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Leaf spectroscopy reveals post-fire phenolic chrono sequence in the Fynbos biome

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ABSTRACT

Fire is a key physical driver of ecosystem change, while plants rely on secondary metabolites such as phenolic compounds for chemical defense. Despite their importance, fire effects on secondary metabolites are often studied separately. Consequently, the mechanisms by which post-fire conditions shape the levels and diversity of secondary compounds in plant communities remain poorly understood. In this study, we used leaf spectroscopy to investigate how post-fire affects secondary metabolites in the fire-driven fynbos biome, a system rich in secondary metabolites. Results showed that both fire and secondary metabolites contribute to ecosystem rejuvenation. Areas subjected to recent fires in 2015, 2016, and 2021 exhibited high phenolic levels, whereas regions with limited fire in 2010 or no recent fire ("never_exp") had low phenolic concentrations. Using continuum removal, we identified the strongest spectral features for total phenols at 1660 nm and for flavonoids at 415 nm. Because chlorophyll also absorbs strongly in the 410–430 nm region, we evaluated and adjusted multiple spectral indices to improve flavonoid sensitivity. The Flavonoid Index (FI)_{415,710} and FI_{420,710} showed the strongest relationships with phenolic content ($R^2 = 0.55$; RRMSE ≈ 1.98 – 1.99%). Phenolic concentrations were also estimated using random forest regression. Prediction of total phenols was generally more accurate than flavonoids (total phenols: $R^2 = 0.82$, RRMSE = 12.54% ; flavonoids: $R^2 = 0.81$, RRMSE = 10.75%). Total phenols were best modeled using short-wave infrared (SWIR) spectra, while flavonoids were best modeled using full (VSWIR) spectra. Overall, our findings clarify the coupled roles of fire and secondary metabolites in supporting fynbos resilience and regeneration.

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1. Introduction

Globally, fire and plant secondary metabolites (PSMs) play a crucial role in maintaining ecosystem health and functioning. Fire is an essential ecological process that plays a huge role in numerous ecosystems such as fynbos, chaparral, sclerophyll communities, grassland, and savanna (Collins 1990; Keeley et al. 1981; Kraaij and van Wilgen 2014; Morgan 1999). It helps break down dead plants by removing old vegetation, which then allows new, nutrient-rich plants to grow (Smit, Baard, and van Wilgen 2024). In environments where natural decomposition is slow, causing litter to accumulate over time, fire breaks down the build-up and creates favourable conditions for seed germination and nutrient cycling (Bengtsson et al. 2011). This process enhances forage quality for herbivores and is vital for the regeneration of ecological communities (Neary and Leonard 2020).

Similarly, PSMs serve as defence compounds that help plants survive various biotic and abiotic stressors, including herbivory, pathogens, heavy metals, drought, UV radiation, and air pollution (Campbell et al. 2004; Kumar et al. 2020; Nandave et al. 2009; Porter 1992; van der Merwe, Van der Walt, and Marais 1994; Wand 1995). Plants respond to stress by producing specific secondary metabolites, including alkaloids, phenolic compounds, and terpenes (Chomel et al. 2016). For example, research indicates that the production of phenolic compounds increases when plants face stress, which enhances their resilience (Chomel et al. 2016; Xie et al. 2024). Just as fire acts as a physical driver of ecosystem change, PSMs function as a chemical defence mechanism, both contributing to the overall adaptability and survival of plant communities.

While a substantial body of research has explored the significance of fire and PSMs in the functionality of various ecosystems, however, these elements are often studied separately (Kozłowski 2012; Kraaij and van Wilgen 2014; Skidmore et al. 2010; Xie et al. 2024; Zhou et al. 2022). Most studies on post-fire remote sensing tend to emphasize structural recovery, focusing on biomass and vegetation cover (Di Mauro et al. 2014; X. Li et al. 2018; Sato et al. 2016). Therefore, there is still a gap in understanding how post-fire affects the levels and variety of secondary compounds in plant communities using remote sensing technology. In this regard, there is a need to investigate the application of remote sensing technologies to effectively monitor changes in PSM levels following fire events, offering a broader landscape-level perspective.

Arguably, remote sensing is one of the most cost-effective and efficient methods for studying the post-fire phenolic compound sequence over time. Its advantages include being non-destructive, covering large areas, and offering high spectral and temporal resolution, allowing researchers to examine changes over different periods (De Klerk 2008; Skidmore et al. 2010; Tshabalala et al. 2021; Zhou et al. 2023). This capability enables continuous monitoring and analysis of vegetation recovery and chemical responses over time, including revisiting previous conditions to better understand the dynamics of ecosystem resilience.

Furthermore, phenolic compounds possess distinct spectral features that can be detected by advanced sensors like the ASD FieldSpec3 (Sibiya et al. 2024; Zhou et al. 2022). These sensors have highly detailed spatial and spectral resolution due to their narrow bandwidths, enabling them to capture precise spectral information from plant canopies. The primary advantage of hyperspectral sensors is their ability to collect data

that closely resembles laboratory measurements, making them highly effective for detailed analysis of post-fire phenolic chrono sequence.

We therefore conducted this study in the fynbos biome found in the Great Cape Floristic Region (GCFR), South Africa, a fire-driven ecosystem and very rich in phenolic compounds (Bengtsson et al. 2011; Kumar et al. 2020; Nandave et al. 2009). The fynbos's slow decomposition rate leads to significant litter accumulation over time, which is then broken down by fire, facilitating seed germination and the establishment of new vegetation (Mitchell et al. 1986; Stock and Allsopp 1992). This vegetation subsequently grows on nutrient-poor soils, often sandy or stony, with limited nitrogen and phosphorus (Bengtsson et al. 2011; Magadlela et al. 2015). The resilience of native plant species in these harsh, stressful conditions raises questions about their adaptive mechanisms. Literature suggests that plants adapt to such stressors by producing secondary metabolites, particularly phenolic compounds (Chomel et al. 2016).

Xie et al. (2024) note that the level of phenolic compounds would increase if a plant faced stress. These compounds are an important part of the carbon in vascular plants, and their total is usually called total phenols (Vermerris and Nicholson 2007). Flavonoids are a specific subgroup within the broader class of phenolic compounds (Agati et al. 2013; Tshabalala et al. 2021; Vambe et al. 2024). Flavonoids have been widely researched for their important role in helping plants defend against different environmental stresses, such as UV radiation, pathogens, and herbivores (Wu et al. 2020). Furthermore, flavonoids play a crucial role in absorbing shortwave-blue light between 410 and 430 nm, similar to chlorophyll, which also absorbs this light spectrum (Gitelson et al. 2017).

To better detect and quantify flavonoids, it is important to minimize the influence of chlorophylls, to enhance their sensitivity. Consequently, various studies have proposed the formulation of vegetation indices that directly relate to flavonoid absorption characteristics (Gitelson et al. 2017; Zhou et al. 2022; 2023). In this study, therefore, we tested and modified these spectral indices in the fynbos ecosystem to evaluate their effectiveness in this unique, phenolic fire-adapted ecosystem. Additionally, we used hyperspectral remotely sensed data to estimate the concentrations of phenolic compounds (i.e. total phenol and flavonoid) using the random forest model.

2. Materials and methods

2.1. The study area

The research took place in three locations within the Overstrand Local Municipality in the Western Cape, South Africa: Sandberg Nature Reserve (-34.577953° E, 19.693025° S), Smith farm (-34.584551° E, 19.668460° S), and SANParks (South African National Parks) (-34.642366° E, 19.592418° S) (Figure 1). Sandberg Nature Reserve and Smith Farm are privately owned neighbouring areas, while the SANParks oversee national parks and protected areas as per the National Environmental Management: Protected Areas Act of 2003. The region has wet winters and dry summers, typical of a Mediterranean climate, leading to fires every 10 to 15 years (Smit, Baard, and van Wilgen 2024; Van Wilgen et al. 2010). Winter temperatures can fall to -3.2°C , while summer temperatures can increase to 47°C (Deacon, Jury, and Ellis 1992). Average yearly rainfall ranges from approximately 210 mm to more than 3000 mm, usually becoming higher with increasing altitude and as

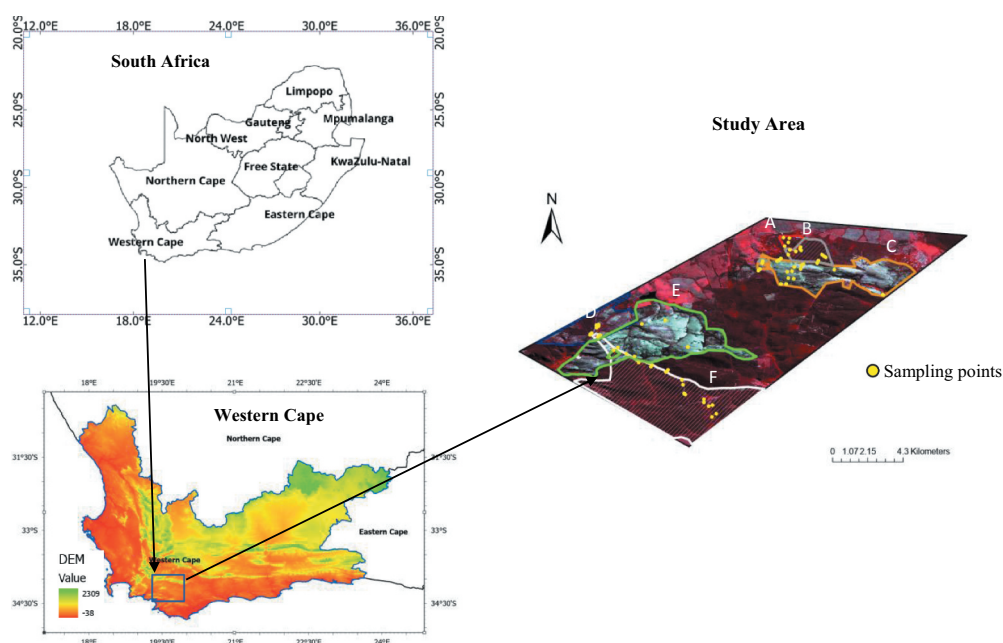


Figure 1. Location of study sites taken from 2016-sentinel-2 image and showing areas that have been burnt between 2010 and 2024: a = 2021 (May), B = never_exp, C = 2016 (February), D = 2015 (March), E = 2016 (April), F = 2010 (January).

one moves eastward (De Klerk 2008). The landscape features fynbos, a type of vegetation that includes small shrubs, evergreen plants, and bulbs, typically found in poor sandy/stony soils (Bengtsson et al. 2012). The dominant species encountered in the study site are shown in Figure 2.

