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Long-term legacy of sowing legume-rich mixtures in Mediterranean wooded grasslands

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Supplementary Material

Long-term legacy of sowing legume-rich mixtures in Mediterranean wooded grasslands

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High-resolution orthophotos processing with eCognition©.

Orthophotos from PNOA (http://centrodedescargas.cnig.es/CentroDescargas/index.jsp#) were processed at the field level with the eCognition© software (ver 9.0.1, Trimble Inc., Sunnyvale, California, USA). Before creating the final open pasture layer in a GIS environment, shapefiles identifying trees (as in this example) and small buildings were generated through algorithms based on shape and reflectance at the red and blue bands. The list of used algorithms, parameters, and their values is reported in Table 1S. An example of object identification output is reported in Fig. 1S.

Table 1S. Algorithms, parameters, and their values as set in the eCognition process tree to identify trees, small buildings, and streets

		Objects	
Algorithm	**Parameter**	**Trees**	**Small buildings and streets**
Multiresolution segmentation	Scale parameter	80	15
	Shape	0.5	0.7
	Compactness	0.5	0.5
Assign class	Threshold condition (Feature: Layer, Mean value)	RED<150	BLUE>135

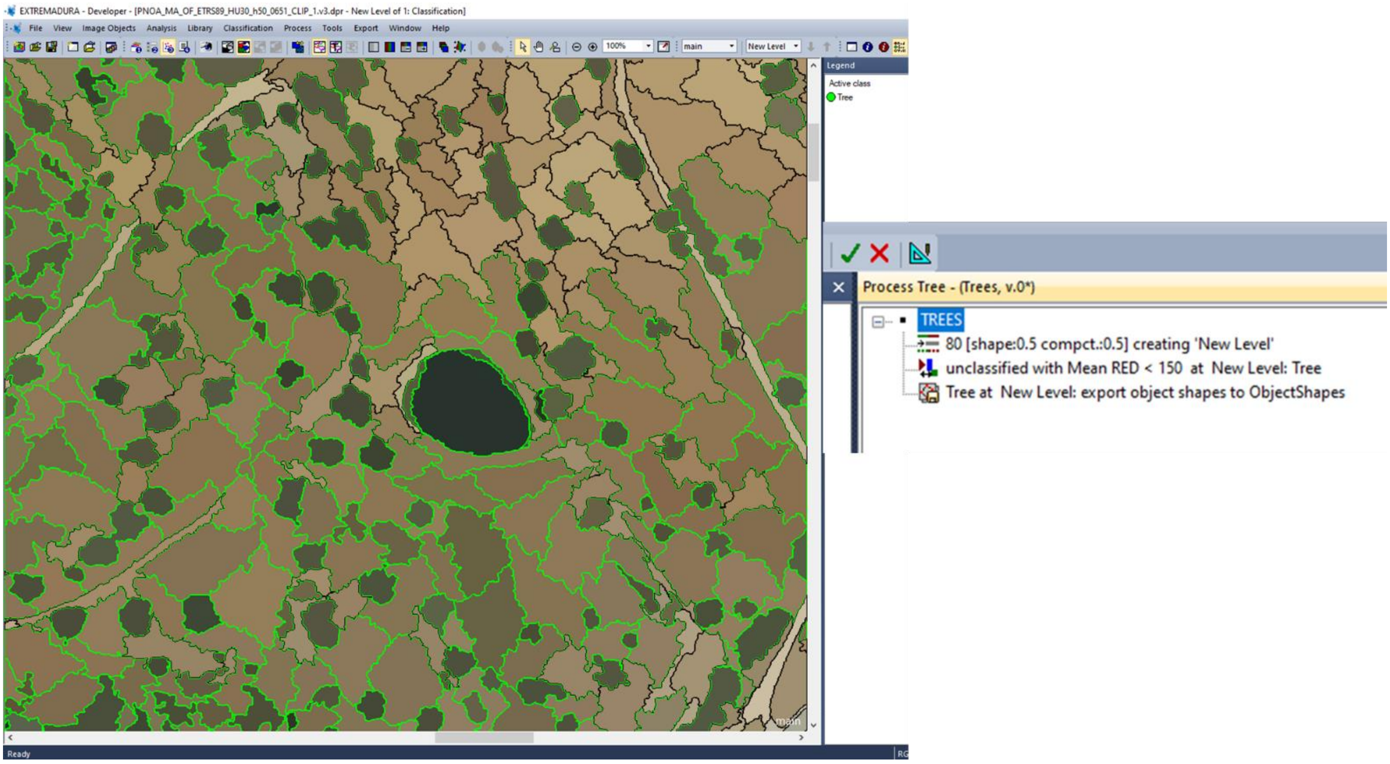

Fig. 1S. Output of a process-tree-based object classification performed with the eCognition software.

Table 2S. Tree-covered and open pasture area (%), available pixels for the remote sensing analysis (%), and area covered by the available pixels (m^2) in the field study.

Farm	Field	Tree cover (%)	Open pasture (%)	Available pixels (%)	Area covered by available pixels (m^2)
Farm1	2010	0.29	0.71	0.44	418569
	2011	0.21	0.79	0.70	391708
	2012	0.29	0.71	0.45	233977
	2013	0.22	0.78	0.72	667038
	2014	0.25	0.75	0.49	89792
	Control	0.21	0.79	0.62	623031
Farm2	2002	0.35	0.65	0.21	30539
	2005	0.29	0.71	0.39	136617
	2011	0.41	0.59	0.15	67256
	Control	0.37	0.63	0.17	29639
Farm3	2005	0.44	0.56	0.10	18374
	2011	0.30	0.70	0.36	34879
	2014	0.37	0.63	0.15	19953
	Control	0.45	0.55	0.20	32077
Farm4	2002	0.33	0.67	0.44	394200
	2007	0.75	.25	0.33	217800
	2012	0.31	0.69	0.28	39600
	2014	0.20	0.80	0.43	90900
	Control	0.20	0.80	0.61	207900
Farm5	2003	0.23	0.77	0.57	149722
	2011	0.09	0.91	0.83	583737
	2015	0.21	0.79	0.44	43039
	Control	0.19	0.81	0.60	148110
Farm6	2002	0.56	0.44	0.03	7012
	2003	0.61	0.39	0.02	2861
	2014	0.31	0.69	0.27	28719
	2015	0.51	0.49	0.06	24287
	Control	0.40	0.60	0.22	27376

Effect of legume-rich-mixtures oversowing on biomass production and Nitrogen canopy content over the ages.

A previous study conducted by Hernández-Esteban et al. (2018) in the same study site reported a significant increase in pasture biomass production and quality in fields oversown with legume-rich mixtures compared to unsown. Data already reported in that study were reworked according to the methodological scheme of the present research by performing a normalization of biomass production (Mg ha^{-1} of DM) and N pasture content (g m^{-2} of N) measured in 2016 in oversown fields with respect to control fields for each *j*th field of the i*th* farm as follows:

$$nrValue_{i,j,k} = \frac{Vsown_{i,j} - Vcontrol_i}{Vcontrol_i}$$

Exponential regression models were fitted to test the effect of age on normalized biomass production and N pasture content. No significant effects of age were observed for both variable. Results are reported in Fig 2S.

