 2005) and wavelet transformation (Jach, 2015). A simpler approach to identify differences between spectra is the application of thick pen transform (TPT) and thick pen measure of association (TPMA) (Fryzlewicz and Oh, 2011). This technique has the additional advantage that it can be applied to analyse non-equispaced spectra, as are obtained when there is high spectral resolution. Other benefits of this method are its relative simplicity and its visual interpretability.

1.2. Solar radiation and climate in high-elevation environments

Mountain environments are typically characterised by high maximum integrated biologically effective UV-B irradiance (mW m^{-2}), and in general unweighted, but particularly biologically effective UV-B irradiance increases with lower latitudes and with increasing elevation (Caldwell et al., 1980). In the Alps for instance, a rise of 1000 m in altitude (altitude effect of annual total global radiation, UV-B measured as sunburn units, SUy^{-1}) may cause a 11-19 % increase in biologically effective UV radiation when compared to UV radiation at 577 m a.s.l (Blumthaler et al., 1992). Hence, mountain environments and the species adapted to live in them have been of interest to researchers in the context of plants' tolerance of high UV-B radiation (e.g., Robberecht et al., 1980), even before concerns about the effects of ozone depletion (Caldwell, 1968). This remains of importance today, particularly in regions where climate change and ozone depletion interact to further increase the severity of the environment, e.g., in the high Andes (reviewed by Bornman et al., 2019). However, the rate of increase in UV irradiance with increasing elevation is not universal in practice, as this rate may vary among geographical and climatic regions (Blumthaler, 2012). Furthermore, high albedo, particularly found with snow cover, may increase the downward UV radiation which is backscattered by the atmosphere, including clouds, to the ground (Caldwell et al., 1980, Gröbner et al., 2000).

In addition to the described climatic conditions in mountains, high-elevation environments typically have short growing seasons, late snowmelt and frequent temperatures below 0 °C (Körner et al., 2019). They harbour plant species which have adapted physiological

and morphological features allowing them to live under these conditions (Körner et al., 2019). However, environmental changes driven by climate change have already led to observed range shifts within different mountain regions in some species, and this trend is also expected to continue into the future (Rumpf et al., 2018, He et al, 2019). Similarly, range shifts are predicted for plants species from high latitudes and boreal regions (Chen et al., 2011, Wróblewska and Mirski, 2018).

1.3. Common plant responses to changes in received solar radiation

Early studies using particularly high doses of UV-B radiation have led to a realisation that the background PAR and UV-A radiation may play an important part in UV-B induced responses (Caldwell et al., 1994, Jansen et al., 1998, Krizek, 2004). For instance, plants grown under low PAR may have increased sensitivity to UV-B radiation (Teramura, 1986) and UV-B-induced DNA damage may be alleviated by photolyase activated with UV-A radiation and blue light (Sancar, 1994, Jansen et al., 1998, algae: Pescheck, 2019). In recent years, the research emphasis has shifted from damage to consider UV-B radiation as a trigger of regulatory responses (reviewed by Björn, 2015). Furthermore, the concept of eustress has been proposed with respect to UV-B radiation, whereby low UV-B irradiance may provide cross-tolerance to high irradiance (Klem et al., 2015) and for drought (partial amelioration of water stress by UV: Robson et al., 2015a). Studying understorey species adapted to environments with generally low solar radiation but occasional rapid increases, may provide further answers to questions about the role of solar radiation in provoking cross-tolerance.

The photoreceptor UV RESISTANCE LOCUS 8 (UVR8) mediates the perception of UV-B radiation (and UV-A radiation up to 350 nm) in plants (Brown et al., 2005, Rizzini et al., 2011, Morales et al., 2013, Rai et al., 2020). UVR8 mediates acclimation and photomorphogenesis in response to UV, as well as gene expression participating in, e.g., flavonoid biosynthesis (Morales et al., 2013, Robson et al., 2015b, Jenkins, 2017, Rai et al, 2018, Rai et al., 2020). Furthermore, signalling downstream of UVR8 shares components with signalling downstream of phytochromes, and UVR8 plays a role in the moderation of the shade-avoidance response by sunflecks (Moriconi et al., 2018). There is also evidence of multiple antagonistic interactions between signalling pathways downstream of UVR8 and cryptochromes in response to UV radiation (Rai et al., 2018 & 2020). Phenotypic changes that are characteristic of photomorphogenesis induced by UV-B radiation often include thicker leaves with short petioles, short stems and increased axillary branching (Robson et al., 2015b). Additionally, cryptochromes and phototropins can absorb within the UV-A region, leading UV-A radiation to affect, for example, plant biomass accumulation and morphology (reviewed by Casal, 2013 and Verdaguer et al., 2017). Interestingly, UV-A radiation can result in an increase in photosynthesis when combined with reduced leaf phenolics and the lack of a cuticle in the sub-alpine shrub *Pimenea ligustrina* Labill. (Turnbull et al., 2013). There are contradictory findings on the effect of UV-A radiation on leaf flavonoid induction and accumulation, and this response appears to be species-specific (reviewed by Verdaguer et al., 2017). Often, it is only possible to identify this effect by considering compositional changes in the flavonoid profile or isolated individual compounds, not necessarily from the total pool (reviewed by Verdaguer et al., 2017). Plant responses to the lowered R:FR ratio perceived through phytochromes in shade have been well studied (Ballaré and Pierik, 2017). Plants utilise this spectral cue as a signal of impending neighbourhood competition, to initiate the shade avoidance syndrome (SAS) (Mazza and Ballare, 2015). However, this response is often lacking

in the shade-tolerant species commonly found in forest understoreys, that are adapted to live in unavoidable shaded conditions (Gommers et al., 2013).

Summarising, changes to the spectral composition of solar radiation may be perceived and utilised differently by plants. A plethora of other interesting light-induced plant responses beyond those mentioned here have been identified, especially many related to photosynthetic adjustments and carbon gain in fluctuating light conditions (e.g., Way and Pearcy, 2012, Yamori, 2016). UV radiation is interesting as it is the shortest spectral region perceived by plant photoreceptors, and it is important in eliciting various plant responses that vary according to complex interactions among spectral regions and with other environmental stimuli, causing interactions between signalling pathways. Gaining knowledge of these responses in realistic spectral irradiances that plants experience in nature is crucial.

1.4. The multiple roles of flavonoids in plants

Flavonoids are a large group of secondary metabolites produced by plants and consequently they are widely distributed in the plant kingdom (Grotewold, 2006). The vast diversity of flavonoid compounds with a carbon skeleton stems mainly from the modification mechanisms e.g., glycosylation and hydroxylation (Grotewold, 2006). In turn, the structure of flavonoids affects how strongly they absorb light at a particular wavelength (Cockell and Knowland, 1999). Flavonoids absorb radiation mainly within the UV region, with absorption maxima of major groups such as flavonols and flavones at the UV-A region (Agati et al., 2013: Figure 1 for flavonoid glycosides, Cerovic et al., 2002: Table 1 for aglycones). Flavonoids are found in vacuoles of epidermal and of adjacent mesophyll cells, but also in epidermal cell walls, trichomes and chloroplasts (Agati et al., 2007, Tattini et al., 2007, Agati et al., 2009). Flavonoids have multiple roles in plants, and they are involved in e.g. photoprotection, insect pollination, plant-pathogen interactions and auxin transport (Winkel-Shirley, 2001, Jansen, 2002, Grotewold, 2006, Agati et al., 2013, Brunetti et al., 2018). Studies with high or supplemental UV-B irradiance have commonly found plants to produce flavonoids and related phenolic compounds in response to UV-B radiation (meta-analysis: Searles et al., 2001). However, when effects of ambient solar UV-B radiation on leaf flavonoid responses are studied in plants, a weak relationship between the accumulation of flavonoids and received UV irradiance is often produced (Liakoura et al., 2001, Coffey et al., 2017). The accumulation of flavonoids may occur in absence of UV radiation, under only PAR (Agati et al., 2009, Barnes et al., 2013), or at low temperatures (Bilger et al., 2007). In leaves the accumulation and biosynthesis of flavonoids in response to UV-B radiation is mediated via UVR8 (Brown et al., 2005). Studies have reported induced synthesis of dihydroxy B-ring-substituted flavonoids upon increased irradiance, and an increase in their ratio compared to monohydroxy B-ring-substituted flavonoids (e.g., Majer et al., 2014, reviewed by Agati et al., 2010). Dihydroxy B-ring-substituted flavonoids are thought to have a better potential to scavenge reactive oxygen species (ROS) (Agati et al., 2010). However, there are still open questions related to potential flavonoid antioxidant functions *in planta* (Hernández et al., 2009, Agati et al., 2020).

In the past, flavonoids and related phenolic compounds have often been studied in context of UV protection, particularly in mountains with high UV-B radiation. These studies revealed that the transmittance of the leaf epidermis, particularly in the UV-B region, is typically relatively low i.e., < 2 % (Robberecht et al., 1980). However, over large environmental gradients the received UV-B radiation has only occasionally been reported to affect leaf flavonoid contents: with simple leaf extracts quantifying UV-absorbing compound

content over elevation with positive relationship (Rozema et al., 1997) or the opposite, with positive relationship over latitude in flavones, applying more sophisticated methods (Tripp et al., 2018). While environmental conditions, such as light and temperature, are typically found to affect leaf flavonoid synthesis and accumulation, in some species only small changes has been detected (Barnes et al., 1987, Ziska et al., 1992, Nybakken et al., 2004, Barnes et al., 2017). Furthermore, recent studies suggest there may be short-term transgenerational effects of UV radiation (Yan et al., 2020, Jiang et al., 2021, Zhang et al., 2021) or long-term effects whereby e.g., a characteristic association of leaf flavonoids with specific *Pinus sylvestris* L. populations remained after almost a century (Oleszek et al., 2002).

There are still many unknowns about the roles of flavonoids in plants, and their synthesis and accumulation according to environmental stimuli. In this doctoral dissertation, trends in leaf flavonoids were investigated, mainly through optical *in planta* measurements with a leaf-clip instrument, from large set of taxa varying in their growing and original environment, their ecology and relatedness. These results were interpreted based on spectral irradiance measurements done to characterise the growing environments.

1.5. Research questions

The research questions of this PhD study were:

1. How does spectral irradiance change during spring phenology in different forest stands of varying canopy tree species composition (**I**);
2. How do leaf flavonoids and the optically measured index based on absorbance, change during the growing season in understorey plant communities and individual species (**II**);
3. Are changes in spectral irradiance, especially those within UV region, important in defining changes in leaf flavonol/flavone accumulation of plant taxa from open high-elevation environments (**III**) and shaded forest environments (**II**);
4. Do patterns in optically measured leaf traits from a large set of taxa, mostly adapted to high UV environments, follow the phylogenetic relatedness of the taxa (**III**);
5. Is there a relationship between the leaf traits of plants growing in a botanical garden and the climate at their original collection site (**III**);
6. How applicable is the optical leaf-clip method for quantifying leaf flavonoids across different plant taxa (**II, III**)?