2.2. Field sampling

The field data collection was conducted from March 3 to 8 March 2024. The experimental sampling design was based on areas that previously experienced fire events between 2010 and 2024. To locate burnt sites, satellite imagery from Landsat-7 (2010–2015) and Sentinel-2 (2015–2024) were employed from Google Earth Engine (GEE). Fire scars were mapped based on visual interpretation of Landsat-7 and Sentinel-2 image false colour composite (Sato et al. 2016) (Figure 1). As in Filipponi (2018) this approach was deemed sufficient, hence, burn indices were not employed. A total of 190 samples were collected from areas that experienced fire, as well as from areas that did not experience fire during this period – designated as ‘never-exp’. The collection dates and the corresponding number of points collected are as follows: 33 points in March 2015, 60 points in February 2016, 12 points in April 2016, 16 points in May 2021, and 37 points in January 2010. Additionally, 32 points were collected from the ‘never-exp’ areas.

To ensure accurate sampling, a high resolution submeter (50 cm) accurate handheld Trimble Global Navigation Satellite System (GNSS) was employed to mark the sampling points, with each point situated at least 200 metres from the

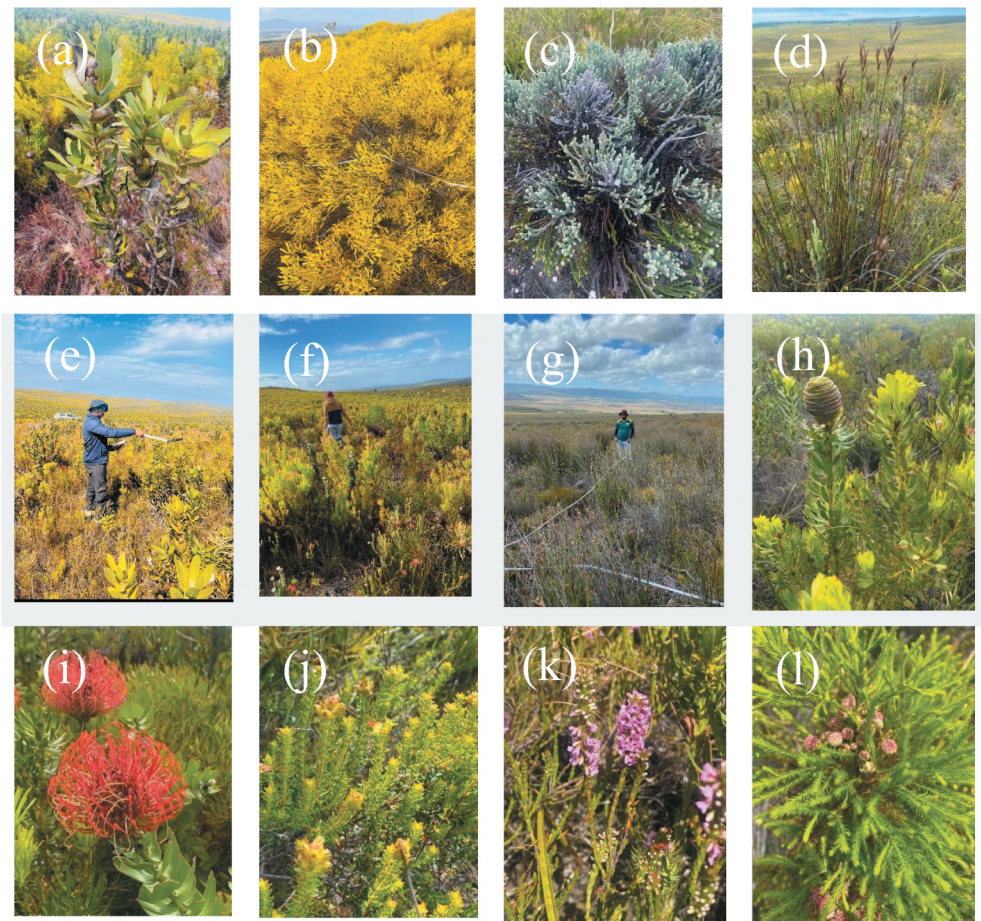


Figure 2. Typical vegetation in the study area. The plant species are as follows: (A). *Proteaceae* (B). *leucadendron platyspermum* (C). *brunia laevis* young (*Silver brunia*) (D). *elegia mucronate* (E). mixture of *Proteaceae* and *elegia mucronate* (F). *leucadendron* (G). *Restionaceae* (H). *leucadendron microcephalum* (I). *leucospermum cordifolium* (J). *thesium viridifolium* (K). *erica species* (L). *Berzelia Lanuginosa*.

road to mitigate road edge effects (Delgado et al. 2007; Lottering and Mutanga 2012). Upon arrival at each plot, two 10-metre tapes were laid perpendicularly as shown in Figure 3. Within each querent, leaf Area Index (LAI) was measured using a LiCOR 2200C plant canopy analyser (LI-COR GmbH, Germany) and Soil moisture content was measured using HH2 moisture probe (Delta-T soil moisture sensors, United states) recorded as a percent at each sample point. Additionally, leaf samples were randomly harvested from various canopy levels, top, middle, and lower branches, to capture intra-canopy variability from all four quadrants (Figure 3). In total 17,922.2g of leaves were harvested from dominant species and were placed in zipped plastic bags and then preserved in a cooler box with ice. The leaf samples were then transported back to a lab for spectral measurements. The spectral measurements were taken on fresh leaves harvested.

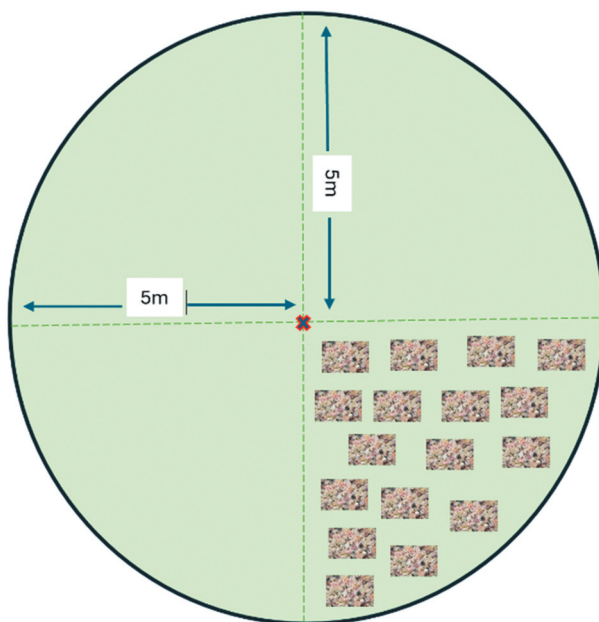


Figure 3. Sampling protocol depiction.

Thereafter, the sample leaves were dried in the oven at 80 °C for about 48 h until constant weight.

2.3. Biochemical analysis

After leaf spectra measurement, the sample leaves were dried in an oven at 80 °C for 48 h. Thereafter, the preserved samples were transferred to labelled 30 ml vials following the grinding process conducted with a coffee grinder. Subsequently, 0.5 g of the leaf powder was added to a centrifuge tube, along with 5 mL of a solution consisting of 50% water and 50% methanol (MeOH), which had been prepared in a reagent bottle. The samples were then sonicated in cold water for 20 min using an ultrasonic bath to extract compounds. Thereafter, the samples were centrifuged for another 20 min to separate the solid leaf powder from the liquid extract. The liquid extract was then carefully removed from the centrifuge tube and stored in a new labelled centrifuge tube. This extracted liquid was used for analysing total phenols and flavonoids as outlined by Vambe et al. (2024). The total phenols and flavonoids were assessed using the Folin-Ciocalteu and aluminium chloride assays (Makkar 1999). Table 1 outlines the specific assay procedures followed for quantifying the total phenol and total flavonoid in the leaf extracts.

2.4. Leaf reflectance measurements

Directional hemispherical reflectance measurements were performed on leaf samples using a FieldSpec 3® spectrometer, which is capable of detecting wavelengths from 350 to 2500 nm and recording data at 1 nm intervals (ASD Analytical Spectral

Table 1. Chemical assay procedures for quantifying total phenol and flavonoid.

Total phenol	Flavonoids
• In a test tube placed in a rack, mix 50 µL of the sample, 950 µL of H₂O, 500 µL of Folin-C, and 2.5 mL of sodium carbonate using pipette. • Garlic acid (0.1 mg/ml) was used as a standard (Figure 4(a)). • Incubate the reaction mixtures in the dark for 30 minutes. • Absorbance was then read at 725 nm using a spectrophotometer. • The assay was done in triplicate and results expressed as mean ± standard error.	• In a test tube placed in a rack, mix the following using a pipette: 250 µL of the sample, 1 mL of H₂O, 75 µL of sodium nitrite (NaNO₂), 75 µL of Aluminum chloride (AlCl₃), 0.5 mL of sodium hydroxide (NaOH). • Catechin (0.1 mg/mL) was used as a standard (Figure 4(b)). • Absorbance was read at 725 nm using a spectrophotometer. • The assay was done in triplicate and results expressed as mean ± standard error.

Devices 2016). This broad spectral range enables the collection of detailed information about the optical properties of the leaves. A total of 190 leaf samples were collected and stored in a dark room to ensure a controlled and consistent measurement environment. Spectral measurements were conducted promptly to minimize any deterioration of the samples. A halogen lamp was used as the light source, with a dark board placed in the background to eliminate interference from extraneous light.