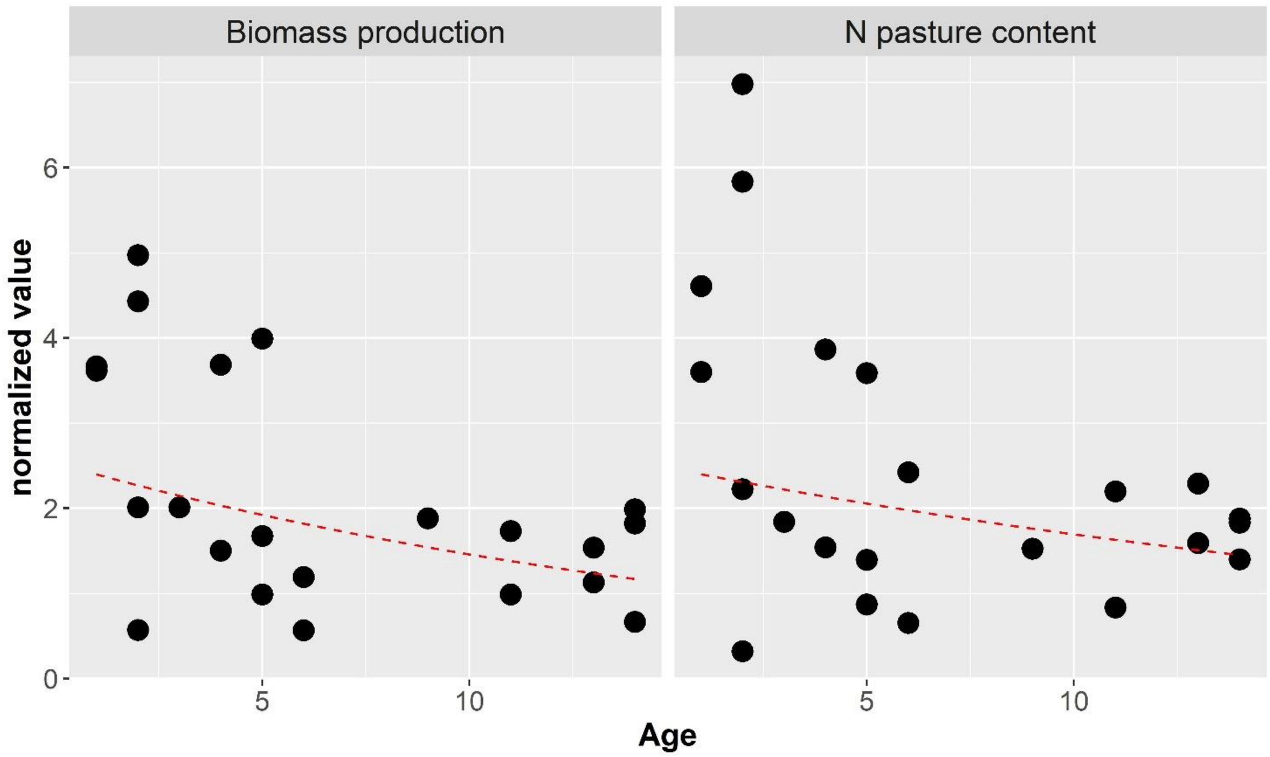

Fig. 2S. Relationships between the normalized biomass production, normalized N pasture content, and the age of sown pastures. The red lines represent the fitted exponential model.

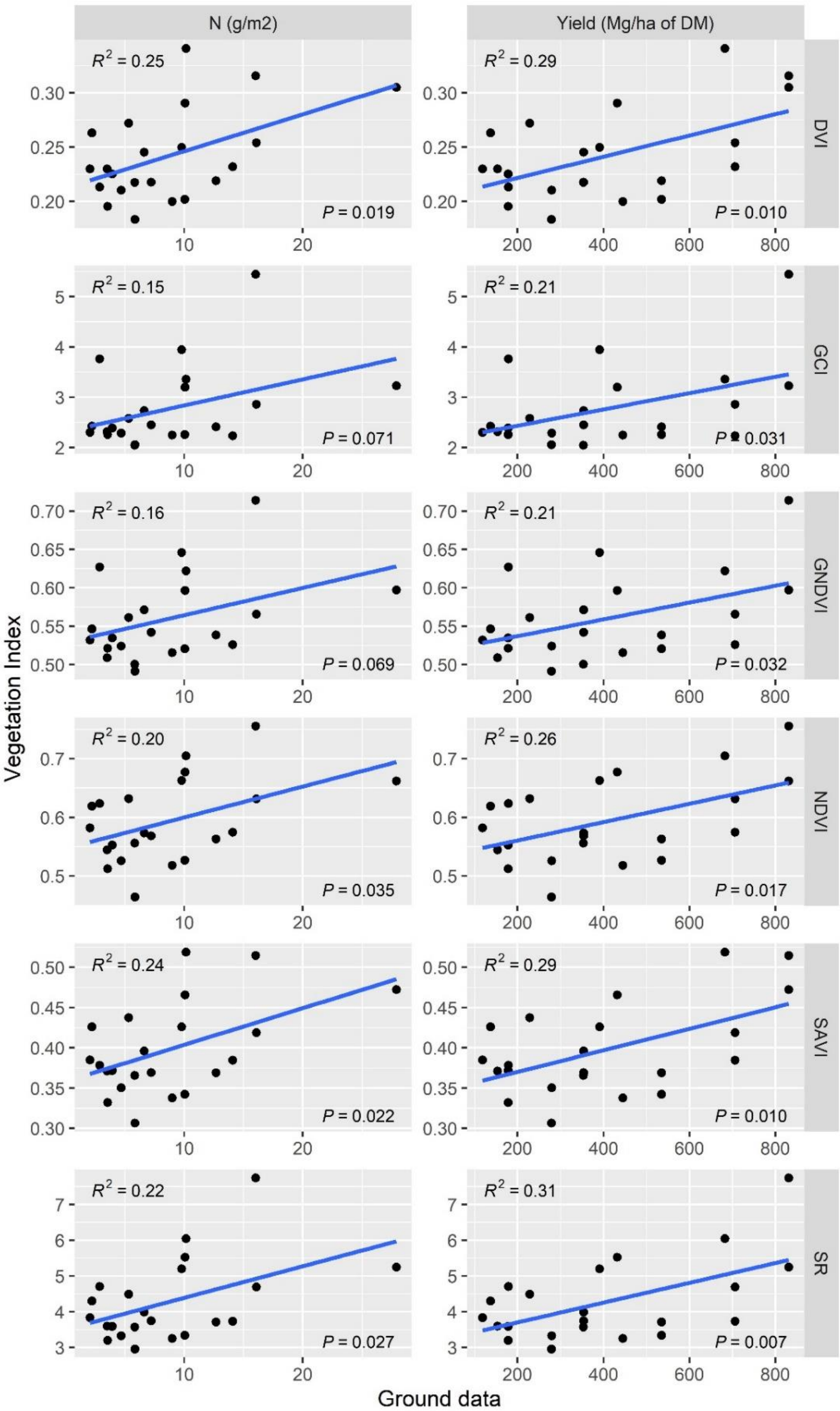

Fig. 3S. Relationships between Vegetation Indices and N pasture content (g m^{-2} of N) and biomass production (Mg ha^{-1} of DM) observed in the study fields in 2016.

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Long-term legacy of sowing legume-rich mixtures in Mediterranean wooded grasslands

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Abstract

The oversowing of legume-rich mixtures is a management practice gaining importance in Mediterranean silvopastoral farms due to enhanced forage production, quality, and ecosystem service provision. However, the oversowing of legume species can be expensive in silvopastoral farms due to the high cost of legume seeds and seeding operations and low persistence under inadequate management. The study aimed to assess the long-term impacts of legume-rich mixtures oversowing on pasture production of Mediterranean wooded grasslands with a remote sensing approach. The study was conducted on six Dehesa farms in Western Spain, where legume mixtures had been sown in different years from 2002 to 2015 in part of the farms. The effects of legume species oversowing were assessed by analyzing the variation across years of a set of derived vegetation indices in sown and unsown areas of each farm. Among all considered vegetation indices, the Simple Ratio (SR) and the Normalized Difference Vegetation Index (NDVI) showed the highest capacity in estimating grassland yield (Pearson's $r = 0.58$ and 0.52, $P<0.01$, respectively) and N content ($r = 0.52$ and 0.48, $P<0.05$, respectively). All normalized vegetation indices increased after the sowing and remained significantly higher than before sowing ($P<0.001$) for the whole monitoring period. Furthermore, the sowing also positively affected the temporal stability of each vegetation index, being interannual variability significantly higher ($P<0.01$) before the sowing than after. The study confirmed the hypothesis that the oversowing of legume-rich mixtures in Mediterranean wooded grasslands effectively improves intra- and interannual biomass production and stability. Remote sensing using Landsat images proved to be an effective tool to assess the impacts of grassland management practices in the long term in silvopastoral systems.