2. MATERIALS AND METHODS

2.1. Study sites and experimental designs

2.1.1. Forest stands in Finland

Forest stands at Lammi Biological Station (61°3'14.6" N, 25°2'13.8" E) were chosen for this study according to their various canopy species, age and structure, as well as the richness of their understorey plant communities (I, II). Three different-aged deciduous *Betula* sp. L. - dominated stands (henceforth: *Betula* old, young and mixed with other canopy species), one deciduous *Quercus robur* L. stand and one evergreen *Picea abies* (L.) H. Karst. stand were chosen. All stands could be considered typical of Finnish forests and their respective understorey communities, apart from the *Q. robur* stand, an atypical canopy species in Finland which was planted in the 1950's. In each stand, four measurement points (radius of 3 m) were established approximately equidistance between the nearest trees, while minimising the

potential mixing effects from e.g. abundant high-grown tree seedlings. Prior testing of the spatial heterogeneity in spectral irradiance led us to consider four locations to be the minimum number of replicate patches required to describe stand-level variation in irradiance (Hartikainen et al., 2018: I) and in the plant communities, albeit enabling measurements close to solar noon.

The study commenced over spring and summer of 2015 with 4-5 repeated measurements during this time to capture the periods when irradiance in the forest understorey was subject to the greatest changes. A time course of spectroradiometer measurements was taken on day of the year (DOY) 115, 142/144, 156 and 202, and corresponding optical leaf trait measurements were taken on DOY 114, 142-144, 156/157, 202/206, and additionally on DOY 125 when due to partly cloudy weather no irradiance measurements were recorded. Since the fibre-optic cable to the diffuser broke during the measurements on DOY 202, no data from the *P. abies* stand or three measurement points from the *Betula* young stand were obtained on this day. The study continued in 2016, allowing a further dataset consisting of optical leaf trait measurements, from DOY 120 extending further into autumn on DOY 292, to be collected for six selected understorey species from the same stands. These understorey species were among the most abundant and were selected based on their contrasting leaf retention strategies and phenological timing. Plants measured for this dataset were growing within the stand, outside the established measurement points. Additionally, records of daily temperature (max, min, mean) and snowpack depth were obtained from Lammi Biological Station's weather station managed by the Finnish Meteorological Institute.

2.1.2. Botanical gardens in the French Alps and in Helsinki, Finland

Our other study site at the Lautaret Pass in the French Alps (2100 m a.s.l.; 45°2'9" N, 6°23'59" E) was chosen to study species mostly adapted to high UV radiation environments, and for its high diversity of taxa originating from around the world (III). The plants established in the Joseph Fourier Alpine Station's alpine botanical garden (Université Grenoble Alpes, France) originate from seeds collected either directly from their original high-elevation habitat, or from the collections of other botanical gardens. Before they are planted in the garden, their seeds are germinated in the university facility in Grenoble and acclimated at the outdoor seed nursery of the alpine botanical garden. Nevertheless, most species measured were planted in the garden several years prior to our sampling so could be considered well established and acclimated to their growing environment. Sampling in the alpine botanical garden was made over two consecutive summers (19.6.-1.7.2014 and 21.6.-6.7.2015). Temperature (mean, max, min) and daily maximum photosynthetically active radiation data were obtained from e-METSYS/JFAS weather station (Vantage Pro 2 Plus, Davis Instruments, Hayward, CA, USA) located at the study site.

Additional measurements were made of plants growing in the botanical garden at Kumpula, Helsinki in southern Finland during June of 2015 (LUOMUS, University of Helsinki, Finland) (III). There, 27 of the same species and in total of 86 taxa from the same genera or families as in the alpine botanical garden were sampled, in order to compare plants growing at high elevation with those growing in a different environment at high latitude but low elevation. These measurements were made to assess the flexibility of optically measured leaf traits. The species in Kumpula Botanical Garden originate from the Northern Hemisphere and from environmental conditions resembling those found in Finland. Temperature (max, min, mean) and solar radiation (direct, diffuse, global) data were obtained from a nearby weather station (within ~ 500 m) in Kumpula maintained by the Finnish Meteorological Institute.

Coordinate data of the plants' original collection sites were provided by the courtesy of Rolland Douzet from the alpine botanical garden, and from LUOMUS database for Kumpula Botanical Garden. Those species lacking coordinate data but including specific information of their original location and elevation, were given approximate coordinates according to the expected maximum exposure, usually southern slope, at their original collection sites.

2.2. Methods for depicting and analysing spectral irradiance in forest understoreys

To accurately quantify solar spectral irradiance reaching the forest understorey, from the UV to near-infrared regions in rapidly changing conditions, we used a portable CCD array spectroradiometer Maya 2000 pro (Ocean Optics, Dunedin, FL, USA) with a spectral range of 200-1100 nm attached to a D7-H-SMA cosine diffuser (Bentham Instruments Ltd., Reading, UK) with a fibre-optic cable (I, II). To ensure that we made accurate outdoor measurements of solar radiation, especially within UV-B region, the device was calibrated annually by Finnish Radiation and Nuclear Safety Authority (Ylianttila et al., 2005, Aphalo et al., 2016, Aphalo, 2017). Further technical details on the spectral irradiance analysis may be found in Aphalo et al. (2016 and 2017).

Each measurement set (finished within c 20 sec) contained up to 100 consecutive irradiance spectra, i.e., ideally 40 spectra in the darkest environments with the longest integration times and 100 spectra in the brightest environments with the shortest integration times. Integration time was set manually to maximise signal-to-noise ratio while avoiding saturating the array. Directly after each set of measurements, control measurements without any UV (blocked with a polycarbonate filter) and without any UV radiation or visible light (by entirely obscuring the diffuser) were made. The first control measurement corrects for stray visible light on the array recorded incorrectly as UV radiation, and the latter obtains a baseline of temperature-dependent dark noise and identifies any bad pixels (further details in Aphalo et al., 2016 and Aphalo, 2017). A spirit level on a tripod was used to ensure the horizontal alignment of the diffusor in the field at a standard 40-cm height from the ground. In addition, the spectroradiometer was covered with a white cloth to minimise heating/cooling of the equipment. Field measurements were timed around solar noon (within max. 3 hours) and made under as close to clear-sky conditions as weather in the field allowed, to capture the maximum solar irradiance. At each measurement point, three sets of measurements were made to record the variation in understorey irradiance: 1) in a sunfleck with mostly direct radiation (Smith and Berry, 2013), 2) within the umbra (shade) of a tree trunk capturing diffuse radiation, and 3) at a point in the understorey where radiation penetrated through the leaves in the upper canopy creating penumbra (henceforth understorey position *leaf*). All details related to the post-processing protocol of the solar irradiance data may be found in Hartikainen et al. (2018) (I).

Using a similar protocol, measurements from 29 locations within the alpine botanical garden were made in 2015 to compare the relationship between solar spectral irradiance in the microhabitats of measured plants and their mean flavonol/flavone index (I_{flav}) (III). Additionally, one measurement from near to Kumpula Botanical Garden, from an open environment (Viikki campus, University of Helsinki) was used to include data from Finland to the analysis. Spectral irradiance measurements were combined with mean I_{flav} of the adjacent plants according to their categorised light exposure.

Spectral analyses of Lammi forest understorey irradiance data was done by first applying TPT, utilising upper and lower boundaries of areas obtained from differing thicknesses of "pens" (I). Subsequently, TPMA for spectra normalized according to maximum

values was used to make bivariate and multivariate comparisons between spectra from differing stands, understorey positions and dates. TPMA quantifies cross-dependence between spectra, whereby the overlap or lack of overlap of the intervals (of normalized TPT) results in either positive (0 to 1) values revealing the ratio of the intersection to the union, or negative (-1 to 0) values indicating the ratio of the gap to the shortest interval containing union respectively. All further details related to our spectral analysis may be found in Hartikainen et al. (2018) (I).

2.3. Climate data from the original collection sites of plants

To study whether there was a significant relationship between climate at the original collection site of the plants growing in two botanical gardens and their measured I_{flav}, we obtained common climate variables for these locations from databases (III). Temperature and precipitation (annual mean temperature and precipitation, and their seasonality, min and max temperature of the coldest and warmest months) and solar radiation variables (annual mean solar radiation, its seasonality, highest and lowest weekly solar radiation, solar radiation of the wettest, driest, warmest and coldest quarters) were obtained from 59 original collection sites of the plants from WorldClim (Bio 1-15, Fick and Hijmans, 2017) and CliMond (Bio 20-27, Hutchinson et al., 2009, Kriticos et al., 2014) databases. Mean monthly solar radiation was acquired from WorldClim (Fick and Hijmans, 2017). WorldClim data are based on observations from years 1970-2000 with resolution of 30 arc seconds, and CliMond from years 1961-1990 with resolution of 10 arc minutes. A global UV-B radiation climatology based on observations over 2004-2013 with resolution of 15 arc minutes (Beckmann et al., 2014) was used to obtain the UV-B radiation (products 1-6 and mean monthly UV-B radiation) from the plants' original collection sites.

2.4. Quantifying leaf flavonoids and optically measured leaf traits in plant taxa from different habitats

2.4.1. Optically measured leaf traits

Optical leaf trait measurements were made by using leaf-clip Dualex Scientific[+] which compares chlorophyll fluorescence (measured between 710-900 nm) stimulated by light beams of differing wavelengths (375 nm for flavonols/flavones, I_{flav}, 515 nm for anthocyanins, I_{ant}) and that of a reference beam at 635 nm to give an index value (Goulas et al., 2004, Cerovic et al., 2012). The beam wavelength of 375 nm is chosen to gain a strong signal (Goulas et al., 2004), and the major absorbers at 375 nm are suggested to be flavonols in dicotyledons and flavones in monocotyledons (Cerovic et al., 2002 & 2012). These compounds are commonly found among flowering plants (Tripp et al., 2018 and references therein). The chlorophyll index (I_{chl}) is acquired by comparing the transmittance at 710 nm and at reference wavelength of 850 nm (Cerovic et al., 2012) where chlorophyll does not absorb.

From Lammi forest stands, at least four individual plants of each species present at each measurement point were measured (II). While we intended to always compare four different plants, we cannot exclude the possibility that sometimes measurements were unwittingly made from clonal plants which were not distinguishable in the forest understorey. In many of the understorey species we measured, vegetative propagation is common (e.g., Stehlik and Holderegger, 2000, Berg, 2002, Vandepitte et al., 2010). As a standard procedure the first mature, uncovered and typically the 3rd or 4th distal leaf (adaxial side) was measured to exclude major differences in leaf age. For this reason, typically only one leaf was measured per plant.

Additional comparisons of younger and older leaves were made for a set of species: *Fragaria vesca* L., *Hepatica nobilis* Schreb., *Oxalis acetosella* L., *Vaccinium vitis-idaea* L. (species with overwintering leaves) and *Campanula persicifolia* L., *Convallaria majalis* L. (summer green species). In 2016, we further extended the standard leaf measurements and measured both leaf sides (adaxial and abaxial) to gain knowledge on the functionality of the optical method. To minimise the effect of potential diurnal changes in leaf flavonoids (Barnes et al., 2016) and chloroplast movement (Williams et al., 2003) on optical measurements, we timed the sampling as close to solar noon as possible ($\pm$ 3 hours). Visibly healthy leaves were selected for measurements to avoid herbivory or other damage that can stimulate secondary metabolism from influencing the results.