Figure 5 illustrates the zenith angles of the light source and the viewing angle, along with the field of view (FOV) for the bare fibre optics. The orange colour bar indicates the light source used in conjunction with the ASD leaf clip, while the blue colour bar represents the bare fibre optics of the ASD spectrometer. In cases where a leaf sample was too small to cover the sensor port adequately, a leaf mat was created by stitching together multiple leaves side by side, ensuring minimal overlap. For the spectral measurements, data from five randomly selected leaves were averaged. Three scans were taken for each leaf to reduce spectral noise, and these averaged values were then used as the final leaf spectra (Lu, Proctor, and He 2021). All spectral measurements were conducted promptly to ensure the integrity of the leaf samples.

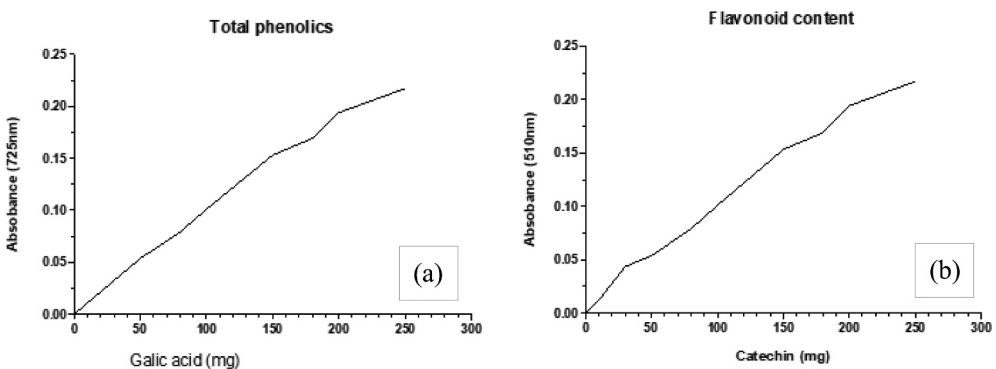


Figure 4. Standard calibration curves used to estimate the concentrations of phenolic compounds in plant extracts: (A) total phenol and (B) flavonoids.

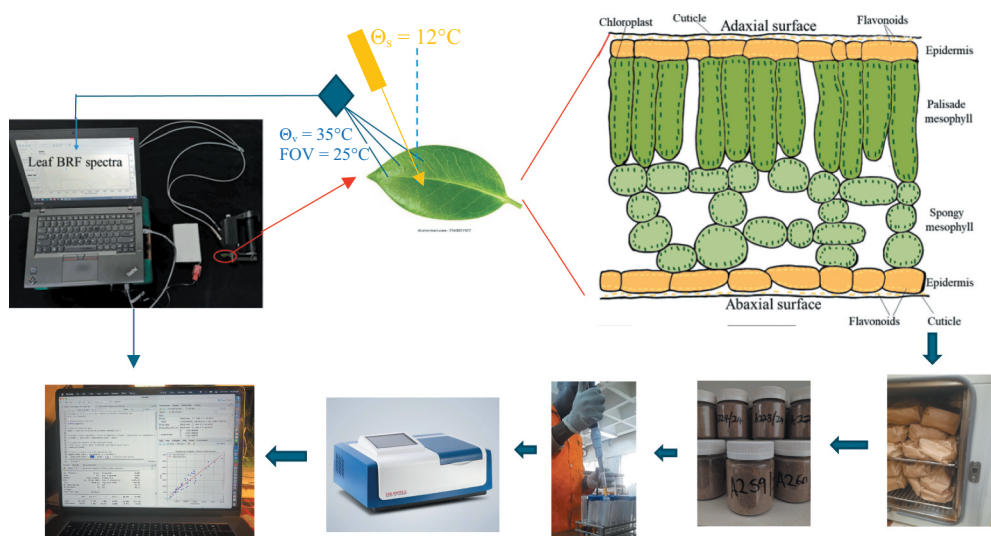


Figure 5. Illustrates the modeling of the relationships between the acquired leaf bidirectional reflectance factor (BRF) spectra and the measured phenolic compounds.

2.5. Spectral data processing

Prior to conducting spectral pre-processing on the leaf reflectance data, it was crucial to tackle the inadequate signal observed within certain spectral bands, specifically, the spectral range between 350–399 nm and between 2200–2500 nm were removed. Following the removal of the low-quality spectral regions, to enhance the smoothness, the Savitzky-Golay method with a second-degree polynomial was used to make the data smoother, using a moving window of 15 (Savitzky and Golay 1964; Xie et al. 2024). Thereafter, we used the Continuum removal method to preprocess the spectra (Clark and Roush 1984). The selection of the continuum removal technique for this study is underscored by its demonstrated efficacy in accurately estimating phenolic compounds. Numerous studies have validated the robustness of this method in diverse applications.

For instance, Tshabalala et al. (2021) conducted a comparative analysis involving first-order and second-order derivative techniques alongside continuum removal. Their findings revealed that the continuum removal approach consistently yielded the most reliable results in the accurate estimation of phenolic compounds. Furthermore, Xie et al. (2024) employed the continuum removal technique in their investigations, achieving successful results in the quantification of phenolic compounds. The adaptability and effectiveness of continuum removal can be attributed to its ability to suppress baseline noise and correct for scattering effects, thereby improving signal detection in complex mixtures.

The continuum was calculated using the ConvexHull approach, which involves fitting a convex polygon to the upper envelope of the smoothed spectrum. Using Python the ConvexHull function from the `scipy.spatial` module, the ConvexHull was imported. The application of Savitzky-Golay filtering and continuum removal techniques is the well-known pre-processing workflow to enhance the quality and interpretability of the spectral data by reducing noise, preserving spectral features, and isolating distinct absorption

traits associated with the biochemical and structural attributes of the leaves (Kokaly and Clark 1999; Mutanga et al. 2005). The application of these pre-processing steps is crucial for subsequent feature extraction, spectral modelling, and the development of robust predictive algorithms for various leaf and plant-related applications.

2.6. Calculation of vegetation indices

Flavonoids absorb shortwave blue light in the 410 to 430 nm range but do not absorb red or red-edge light (Gitelson et al. 2017). Conversely, chlorophyll absorbs blue, red, and red-edge light, with the red-edge spectrum shown to be particularly sensitive to chlorophyll content (Gitelson et al. 2017). To improve the detection of flavonoids and minimize the influence of other pigments, especially chlorophyll, specific formulas have been developed as shown in Table 2. In this study, we tested and modified these formulas from Zhou et al. (2022) to enhance their effectiveness. This was achieved by replacing 420 nm wavelength with 415 nm. A simple linear regression model was used to find the relationship between vegetation indices and flavonoid content.

2.7. Modelling of foliar phenolic compounds

In this study, the random forest algorithm was used to estimate total phenol and flavonoid concentrations using leaf spectroscopy in the Cape Floristic Region. Random forest is a robust machine learning method that excels in feature selection and predictive modelling effective in reducing overfitting, which is crucial for high-dimensional hyperspectral data (Chan and Paelinckx 2008). The random forest algorithm builds multiple decision trees by bootstrapping training data and randomly selecting input variables at each split (Ließ, Schmidt, and Glaser 2016). Each

Table 2. Utilized vegetation indices.

Group	Index	Formulation	Reference
$Fl_{\lambda1,\lambda2}$	$Fl_{420,710}$	$R_{420}^{-1} - R_{710}^{-1}$	Zhou et al. (2022) modified from Gitelson et al. (2017)
	$Fl_{415,710}$	$R_{415}^{-1} - R_{710}^{-1}$	Modified from Zhou et al. (2022)
$Fl_{\lambda1,\lambda2,800}$	$Fl_{420,710,800}$	$(R_{420}^{-1} - R_{710}^{-1}) \times 800$	Zhou et al. (2022) modified from Gitelson et al. (2017)
	$Fl_{415,710,800}$	$(R_{415}^{-1} - R_{710}^{-1}) \times 800$	Modified from Zhou et al. (2022)
	FRI	$(R_{410}^{-1} - R_{460}^{-1}) \times 800$	Merzlyak et al. (2005)
$mFl_{\lambda1,\lambda2}$	$mFl_{420,710}$	$(R_{420} - R_{445})^{-1} - (R_{710} - R_{445})^{-1}$	Zhou et al. (2022) modified from Gitelson et al. (2017)
	$mFl_{415,710}$	$(R_{415} - R_{445})^{-1} - (R_{710} - R_{445})^{-1}$	Modified from Zhou et al. (2022)
	$mFl_{420,690}$	$(R_{420} - R_{445})^{-1} - (R_{690} - R_{445})^{-1}$	Zhou et al. (2022)
	$mFl_{415,690}$	$(R_{415} - R_{445})^{-1} - (R_{690} - R_{445})^{-1}$	Modified from Zhou et al. (2022)
$mFl_{\lambda1,\lambda2,800}$	$mFl_{420,710,800}$	$[(R_{420} - R_{445})^{-1} - (R_{710} - R_{445})^{-1}] \times (R_{800} - R_{445})^{-1}$	Zhou et al. (2022) modified from Gitelson et al. (2017)
	$mFl_{415,710,800}$	$[(R_{415} - R_{445})^{-1} - (R_{710} - R_{445})^{-1}] \times (R_{800} - R_{445})^{-1}$	Modified from Zhou et al. (2022)
	$mFl_{420,690,800}$	$[(R_{420} - R_{445})^{-1} - (R_{690} - R_{445})^{-1}] \times (R_{690} - R_{445})^{-1}$	Zhou et al. (2022)
	$mFl_{415,690,800}$	$[(R_{415} - R_{445})^{-1} - (R_{690} - R_{445})^{-1}] \times (R_{690} - R_{445})^{-1}$	Modified from Zhou et al. (2022)
$DDblue_{\lambda1,\lambda2}$	$DDblue_{421,733}$	$(R_{445} - R_{421}) / (R_{455} - R_{433})$	Zhou et al. (2022)
$DDnir_{\lambda1,\lambda2}$	$DDnir_{434,421}$	$(R_{800} - R_{434}) / (R_{800} - R_{421})$	Zhou et al. (2022)

tree is created through recursive binary splitting, with the best split determined by criteria like mean squared error (Breiman 2001). Predictions for new samples are made by aggregating outputs from all trees, either through voting or averaging. To assess the performance of random forest, we tested various options: the full spectrum (400–2200 nm), visible to near-infrared (VNIR, 400–1000 nm), shortwave infrared (SWIR, 1000–2200 nm), and continuum-removed spectra from VNIR and SWIR. We selected the input yielding the most accurate predictions for further regression analyses. The random forest algorithm was implemented in Python 3.9.13 version.