Keywords. Legume oversowing; agroforestry systems; remote sensing; temporal stability; vegetation indices; Landsat; sustainable intensification

1. Introduction

Grassland ecosystems cover about 25% of the Earth's surface and 70% of the total agricultural terrestrial area (Conant, 2010). About 17M km^2 of grasslands worldwide are identified as silvopastoral systems (Zomer et al., 2009). Grasslands are the most important sources of food worldwide as they support livestock activities, such as beef and milk production, which offer livelihoods for about 1 billion people among the poorest countries and one-third of the global protein intake (Steinfeld et al., 2006). Wooded grasslands provide a wide range of ecosystem services (Torralba et al., 2016), such as fodder production (Porqueddu et al., 2016; Seddaiu et al., 2018), biodiversity conservation (Bagella et al., 2020), C sequestration (Mosquera-Losada et al., 2011; Pulina et al., 2022), and water and nutrient cycles regulation (Lozano-Parra et al., 2018; Nair et al., 2019), among others.

The increasing demand for high-quality animal products complies with the need for sustainable and resource-efficient pasture-based farming systems such as silvopastoral farms (Moreno et al., 2018). However, these grazing systems are threatened by low profitability (Palomo-Campesino et al., 2018), which is partly associated with abandonment and intensification trends. Abandonment trends can lead to undergrazing, which, combined with climate change, contributes to land degradation and the loss of palatable vegetation (Mahyou et al., 2016). On the other hand, intensification through overgrazing can hamper tree regeneration processes compromising the long-term preservation of silvopastoral systems (Rossetti and Bagella, 2014) and soil and herbaceous cover degradation (Kairis et al., 2015). For these reasons, the sustainability of these agroecosystems requires management practices and tools to reverse the abandonment and degradation in marginal areas.

Pasture improvement by sowing legume-rich mixtures in extensively managed grasslands is gaining importance due to enhanced forage production and quality (Nichols et al., 2012; Teixeira et al., 2015; Sanna et al., 2018; Edwards et al., 2019). Legume oversowing can expand the forage growing season (Mason et al., 2019) and improve soil functions by strengthening fertility and N and C cycling (Bondaruk et al., 2020). Legumes sowing can modify soil microbial community composition playing a crucial role in their persistence (Collins et al., 2017). This mechanism can trigger the increase of microbial biomass in the soil, enhancing the overall soil functional diversity and N fixation and C sequestration (Moreno et al., 2021). However, the oversowing of legume species can be expensive due to the high cost of legume seeds and seed mixtures (Schaub et al., 2021) and low persistence under grazing mismanagement (Muir et al., 2011; Hayes et al., 2019). Nevertheless, legume-based forage systems can represent a solution facing the rising costs for inputs (e.g. fertilizers) whenever proper management can ensure their persistence. For this reason, the sustainability of such practices is highly related to the persistence over years of the most important species introduced with the sowing.

Assessing the long-term persistence of legume sown fields requires monitoring large spatial and temporal scales. There is great interest and numerous attempts to use satellite imagery (e.g. Landsat) for monitoring grassland productivity and quality (Wachendorf et al., 2018; Reinermann et al., 2020). Remote sensing data offer a cost-effective solution to monitoring phenology, productivity, and quality of grasslands over relatively large areas and long time scales. However, finding an appropriate spatial and temporal scale to detect changes remains a significant challenge in most circumstances and pasturelands (Liu et al., 2016; Berra et al., 2019).

Understanding the impacts of both abiotic and biotic drivers of primary production of the herbaceous component in wooded grasslands represents a challenge because of the presence of trees. In fact, in the case of such agroforestry systems, the intimate mixing of plant strata with very different phenological patterns is complex, making it challenging to discern the contribution of each stratum to the overall phenological phases detected with satellite imagery. Different approaches aiming to exclude and isolate the effect of trees emerge from the literature. For instance, based on the differences between Normalized Difference Vegetation Index (NDVI) dynamics, the spectral signal from trees can be isolated from those attributable to grassland layers (Lu et al., 2003; Gómez-

Giráldez et al., 2019). In wooded grasslands where tree vegetation is dominated by evergreen mature-scattered trees, such as Spanish Dehesas, tree pixels can be isolated based on the assumption that they keep a constant value throughout the year (González-Dugo et al., 2021). The availability of higher-spatial-resolution images (e.g. those from the Sentinel2 satellite) allows for adopting more detailed techniques, such as using a GPS device to reference the trees and exclude pixels including them (Fernandez-Habas et al., 2021) or applying classification techniques of the landscape such as the Object-based image analysis (OBIA) integrated with LiDAR (Light Detection and Ranging) data (Hellesen and Matikainen, 2013). However, its relatively small temporal availability, as compared to Landsat, hinders the assessment of long-term effects.

A previous study conducted by Hernández-Esteban et al. (2018) reported a significant increase in pasture yield and quality in fields sown with legume-rich mixtures compared to unsown. However, it is still uncertain if these improvements are sustained in the long term or if it is a transient effect. This study hypothesized that the oversowing of legume-rich mixtures could improve pasture productivity and stability in the long term and that remote sensing could represent a tool to assess the effectiveness of this practice in Mediterranean wooded grasslands. The aims of the study were: (i) to assess to what extent pasture production and quality (N content) can be monitored by means remotely sensed vegetation indices in Mediterranean wooded grasslands; (ii) to evaluate the long-term effects of legume oversowing on the temporal trends of these indices, and on (iii) their temporal variability as an indicator of the stability of the forage availability and quality.

2. Materials and Methods

2.1. Study Site

The study was conducted on six Dehesa farms located in central-western Spain, in the Extremadura community (39°57'/39°46' N – 6°28'/5°56' O). The climate is Mediterranean pluviseasonal continental (Rivas-Martínez et al., 2011), characterized by hot, dry summer and cold, rainy winter. The average annual temperature in the farms ranges from 16.0 °C to 17.5 °C, while the average annual rainfall ranges from 500 mm to 700 mm (Hernández-Esteban et al., 2019). The Dehesa vegetation within the farms is composed of scattered trees, primarily evergreen oaks such as *Quercus suber* L. and *Q. ilex* L., sparse shrubs species, and herbaceous annual self-seeding species that form the grassland system. Grasslands are managed through continuous grazing of cattle, pigs, sheep, and some wild cervids.

In each farm, different legumes-rich mixtures (*Trifolium subterraneum* L. subsp *brachycalycinum* and *yanninicum*, *Ornithopus sativus* L., *T. incarnatum* L., *T. michelianum* Savi var balansae, *T. resupinatum* L., *T. vesiculosum* Savi, and *T. glanduliferum* Boiss) have been sown over the years (from 2002 to 2015) in multiple large areas within each farm, aiming to improve the overall grassland productivity and forage quality. As a result, each farm comprises a chronosequence of several legume-sown fields, remaining the rest of the farm as unsown areas. These large unsown areas were considered as control. Details of the size and number of sown areas in each farm are reported in Table 1. The age of sowing, location, and limits of each sown field was gathered by interviewing farmers. Further details on sowing and overall grassland management are reported by Hernández-Esteban et al. (2018) and Hernández-Esteban et al. (2019).

2.2. Ground data collection

Pasture production and quality were characterized by measuring the annual grassland yield (kg m^{-2} of dry matter, DM), biomass nitrogen content in spring or peak of content (kg m^{-2} of N), and plant functional group (relative abundance of grasses, legumes, and forbs) composition.

Grassland yield was measured in 2016. Six exclusion cages (1 m x 1 m) were placed per field, allowing estimating the biomass production under grazing during the growing season. The exclusion cages were placed within the sown fields and in areas where sowing was never performed before, identified as control unsown fields. At the end of the growing season (end of spring), biomass was cut at ground level inside each cage within a 50 cm x 50 cm square quadrat. Biomass was then oven-dried until it reached constant weight to determine the DM content. The N content on plant biomass was measured using the Dumas Method in a DUMATHERM Gerhardt analyzer.