A similar protocol for optical leaf trait measurements to this was used in the alpine botanical garden and Kumpula Botanical Garden with the difference that no measurement points were established (III). Instead, all those taxa growing in the alpine botanical garden that were feasible to measure with Dualex were sampled (plants from 680 taxa and developmental stages), with a minimum of four individuals (or ramets) per taxa (10 266 measurements). From Kumpula Botanical Garden, 27 of the same species were sampled as those from the alpine botanical garden, and in total of 86 taxa (504 measurements) representing same genera or families as those measured in the French Alps. In addition, plants from the alpine botanical garden were categorised according to their phenology (scale of 1-10; not fully opened leaves = 1, senescent = 10), and approximate leaf inclination angle (the angle between the horizon and leaf blade, scale of 1-9; for erect = 1, downward leaves = 9). Locations within both botanical gardens were categorised according to their solar radiation exposure (scale of 1-4; mostly shaded during the day by other plants = 1, exposed to full sunlight throughout the day = 4) to give an indication of their micro-environment.

2.4.2. Whole leaf extracts

Quantifying total or individual flavonoids present in leaves can be done by using a variety of techniques (Julkunen-Tiitto et al., 2015), and crude leaf extracts in acidified methanol are the simplest. Leaf extracts were made from leaves sampled from selected taxa at both sites (5 species from Lammi forest stands and 50 taxa from the alpine botanical garden). The absorbance of these extracts was compared with optically measured $I_{flav.}$ The selection of taxa for these comparisons was based on previous year's optical leaf trait surveys.

In Lammi, five common understorey species were chosen for sampling on 5-6 occasions ($n = 15$ for each species and sampling time) during the spring and summer from two forest stands with contrasting tree canopies (*Q. robur* and *P. abies*) (II). *Anemone nemorosa* L. from both stands, *Aegopodium podagraria* L. and *Convallaria majalis* from the *Q. robur* stand, and *Hepatica nobilis* and *Oxalis acetosella* from the *P. abies* stand. The same leaves were first measured with Dualex from the middle section of the lamina avoiding major veins, and subsequently two leaf-disks (2×0.28 cm^2 area) from the same part of each leaf were punched directly into 3 ml of acidified methanol (99.9 % MeOH acidified with HCl 1:200). Extracts were then directly placed into a cool box on ice and in darkness, where they were kept for the whole daily sampling period, until subsequent transfer into +6 $^\circ$ C overnight in a fridge. Leaf extracts were analysed with a spectrophotometer (Shimadzu UV-2501 PC UV-VIS, Kyoto, Japan) using a quartz cuvette and obtaining absorption spectra from 190-900 nm. Samples were diluted with methanol where necessary to keep absorbance values ≤ 2. Leaf extract absorbance at 375 nm, mean absorbance integrated over the UV-B region, UV-A region and the whole UV

(UV-B plus UV-A) region were used to compare the relationship between leaf extracts and optically measured I_{flav}. Extra leaf disks were collected from the same or adjacent plants to obtain fresh and dry weights. Fresh weight was measured shortly after field sampling and dry weight after drying at +50 °C for > 24 hours.

Leaf extracts from plants growing in the alpine botanical garden were made from 50 selected taxa during summer, 2015 (III). Leaves from four individuals (or potentially ramets) from each taxon were collected in sealed plastic bags placed in the cold and dark for subsequent sampling within approximately 1 hour. Dualex and leaf disk sampling, and preparation of leaf extracts, were done as described previously. Leaf extract absorbance was measured at 305 and 375 nm with spectrophotometer (Beckman DU series 64 UV-VIS, Brea, USA) using a quartz cuvette, and sample volume was diluted to keep the absorbance values ≤ 2.7. Two extra leaf-disks were taken for fresh and dry weight: fresh weight was measured immediately, and dry weight after drying the samples for 48 hours at +60 °C. Due to malfunction of the spectrophotometer, dried samples were analysed in Finland to obtain absorption spectra for the samples. The dried leaf samples were diluted after a week to 3 ml of acidified methanol, and after 24 hours leaf extracts were analysed same way as those from Lammi forest stands. The relationship between optically measured I_{flav} and leaf extracts was assessed by using sample absorbance at 305 and 375 nm measured from *in situ* leaf extracts, and mean absorbance over each UV region from dried sample extracts, as described above. The absorbances of all leaf extracts from both sites were normalized for sample volume and leaf fresh weight. We also used TPT and TPMA analysis to investigate the UV absorbance spectra of the leaf extracts (SM A3 from III).

2.5. Using digital hemispherical photos to estimate plant area index

To better understand the changes in spectral irradiance during canopy leaf-out, a quantitative characterisation of canopy (leaf) coverage is needed. We chose to do this by using digital hemispherical photography to calculate the plant area index, PAI ($m^2 m^{-2}$) (I, II). Pictures were taken in tandem with spectral irradiance measurement during spring and summer 2015 from all measurement points from Lammi forest stands. To achieve this, we used a Sigma 4.5 mm f2.8 EX DC HSM circular fisheye lens (Sigma Corporation of America, USA) combined with Nikon D7100 (Nikon corporation, Japan) camera body, which has a 24.1 MP CMOS sensor. Three to five pictures in RAW format aligned to the north were taken from the exact position of each measurement point each time, during overcast weather to maximise homogeneity of the sky, and contrast between the sky and canopy. For these three to five pictures, same settings with aperture f 20/22 and ISO 200 were used. However, to capture the correct range of PAI, exposure time was manually set to obtain potential variation in PAI stemming from different exposure times. The longest exposure time was used to maximise small gaps especially in the periphery, while the shortest exposure time was used to exclude overexposure of the top canopy. Shorter exposure times compared to automated exposure settings were deliberately used throughout.

To reduce the variation related to different exposure times or inconsistent results with the automated binarization algorithm (Nobis and Hunziker 2005); 1) pre-processing was done according to Macfarlane et al. (2014) and, 2) two additional binarization methods were used: Floyd-Steinberg dithering option (IrfanView, version 4.44, Irfan Skiljan, Wiener Neustadt, Austria) and the standard binarization algorithm in IrfanView. These three versions of each photo (one grey-scale image obtained with Macfarlane procedure, and two using the two

different binarization algorithms) were then analysed with Hemisfer 2.16 (Patric Schleppi, Swiss Federal Institute for Forest, Snow and Landscape Research WSL). For grey-scale images, an automatic threshold algorithm by Nobis and Hunziker (2005) was used. The PAI estimates were initially calculated using all methods provided in the software, but Miller et al. (1967) method was chosen for final comparisons. Potential issues with foliage clumping were addressed by using Chen and Cihlar (1995) correction. In addition to PAI, sunfleck duration in minutes on each measurement day was calculated with Gap Light Analyser (GLA, version 2.0, Simon Fraser University, BC, Canada) using the same thresholds determined during image processing with Hemisfer. Finally, mean PAI and sunfleck duration were calculated for each measurement point and date from photos with differing exposure times.

2.6. Chlorophyll fluorescence measurements from the alpine botanical garden

Based on the results of the survey of optically measured leaf traits done for 680 plant taxa and developmental stages during previous year (2014) in the alpine botanical garden, 88 taxa representing a diversity of leaf traits and taxa were chosen for sampling in the following year, 2015 (III). Measurements of the operating efficiency of PSII photochemistry (henceforth ΦPS II) from light-adapted leaves, and maximum quantum efficiency of PSII photochemistry (henceforth F_v/F_m) from dark-adapted leaves were measured with a PAM fluorometer (mini-PAM, Heinz-Walz GmbH, Effeltrich, Germany). To catch any diurnal fluctuations in ΦPS II functioning, measurements were taken at mid-morning (between 2-4 h after the beginning of daybreak) and during maximum solar irradiance at midday ($\pm$ 1 h around solar noon). Likewise to capture any diurnal trends, F_v/F_m was measured pre-dawn (1-3 h before local sunrise), and from 30 minutes dark-adapted leaves at midday (approximately $\pm$ 3 h from solar noon). Fluorescence parameter, non-photochemical quenching (NPQ), was calculated according to Murchie and Lawson (2013) as $(F_m-F_m')/F_m'$. Furthermore, parameters Y(NO) and Y(NPQ) were calculated whereby the first parameter describes the fraction of energy passively dissipated in form of heat and fluorescence, and the latter describes the fraction of energy dissipated in form of heat through the regulated photoprotective NPQ mechanism (Klughammer and Schreiber, 2008). The means of these parameters among plants of the same species were used to avoid any potential calculation errors arising from measuring different individual plants or leaves. All measurements were made by following same protocol i.e. leaves were selected by applying the same criteria used with optically measured leaf traits.

2.7. Forest inventories for stand characteristics and understorey plant communities

The phenology of three to four trees adjacent to each measurement point in forest stands was recorded using a categorical scale between 1-7, where the first category is unswollen closed buds and the last category is for fully opened expanded leaves (I, II). Surveys were done in tandem with spectral irradiance measurements in Lammi forest stands during spring and summer, 2015. The phenology of individual trees was surveyed from the lowest branches, middle and upper canopy. From each tree, the circumference at breast height was measured and respective diameter (DBH) was calculated. All trees and tree seedlings were inventoried in the area around (radius 3.99 m) each measurement point to obtain the density. The basal area of trees around each measurement point was surveyed using a relascope.

The abundance of all understorey species present at each measurement point, and their phenology (i.e. timing of emergence, leaf opening, flowering, seed production and senescence) at the stand level were estimated (II). The relative abundance data was multiplied by the I_{flav}

value to obtain the community weighted means (CWM) for I_{flav}, for each measurement point on each DOY of measurement.

2.8. Main data analysis

Differences in I_{flav} trends were compared between years, stands, species, and between adaxial and abaxial leaf sides, by inspecting if any overlap occurred between 95 % confidence intervals (CI) of loess-based fits (R function loess) (II). A similar approach was used to compare differences between trends in spectral irradiance from forest stands. Pearson's correlation was used to compare the relationship between spectral irradiance or temperature and mean I_{flav} obtained from each measurement point. Students t-test, or equivalent non-parametric Wilcoxon rank sum test, was used to compare differences between adaxial and abaxial I_{flav}, and between I_{flav} of newly produced and mature leaves. Separate models were made explaining changes in mean I_{flav} for each integral of spectral irradiance (UV-B, UV-A, PAR), and likewise for effective dose calculated according to biological spectral weighting functions (BSWFs: GEN(G), FLAV, PG) from different understorey positions. Comparisons of the AIC, R^2-values and visual inspection of the fit were used to select the best models. A similar approach was used for comparing changes in I_{flav} and whole leaf extracts (absorbance at 375 nm, mean absorbance within UV-B, UV-A or UV regions) of each understorey species.