2.8. Model validation and accuracy assessment

The accuracy of the random forest model was evaluated by comparing predicted values to the measured values in the test set. Validation metrics included the Normalized Root Mean Square Error (NRMSE (%)) and the coefficient of determination (R^2). R^2 (Equation 1) measures the correlation between the observed and predicted samples, and it ranges from 0 to 1. Where values closer to 1 depict a high accuracy (Singh et al. 2017), while $RRMSE\%$ (eq.2) is used to measure the scale of error from the measured values, expressed as a percentage (J. Li 2017). The dataset was divided using the `train_test_split` function from the `sklearn.model_selection` module in Python, allocating 70% of the data for training and 30% for testing. R

$$R^2 = 1 - \frac{\sum (Y_{measured} - Y_{predicted})^2}{\sum (Y_{measured} - Y_{measured})^2} \quad (1)$$

$$RRMSE\% = \frac{\sqrt{\frac{1}{n} \sum_{i=1}^N (Y_{measured} - Y_{predicted})^2}}{Y} \times 100 \quad (2)$$

3. Results

3.1. Changes in phenolic content post-fire

To locate areas affected by fire between 2010 and 2024, we used satellite data from Sentinel-2 and Landsat. This analysis revealed fire events in January 20 March 201015, February 20 April 201616, and May 2021, with the burnt areas clearly visible in [Figure 6](#). Furthermore, we identified one area, labelled 'never_exp', that remained unburnt throughout the study period ([Figure 3\(b\)](#)). Among areas that experienced fire, the most fire events occurred in January 2010 and in February and April of 2016, covering large areas. These months coincide with the dry period in the GCFR, which typically extends from late spring to early autumn. During this time, the region experiences its lowest rainfall, creating conditions conducive to fire outbreaks. This is evidenced by the high frequency of fire events recorded during these months.

[Figure 7](#) demonstrates the vertical distribution of total phenol and flavonoid concentrations subsequent to post-fire events. The data reveal that the concentrations of these phenolic compounds exhibited comparable trends throughout the post-fire years.

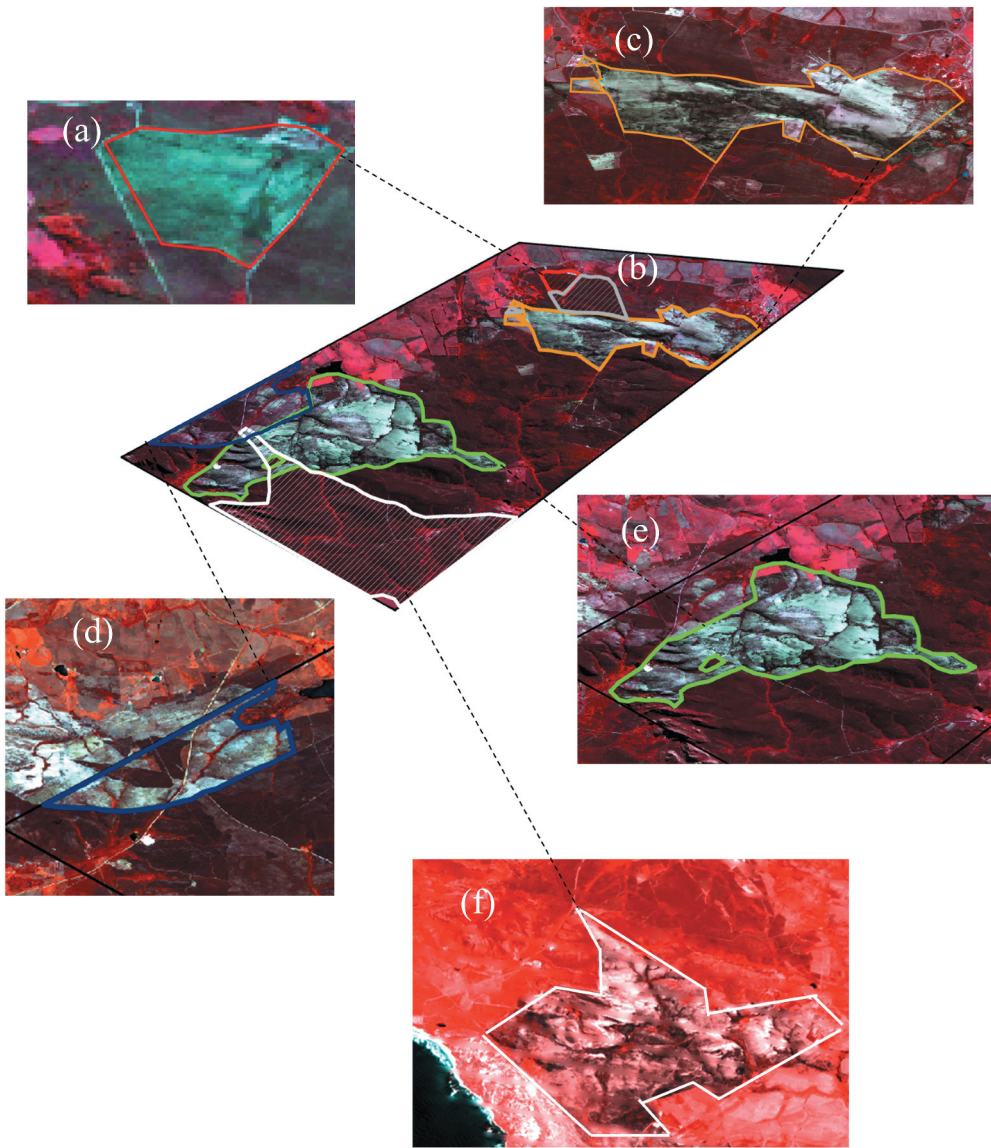


Figure 6. Location of areas that have been burnt between 2010 and 2024 using false colour composite: a = 2021 (May), B = never_exp, C = 2016 (February), D = 2015 (March), E = 2016 (April), F = 2010 (January).

Specifically, the highest concentrations were recorded in 2021 (May), followed by those in 2016 (April).

3.2. Evaluation of leaf spectra

3.2.1. Comparison of the spectral characteristics

We observed notable differences in mean reflectance and spectral variation (%) between areas previously affected by fire and those unaffected [Figure 8](#)). The

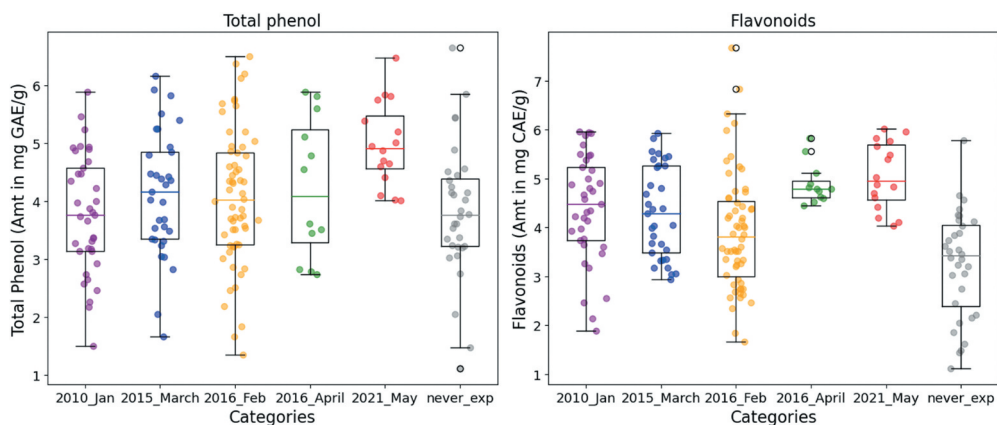


Figure 7. Variation in total phenols and flavonoids in five areas that had fires and areas that have not experienced fires from 2010 to 2024.

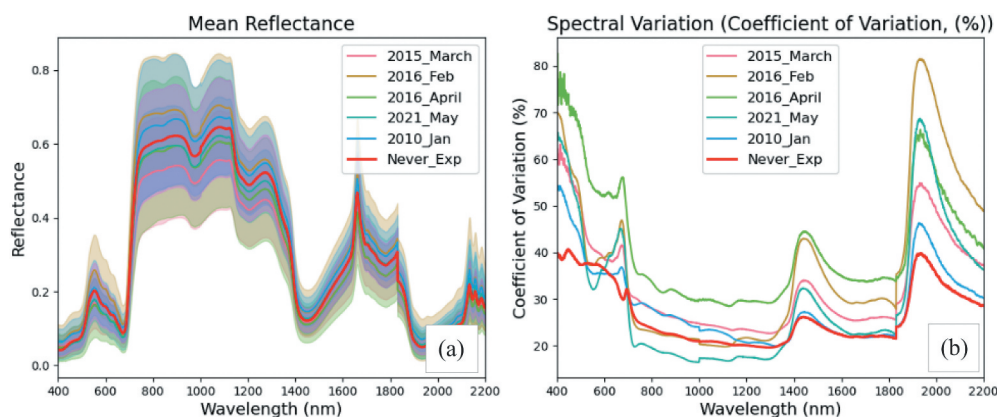


Figure 8. Comparing the average reflectance and spectral coefficient of variation of five areas that experienced fire with areas that have not experienced fire during this period (A and B).

results show that the highest mean reflectance was recorded in January 2010 and February 2016. Conversely, March 2015 exhibited the lowest mean reflectance. In terms of spectral variation (coefficient of variation %), the highest levels were observed in April 2016 and February 2016, while the lowest levels were found in January 2010 and in the 'never_exp' areas. Notably, peaks were identified in the visible region of the spectra, especially between 400–430 nm, while the highest was observed in the Short-Wave Infrared (SWIR) region; indicative of phenolic compounds.