Plant inventory was carried out through the Point Transect method (Southwood and Henderson, 2009), recording plant species every 100 cm in eight random 25 m transects within each field (200 individuals per field). The relative abundance of the three main functional groups (grasses, legumes, and forbs) was then calculated. Detail on biomass, plant inventory, and N measurements are reported in Hernández-Esteban et al. (2018).

2.3. Remote sensing method to assess the long-term impacts of legume-rich mixtures oversowing

Freely available high-resolution orthophotos (PNOA, http://centrodedescargas.cnig.es/CentroDescargas/index.jsp#) were used to distinguish between trees, built-up areas (i.e., streets, houses or rural buildings), and small water ponds from pasture areas in sown and control fields. The images were processed at the field level with the eCognition© software (ver 9.0.1, Trimble Inc., Sunnyvale, California, USA). A tree-decision-based algorithm was set up to identify trees and built-up areas, resulting in vectorial shapefiles delineating trees and other elements from pasture. An example of object classification is reported in Supplementary Material (Fig. 1S). The few water ponds were identified and mapped using the QGIS software (ver. 3.6.0-Noosa, QGIS Development Team, https://qgis.org/it/site/). Pasture layers were derived as the difference between the borders of each field and the identified objects (trees, streets, buildings, water ponds).

Multispectral images from Landsat satellites (https://www.usgs.gov/landsat-missions) were acquired among those available and free from clouds in the study areas. For each farm, images were acquired from four years before the first sown to four years after the last sown, in the time window from March 20th to May 31st, corresponding to spring. In the study areas, the maximum pasture biomass growing rates and the highest forage availability can be observed in this period.

A total of 125 images were obtained from 1998 to 2019. All images were downloaded and then pre-processed to perform the DOS1 (Dark Object Subtraction) method for atmospheric correction and get the TOA (Top Of Atmosphere) reflectance values using the QGIS Semi-Automatic Classification Plugin (Congedo, 2020). The characteristics of satellites, sensors, and spectral bands used for the study are reported in Table 2.

Spectral reflectance at the green, red, and NIR bands was extracted from images using the raster package (Hijmans, 2020) within the R (version 4.0.5) environment (R Core Team, 2021). The extraction was performed by using the shapefiles of the pasture layer previously built as extracting layers. Moreover, the percentage of the total pixel area (30 m x 30 m) covered by the pasture layer was calculated to exclude pixels with less than 70% (about 630 m^2) of pasture coverage.

A set of Vegetation Indices (VI) in the range of the selected bands (SR, GCI, DVI, NDVI, GNDVI, SAVI) was calculated from the extracted reflectance values. Details on indices, formulas and their description are reported in Table 3.

A flowchart of the remote sensing methodology adopted in this study is provided in Figure 1.

2.4. Assessment of the long-term impacts of legume-rich mixtures oversowing

To assess to what extent remotely sensed vegetation indices (VIs) could be used to monitor pasture yield and quality, a correlation analysis was performed between VIs and the observed data for yield (g m^{-2} of DM), N pasture content (g m^{-2} of N), grasses (%), legumes (%), and forbs. The significance of Pearson's correlation index (r) was tested by performing a two-tails Student's t-test. Furthermore, the root-mean-square error (RMSE) of the linear regression between VIs and ground data was calculated. Previous to these analyses, both ground data and VIs were averaged at the field level.

To assess the long-term effects of legume sowing and to minimize any influence of the interannual variability of remotely sensed images and the effect of the different satellite sensors, VI values of sown fields were normalized with respect to the control fields (nrVI) for each *j*th field of the i*th* farm at each *k*th sensing date as follows:

$$nrVI_{i,j,k} = \frac{VIsown_{i,j,k} - VIcontrol_{i,k}}{VIcontrol_{i,k}}$$

where the *VIsown* and the *VIcontrol* were the VIs values in the sown and control areas averaged at the field level, respectively. Furthermore, to assess if this effect was dependent on the time since sowing, we calculated the temporal distance (years) between the VI sensing and the year when the sowing was carried out in that field. This variable was called age. A positive age indicates years after sowing, and a negative age indicates years before the sowing, being zero in the year of sowing.The temporal stability of the remotely-sensed data was calculated by analyzing the temporal dynamics of the standard deviation of nrVIs (sdVI) across ages, adopting a mobile time windows approach to calculate moving standard deviation. For each nrVI, the standard deviation at the *j*th field and the *k*th age (sdVI) was calculated within a window of three years (*m*=3) as follows:

$$sdVI_{j,k} = \sqrt{\frac{1}{m} \sum_{i\,=\,k-m+2}^{k+2} \left(nrVI_{i,j} - \overline{nrVI_j}\right)^2}$$

where $nrVI_{i,j}$ is the nrVI value at the *i*th age, and $nrVI_j$ is the average value within the window.

The effect of sowing was assessed by fitting a linear mixed-effect model (lme), with both nrVI and sdVI as response variables and period (before sowing *vs.* after sowing) and age (years to/from sowing) as predictors, assigning the field as a random factor. If the effect of the age on nrVIs was not significant, highlighting the absence of any significant trend, a one-tail Student's t-test was performed to compare before and after sowing periods in order to test differences between the average nrVI and nrVI = 0. If the effect of age on sdVIs was significant, linear or 2nd-degree polynomial regressions were fitted to test for temporal trends in each period (i.e., before and after the sowing).

Data analysis and statistical computations were performed using the RStudio application of R software (version 4.0.5) (R Core Team, 2021). The significance of statistical computations was evaluated at $P<0.05$ unless otherwise stated.

3. Results

3.1. Pasture pixels' availability in the wooded grasslands

The mean, maximum, minimum, and standard deviation of each VI in control and sown areas both before and after the sowing are reported in Table 4.

As expected, a negative exponential relationship (R^2=0.77, data provided in Supplementary Material, Table 2S) was found between the field tree cover and the number of treeless pixels available for the analysis (i.e., pasture areas). The adopted method in selecting useful pixels allowed us to explore, on average, 37% of the total field area, ranging from values lower than 5% in the high-covered fields (tree cover > 50%) to a useful surface higher than 60% in the low-covered fields (tree cover < 20%).

3.2. Relationships between ground and remote sensing data

The mean and the standard deviation of grassland yield (Mg ha^{-1}), the relative abundance (%) of grasses, legumes, and forbs, and the N pasture content (g m^{-2} of N) are reported in Table 5. All the VI had a significant positive correlation ($P<0.05$) with the observed grassland yield (Mg ha^{-1} of DM) in 2016. The highest correlation indices were observed between yield and SR (r=0.56; RMSE = 1.95 Mg ha^{-1}) and NDVI (r=0.54; RMSE = 2.03 Mg ha^{-1}). The N content (g m^{-2} of N) had a significant positive correlation with all indices except for GCI and GNDVI (both r=0.39, P=0.07). No significant correlations between the relative abundance of the considered functional groups and the VI were observed. Results are in detail reported in Table 6.

Further information about the pasture biomass production and N pasture content over ages and the linear regressions between each VI and ground data showing significant correlations are reported in the Supplementary Material (Fig. 2S, Fig. 3S)

3.3. Effect of sowing legume-rich pastures on vegetation indices

The period significantly ($P<0.001$) affected each nrVI, which were significantly higher after sowing than before. The dynamics of each nrVI are reported in Figure 2. The age within the period did not affect the nrVIs, highlighting the absence of significant trends before and after sowing periods.

The mean values of nrVIs were significantly different from 0 (sown VI > control VI) after the sowing, while no significant differences were observed before ($P<0.001$).

3.4. Stability of NDVI across years

Each sdVI was affected by period ($P<0.01$) and age within the period ($P<0.001$). The average sdVI was always higher before the sowing than after, regardless of the VI used. Furthermore, significant increasing trends of sdVI were observed before the sowing, while no trend was observed after sowing (Figure 3).