Kohonen self-organising maps (SOM; Kohonen, 1982, Wehrens and Kruisselbrink, 2018) were used to segregate groups of taxa from the alpine botanical garden based on optically measured leaf traits and categorised light condition, approximate leaf inclination angle and phenology of the plant, and chlorophyll fluorescence and quenching parameters (III). To investigate whether optically measured leaf traits followed patterns of phylogenetic relatedness among plant taxa, we used a previously published updated mega-tree (GBOTB.extended.tre, Jin and Qian, 2019) which is based on phylogenies from Smith and Brown (2018, GBOTB), and clade of pteridophytes from Zanne's et al. (2014) phylogeny. A V.PhyloMaker tool for generating phylogenetic trees utilising this mega-tree as a backbone (Jin and Qian, 2019) was then used for our set of taxa measured at high elevation (France, 629 taxa) and at high latitude (Finland, 86 taxa). We then calculated a phylogenetic signal for optically measured leaf traits by using Pagel's lambda (λ) test (Pagel, 1999, phytools R package; Revell, 2012 & 2013) for both datasets. In this test, the phylogeny is transformed according to the optimized λ value (from 0 to 1) moving internal nodes more basal, whereby the distance to most recent common ancestor of sister lineages increases (Pagel, 1999). The selected λ value best explains the trait values at the tips under a Brownian motion model of trait evolution (Pagel, 1999). A likelihood ratio test is used to test this transformation against a transformation with $\lambda = 0$ (a star phylogeny) (e.g., Revell, 2012 & 2013). A local indicator of phylogenetic association (local Moran's I: Anselin, 1995; R function lipaMoran from R package phylosignal: Keck et al., 2016) was used to distinguish local patterns in leaf traits and particularly interesting taxa. Spearman's rank correlation was used to estimate the relationship between mean I_{flav} of plants and climatic conditions at their original collection site. Most plants used in this analysis were growing in the Kumpula Botanical Garden and fifty of the sites had mean leaf trait value from single species, while mean traits from 2-6 species were calculated for the rest of the sites of origin.

The differences between predawn and midday F_v/F_m were compared using Student's or Welch t-test, or non-parametric Wilcoxon rank sum test (III). All further details of data analysis may be found from the publications and their supplementary materials (referred in the text as SM A#).

3. MAIN RESULTS AND DISCUSSION

3.1. Spectral irradiance in the understorey

By applying TPT and TPMA to compare the shape of irradiance spectra from understoreys, we found that understorey position (i.e., understorey shade, leaf semi-shade or sunfleck) was the most important determinant of the shape similarity of spectra (I, Figure 2). When spectra from all understorey positions (per stand and date) were combined, this resulted in low mean TPMA with a maximum of 0.5 and which decreased with date i.e., these spectra from different understorey positions differed in their shape and became less similar over the course of the spring (I). In early spring, similarities among the spectra from different understorey positions mainly stemmed from high cross-dependence at wavelengths < 500 nm, while by June there was a particularly low cross-dependence in these comparisons between wavelengths 400-650 nm in all stands, indicating major differences over spring within PAR region in these spectra (I).This likely stems from the canopy closure which produced seasonal differences in shade spectra (compare to low similarity in early spring of spectra from *P. abies*, Figure 2) with marked differences in PAR absorbed by the canopy, and larger relative differences in PAR than in UV region due to higher direct proportion. In contrast with the extent of differences due to understorey position, differences in the shape of the spectra were smaller 1) when spectra from all stands with different canopies were compared (per position and date), and smaller still 2) when spectra from all measurement dates over spring were compared (per position and stand) (I). This finding seemed to stem from similarities among sunfleck spectra (across stands and dates), that tended to increase the overall similarity of the spectra among these comparisons. We also found that on average the degree of cross-dependence increased with increment in pen thickness (capturing more coarse features of the spectrum), although this did not always result in positive mean TPMA values (I). Some of the intricate differences between spectra identified using TPMA may have been difficult to detect by other approaches. For example, differences in spectral irradiance from the *leaf* semi-shade of *Betula* stands that likely stemmed from earlier phenology or more closed canopy structure in *Betula* mixed compared to other *Betula* stands (Figure 4 in article I). When measuring direct beam sunflecks, we found that the shape of the spectra was quite consistent (i.e., high cross-dependence) among sunflecks across all stands, dates, and in comparison with open control measurements (I). This finding is supported by earlier studies where the spectral composition of sunflecks has been found to resemble that above the canopy (Dengel et al., 2015 and references therein). We found that by using TPT and TPMA we could extract informative multivariate comparisons from our dataset, and we see the potential for this approach to be equally informative if applied for other spectral datasets. Our results further underlined the importance of considering both the spatial and temporal dynamics when quantifying understorey spectral irradiance. These dynamic patterns in irradiance have often been overlooked or have proven difficult to capture technically. For instance, in the past many interesting studies were made when these complex interactions between fluctuating light caused by sunflecks in forest understoreys were initially described along with the corresponding photosynthetic responses of plants (reviewed by Pearcy and Way, 2012) Studying plant responses to more realistic heterogenous light conditions (such as those found in dynamic understoreys) has gained more interest recently (e.g., Kono et al., 2020, review by Tanaka et al., 2019), partially because new lighting technologies have enabled easier manipulation of the solar spectrum (e.g. in Vialet-Chabrand et al., 2017). For example, low

UV-A and blue irradiance, similar to that found in forest understoreys did not induce effective protection in *Arabidopsis* mutants against stress imposed by acute high light exposure (Brelsford et al., 2019). However in the same experiment, functional cryptochromes were found to mediate responses that partially ameliorate this stress improving plants photosynthetic performance compared with mutants lacking this photoreceptor (Brelsford et al., 2019). Results of these studies, underline the importance of knowing the realistic spectral irradiance experienced by plants in nature to investigate complex plant responses.

In our comparisons between integrals of understorey irradiance over spectral regions, we found that the trends in spectral irradiance measured from understorey shade during spring, differed (non-overlapping 95 % CIs) between evergreen (*P. abies*) and deciduous (*Betula*, *Q. robur*) stands (II). For different evergreen stands with fairly consistent PAI, seasonal differences in understorey solar irradiance usually follow changes in solar elevation angle (e.g., Leuchner et al., 2011). This is consistent with the trend for increasing irradiance measured from sunflecks towards the summer solstice found in our data from the *P. abies* stand (II). In general, we found that the range of spectral irradiance values integrated over wavelength regions was larger in sunflecks compared to irradiance in the shade or *leaf* positions, which likely stemmed from differences in sunfleck size (II). We also found that later timing of the canopy leaf-out within the *Q. robur* stand in comparison to the *Betula* stands might contribute to the increase in UV irradiance on DOY 142/144. However, this difference in trends between the stands was not detected for PAR region (II). As boreal and temperate tree phenology is often driven by air temperatures after meeting the chilling requirement (Hänninen, 2016, Zohner et al., 2016), while especially early understorey spring species are affected by soil temperature (Augspurger and Salk, 2017), a potential mismatch has been sometimes proposed between tree canopy and understorey phenology due to their differing responses under future climate change scenarios (Augspurger and Salk, 2017, Heberling et al., 2019b). This could potentially result in a reduction in the annual, and particularly the spring carbon budget of some understorey species (Heberling et al., 2019a, b). Furthermore, open questions remains whether climate change will increase the occurrence of asynchronies among trophic levels (Renner and Zohner, 2018). For instance, phenological mismatch between pollinators and early ephemeral forest species may occur, with a potential negative impact on seed production (Kudo and Cooper, 2019). Although species' responses and interactions may be region-specific (Zohner et al., 2016, Renner and Zohner, 2018), earlier canopy closure would mean that maximum solar elevation angle is lower and day-length shorter at the time of canopy leaf-out. The findings of these studies allow us to speculate that solar radiation available to understorey species could conceivably be reduced by climate change, and this reduction would be canopy species-specific.
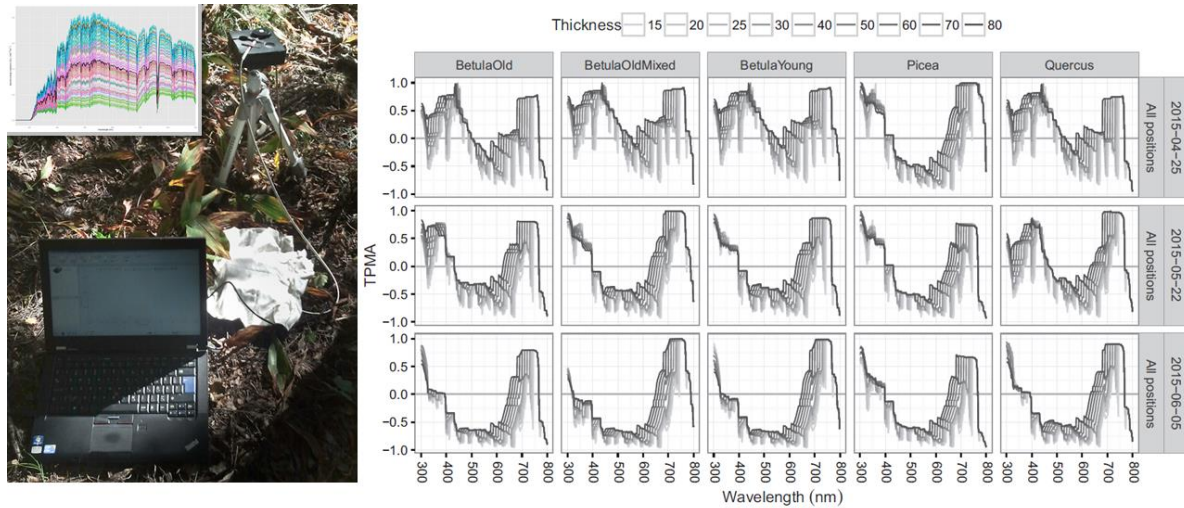
Figure 2. The image on the left shows, the set up for spectroradiometer measurements with plotted solar spectra (across UV to NIR regions, and expressed as spectral photon irradiance) during dynamic light conditions (over measurement of 100 spectra). On the right, Thick Pen Measure of Association (TPMA, negative values indicate dissimilarity and positive values similarity among spectra) where normalized spectra from differing understorey positions are pooled and compared per different stand and date during spring. The effect of using differing thicknesses of "pens" is shown as different shades of grey. The figure shows a decrease in similarity of the spectra over time, and the lowest cross-dependence between spectra from differing understorey positions in the evergreen *Picea abies* stand during early spring in comparison to other deciduous stands. Detailed in article I, TPMA figure from article I.

3.2. Seasonal, stand- and species-specific trends in the I_{flav} of understorey species

All results in this section concern findings in article II, which are discussed in the context of my research questions. We found similar seasonal trends in the I_{flav} of understorey plants from two consecutive years, although these trends deviated from each other during spring (with non-overlapping 95% CI) and I_{flav} values were slightly higher in 2015 than 2016 (Figure 3). Trends in I_{flav} from understorey plants growing in different forest stands differed, whereby the I_{flav} from the *P. abies* stand was lowest, but seasonal trends in I_{flav} from the three *Betula* stands were indistinguishable (overlapping 95% CI). These trends in I_{flav} were similar between the two years studied, but the differences were slightly less distinct for 2016. Furthermore, the I_{flav} trend of plants in the deciduous *Q. robur* stand declined more gradually after the spring peak I_{flav} values (from DOY 142-144 onwards) compared to I_{flav} trends from all the other stands. However, when considered as averages across measurements points, as community weighted means and using 2016 measurements, the I_{flav} from the *Q. robur* stand was no longer distinguishable from the *Betula* stands. There are no published time-series of changes in the I_{flav} or flavonoid content of understorey plants for comparison with our results, but previous studies reporting seasonal patterns in leaf flavonoids and related phenolics in trees, bear some resemblance to the patterns found from our data (Kotilainen et al., 2010). Differing seasonal patterns in various Mediterranean species have been associated with species-specific ontogenic changes in leaf surface features, whereby, for instance, young leaves dense in trichomes had high concentration of UV-absorbing compounds (Liakoura et al., 2001). Furthermore, there is some experimental evidence that differences in the interception of UV-B radiation among leaves within an individual plant may be connected with differences in the total flavonoid

content of leaves of kale (*Brassica oleracea* L. var. *Acephala*) (Yoon et al., 2021). Some of our sampled species have peculiar leaf features (e.g., the convex epidermal cells of *Oxalis acetosella*: Myers et al., 1994, and the hairy winter leaves of *Fragaria vesca*: Åström et al., 2015) which could potentially affect their seasonal patterns in optically measured I_{flav}. However, the quite consistent seasonal patterns that we recorded from various species displaying differing leaf anatomical features and leaf retention strategies suggests that leaf morphological divergence and features, such as those mentioned above did not determine the seasonal I_{flav} trends.