3.2.2. Influence of leaf area index and soil moisture on phenolic compounds in plants

Figure 9 illustrates the relationships between phenolic compounds and leaf area index (LAI), and phenolic compounds with soil moisture content (%), using linear modelling. The data reveal a linear relationship between both phenolic compounds and LAI, indicating that as LAI increases, the concentration of phenolic compounds

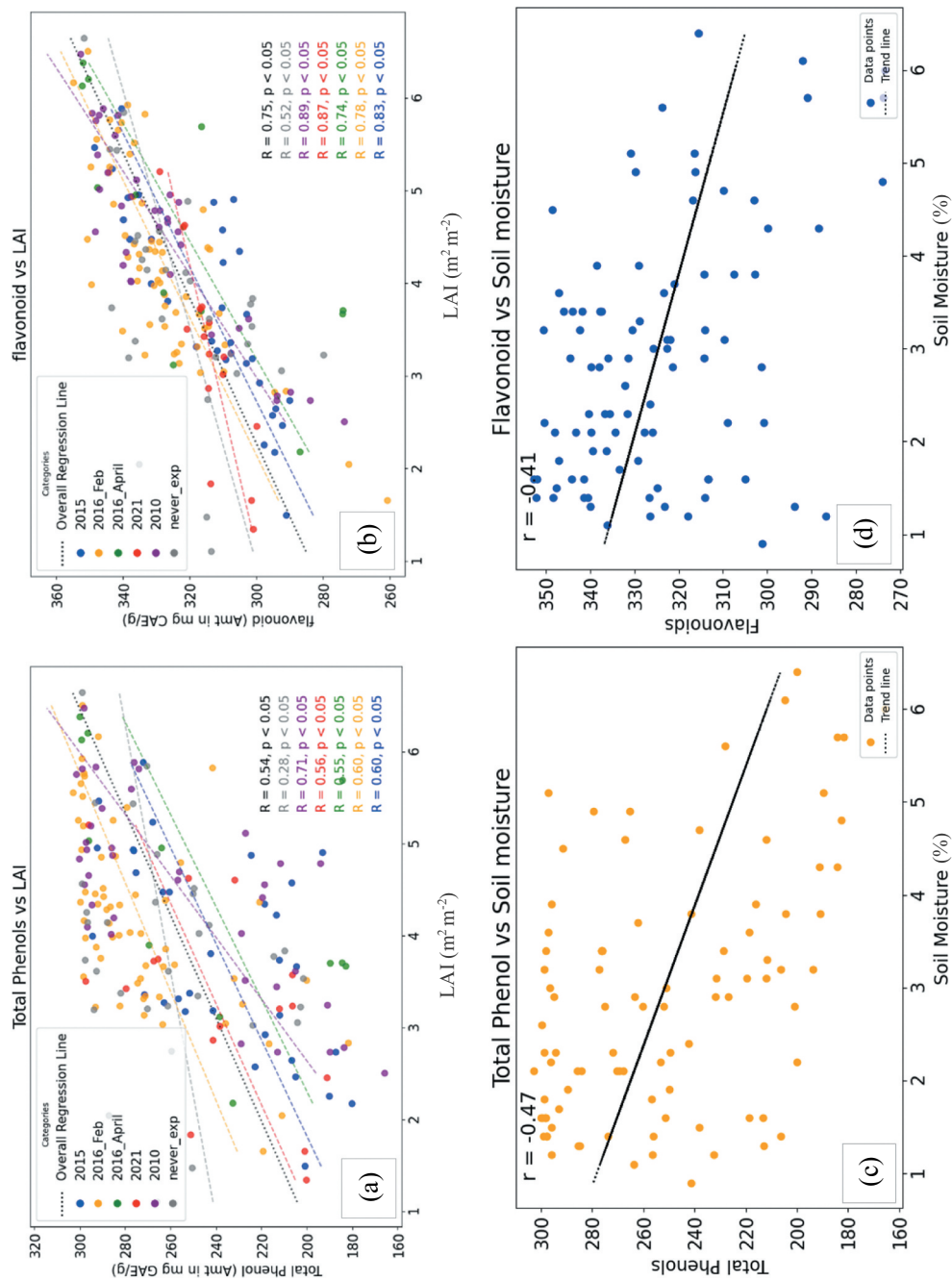


Figure 9. The relationships of leaf area index (LAI) and phenolic compounds (A-B) and relationships of soil moisture and phenolic compounds (C-D).

also tends to rise. Particularly, flavonoids produced higher correlation coefficients of $r=0.75$ than phenolics with r of 0.5. For both phenolic compounds, areas that experienced fire in 2010 showed the highest correlation coefficient, $r=0.71$ for total phenols and $r=0.89$ for flavonoids. Conversely, regions that remained unburned during the period from 2010 to 2024 exhibited much lower correlations of $r=0.28$ and $r=0.52$, respectively.

Regarding soil moisture, due to a limited number of samples, the study did not classify samples based on post-fire occurrence. Nonetheless, the observed trend demonstrates an inverse correlation between soil moisture levels and phenolic compound concentrations, indicating that as soil moisture decreases, phenolic content tends to increase. This pattern aligns with plant responses to water stress, wherein phenolic compounds often serve as protective agents against oxidative stress during drought conditions.

3.3. Phenolic absorption features

By analysing the average continuum-removed spectra, we determined that the lowest spectral pit occurs at 1660 nm for total phenols and 415 nm for flavonoids (Figure 10). The depth of these spectral features demonstrated a positive correlation to the amounts of total phenol and flavonoid in our dataset, yielding correlation coefficients of $r=0.59$ ($p<0.05$) for total phenol and $r=0.47$ ($p<0.05$) for flavonoid (Figure 11). Notably, the correlation was stronger for total phenols than flavonoids. Additionally, existing literature suggests that flavonoids possess characteristic absorption bands in the 410–430 nm range (Gitelson et al. 2017). However, in this region (410–430 nm) chlorophyll absorbs light. Therefore, to minimize the effect of Chlorophyll, we tested and modified the existing flavonoid indices.

3.4. Relationships between flavonoid and vegetation indices

Table 3 explores the relationship between flavonoids and spectral indices. We evaluated existing spectral indices from Gitelson et al. (2017) and Zhou et al. (2022) and then enhanced these indices by incorporating a spectral feature at 415 nm. Generally, spectral indices showed weak correlations with flavonoid levels, with R^2 values below 0.40. However, several indices, including $FI_{415,710}$, $FI_{420,710}$, $mFI_{415,710, 800}$, and $mFI_{415,690, 800}$, exhibited improved correlations (Figure 12). Notably, $FI_{415,710}$ and $FI_{420,710}$ achieved the highest correlations, with an R^2 of 0.55 and an rRMSE of approximately 1.98–1.99%. Interestingly, the indices modified in this study, specifically those incorporating the 415 nm wavelength, outperformed the original indices from Gitelson et al. (2017) and Zhou et al. (2022), leading to more accurate flavonoid predictions. This highlights the potential importance of the 415 nm spectral region in estimating plant flavonoid content.

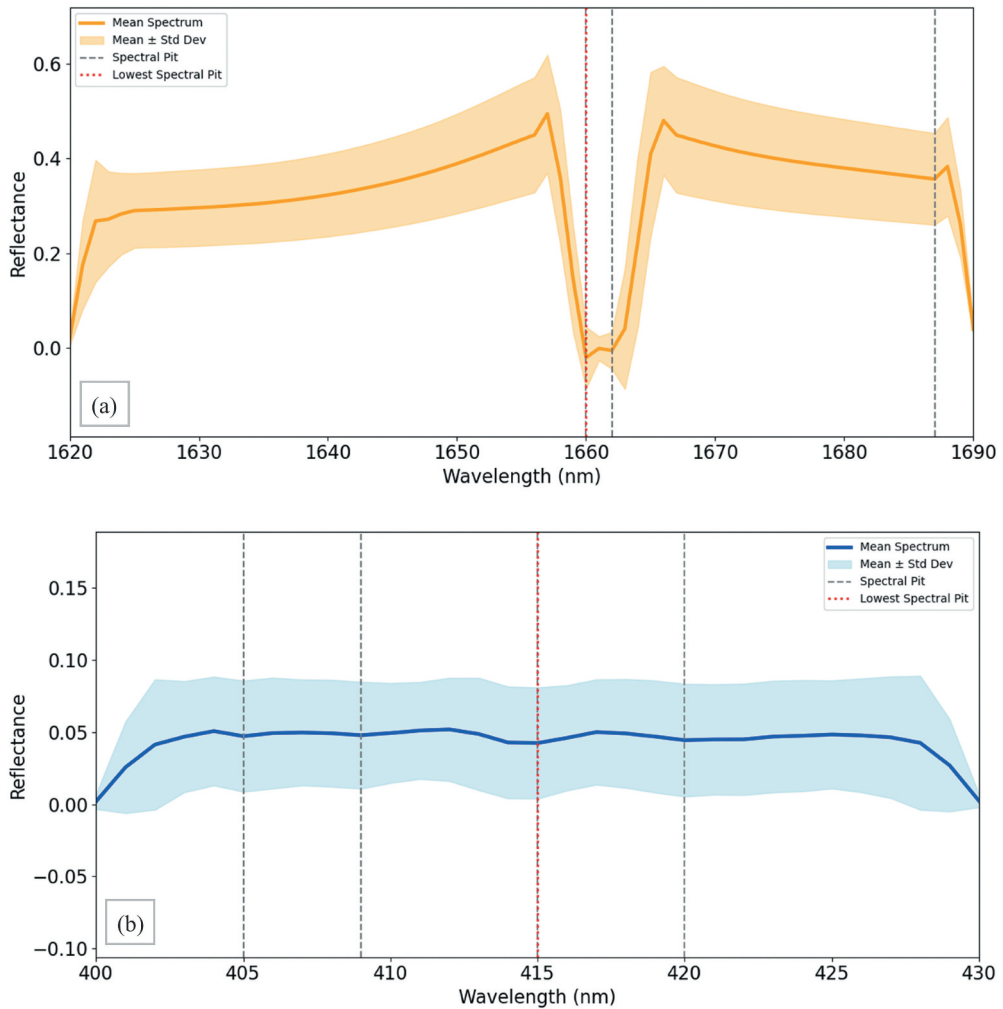


Figure 10. Continuum-removed spectral features for the hotspot region: (A) total phenol in the range of 1650–1670 nm, and (B) flavonoid in the range of 400–430 nm. A red dotted line highlights the lowest absorption.