4. Discussion

4.1. The challenge of assessing wooded pastures by satellite images

In this study, identifying and limiting pasture areas allowed reaching an adequate level of completeness of information about the pasture contribution to primary productivity. However, the lower availability of pixels under high-covered fields implies a loss of spatial information from multispectral data. Nevertheless, the information from the treeless pixels can be more accurate than those deriving from the raw dataset in wooded grassland (Franklin, 1991). The higher spatial accuracy can result in higher precision in estimating primary production (Seaquist et al., 2003;

Duncanson et al., 2020). Furthermore, the integrated approach adopted in this study allowed distinguishing trees from pasture without analyzing spectral differences during drier periods, as proposed by Gómez-Giráldez et al. (2019), minimizing the amount of data to manage in performing the long-term analysis. On the other hand, the adopted methodology required integrating different types of images – the high-resolution RGB orthophotos with the Landsat long-term series – resulting in a two-step workflow that requires access to different databases. Despite this could hamper the automatic deployment of a decision-support system, the automatic separation performed with eCognition, even if challenging, guarantees a high accuracy (Gomes et al., 2018; Arenas-Corraliza et al., 2020; Pearse et al., 2020).

The observed significant correlations between VIs and ground data on grassland biomass production confirmed a large body of literature reporting the effectiveness of these indices derived from the green-red-NIR regions in predicting forage availability (Muñoz et al., 2010; Maselli et al., 2013; Gómez-Giráldez et al., 2019; Rezende et al., 2020). The VIs proved their reliability in describing the overall biomass patterns, even if their performances can be limited due to the saturation issues, as observed by Ullah et al. (2012) using NDVI and SAVI. Although it is recognized the ability of NDVI and SR to estimate foliar N content and chlorophyll in grassland ecosystems (e.g., Gamon et al., 1995), the lower effectiveness of VIs in predicting the N content was expected. The chlorophyll concentration, its distribution, and other chemical constituents are functions of the spectral reflectance at the leaf level (e.g., Sims and Gamon, 2002), while spectral reflectance is a function of leaf area index and other biophysical properties, such as overall vegetation cover, at the pasture canopy level. Factors affecting vegetation reflectance can vary according to the scale (Zarco-Tejada et al., 2003). For this reason, VIs that can adequately predict nitrogen content at the leaf scale might underperform at the pasture canopy level, limiting their effectiveness in predicting nitrogen patterns. Another limitation could be represented by the influence of weather fluctuation on relationships between VIs and ground data, i.e., under drought conditions (Wagle et al., 2014; Zhao et al., 2018). Even if only the relationships derived from only one year of ground data, a large literature supports the effectiveness of used VIs in predicting biomass patterns in Dehesa agroecosystems (e.g., Serrano et al., 2018; Gómez-Giráldez et al., 2019; Serrano et al., 2021), and a large range of conditions in terms of productivity and nitrogen pasture content was explored. Furthermore, the study was oriented to describing the relative patterns of oversown *vs.* unsown areas without the purpose of predicting the actual biomass production or nitrogen.

4.2. The effectiveness and persistence of sown legume-rich pastures

The observed VIs dynamics confirmed the hypothesis that legume oversowing could enhance the spring forage availability of wooded grasslands. The results confirmed what was already observed in the same study fields in the experiments conducted by Hernández-Esteban et al. (2018), aiming to assess, among others, the effect of legume oversowing on forage availability. The increased forage availability after sowing can be attributable to the enhancement of N_2-fixing-species in plant communities (Bondaruk et al., 2020), as well as to the synergy that can occur between legume and no-fixing-species which may promote grasses growth and biomass production (Nyfeler et al., 2009). This can induce, at the same time, a potential loss of species richness, as observed by Jaurena et al. (2016). Nevertheless, this undesirable side effect was not observed within the study side, according to what was reported by Hernández-Esteban et al. (2019).

The positive effect of legume oversowing on forage productivity associated with the enhanced N availability is also reflected in the long-term persistence of higher values of each VI after the sowing. Although the remote-sensed dataset did not allow to fully confirm the findings of Hernández-Esteban et al. (2018), which observed an increasing pattern of soil N content, the absence of any progressive decrease of VIs after sowing can be considered as an indicator of an improved soil N cycling (Gómez-Rey et al., 2012; Teixeira et al., 2015) thanks to a triggered activity of *Rhizobium* bacteria (Moreno et al., 2021). In addition, legume oversowing may have had a robust positive effect also on the other native plant species composing the floristic communities of grassland, especially grasses, which have benefited from enhanced N availability over time and which maximized their N use efficiency under these specific conditions induced by oversowing (Suter et al., 2015; Cong et al., 2017). Furthermore, the oversown legume-rich mixtures were composed of hard-seeded species that directly affected the grassland seed bank. The long-lasting dormancy that can be induced through this biological mechanism represents an adaptation strategy facing interannual weather variability, guaranteeing the persistence over time of legume species (Beuselinck et al., 1994; Teixeira et al., 2015; Hayes et al., 2019).

4.3. The effect of legume oversowing on temporal variability

The evidence emerging from the remotely sensed dataset confirmed the hypothesis that the legume oversowing could smooth the interannual variability of forage production. The increasing temporal variability that was observed before sowing could be related to an overall degradation of grassland without proper agronomic management leading to the restoration of soil fertility and pasture quality. This interpretation can explain the choice to improve those fields through an oversowing with legume-rich mixtures. All the factors leading to an increased and more persistent biomass productivity (improvement of C and N cycling, increased N availability through N fixation, dormancy mechanism associated with the hardseedness) are expected to play a crucial role in reducing the temporal fluctuations, which are mainly associated with environmental and weather variability (Gea-Izquierdo et al., 2009). The decrease in temporal variability of biomass production can be attributed to the increase in species richness (Caldeira et al., 2005), which can enhance the stability of biomass availability through the compensatory effect induced by the reduction of synchrony (defined as the extent to which species population density covary positively over time) between species (Valencia et al., 2020). However, this mechanism can only partially explain what was previously found in the same study site by Hernández-Esteban et al. (2019), which observed an increase of species richness only for legumes and a decrease of forbs richness under drought conditions in the ages soon after sowing. These findings highlight that in these agroecosystems, biomass temporal variability after sowing is mainly driven by the enhanced fertility (i.e., C cycling) and nutrient (i.e., nitrogen) availability induced by oversowing, which in turn could also improve the efficiency in using yield-limiting factors such as nitrogen itself and water (El- Madany et al., 2021).

5. Conclusions

This study confirmed the hypothesis that the oversowing of legume-rich mixtures in Mediterranean wooded grasslands could effectively improve intra- and interannual biomass production of the herbaceous vegetation component. The long-term dynamics of the derived vegetation indices highlighted the positive role of legume oversowing in guaranteeing higher forage availability compared to unsown areas. Furthermore, legume enrichment proved to be an effective solution to reduce the temporal fluctuations of forage provision mainly due to the interannual variability of factors driving biomass production, i.e., water availability and meteorological conditions.

Remote sensing through Landsat images analysis resulted in a suitable tool to assess the impact of grassland management in the long term under the specific environmental conditions characterized by the presence of the trees. Furthermore, combining different image analysis tools allowed reaching an adequate level of information about the grassland contribution to the primary productivity during spring in wooded grasslands, even if the availability of valuable pixels is affected by the tree cover. Further insights may concern the study of the effect of weather patterns on the relationships between vegetation indices and field data to explore the broadest range of conditions and strengthen the predictive capacity of remote sensing.

The results of this study highlight that the combination of new software resources and big-data analytics for predictive modeling can provide cost-effective, integrated farm-level decision support tools for sustainable grassland management.