For two of the species with overwintering leaves (*F. vesca* and *O. acetosella*), the spring-time stand-related differences in I_{flav} were no longer evident during summer in mid-July when I_{flav} reached its seasonally lowest values, whilst in autumn significant differences in I_{flav} were once again found. These temporal patterns were consistent with the results from comparing species-specific trends in adaxial and abaxial I_{flav}, whereby the adaxial I_{flav} was typically higher but where we found no differences in I_{flav} values between leaf sides for *F. vesca* and one other species, *H. nobilis*, at the time of their seasonal minimal values. We also found that leaf adaxial and abaxial I_{flav} changed in a species-specific manner over growing season. Furthermore, the I_{flav} of understorey species differed in leaves according to their age in two summer green species, *C. persicifolia* and *C. majalis*, and the timing of the new leaf production contributed to differences in the I_{flav} of leaves in four species with overwintering leaves (*F. vesca, H. nobilis, O. acetosella* and *V. vitis-idaea*). In these species, new leaves produced after the start of the growing season had lower I_{flav} values compared to mature leaves at the same DOY. A higher investment in stress-tolerance among shade-tolerant species (reviewed by Valladares and Niinemets, 2008), as well as investment in protection from photodamage in long-lasting leaves, has sometimes been proposed particularly in tree species (Close and McArthur, 2002). Furthermore, there is some indication that leaf flavonoids can differ between sun and shade species of Brassicaceae growing under similar light treatments (Reifenrath and Müller, 2007). We found that patterns of investment in leaf flavonoids were complex, with stand- and species-specific differences in understorey species. For instance, in those species with long-lasting leaves (e.g., *Hepatica nobilis*) there were often detectable seasonal changes, or potentially even down-regulation of leaf flavonoids during spring and summer, among leaves produced in early spring. However, our understorey setup lacks an I_{flav} reference against real open-habitat species and conditions, and hence we are unable to make a direct comparison between the responses of species with shade-tolerant and shade-intolerant strategies. For tree species, general phenotypic plasticity of leaf traits from shade-tolerant species is suggested to be lower compared to shade-intolerant species (Portsmuth and Niinemets, 2007, Valladares and Niinemets, 2008). On the contrary, a combination of traits, including I_{flav}, in 12 shade-tolerant understorey forb species were more plastic than 11 shade-intolerant species traits in response to changes in various spectral regions in an experimental study using filters to attenuate solar radiation (Wang et al., 2020). We found plasticity in optically measured I_{flav} from mostly herbaceous understorey species, however this should be considered as a finding specific to this leaf trait, not scalable to other leaf traits of understorey species, commonly measured in plants.
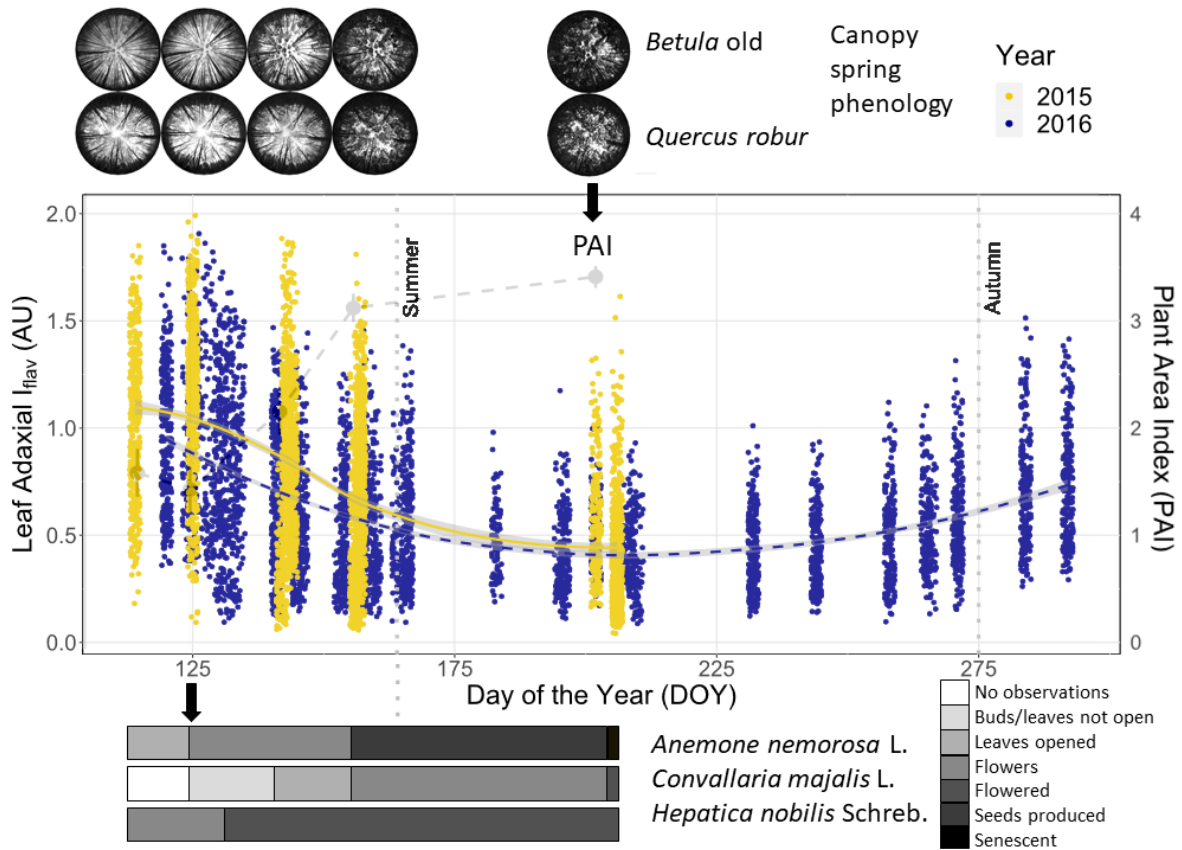
Figure 3. Seasonal trends in leaf adaxial I_{flav} from two consecutive years (2015 & 2016) measured from understorey plants growing in five forest stands. Trendlines are given by loess fit to the cloud of points annually with grey 95 % CI. Vertical dashed lines marking the approximate beginning of summer (mean daily air temperatures continuously above +10°C) and autumn (subsequent mean daily air temperatures continuously below +10°C). The average development of mean ($\pm$ SE) plant area index (PAI, m^2 m^{-2}) of all deciduous stands during spring is shown as grey points with dashed line (y-axis on the right). Above, is a time-course of hemispherical photographs for two example stands (*Betula* old & *Quercus robur*). Below, grey shaded areas represent the time course of spring phenology of three common understorey species. Here, each category starts from the time of its first observation (phenology surveyed five-six times during spring). Figure modified from the original in article II.

3.3. Comparing the trends in spectral irradiance in the understorey with the I_{flav} of understorey species

We found a strong positive relationship among integrals of spectral irradiance measured in understorey shade and the mean I_{flav} of understorey plants (II). However, this relationship was often compounded by the stand-specificity. For the tested integrals of spectral irradiance, and effective UV doses calculated according to BSWFs (FLAV, PG and GEN(G) action spectra), we found the model using PG and unweighted UV-A irradiance measured from shade gave the best fit for explaining the changes in mean I_{flav} of understorey plants (SM A2 in II). However, these results fail to fully explain the higher I_{flav} in early spring in the evergreen stand, especially for sunfleck irradiance where there was a negative relationship between mean I_{flav} and spectral irradiance throughout the spring and summer season. It is noteworthy that we tested these co-linear variables separately and these results should hence be treated with some caution.

Nevertheless, previous research has found similar seasonal trends to ours in leaf flavonoids from the leaves of *Betula* trees in an open field (Kotilainen et al., 2010), whereby high early season values could not be explained by UV radiation alone. Seasonal trends in the accumulation of UV-absorbing pigments in *Arabidopsis* were also present under attenuated solar UV radiation following short-term monthly exposure outdoors (Coffey et al., 2017). Recent studies further suggest that perception of differing wavelengths of solar UV radiation (below and above 350 nm) and blue light is mediated through different photoreceptors with interactions in their signalling pathways (Rai et al., 2018 & 2020). This suggests that there may be important differences e.g., in phenolic accumulation in response to UV below and above 350 nm or a combination of these wavelengths.

Our models using daily minimum temperature as the explanatory variable managed to only poorly explain the changes in adaxial or abaxial mean I_{flav} of understorey plants (SM A2 in II), but we recognise that many studies have found strong evidence for low temperature induced flavonoid accumulation (e.g., Bilger et al., 2007) and that temperature likely played some role in the trends in I_{flav} that we recorded. Overall, past studies of seasonal trends in leaf phenolics (e.g., Kotilainen et al., 2010, Nenadis et al., 2015, Coffey and Jansen, 2019) in combination with ours suggest that other environmental factors and developmental processes, that often change seasonally in addition to UV radiation, are also likely to contribute to the trends we report in the I_{flav} of understorey species.

3.4. Comparison of optically measured leaf I_{flav} and leaf extracts

We compared the relationship between I_{flav} and leaf extracts from five understorey species in Lammi forest stands throughout spring and summer, and from 50 taxa from the alpine botanical garden. The strength of this relationship was tested across several spectral regions: mean absorbance within the UV-B, UV-A, and UV regions, plus at 375 nm wavelength corresponding to that used by the Dualex for I_{flav} (Cerovic et al., 2012). Of these comparisons, the absorbance of leaf extracts at 375 nm produced the weakest relationship with mean I_{flav} of understorey plants (II, SM A2 of II, Figure 4). On the contrary, for plants from the alpine botanical garden absorbance at 375 nm was found to have the strongest relationship with I_{flav} among all the tested wavelength regions (those above and additionally at 305 nm) (SM A3 of III, Figure 4). Regardless of the ostensibly differing results from the two locations, at both study sites the relationship between I_{flav} and absorbance of leaf extracts at 375 nm was similar (Lammi $r = 0.36$, $p \leq 0.0001$, 95 % CI: 0.28-0.44, $n = 495$; alpine botanical garden $r = 0.41$, $p \leq 0.0001$, 95 % CI: 0.28-0.52, $n = 204$ for fresh leaf samples).