3.5. Model performance

3.5.1. Retrieval of phenolic compounds using random forest model with different spectral subsets

Table 4 presents a summary of the predictive capabilities of various spectral domains of leaf reflectance/continuum-removed spectra in estimating phenolic compound concentrations using random forest model. Overall, the predictive accuracy for total phenol was consistently higher than that for flavonoids across the majority of spectral ranges. Notably, the SWIR range (1000–2200 nm) yielded the most accurate predictions for total phenol, however, it demonstrated lower predictive accuracy for flavonoids. The application of continuum-removed full-spectrum data significantly enhanced the accuracy of predictions for both phenolic compounds. However,

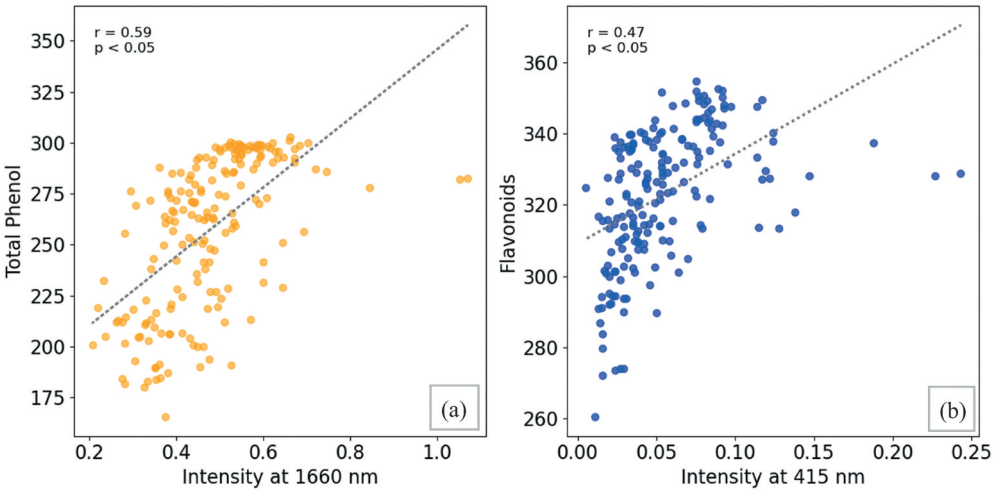


Figure 11. The correlation between total phenolics concentration and the spectral feature at 1660 nm (A), as well as the correlation between flavonoids concentration and the spectral feature at 415 nm (B).

Table 3. Accuracy of various flavonoids spectral indices.

Group	Index	CV-R2	RMSE	rRMSE (%)
FI _{λ1, λ2}	FI _{420,710}	0.55	6.94	1.99%
	FI _{415,710}	0.55	6.89	1.98%
FI _{λ1, λ2,800}	FI _{420,710,800}	0.21	8.71	2.70%
	FI _{415,710,800}	0.22	8.66	2.68%
	FRI	0.27	8.35	2.59%
mFI _{λ1, λ2}	mFI _{420,710}	0.14	9.01	2.79%
	mFI _{415,710}	0.33	8.02	2.49%
	mFI _{420,690}	0.14	8.98	2.51%
	mFI _{415,690}	0.33	7.99	2.48%
mFI _{λ1, λ2,800}	mFI _{420,710,800}	0.15	8.96	2.78%
	mFI _{415,710,800}	0.42	7.49	2.27%
	mFI _{420,690,800}	0.16	8.915	2.76%
	mFI _{415,690,800}	0.42	7.47	2.27%
DDblue _{λ1, λ2}	DDblue _{421,733}	0.38	7.69	2.39%
DDnir _{λ1, λ2}	DDnir _{434,421}	0.33	8.03	2.49%

flavonoids achieved the highest predictive accuracy, reflected by an R^2 value of 0.81 and an RRMSE of 10.75% (Figure 13(b)). Meanwhile, total phenol also demonstrated strong performance in the SWIR range, with an R^2 of 0.82 and RRMSE of 12.54% (Figure 13(a)).

3.5.2. Informative wavebands selected by the random forest model

Figure 14 illustrates the significant waveband features identified by the Random Forest model for the analysis of total phenols and flavonoids, highlighting specific wavelength regions that are critical for this assessment. Notably, the essential wavebands for total phenols were determined to be within the ranges of 1000–1100 nm and 2100–2200 nm. In contrast, the analysis of flavonoids revealed multiple significant wavelength regions,

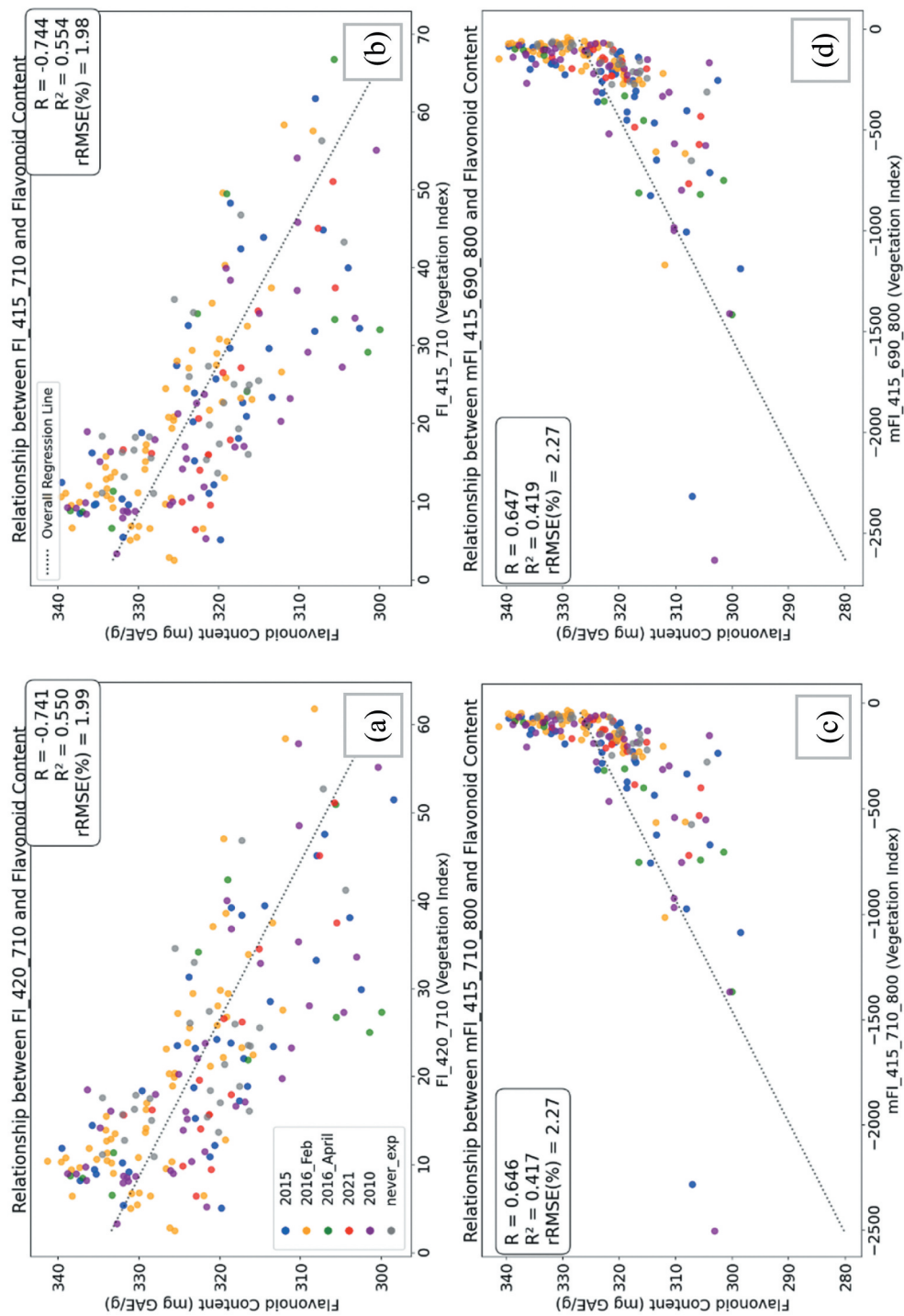


Figure 12. Top four best-performing flavonoid spectral indices.

Table 4. Random forest performance statistics for the quantification of total phenol and flavonoid content are derived from reflectance data gathered across various spectral ranges: visible and shortwave Infrared (VSWIR, 400–2200 nm), visible and Near-Infrared (VNIR, 400–1000 nm), shortwave Infrared (SWIR, 1000–2200 nm), and continuum-removed SWIR spectra.

Input data	Total phenol		Flavonoids	
	R^2	RRMSE (%)	R^2	RRMSE (%)
VSWIR (400–2200 nm)	0.76	14.70%	0.77	11.63%
VNIR (400–1000 nm)	0.73	13.37%	0.79	11.43%
SWIR (1000–2200 nm)	0.75	13.08%	0.61	11.69%
Continuum removed VSWIR	0.70	13.72%	0.81	10.75%
Continuum removed SWIR	0.82	12.54%	0.45	12.22%

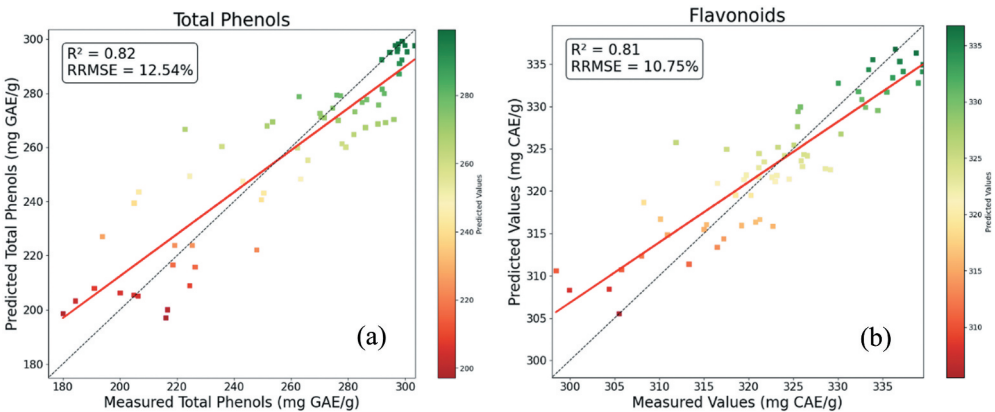


Figure 13. Illustrates the comparison between observed and predicted values for total phenols (A) and flavonoids (B) utilizing pooled data. The predictions were generated using continuum-removed SWIR and VSWIR spectra using random forest model. A dashed grey line indicates the 1:1 reference line and red regression line.

with prominent peaks occurring in the ranges of 400–500 nm, around 750 nm, and 1900–2200 nm.