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Tables

Table 1. Farms, soil classification (Soil Survey Staff, 2014), year of sowing, and sown species within the study area

Site code	Farm	Coordinates and elevation	Soil	Year of sowing	Size of the sown field (ha)	Tree cover (%)	Mixture species
Farm1	Atoquedo	39.77 N; 5.92 W 371 m asl	*Eutric Cambisol*	2010 2011 2012 2013 2014 Control	83.34 48.51 45.42 81.48 14.49 80.37	0.29 0.21 0.29 0.22 0.25 0.21	*Trifolium subterraneum*; *T. subterraneum* ssp *yanninicum; T. subterraneum* ssp. *brachycalycinum; T. incarnatum; T. vesiculosum; T. resupinatum; T. michelianum; T. glanudliferum; Ornithopus sativus; Lupinus luteus*
Farm2	La Caña	39.29 N; 6.13 W 265 m asl	*Eutric Cambisol*	2002 2005 2011 Control	11.56 30.62 40.12 14.03	0.35 0.29 0.41 0.37	*Trifolium subterraneum*; *T. subterraneum* ssp *yanninicum; T. subterraneum* ssp. *brachycalycinum; T. incarnatum; T. vesiculosum; T. resupinatum; T. michelianum; T. glanudliferum; Ornithopus sativus; Lupinus luteus*
Farm3	La Ciervina	39.71 N; 6.13 W 373 m asl	*Eutric Cambisol*	2005 2011 2014 Control	14.89 7.34 10.00 12.76	0.44 0.30 0.37 0.45	*Trifolium subterraneum*; *T. subterraneum* ssp *yanninicum; T. subterraneum* ssp. *brachycalycinum; T. incarnatum; T. vesiculosum; T. resupinatum; T. michelianum; T. glanudliferum; Ornithopus sativus; Lupinus luteus*
Farm4	Las Casillas	39.50 N, 6.48 W 433 m asl	*Dystric Cambisol*	2002 2007 2012 2014 Control	75.21 24.44 11.58 24.96 29.34	0.33 0.75 0.31 0.20 0.20	*Trifolium subterraneum*; *T. subterraneum* ssp *yanninicum; T. subterraneum* ssp. *brachycalycinum; T. incarnatum; T. vesiculosum; T. resupinatum; T. michelianum; T. glanudliferum; Ornithopus sativus; Lupinus luteus*
Farm5	La Villa	39.85 N; 6.48 W 404 m asl	*Chromic Cambisol-Luvisol*	2003 2010 2015 Control	22.54 64.01 7.30 20.97	0.23 0.09 0.21 0.19	*Trifolium subterraneum*; *T. subterraneum* ssp *yanninicum; T. subterraneum* ssp. *brachycalycinum; T. incarnatum; T. vesiculosum; T. resupinatum; T. michelianum; T. glanudliferum; Ornithopus sativus; Lupinus luteus; Lolium multiforum* (2015); *Cynodon dactylon* (2003-2010)
Farm6	Valdelacasa	39.96 N; 5.95 W 288 m asl	*Dystric Cambisol*	2002 2003 2014 2015 Control	22.40 13.65 8.14 34.09 9.73	0.56 0.61 0.31 0.51 0.40	*Trifolium subterraneum*; *T. subterraneum* ssp *yanninicum; T. subterraneum* ssp. *brachycalycinum; T. incarnatum; T. vesiculosum; T. resupinatum; T. michelianum; T. glanudliferum; Ornithopus sativus; Lupinus luteus*

Table 2. List of satellites and sensors, interval between scenes detection, and characteristics of spectral bands used in the study

Satellite	Repeat interval	Bands	Wavelengths (μm)	Spatial resolution (m)
Landsat 4-5 TM; Landsat 7 ETM+	16 days	Band 2 – green	0.52-0.60	30
		Band 3 – red	0.63-0.69	30
		Band 4 – near-infrared	0.77-0.90	30
Landsat 8 OLI	16 days	Band 3 – green	0.53 - 0.59	30
		Band 4 – red	0.64 - 0.67	30
		Band 5 – near-infrared	0.85 - 0.88	30

Table 3. Vegetation indices derived from Landsat green, red, and near-infrared bands used in the study.

Vegetation Index	Equation	Description	Reference
Simple Ratio (SR)	$SR = \frac{\rho_{nir}}{\rho_{red}}$	The SR allows distinguishing green leaves from other objects in the scene or stressed from no-stressed vegetation. When SR value is close to 1, the object has similar reflectance in red and NIR bands (e.g. soil). Green objects may have an SR value much higher than 1.	Pearson and Miller (1972)
Green Chlorophyll Index (GCI)	$GCI = \frac{\rho_{nir}}{\rho_{green}} - 1$	The GCI is a chlorophyll content proxy index based on NIR and green reflectance. In general, the chlorophyll content is directly related to vegetation. It allows distinguishing high-biomass hotspots better than NDVI.	Gitelson et al. (2005)
Difference Vegetation Index (DVI)	$DVI = \rho_{nir} - \rho_{red}$	The DVI can distinguish soil from vegetation in a scene, being sensitive to the amount of photosynthetically active vegetation in the plant canopy.	Tucker (1979)
Normalized Difference Vegetation Index (NDVI)	$NDVI = \frac{\rho_{nir} - \rho_{red}}{\rho_{nir} + \rho_{red}}$	The NDVI is a dimensionless index, that describes the difference between visible and near-infrared reflectance of vegetation cover,. It ranges from -1 to 1. A densely green-leafed area tends toward values close to 1, while water and built-up areas tend to zero or negative values, the latter likely indicating water.	
Green Normalized Difference Vegetation Index (GNDVI)	$GNDVI = \frac{\rho_{nir} - \rho_{green}}{\rho_{nir} + \rho_{green}}$	The GNDVI is a photosynthetic activity proxy index commonly used to determine water and nitrogen uptake into the plant canopy. Compared to the NDVI index, it is more sensitive to chlorophyll concentration, considering the green instead of the red reflectance.	Gitelson et al. (1996)
Soil-Adjusted Vegetation Index (SAVI)	$SAVI = \frac{\rho_{nir} - \rho_{red}}{\rho_{nir} + \rho_{red} + 0.5}(1 + 0.5)$	The SAVI is an index derived from NDVI that minimizes soil brightness influences using a correction factor. A correction factor of 0.5, which is used in arid regions where vegetative cover is low, is adopted in this study	Huete (1988)

Table 4. Mean value, standard deviation, and range of variation of the Vegetation Indices in control zones and sown fields before and after the sowing

Zone	Period	Index	mean	sd	max	min
Control		DVI	0.18	0.05	0.32	0.08
		GCI	2.16	1.06	5.48	0.86
		GNDVI	0.49	0.11	0.72	0.30
		NDVI	0.48	0.11	0.76	0.25
		SAVI	0.31	0.08	0.52	0.15
		SR	3.11	1.08	7.27	1.67
Sown	Before	DVI	0.17	0.05	0.41	0.08
		GCI	1.90	1.17	9.22	0.66
		GNDVI	0.45	0.11	0.82	0.25
		NDVI	0.45	0.12	0.84	0.18
		SAVI	0.29	0.08	0.62	0.13
		SR	2.95	1.37	12.17	1.45
	After	DVI	0.21	0.06	0.41	0.09
		GCI	2.75	1.35	7.95	0.70
		GNDVI	0.55	0.12	0.80	0.26
		NDVI	0.55	0.13	0.84	0.25
		SAVI	0.35	0.09	0.60	0.16
		SR	3.90	1.69	11.80	1.69

Table 5. Grassland biomass production (Mg ha^{-1} of DM ± standard deviation), relative abundance of legumes, forbs, and grasses (%), and Nitrogen pasture content (g m^{-2} of N) observed in 2016 in the field study.