For the changes in the I_{flav} of understorey species over the spring and summer, the best model fit was obtained using mean absorbance of leaf extracts integrated over the UV-B region as the explanatory variable for three species (*A. podagraria, C. majalis, O. acetosella*), while mean absorbance integrated over the UV-A region gave the best fit for two species (*A. nemorosa, H. nobilis*) (II, SM A2 of II). Hence, the seasonal relationship between I_{flav} and absorbance of leaf extracts differed in species-specific manner, with respect to which spectral region gave the best relationship, and in some cases no region produced a strong relationship e.g., in *C. majalis* (II). Low I_{flav} values which expressed little seasonal variation may have contributed to this result for *C. majalis*. Our results further indicated that there might have been seasonal differences in the relationship of I_{flav} with the absorbance of leaf extracts, whereby high values of I_{flav} during spring were not apparent as clear increase in the absorbance in leaf extracts in all cases (II, Figure 4: *Aegopodium podagraria*). We can speculate that a there could

be a differing contribution of compounds measured by Dualex, possibly changing seasonally, to the whole leaf phenolics pool. Some studies have found that compounds measured with Dualex may contribute only a small portion of the whole leaf flavonoid content, e.g., when monitoring diurnal changes in *Vitis vinifera* leaves (Csepregi et al., 2019), while in some other cases epidermal flavonoids, estimated via optical measurement, have been suggested to constitute a large proportion of all leaf UV-absorbing compounds in leaf extracts (e.g., Bilger et al., 2007). One commonly reported qualitative change is an increase in ratio of quercetin to kaempferol glycosides when solar irradiance increases, and this may reflect as an increase in the absorbance of leaf extracts within the UV-A region relative to the UV-B region (Agati et al., 2010, Majer et al., 2014). These types of qualitative changes might partly explain the differences in the relationship between I_{flav} and leaf extracts that we found. Results from the alpine botanical garden showed there were differences between monocotyledon and dicotyledon taxa in the relationship between their I_{flav} and absorbance of leaf extracts at 375 nm, whereby this relationship was significant only for dicotyledon taxa (SM A3 of III). Particularly, two *Lilium* species with the highest I_{flav}, yet low absorbance of leaf extracts may have been partially responsible for the lack of a significant relationship (Figure 4, SM A3 of III). However, we did not find a difference in this relationship among groups of taxa with maximum UV absorbance around 290 nm or around 330 nm, distinguished according to the maximum of normalized UV spectra (SM A3 of III). This may imply that in terms of optical leaf-clip method, qualitative differences between groups of taxa (those with maximum ~290/~330 nm) were less important than those between mono- vs dicotyledon taxa. Normalization of the UV spectra distinguished four groups in total: maximum UV absorbance ~290 nm, ~330 nm, ~350 nm and ~360 nm (Figure 4, SM A3 of III). However, our further inspection of the UV absorbance spectra using TPT and TPMA found that there was mostly high dissimilarity in the shape of the UV spectra (low TPMA) among these groups, apart from two *Allium* species (maximum ~350 nm) (SM A3 of III). Although TPMA indicated dissimilarity of the spectra among species from same genus in most cases, they often displayed a clear visual resemblance (SM A3 of III). We also tested for a phylogenetic signal (Pagel's λ) from these data to investigate how absorbance of leaf extracts in different wavelength regions compares to optically measured leaf traits along the phylogeny (Figure 4, SM A3 of III). We found slightly higher significant λ values (λ = 0.65-0.70, $p \leq 0.0001$ mean abs. 305 nm & UV-B region; λ = 0.68, $p < 0.05$ mean abs. at 375 nm; λ = 0.72, $p \leq 0.0001$ max. UV wavelength) compared to our larger optically measured dataset from the alpine botanical garden (presented in the sections to follow), and insignificant results for all optically measured leaf traits among these 49 taxa (Figure 4, SM A3 of III). This suggests that the phylogenetic relatedness of these taxa may more clearly explain their UV absorbance of leaf extracts than their optically measured I_{flav}. This may be down to taxonomic divergence in the composition of the leaf phenolics, expressed in the shape of the absorbance spectra. Although some caution should be exercised when using I_{flav} across species to estimate leaf flavonols/flavones, the leaf-clip method is beneficial in allowing repetitive *in vivo* estimates of leaf UV-A absorbance.

Multispectral- and hyperspectral methods have potential as future non-destructive methods for screening and studying leaf traits in ecosystems (e.g., Asner et al., 2011 and 2012, Schweiger et al., 2018) and under experimental setups (e.g., Pandey et al., 2017, Behmann et al., 2018). At the leaf level, some studies suggest that application of previously used reflectance-based phenolic indices, such as R_{460}/R_{410} and $FLAVI_{700,760}$, may be inadequate to describe a change in flavonoids particularly from medium to high concentrations in comparison

to chlorophyll fluorescence-based methods (Sytar et al., 2020). Hence, there may still be a need for a variety of *in vivo* and *in vitro* approaches, which can be combined to describe functional and quantitative changes in UV-absorbing compounds under differing research scenarios.
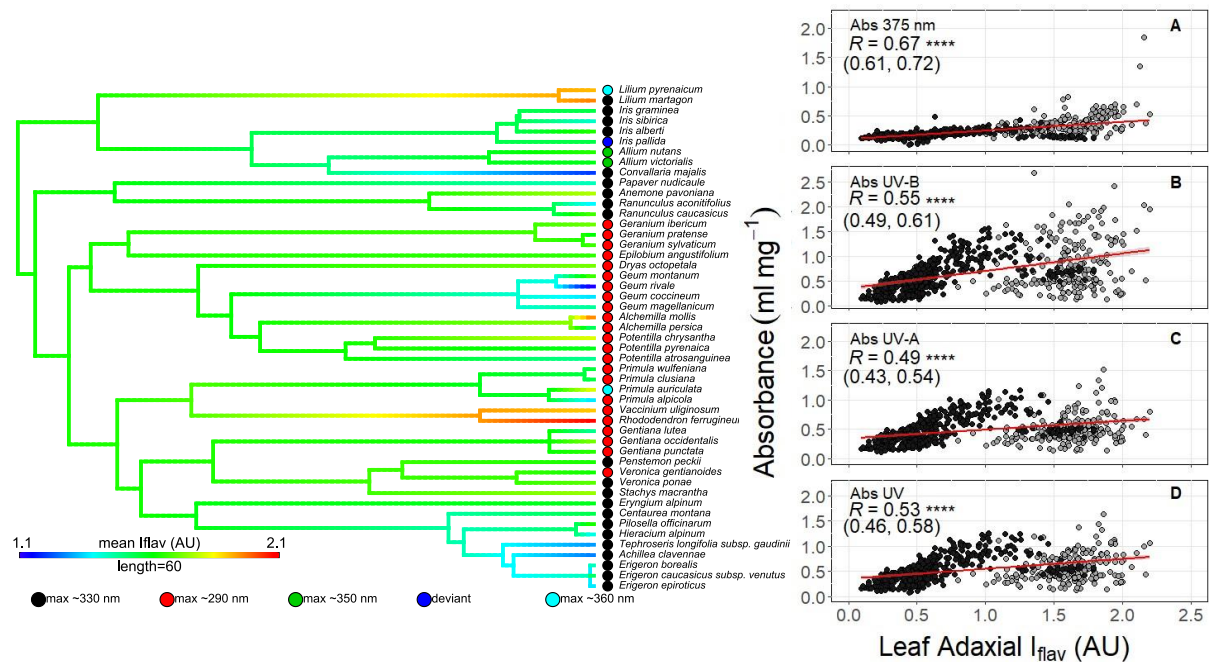
Figure 4. On the left, the phylogeny used for the 49 taxa studied (one genus level measurement was omitted) from the alpine botanical garden (Col du Lautaret, French Alps) based on a published mega-tree (GBOTB.extended.tre) and methodology (Scenario 1) from Jin and Qian (2019). It is plotted with a colour scale based on mean leaf trait values (I_{flav}, Arbitrary Unit) by using R package phytools (Revell, 2012 & 2013). Colour bar length is a scale for branch lengths (million years). Grouping is based on the maximum absorbance values within UV region (290-400 nm) of the leaf extracts, indicated as a coloured circle at the tip of each branch. The plotting method estimates ancestral trait values, but the estimate of uncertainty is not shown - these ancestral values are displayed largely to aid visual clarity. Infraspecific taxa (i.e., subspecies and variety) were included in the phylogeny by combining them with their parental species. The panels on the right show scatterplots with Spearman's rank correlation and respective significance level (* <0.05, **≤0.01, ***≤0.001, ****≤0.0001) for absorbance of leaf extracts from forest understoreys (Lammi, Finland = **FI**, 2016, 5 species through spring and summer) and the alpine botanical garden (French Alps = **FR**, 2015, 50 taxa during summer). Panel (**A**) absorbance at 375 nm, (**B**) mean absorbance within UV-B region & (**C**) UV-A region, and (**D**) whole UV region respectively with the linear trend lines plotted to the cloud of points with 95% CI (grey band). The 95 % CI for Spearman's rank correlation (in parenthesis) were computed by bootstrapping (R function spearman.ci from R package RVAideMemoire). The highest I_{flav} values from Lammi (black dots on x-axis, seen clearly in **B-D**) which have low absorbance of leaf extracts, represent those from early spring *A. podagraria*. Figure of the phylogeny from article III.

3.5. General patterns in optically measured leaf traits from a large diversity of plants

All results in this section concern findings in article III, which are discussed in the context of my research questions. We found generally high I_{flav} to be common in our survey of optically

measured leaf traits (I_{flav}, I_{chl} and I_{ant}) from 680 sampled taxa (and developmental stages) growing in the alpine botanical garden (Figure 5). The Kohonen SOM, used to segregate these taxa based on their measured traits, produced six nodes with the highest loadings (in total of 249 taxa) with relatively high I_{flav}, while other leaf trait values varied among these nodes. Only 3.8 % of taxa measured from the alpine botanical garden had a mean $I_{flav} < 1.0$ AU, while low I_{flav} among 86 taxa growing in high latitude was more common (French Alps: mean $\pm$ SE I_{flav} = 1.53 $\pm$ 0.003, Finland: mean $\pm$ SE I_{flav} = 1.38 $\pm$ 0.017). At both sites, I_{flav} was lower in plants categorised as experiencing extended shading over the day compared to the mean I_{flav} of all plants. Furthermore, mean I_{flav} of plants showed a weak positive relationship with UV irradiance (UV-B & UV-A: r = 0.45-0.47, $p \leq 0.042$) and biologically effective UV doses calculated according to various BSWFs (e.g., FLAV: r = 0.46, p = 0.042). Despite its acknowledged role as a driver of flavonoid accumulation (Jenkins, 2017), received UV radiation failed to explain all detected differences among taxa. We found some species (e.g., *Sanguisorba dodecandra* Moretti) sampled from both botanical gardens, in which leaf I_{flav} did not differ between the two sites and maintained a narrow range of values. This might suggest that these species may have less flexibility in I_{flav} according to their environment. Earlier studies have also reported that some species are relatively unresponsive to changes in their environment regarding leaf flavonoids or epidermal UV-transmittance (Nybakken et al., 2004).

