EVALUATING REMOTE SENSING DATA AS A TOOL TO MINIMIZE SPATIAL AUTOCORRELATION IN IN-SITU VEGETATION SAMPLING

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With 7 figures, 1 table and appendix
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Summary: The distinction between geographical patterns caused by underlying environmental factors and inherent spatial autocorrelation is a general challenge for field research. The quality and validity of phytogeographical studies is strongly dependent on disentangling spatial and ecological proximity. This is also crucial for applied studies in nature conservation. One key assumption for many statistical analyses is the independence of observations. In this study we first identify the range of spatial autocorrelation in managed grasslands based on field data. Along a gradient in a valley bottom, we set up five 60 m x 60 m squares, segmented in 36 10 m x 10 m square grid cells. In 20 of the 10 m x 10 m grid cells, we sampled vegetation along a 10 m line with a buffer of one meter resulting in a 20 m² sampling plot. In a second step, we matched Sentinel-2 images for the same locations and calculated the normalized difference vegetation index NDVI and the normalized difference red edge index NDRE. For both, field data and satellite data, Mantel correlograms for floristic distances and spectral indices were used to analyse the spatial autocorrelation. We found the vegetation in the studied grasslands to be spatially correlated up to 25 m. At none of the studied sites the positive spatial autocorrelation reaches beyond. The spatial autocorrelation of spectral indices correlates well with the correlations observed field data. The correlograms of NDVI resembled the ones of the field data slightly better compared to the correlograms of NDRE and RGB. We conclude that employing remote sensing to assess the role of spatial autocorrelation for grasslands is a valid approach. We show that it reflects similar patterns as the field data. The spatial resolution of freely available satellite data proved sufficient to test for the minimum distance between vegetation samples to avoid spatial autocorrelation.

Keywords: Altmuehl Valley, grasslands, monitoring, nature conservation, NDVI, Sentinel-2, spectral reflectance

1 Introduction

Spatial patterns are omnipresent in ecology and biogeography, identifying them provides the basis to understanding spatial ecological data and underlying drivers (MacArthur 1972, Levin 1992, Beierkuhnlein 2007). An important concept in ecology is spatial dependence, best described by Tobler's first law of geography "everything is related to everything else, but near things are more related than distant things" (Tobler 1970). It implies that covariance and correlation of measures close to each other should be high and decrease with increasing spatial distance. This can be a result of population or community dynamics also referred to as inherent autocorrelation or stochasticity (Shoemaker et al. 2020). However, it also can be the expression of an underlying ecological driver that contains a spatial dependence (e.g., elevational gradient) or interactions amongst neighbouring organisms (Wagner & Fortin 2005). Intricately linked to spatial patterns in ecology is the term spatial autocorrelation, which is often used interchangeably with spatial dependence. However, spatial autocorrelation only refers to spatial patterns driven by endogenous mechanisms (occurring directly from response variable considered) while spatial dependence refers to both, endogenous and exogenous (also occurring outside of response variable, such as environmental gradients) mechanisms (Bolker 2003, Dale & Fortin 2014). And while detecting an underlying environmental gradient that drives spatial patterns in ecology is desirable, spatial autocorrelation is considered "noise" that diffuses the observed pattern. Understanding this distinction is critical for ecologists, as it allows for a more nuanced interpretation of ecological patterns and informs the selection of appropriate analytical methods. Therefore, distinction between the terminologies is also important when analysing spatial patterns.

Spatial autocorrelation received considerable attention from ecologists, mainly either population geneticists analysing small-scale spatial genetic structures of populations (Guillot et al. 2009) or biogeographers interested in macroecological patterns (Kissling & Carl 2008). It is a much-discussed topic in ecology (e.g., Goslee 2006, Roe et al. 2012) generally described as a pair of observations depending on their distance from each other are more (posi-

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tive spatial autocorrelation) or less (negative spatial autocorrelation) similar compared to the expected similarity of a randomly associated pair (Legendre 1993). Spatial autocorrelation is defined as the lack of independence between observations because of their spatial closeness (Legendre & Legendre, 1998). However, independent observations are a requirement for many common statistical analyses (Sokal & Oden 1978, Haining 1990, Diniz-Filho et al. 2003, Dormann et al. 2007, Dray et al. 2012, Pouget et al. 2021). Ignoring spatial autocorrelation in ecological data can lead to artificially inflating the sampling size with pseudo-replication and significantly alter the statistical results (Hurlbert 1984). Inherently, any distance matrices or comparisons between observations are under the influence of spatial autocorrelation (Legendre & Fortin 2010, Meirmans 2012) and species distribution models have been found to benefit from incorporating spatial autocorrelation (Dormann 2007). Measuring spatial autocorrelation is relevant on different scales, from local and regional (Bini et al. 2000) to latitudinal gradients (Jetz & Rahbek 2001). It is present in all vegetation studies and should be considered when planning field sampling.