Farm	Year of sown	Grassland yield (Mg ha^{-1} of DM)	Legumes (%)	Forbs (%)	Grasses (%)	N pasture content (g m^{-2} of N)
Farm1	2010	2.79±2.17	50.0	29.8	20.2	5.8
	2011	3.54±2.79	28.8	29.8	41.3	6.6
	2012	4.45±2.41	45.2	15.4	39.4	9.0
	2013	5.35±3.55	41.3	25.0	33.7	10.0
	2014	2.79±2.00	34.6	33.7	31.7	4.7
	Control	1.78±0.57	15.2	45.1	39.7	3.5
Farm2	2002	2.28±1.40	31.3	32.9	35.8	5.3
	2005	3.74±2.07	39.4	20.2	40.4	7.1
	2011	6.82±1.44	35.6	21.2	43.3	10.1
	Control	1.37±0.58	19.2	37.8	43.0	2.2
Farm3	2005	3.54±1.90	51.0	37.5	12.5	7.2
	2011	4.76±1.90	36.5	41.3	28.8	9.4
	2014	5.35±2.11	67.3	26.9	11.5	12.7
	Control	1.78±0.50	54.4	28.6	20.2	3.9
Farm4	2002	3.53±1.98	26.9	26.9	46.2	5.8
	2007	3.41±2.29	26.9	25.0	48.1	5.2
	2012	5.54±1.55	39.4	29.8	30.8	10.0
	2014	7.06±1.75	49.0	28.8	22.1	14.1
	Control	1.18±0.50	34.8	27.8	37.4	2.1
Farm5	2003	3.79±2.34	25.0	48.1	26.9	9.4
	2010	3.91±1.53	44.2	40.4	15.4	9.8
	2015	8.31±2.13	26.9	33.7	39.4	16.0
	Control	1.78±0.74	24.1	43.3	32.7	2.9
Farm6	2002	4.32±1.75	36.5	42.3	25.0	10.1
	2003	3.88±2.44	27.9	27.9	35.6	9.1
	2014	8.31±1.75	90.4	27.9	14.4	27.9
	2015	7.06±2.26	76.9	7.7	16.3	16.1
	Control	1.53±0.42	44.6	40.5	24.5	3.5

Table 6. Pearson's correlation index (r), p-value of the correlation analysis, and root mean square error (RMSE) of the relationships between the Vegetation Indices derived from multispectral Landsat remote sensed data and the field ground data (Yield, pasture N, grasses, legumes, and forbs) collected in 2016 in the study fields.

		Vegetation Indices					
Ground data		**DVI**	**GCI**	**GNDVI**	**NDVI**	**SAVI**	**SR**
Yield (Mg ha^{-1})	r	0.54	0.46	0.46	0.50	0.54	0.56
	p-value	0.010	0.031	0.032	0.017	0.010	0.007
	RMSE	1.98	2.08	2.09	2.03	1.98	1.95
N (g m^{-2})	r	0.50	0.39	0.39	0.45	0.48	0.47
	p-value	0.019	0.072	0.069	0.035	0.022	0.027
	RMSE	5.41	5.73	5.72	5.55	5.44	5.49
Grasses (%)	r	0.11	-0.05	-0.05	0.05	0.10	0.05
	p-value	0.581	0.794	0.817	0.807	0.63	0.797
	RMSE	11.00	11.05	11.05	11.05	11.02	11.05
Legumes (%)	r	0.08	-0.08	-0.08	-0.03	0.03	-0.04
	p-value	0.696	0.685	0.703	0.894	0.87	0.853
		17.17	17.17	17.17	17.22	17.21	17.21
Forbes (%)	r	-0.11	0.25	0.23	0.051	-0.05	0.07
	p-value	0.588	0.206	0.240	0.795	0.800	0.723
	RMSE	9.37	9.14	9.17	9.41	9.41	9.40

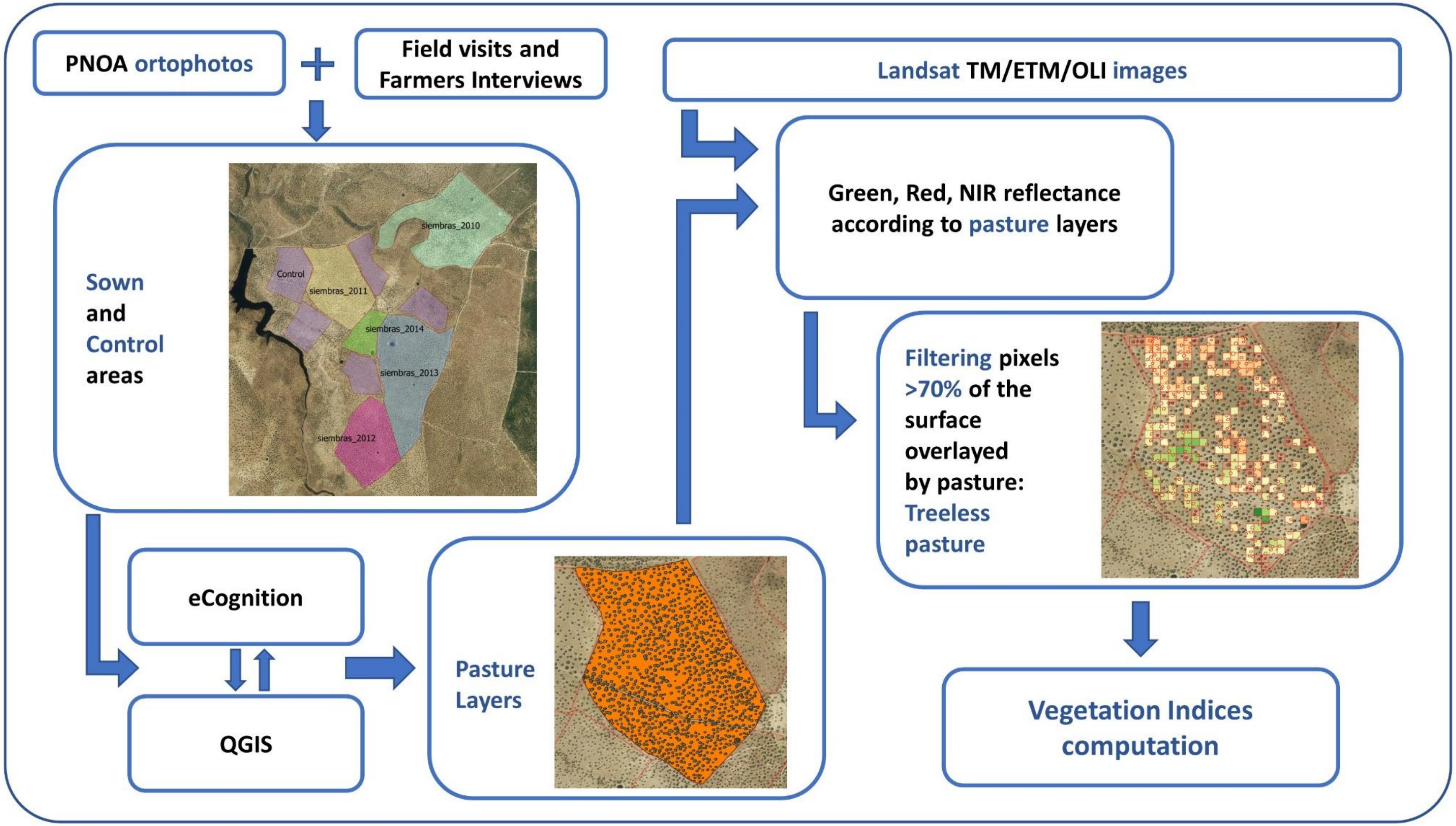

Figure 1. Flowchart of the remote sensing methodology adopted in the study to calculate the vegetation indices in the pasture areas.