We also found significant interannual differences in I_{flav}, although the correlation (r = 0.58 and $p < 0.001$) between the values from two consecutive years indicated prevailing similarities in responses of the taxa from year to year. A weak negative relationship was found between I_{flav} range (max-min) and mean I_{flav} of plants, suggesting that taxa with high mean I_{flav} generally expressed slightly less flexibility in their I_{flav} or alternatively accumulated flavonols/flavones relatively readily to a ceiling level beyond which they did not further increase. Some studies suggest that species may differ in the flexibility of their epidermal UV-A transmittance, whereby less flexibility along an elevational gradient was found from the native mountain species *Vaccinium reticulatum* compared to the invasive species *Verbascum thapsus* L. (Barnes et al., 2017). Furthermore, two *Vicia faba* L. accessions, one originating from a northern latitude with low UV radiation (Sweden) and the other from the Andean mountains with high UV radiation (Ecuador), under experimental setup had differing flavonoid profiles in response to blue and UV-radiation when grown together in a filter experiment in Helsinki (Yan et al., 2019). These types of compound-specific differences may be important for leaf flavonoid responses of plants of differing origin and were potentially present in our setup, albeit they were not the focus in our study.

Of the other optically measured traits monitored, contrary to I_{flav}, only a minority of taxa from alpine botanical garden had relatively high I_{chl} or I_{ant} in Kohonen SOM. Plants with a relatively high I_{ant} also had the highest NPQ, although relatively high I_{ant} was consistent interannually only in e.g., some *Penstemon* species. In flowers, petal colours containing anthocyanins have been associated with concentration of flavones suggested to stem from enzyme sharing in flavonoid pathway (Tripp et al., 2018). We did not find general common patterns between leaf I_{flav} and I_{ant} except for two *Penstemon* species identified with both high I_{flav} and I_{ant}, suggesting that this was not a common strategy among taxa we sampled. Most of the sampled 88 taxa did not show significant drop from predawn to midday in F_v/F_m, and mean predawn F_v/F_m was 0.80 $\pm$ 0.003. However, among 38 taxa one or more predawn F_v/F_m value was below 0.78 compared to the optimal 0.83. Previous studies have reported depressed F_v/F_m values in mountain species (Fernández-Marín et al., 2020, Fernández-Marín et al., 2021),

which may explain some of these finding. Finally, the NPQ was generally high (mean ± SE at midday 3.74 ± 0.12) among the studied taxa. Most plants did not show indication of long-term photoinhibition potentially leading to photodamage, apart from a group of taxa with relatively low predawn F_v/F_m, relatively high Y(NO) and interestingly, without evident accumulation of I_{flav}. As mountain plant species are often found to use specific array of photoprotection mechanisms (Streb and Cornic, 2012, Fernández-Marín et al., 2021), the lack of accumulation of leaf flavonols/flavones among this group of taxa could either point to their employment of other protective mechanisms, or that photodamage was partially due to a lack of leaf flavonols/flavones. In conclusion, it seems that most studied taxa accumulated relatively high concentrations of leaf flavonols/flavones, employed high NPQ, and did not suffer from long-term photoinhibition, suggesting that these taxa were in general well adapted to their current environment.
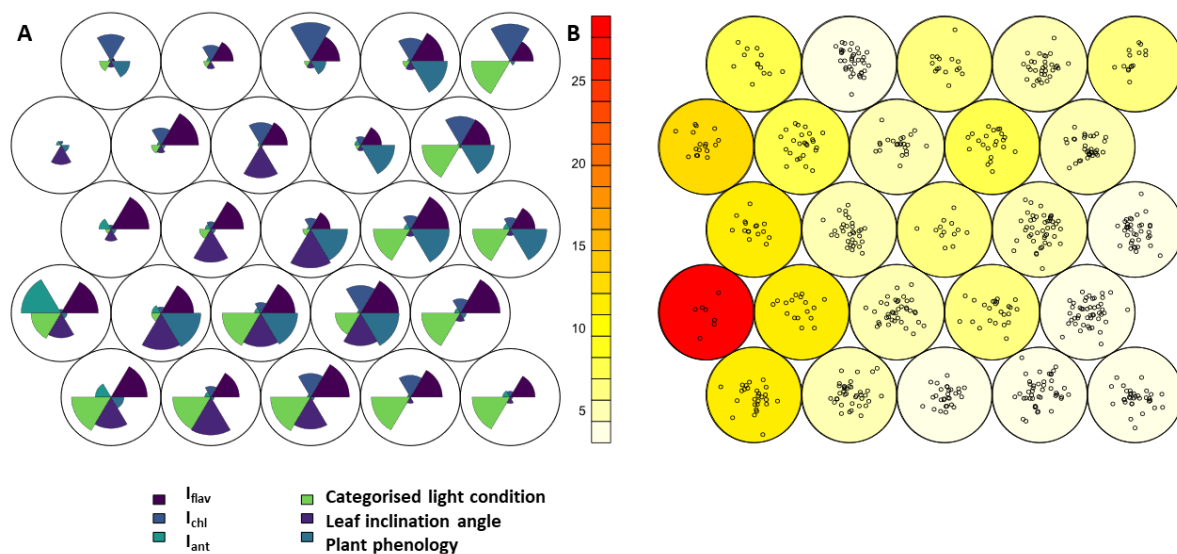
Figure 5. Relative differences in leaf traits from 680 taxa in the alpine botanical garden (Col du Lautaret, France) in 2014 shown in a hexagonally arranged Kohonen self-organising map (SOM) with 25 nodes. The Kohonen SOM produces a simple low-dimensional visualisation of patterns found in high-dimensional data using unsupervised learning (Kohonen, 1982, Wehrens and Kruisselbrink, 2018). Panel **A** shows codebook vectors associated to each unit. The relative differences according to the radius of each wedge in the three leaf traits in the upper half circle (I_{flav}: dark blue, above right wedge, I_{ant}: turquoise, above left wedge, I_{chl}: blue, above middle wedge). The lower half circle contains relative differences in categorised light condition of the plants, categorised phenological advancement, and categorised leaf inclination angle. Panel **B** shows loadings of the nodes i.e., the number of taxa grouped in the node as points, and neighbour distance (the sum of distances to all immediate neighbours) as a colour scale with red for the greatest distance. These analyses allowed those species sampled at different developmental stages to be distinguished when their trait values differed. Figure from manuscript III.

3.6. Comparing optically measured leaf traits and climatic conditions at plants' origin

All results in this section concern findings in article III, which are discussed in the context of my research questions. We did not find a significant relationship between the tested climatic conditions at plant origin and mean I_{flav} of plants, while for I_{chl} many relationships were

significant, and for I_{ant} the sole relationship was with elevation (r = -0.35, p = 0.0086). The weak positive relationship of I_{chl} with elevation (r = 0.47, p = 0.00031) and the weak negative relationship of I_{chl} with latitude (r = -0.35, p = 0.0084) may have contributed to significant relationships found between I_{chl} and the other climatic variables that vary along these gradients. However, previous studies have found long-lasting associations between leaf flavonoids and population origin, pointing to their local adaptation in *Pinus sylvestris* (Oleszek et al., 2002). In general, trends in phenolic compounds over elevational gradients have been reported specifically in tree species (positive relationship with latitude in *Juniperus*, Martz et al., 2009, positive relationship with latitude in quercetin derivatives and negative relationship with latitude in apigenin in *Betula pubescens,* Stark et al., 2008). It may be that, given the long generation time of trees, effects of past climate could be more important and more easily detected in trees than in herb species. For instance, studies show that the epidermis of conifer needles often transmit less UV-B radiation compared with leaf epidermis of herb species (Day et al., 1992). Beyond long-term associations, other transgenerational effects in response to UV radiation have also been reported lately (Jiang et al., 2021, Zhang et al., 2021), and for instance a northern-latitude accession of *Vicia faba* had a higher induction of quercetin under UV-B radiation in the generation following parental exposure to UV radiation below 350 nm (Yan et al., 2020). Although these long- and short-term effects reported by previous studies may be explained by different underlying mechanisms, it remains possible that these associations could potentially condition the environmental responses of plants (such as accumulation of leaf flavonoids), and hence when present, potentially constraining or facilitating species future success and migration in mountains.

The lack of relationship between I_{flav} and climatic conditions at the plant original collection site may relate to too few observations (n = 59), or the resolution of the climate data may have failed to correctly represent the conditions plants experience. For instance, variation in temperature micro-environments is known to occur even within short distances in mountains (Scherrer and Körner, 2010) and micro-environmental temperatures may differ from temperatures at the adjacent weather stations (Körner and Hiltbrunner, 2018). Overall, the lack of a relationship between climatic conditions at original collection site of plants and their mean I_{flav}, and the relationship found between mean I_{flav} and UV irradiance at the microhabitats of plants, suggests that most taxa adjusted their I_{flav} according to the prevailing conditions where they were growing.

3.7. Comparing optically measured leaf traits and relatedness of taxa from a large diversity of plants

All results in this section concern findings in article III, which are discussed in the context of my research questions. Our tests considering the patterns in optically measured leaf traits across the whole phylogeny, i.e., phylogenetic signal and correlograms both gave significant results, yet respectively low fitted λ values ($0 < λ < 1$) and low autocorrelation. However, we were able to identify local patterns (local Moran's *I*) within the phylogeny, where significant positive local autocorrelation revealed taxa with mainly high leaf trait values (high mean I_{flav}: *Alchemilla, Penstemon* and *Rhaponticum*) and taxa with mainly low leaf trait values (low mean I_{flav}: *Cerastium, Hieracium,* some *Sedum* and many Poaceae species) (Figure 6). These patterns in closely related taxa were in line with the findings from phylogenetic correlograms used to assess the signal depth, where positive autocorrelations were found for relatively short lags of distance in I_{flav}. Identified taxa with local positive autocorrelations, could imply lower variation

in their leaf flavonol/flavone accumulation. If so, particularly those taxa with mainly low mean I_{flav} could be constrained, potentially hindering their migration to habitats with increased UV radiation, unless other photoprotection mechanisms compensate for the low I_{flav}. On the contrary, taxa with high mean I_{flav} may better tolerate an increase in UV radiation. While UV-B is rarely considered to cause damage and stress to plants in nature (Paul and Gwynn-Jones, 2003), acute exposure to high irradiances of UV-B can induce non-specific stress signalling pathways, besides those specific regulatory responses to UV-B radiation (Jenkins, 2017). It is speculated that fluctuations in UV-B radiation could activate non-specific signalling pathways, especially if plants acclimated to lower levels of radiation are subjected to markedly higher levels of UV-B (Jenkins, 2017). Hence, these effects of acute high solar irradiances enriched in UV-B radiation might still be worth studying in context of future range shifts (e.g., invasive species: Watermann et al., 2020).

The higher occurrence of positive autocorrelations (I_{flav}: 79.7 %, I_{chl}: 77.1 %, I_{ant}: 86.2 %), indicated that closely related taxa mostly had similar patterns in I_{flav} values, rather than differing ones (Figure 6). Similar local analysis performed for flavones in numerous species from one genus *Ruellia* was able to reveal high flavone content among lineages from xeric habitats which are also known for their phylogenetic niche conservatism (Tripp et al., 2018). Similarly, our analysis may have revealed more detailed patterns if it had been possible for us to include more species and infraspecific taxa per genus. Genera with mainly high and low mean I_{chl} and I_{ant} were also identified (high I_{chl}: *Allium, Narcissus, Iris*; high I_{ant}: *Penstemon, Lonicera, Ribes, Vaccinium,* low I_{chl}: *Lonicera, Ribes, Heuchera*; low I_{ant}: *Allium, Iris*). The local analysis (Moran's *I*) did not identify any of the same taxa as in the alpine botanical garden for their I_{flav} when same analysis was done for the smaller phylogeny using plants from Kumpula Botanical Garden, although they had 27 common species. This may be because taxa from genera producing the clearest result in local analysis (Moran's *I*) from the alpine botanical garden, were not present in Kumpula Botanical Garden.