4. Discussion

Spectral analyses conducted on 190 leaf samples revealed significant variability in the concentrations of total phenols and flavonoids based on post-fire events. Notably, distinct spectral features were observed at 1660 nm corresponding to total phenols and at 415 nm associated with flavonoids. By employing leaf spectroscopy, we effectively captured this variability.

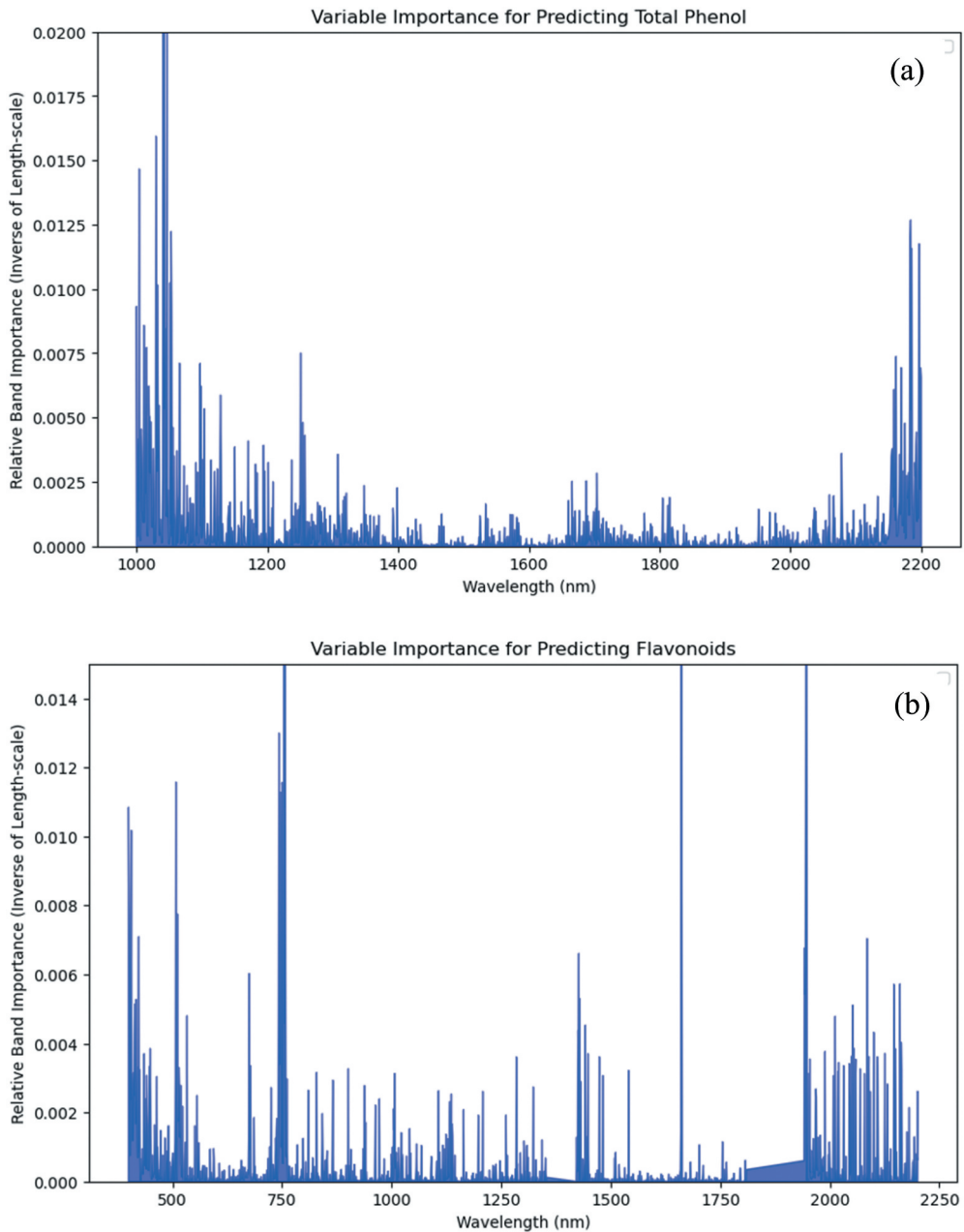


Figure 14. Shows the relative importance of different bands selected by the random forest model for predicting total phenol (A) and flavonoids (B). Remove titles from the figures.

4.1. Changes in the concentration of phenolic compounds

Figure 7 shows higher concentrations of phenolic compounds in areas that experienced recent fire events, specifically in 2021 (May), 2016 (February and April), and 2015 (March) compared to older, unburned vegetation ('never_exp' and 2010). This could be due to

newly sprouted plants to establish themselves and defend against stress factors. Fires create a nutrient-rich environment through the decomposition of organic material and the release of nutrients previously locked in dead biomass (Mitchell et al. 1986; Stock and Allsopp 1992). This availability can further support heightened growth rates and associated increases in secondary metabolite production. This is in line with Zhou et al. (2022), who found that younger Ginkgo trees are significantly higher in flavonoids than older.

In contrast, older vegetation may indicate plants that are experiencing nutrient stress, as fynbos is a fire-driven ecosystem that typically burns every 10 to 15 years (Kraaij and van Wilgen 2014). The period from 2010 to 2024 is significant; vegetation that has not experienced fire during this time may have reached full maturity, leading to senescence. These plants could potentially experience fire at any point within this timeframe. As plants age, their ability to produce metabolic compounds may diminish, particularly in the fynbos, where they compete for limited resources over extended periods. According to Qaderi, Martel, and Strugnell (2023), new leaves typically accumulate metabolites more rapidly than older leaves.

Furthermore, observations from the current study indicate notable differences in mean and spectral variation (%) in spectroscopy reflectance across different years (Figure 5). In terms of the mean reflectance, 2016 (February), 2010 (January), and 'never_exp' areas exhibited the highest mean reflectance (Figure 5(a)). This could be attributed to differences in vegetation density and health, with more recently burned areas typically showing lush, green growth that reflects more light (De Klerk 2008; Smit, Baard, and van Wilgen 2024). On the other hand, older or well-established plants ('never_exp' and 2010) have thicker, more lignified leaves with a higher proportion of structural components like cellulose and lignin, which can increase the overall reflectance (Chen et al. 2022; Mediavilla et al. 2011).

On the other hand, for spectral variation (%), unlike mean reflectance, recently burnt areas such as 2016 (February and April) and 2015 show the highest spectral variation (Figure 5(b)). In contrast, never_exp areas and 2010 displayed the lowest spectral variation for phenolic compounds. This lower variation is likely due to the presence of older, more mature vegetation, which tends to have a more uniform structure and composition, resulting in less spectral variability (Mediavilla et al. 2011).

Furthermore, Figure 9 reveals a linear relationship between phenolic compounds and LAI. This finding aligns with the work of Zhou et al. (2023), who utilized the SAIL model with hyperspectral data to evaluate the flavonoid content in *Ginkgo* plantations. These studies suggest that phenolic compounds can be accurately estimated by measuring LAI. LAI measures the total surface area of leaves, which helps plants capture more light for photosynthesis. This increased light exposure encourages the production of flavonoids (Ni et al. 2020). In contrast, an inverse correlation between soil moisture and phenolic compounds. This finding aligns with the understanding that plants often produce secondary metabolites, such as phenolics, as a response to unfavourable conditions (Ashraf et al. 2018; Bartwal et al. 2013). When soil moisture is limited, plants may experience water stress. Hence, in response, they may elevate their production of phenolic compounds (Naczka and Shahidi 2004; Reginato et al. 2014; Shen et al. 2022; Varela et al. 2016).

4.2. Absorption features of phenolics

Figure 10 indicates that the lowest spectral pit for total phenol occurs at 1660 nm with a correlation (r) of 59% between total phenolics and 1660 nm (Figure 11(a)). This finding aligns with existing literature, which highlights the significance of the phenolic absorption feature around 1660 nm in relation to other absorption characteristics at 1460, 1930, 2140, and 2170 nm. These other features are closely associated with major plant biochemical constituents such as lignin, cellulose, starch, and protein (Kokaly and Skidmore 2015; Xie et al. 2024). For instance, Xie et al. (2024) observed that the phenolic absorption features occur at approximately 1653 nm for Scots Pine, 1667 nm for European Beech, 1664 nm for English Oak, and 1666 nm for Norway Spruce. Such variations underscore that while phenolic absorption peaks may cluster around 1660 nm, they are not universally fixed. Furthermore, while the study identifies significant spectral features associated with phenolics, it is essential to acknowledge the variability of the absorption bands, as well as the potential confounding factors such as water content, dry matter content, cellulose, and lignin, which may influence spectral interpretations.

In contrast, the lowest spectral pit for flavonoids occurs around 415 nm, with (r) of 47% between flavonoid and 415 nm (Figure 11(b)). This study aligns with Gitelson et al. (2017), who identified the spectral range of 410–430 nm as important for detecting flavonoids. However, Gitelson et al. (2017) also noted that chlorophylls contribute significantly to signals in this range. To reduce the influence of chlorophyll and improve the detection of flavonoids, many researchers have developed special indices, e.g. Gitelson et al. (2017); Zhou et al. (2022); and Zhou et al. (2023). In this study, we tested and modified these spectral indices by focusing on the 415 nm wavelength, which we found to be sensitive to flavonoids.