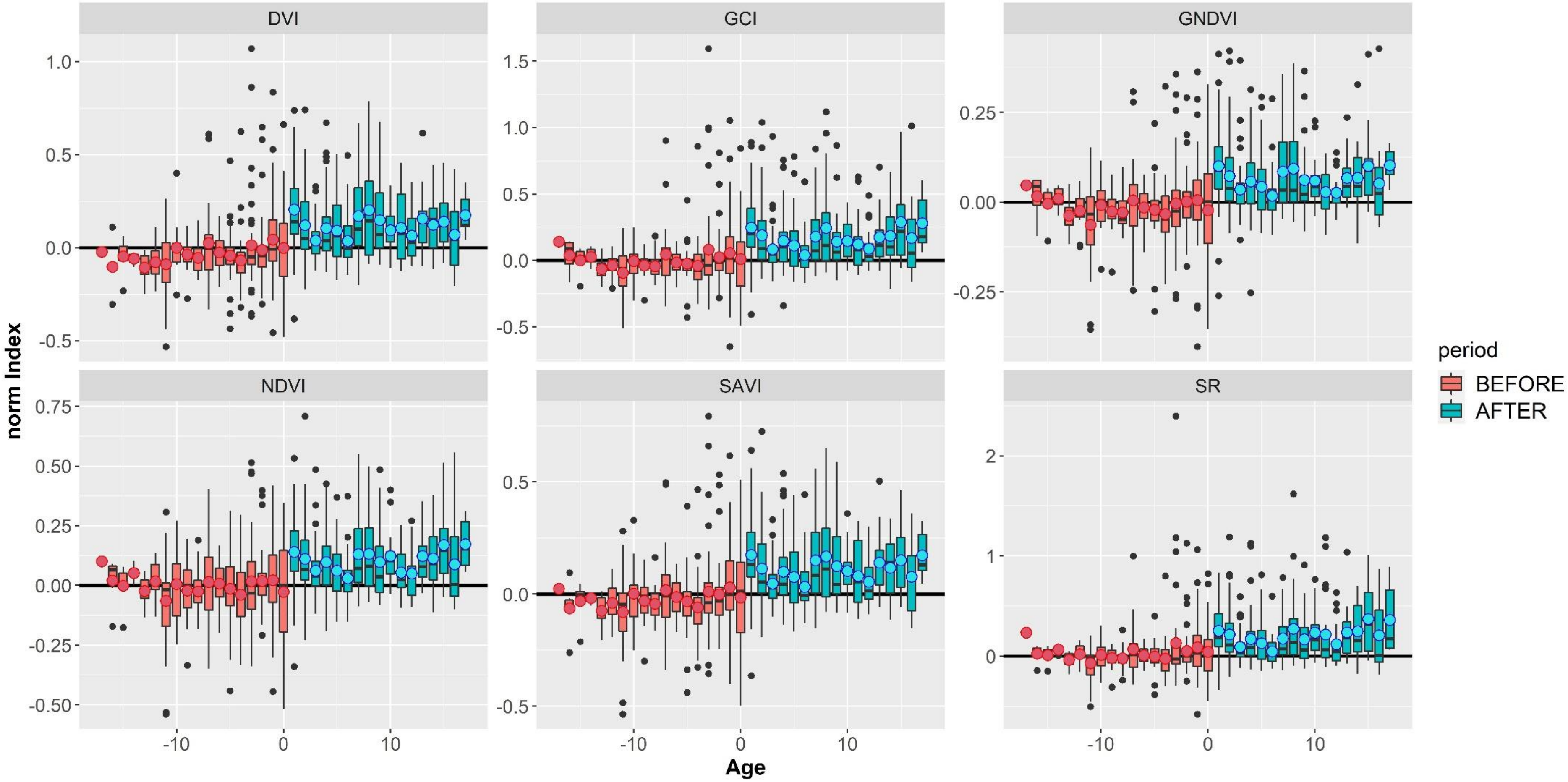

Figure 2. Temporal dynamics of normalized Vegetation Indices before (red) and after (blue) the sowing. The horizontal line at 0 represents the control. The line within the box represents the median value; the box edges represent the interquartile range, the whiskers represent the 95% confidence interval, black dots outside the boxes represent the outliers, and coloured dots represent the average value.

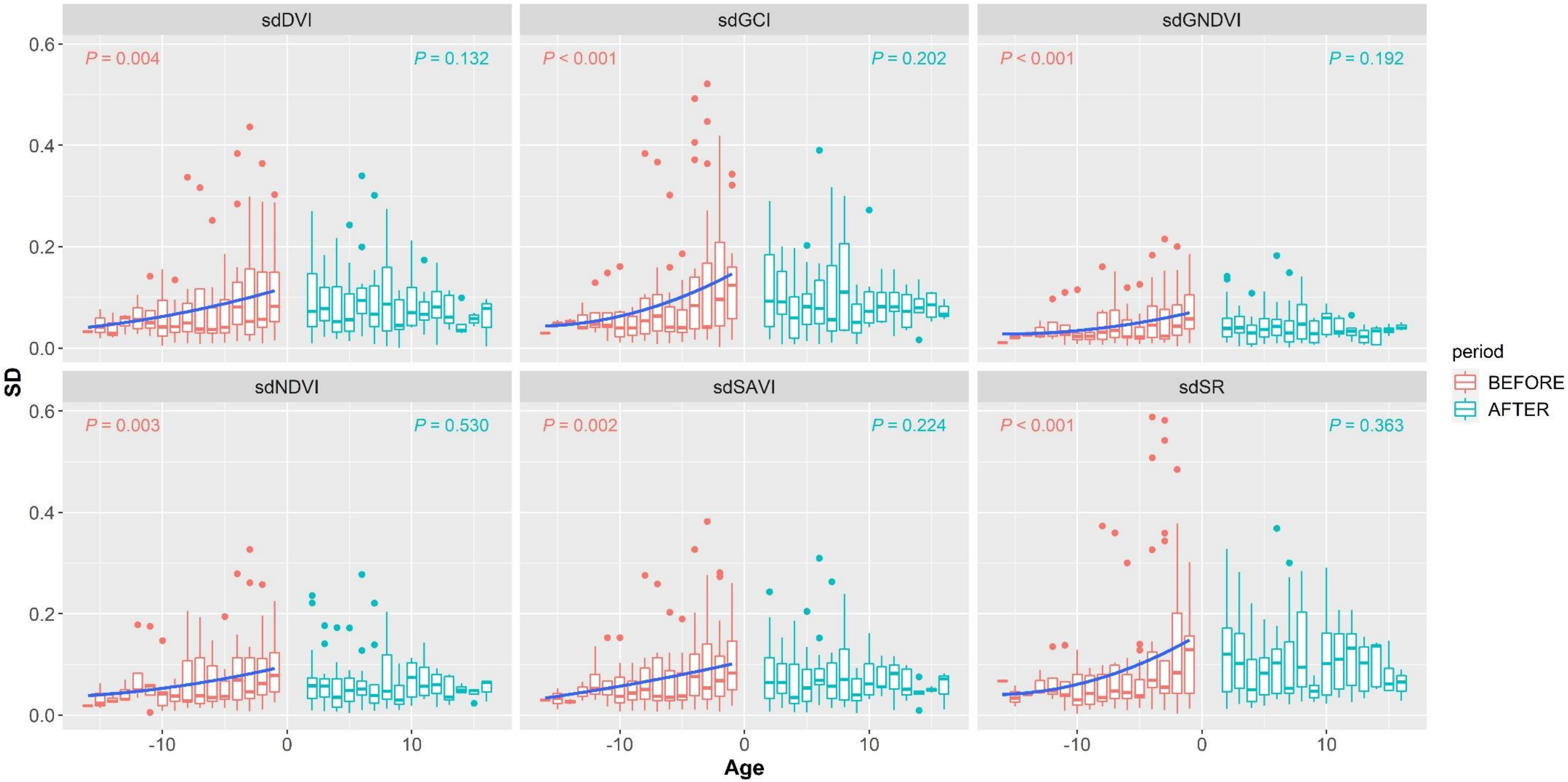

Figure 3. Dynamics of an indicator of temporal stability before (red boxes) and after (blue boxes) the sowing, based on the three-year standard deviation of each normalized Vegetation Indices. The line within the box represents the median value, the box edges represent the interquartile range, the whiskers represent the 95% confidence interval, and the dots outside the boxes represent the outliers. The significance of the fitted linear model before (red, left) and after (blue, right) is reported at the top of each plot. The 2nd order regression model is then represented with a blue line when significant.