As mentioned above, in our tests for a phylogenetic signal we found that the fitted λ value was intermediate, but gave significant results for I_{flav} and I_{ant} (λ = 0.53 & 0.48 respectively, *p* ≤0.0001) from the phylogeny using plants growing in the alpine botanical garden. For the smaller set of taxa growing at high latitude, Pagel's λ test produced significant results only for I_{chl} (λ = 0.51, *p* ≤0.0001) but not for the other two indices. Intermediate (0 < λ < 1) λ values indicate that the observed trait values resembled each other less than expected for this phylogeny under a Brownian motion model of trait evolution (i.e., random walk along the branch length). These significant results, yet low λ values, may partly be explained by testing the λ transformation against a phylogeny with λ = 0, in which relatedness explains none of the similarities in trait values (Revell, 2012, Swenson, 2014). This could imply that some of the patterns we identified by local analysis may have been strong enough to drive significant differences in the phylogenetic signal test. While there were many polytomies present in the phylogeny we used, Pagel's λ should be robust against potential issues stemming from the use of incompletely resolved phylogenies, and phylogenies with suboptimal branch-length information (Molina-Venegas and Rodríguez, 2017). Results from Pagel's λ were mainly similar to Blomberg's K and the autocorrelation-based approach, Moran's *I*. As the relationship between phylogenetic signals and evolutionary processes is complex (Losos, 2008, Revell et al., 2008, Münkemuller et al., 2012), it is not possible to make definitive conclusions about the underlying mechanisms responsible for the observed trait patterns among the taxa without further experiments.

Overall, our results suggest that the observed patterns in I$_{flav}$ among a large diversity of taxa, seemed to be associated with phylogenetic relatedness in some taxa, while in others different drivers appeared to be more important in explaining the patterns in these trait values. Those relatively closely related taxa which we identified for their mainly similar I$_{flav}$, may be interesting to consider for future studies on potential constraints in their responses to environmental changes.
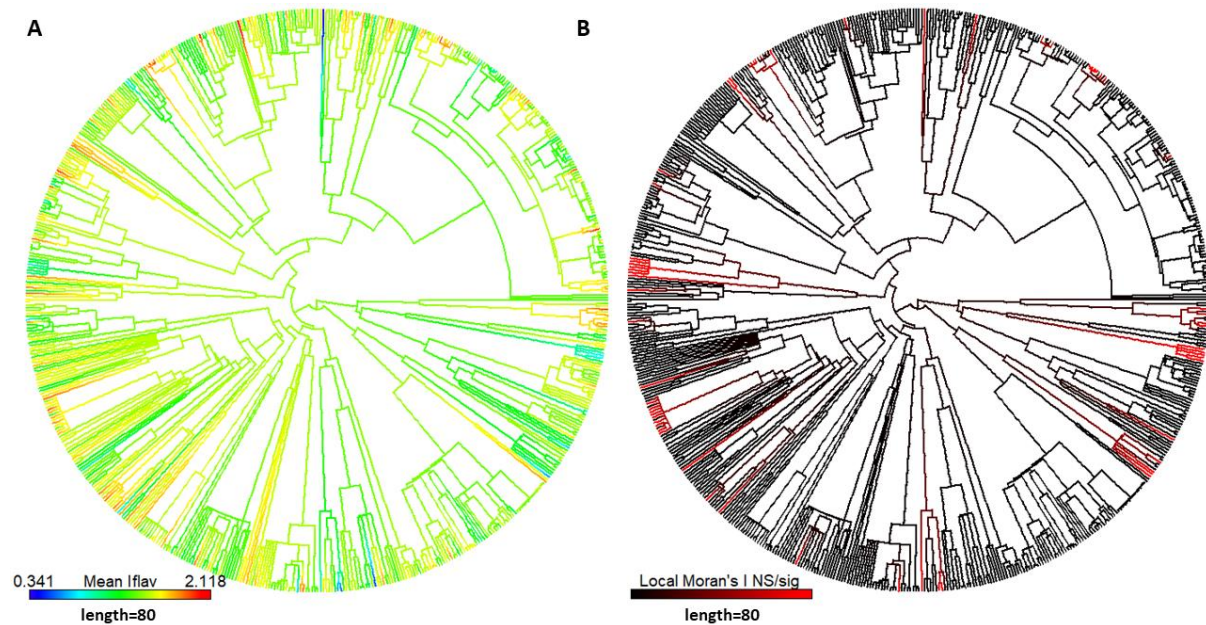

Figure 6. Phylogeny used for the 629 taxa studied from the alpine botanical garden (Col du Lautaret, French Alps) based on a previously published mega-tree (GBOTB.extended.tre) and methodology (scenario 1) from Jin and Qian (2019). Taxa are plotted with mean leaf trait values (panel **A**, I$_{flav}$, arbitrary units) as colours using R package phytools (Revell, 2012 & 2013). Infraspecific taxa (i.e., subspecies and variety) were included in the phylogeny by combining them with their parental species. Colour bar length gives a scale for branch lengths (million years). The plotting method estimates ancestral trait values, but the estimate of uncertainty is not shown, and ancestral values are mainly shown for visual clarity. The panel **B** shows the results (NS: black, p-value < 0.05: red) of testing the local indicator of phylogenetic association (local Moran's I) for I$_{flav}$, identifying local phylogenetic patterns plotted on the same tree. The local Moran's I identifies negative autocorrelations (differing trait values) and positive autocorrelations (similar trait values) in trait values among close relatives. Figure modified from manuscript III.

4. CONCLUSIONS AND FUTURE PERSPECTIVES

In conclusion, our spectral analyses allowed us to identify intricate differences in the shape of the spectra in forest understoreys, and found that the tested main effects responsible for these differences could be organised hierarchically. The major differences stemmed from understorey position, namely spectral irradiance in shade, leaf semi-shade or sunflecks, while differences stemming from stand composition and date over spring were on average smaller (I). We also found the amount of solar spectral irradiance to differ in stands producing stand-specific trends in spring, but this difference was no longer apparent in summer (II). We

designed a repeatable field protocol, combining pre-existing methodologies, as a practical solution to estimate plant area index, allowing a meaningful comparison of time series of hemispherical photographs (II). These results, revealing intricate differences, are important for characterising realistic plant light conditions, and may be utilised in experiments to advance understanding of plant responses under solar spectrum (Research Question 1).

We revealed seasonal stand- and species-specific patterns in optically measured I_{flav}, typically with a seasonal low reached in summer. These trends may be partly explained by forest stand-specific changes in the amount of irradiance in shade, particularly in the UV region. We were also able to point out understorey species-specific trends related to plants' leaf retention strategies and new leaf production, all important in understanding how these leaf traits are regulated producing a particular response to understorey environment (II, Research Questions 2 & 3).

Our study with a diversity of mountain taxa, revealed that generally plants did not express long-term photodamage and exhibited flexibility in their leaf traits according to their current environment, particularly the UV irradiance. We did not find a relationship between mean I_{flav} and climatic conditions at the plants' original collections site, hence no indication that the leaf I_{flav} was constrained as a legacy of climatic conditions at plants' original collection site. We found the phylogenetic signal to be weak for I_{flav}, suggesting that for most species the distribution of optically measured leaf traits did not seem to be confined by their phylogenetic relatedness. However, we were able to identify some genera containing species with mainly similar trait values, indicating similar response amongst species within (III, Research Questions 3-5).

We found that the general relationship between optically measured leaf I_{flav} and absorbance of leaf extracts at 375 nm was in most cases consistent across our datasets covering both alpine and forest understorey species (II, III). However, due to differences in leaf flavonoid composition, there are likely to be species-specific cases where caution should be used when interpreting leaf UV-A absorbance across different species. Consequently, we recommend calibration using crude leaf extracts, or preferably superior methods of analytical quantification (e.g. metabolomics) to establish this relationship when comparing species. The benefits of the optical trait approach are however demonstrated by our studies: we were able to assemble a reference database of optically measured indices of mean leaf traits covering wide range of taxa. These data provide estimates of adaxial leaf flavonols/flavones and anthocyanins, and of chlorophyll content measured from plants representing over 600 taxa growing in a high-elevation environment, and in forest understoreys (II, III, Research Question 6).

Considering future perspectives, the expected and observed advance in timing of spring phenology (e.g., Fitter and Fitter, 2002, reviewed by Parmesan and Hanley, 2015) may extend the period of closed canopy in deciduous forest canopies, and potentially affect the carbon gain of some understorey species (Heberling et al., 2019a, b). Furthermore, the probability of frost damage may increase with reduced snow cover during winter and spring (e.g., Inouye, 2008, Blume-Werry et al., 2016). Reduced snow cover, especially in polar regions is also expected to decrease the surface reflectivity, potentially decreasing back-scattered UV radiation (EEAP, 2019), but increasing the UV irradiance received by exposed plants no longer insulated by snow cover (Robson and Aphalo, 2019). Based on our results, we can speculate that under warmer future conditions with earlier canopy phenology and less snow at high latitudes in

Finland, there may be opposing responses in allocation towards leaf secondary metabolites among understorey species, according to their leaf retention strategy (II). Hence, acute cold temperatures and large temperature fluctuations would cause increased allocation to flavonoids in overwintering leaves, whilst summer green species would reduce their allocation, if not able to coordinate their phenology with the earlier forest canopy closure in deciduous forests (II).

Concerning mountain environments, range shifts of plant species have already been recorded, and this migration is expected to continue in the future for some species (reviewed by Parmesan and Hanley, 2015). Additionally, changes in cloud formation, surface reflectivity and aerosol abundance may affect the terrestrial UV radiation received by plants (reviewed by EEAP, 2019). For instance, cloud pattern changes on large-scales are expected to decrease cloud cover at mid-latitudes and to increase it at high latitudes, hence potentially increasing and decreasing UV radiation respectively (EEAP, 2019). All these effects considered, the capacity of plants to respond to and survive under new conditions can be regarded as critical knowledge. Among these responses it is important that plants can maintain photosynthetic capacity in their leaves, hence leaf traits that express photoprotection and the maintenance of physiological function can be used as an indicator of sufficiency. In this thesis work, flexibility in the optically measured leaf traits was found among most taxa studied, even across very different environments (II, III). The next step in this research would be to better assess the extent of this variation and the functional advantages it confers. Our results further suggests that some mountain plant taxa, but not all, with similar trait values, may reach the limitations of their leaf flavone/flavonol accumulation responses (III). This would have the potential to limit their future success if conditions deteriorate or they are required to migrate to a harsher environments in terms of solar radiation because of climate warming (III). In general across the three studies, these results provide evidence that optically measured leaf traits related to flavonoid accumulation are largely flexible and acclimate to local changes in the environment, as well as adjusting over the growing season.

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