4.3. Flavonoids indices

Table 3 shows that FI-type indices, including $FI_{420, 710}$ and $FI_{415, 710}$, demonstrated the highest accuracy among all the tested indices. This was followed by the mFI-type indices, such as $mFI_{415, 710, 800}$ and $mFI_{415, 710, 800}$, which also performed well. Meanwhile, the spectral indices proposed by Zhou et al. (2022), which replaced 710 nm with 690 nm, did not show improvements in their results, as they produced similar levels of accuracy. This could be due to both 710 nm and 690 nm are close together in the red-edge spectral region, and both wavelengths are strongly related to chlorophyll content (Zhou et al. 2022). Overall, the incorporation of the 415 nm wavelength significantly improved the performance of all indices tested, including both FI-type and mFI-type indices. This suggests that 415 nm is a sensitive wavelength for detecting flavonoids and enhances the accuracy of spectral indices used for this purpose.

In our analysis, we found that only the indices $FI_{420, 710}$ and $FI_{415, 710}$ exhibited predictive power with R^2 values exceeding 0.50, while the remaining indices fell below this threshold. Such low R^2 values indicate a lack of predictive power, reflecting the challenges in accurately correlating leaf reflectance data with the underlying physiological and chemical characteristics of leaves. The spectral measurements were conducted in a controlled environment, specifically a dark room, which aimed to minimize external light interference and enhance measurement consistency. However, several factors still

contributed to the low accuracy. Firstly, the structural characteristics of Fynbos vegetation, particularly the presence of sclerophyllous plants with tough, waxy leaves, created significant interference in spectral measurements.

The limitations of the spectral indices themselves may also play a role; indices developed for other ecosystems might not be fully optimized for the unique characteristics of Fynbos vegetation, where specific leaf traits, such as thickness and composition, differ from those found in other environments. This variability can lead to inconsistent patterns in leaf reflectance, complicating efforts to establish robust correlations.

4.4. Estimating foliar phenolic compounds using random forest regression

Table 4 indicates that spectral data from the SWIR region (1000–2200 nm) provided more accurate predictions for total phenol content compared to data from the VNIR region (400–1000 nm) or the full-spectrum range (400–2200 nm). This finding is in line with Xie et al. (2024), who also reported that predictions of phenolic compounds were more accurate when utilizing shortwave infrared (SWIR) data compared to the full spectral range. Wavelengths related to phenolic absorption are located within the SWIR region (Ben-Dor, Inbar, and Chen 1997; Ferwerda, Skidmore, and Stein 2006; Flinn et al. 1996; Soukupova, Rock, and Albrechtova 2002). Consequently, excluding the redundant spectral information of VNIR effectively reduces the data dimensionality, while retaining the relevant bands in the model for generating accurate predictions. Our results underscored the importance of employing a relevant spectral domain for the variables of interest in model building, as emphasized by previous studies (Asner et al. 2011; Chlus and Townsend 2022; Couture et al. 2016). Using full-spectrum data to predict phenolic compounds may hinder our understanding of phenolic absorption features because the model can leverage indirect correlations between phenolic compounds and other leaf traits (Ferwerda, Skidmore, and Stein 2006; Xie et al. 2024).

On the other hand, our study underscores the value of utilizing a full-spectrum range of 400–2200 nm for the estimation of flavonoid, which has been shown to yield more accurate predictions (Table 2). This is in line with Tshabalala et al. (2021) who highlighted the importance of the SWIR region and visible region in predicting medicinal phytochemicals in *Moringa oleifera* using hyperspectral reflectance of tree canopies. Their findings demonstrated that specific absorption features in the SWIR region are closely linked to the presence and concentration of flavonoid, thereby enhancing predictive models for these compounds. This suggests that the SWIR region contains information that complements the visible spectrum, allowing for a more comprehensive assessment of flavonoid content. Also, Figure 11 shows that the random forest model selected informative bands in the blue region (400–500 nm), green region (510–580 nm), red-edge (approximately 715 nm), and SWIR region (2000–2200 nm). This indicates that both the visible and SWIR regions are useful for estimating flavonoid content. As mentioned above, another factor contributing to the enhanced predictive accuracy is the relationship between flavonoid and other phenolic compounds, which also exhibit significant absorption features within the SWIR region (Williamson, Kay, and Crozier 2018). Since flavonoids are a subclass of phenolic compounds, their spectral characteristics can overlap with those of other phenolics, reinforcing the need to consider a broader spectral range.

The application of continuum removal substantially improved the prediction and yielded the most accurate estimations for total phenol and flavonoid. This improvement demonstrated the importance of this data transformation technique for studying foliar biochemicals with subtle spectral features, such as phenolic compounds in this study (Gao et al. 2019; Ramoelo et al. 2011).

4.5. Challenges and prospects

This study showed that leaf spectroscopy has strong potential for studying the changes in phenolic compounds over time after a fire in the Fynbos Biome. We also tested and adjusted different flavonoid indices to improve accuracy. While our research primarily emphasizes ecosystem functioning, it acknowledges the importance of functional diversity as a key determinant of overall ecosystem performance (Cadotte, Carscadden, and Mirotnick 2011).

However, this focus presents certain limitations, particularly concerning the lack of detailed information on species-level contributions, as well as the age and physiological state of the analysed leaves. These factors can profoundly affect leaf reflectance and chemical composition, potentially constraining the interpretative power of our findings. Therefore, future studies should examine how specific species and their varying developmental stages influence leaf reflectance patterns and phenolic concentrations. By integrating these aspects, researchers can develop a more comprehensive understanding of the interplay between species diversity, leaf characteristics, and ecosystem functioning, ultimately enriching our knowledge of dynamics within the Fynbos ecosystem.

Furthermore, the sample size across the chronological sequence is highly unbalanced, with 33 points collected in March 2015, 60 points in February 2016, 12 points in April 2016, 16 points in May 2021, and 37 points in January 2010, along with 32 points from the 'never-exp' areas. This disparity presents a limitation in our analysis, as comparing datasets of significantly different sizes such as 60 samples against only 12 carries statistical risks. Specifically, the variance in the smaller group may be artificially low due to under-sampling, potentially leading to misleading conclusions. Future studies should aim for more balanced sample sizes to enhance the robustness of comparative analyses.

Although this study highlights that phenolic compounds exhibit sensitivity around 1660 nm, it's important to note that other sensors, particularly those mounted on aircraft or satellites, may not capture this absorption feature effectively. Many of these sensors typically operate with spectral resolutions ranging from 3 to 10 nm, which may not sufficiently resolve the detailed features associated with phenolic absorption. For example, according to Xie et al. (2024), specific wavebands identified by spaceborne sensors, such as 1656 and 1666 nm on the PRISMA satellite (Cogliati et al. 2021), as well as 1653 and 1664 nm on EnMAP (Guanter et al. 2015), are promising candidates for the future operational retrieval of phenolic compounds at the satellite level.

In addition, hyperspectral imaging from aircraft or satellites is different from measuring the reflectance of leaves in controlled settings, as there are several challenges that can affect how we estimate traits. These challenges include the structure of the tree canopy, atmospheric moisture, background light, and varied angles of sunlight and sensors (Darvishzadeh et al. 2011). Additionally, the quality of data in the SWIR range is often lower than in the visible and VNIR ranges, making it difficult to generate accurate

information. Therefore, it's important to consider these factors when mapping phenolic compounds using data collected from the air or space.

In the future, it is essential to explore the relationship between LAI and flavonoids. Although Zhou et al. (2023) assessed this relationship, their study relied solely on the 4-SAIL model, which does not account for other biochemical variables, such as chlorophyll. Therefore, employing models such as PROSPECT-D or PROSPECT-PRO, in conjunction with the 4-SAIL model to create a comprehensive PROSAIL model, would facilitate a more thorough investigation of flavonoids in relation to chlorophyll and LAI. This approach would enhance our understanding of how LAI and chlorophyll influence flavonoid concentrations. Additionally, it is necessary to evaluate the existing flavonoid vegetation indices developed by Gitelson et al. (2017); Zhou et al. (2022); and Zhou et al. (2023) using data from various environmental settings. Such testing will provide insights into the applicability and robustness of these indices across different ecological contexts.

5. Conclusions

This study revealed that areas that experienced recent fires, specifically in 2021 (May), 2015 (March), and during February and April of 2016 exhibited high levels of phenolic compounds. Conversely, locations that had not recently experienced fire and areas that have not experienced fire for the past decade demonstrated significantly lower concentrations. These findings underscore the critical role of fire in the rejuvenation of the fynbos ecosystem.

We identified a specific wavelength at 1660 nm that is important for detecting phenolic compounds, along with another key wavelength at 415 nm for flavonoids. However, because chlorophyll also absorbs light in this region, we tested and adjusted various spectral indices for flavonoids to improve their sensitivity. Our results showed that FI-type indices, including $FI_{420, 710}$ and $FI_{415, 710}$, achieved the highest accuracy among all the indices tested. Followingly, the mFI-type indices, such as $mFI_{415, 710, 800}$ and $mFI_{415, 710, 800}$, also performed well. The Random Forest model accurately estimated phenolic compounds and could identify informative wavelength. The Random Forest model consistently found patterns related to changes in phenolic compounds, like results seen in other ecosystems like savanna, grasslands, tropical forests, and Moringa plants. We believe that the key wavelengths identified in our study could be used for monitoring phenolic compounds on a larger scale in different areas. Furthermore, we established that models tailored to specific species or forest types exhibited lower accuracy compared to a more generalized model applicable across various species (Xie et al. 2024). These findings add to existing research by emphasizing the role of fire and plant secondary metabolites (PSMs) in ecosystem health and function. Additionally, they highlight the importance of spectral indices in improving the detection and measurement of flavonoids from spectral data.

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Data availability statement

No datasets were generated or analysed during the current study.

Ethical statement

All authors have read, understood and complied, as applicable, with the statement on 'Ethical responsibilities of authors' as found in the Instructions for authors.

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