Traditionally, one long standing sampling requirement when sampling vegetation in plots or sampling units is the relative homogeneity throughout the sample stand area (Ellenberg & Mueller-Dombois 1974) and refers to the consistency or similarity of vegetation characteristics within a sampling unit. Homogeneity in this sense is seen as a measure to ensure that sampling takes place in a representative vegetation stand for the landscape investigated and to not include a gradient already in one single sampling site. The selection of a homogenous vegetation stand is a subjective process, an issue that can be addressed by sampling fixed area plots around concrete coordinates within the landscape under investigation (Chiarucci 2007). Even though the topic of the necessity of homogeneity of the vegetation within one sampling area and the different concepts of vegetation sampling behind it are much debated (e.g., Braun-Blanquet 1965, Lájer 2007, Chiarucci 2007), in many vegetation studies, a "homogeneous" sampling area is the standard. In contrast, spatial heterogeneity of vegetation stands is often used as a proxy for beta diversity. High spatial heterogeneity in a landscape is often considered as a crucial component in maintaining ecosystem functions. A reduction of spatial heterogeneity, defined as an increase of uniformity in vegetation is in this concept therefore considered as an indicator for landscape degradation (Bestelmeyer et al. 2011).

Diameters of homogeneous patches may be assessed to analyse the effect of different management or different landscape positions (Meyers et al. 2014). In the context of managed grasslands, the extent of spatial autocorrelation varies from 20 m (Meyers et al. 2014) to 100 m (Podani & Csontos 2006) and is quantified using Mantel correlograms (Legendre & Fortin 1989, King et al. 2004, Caballero et al. 2008, Gallardo-Crúz et al. 2010, Legendre et al. 2015, Wildi 2017). A major issue is the distinction of inherent autocorrelation from spatial dependence induced by underlying environmental factors, e.g., soil moisture (Fortin & Dale 2009) which must be addressed by appropriate sampling approaches (Schweiger et al. 2016). Generally, the species assemblages of hay meadows are influenced by management (uniform per field unit), nutrient availability and soil moisture (Ellenberg & Leuschner 2010, Kollmar et al. 2010). And while agricultural management is uniform per field unit, the sizes vary considerably. In southern Germany for example, small pieces of land prevail (often smaller than 0.3 ha) with potential significant differences in their management intensities on a small spatial scale.

Remote sensing provides valuable tools to monitor landscape dynamics and finding patterns of similarities or differences within and between landscapes (Singh et al. 2010, Pettorelli et al. 2017). Additionally, promising methods are under development for mapping species richness and monitoring and quantifying biodiversity from space (Rocchini et al. 2021, Rocchini et al. 2022). Especially in conservation planning and monitoring, remote sensing techniques and approaches gain importance as a cost and time efficient option for ecological monitoring (Kennedy et al. 2009, Willis 2015, Ali et al. 2016, Reinermann et al. 2020). In satellite remote sensing, indirect measurements and indices are used to relate the spectral signal to plant diversity on the ground. One popular index is the normalized difference vegetation index (NDVI), which is derived from the red to near-infrared reflectance ratios (Equation 1) (Rouse et al. 1974). With the launch of the Sentinel-2 satellite program in 2015, high resolution, multispectral remote sensing data is available free of charge via the Copernicus website and the vast amount of earth observation data available facilitates the development of ecological applications (Ustin & Middleton 2021). While a proper set up for field data collection is crucial, scouting the area of interest via remote sensing methods to exclude bias and potential inherent errors is not widely used yet. As the spatial autocorrelation can differ significantly on a local level in space and time (Herben et al. 1995), in theory, prior to each field sampling the reach of spatial autocorrelation should be checked. In reality, approximations and thresholds based on similar studies or found in literature are usually deemed sufficient. While this might be applicable when studying large scale spatial patterns, this quickly becomes an issue when working on small scale differences within patchy landscapes. Henebry (1993) suggests the detection of changes in grasslands based on measuring changes in spatial dependence based on Landsat data, but rather focuses on monitoring changes in the spatial patterns within grasslands than spatial autocorrelation measurements per se. We suggest employing the freely and readily available data Sentinel-2 provides to a-priori ensure the independence of the sampled vegetation data. With its 10 m pixel resolution it fits the common sampling sizes of grassland vegetation sampling units well.

Therefore, in this study we combine the issue of spatial autocorrelation and its range in managed grasslands with the potential opportunity to quantify spatial autocorrelation from space. With this approach, data quality and its statistical usability of vegetation sampling could be significantly improved. We investigate on the one hand, up to which distance the species combination of managed grasslands in our study area is homogeneous to the point of being spatially autocorrelated. In the next step, we test whether the spatial autocorrelation patterns found in the field are supported by space-borne remote sensing approaches. To do so, we analyse the spatial autocorrelation of the values for the indices derived from remote sensing data at the same spatial resolution for the same study area and plot extents. As the tested indices (NDVI and NDRE) are already proven to be good proxies for biomass (Piñeiro et al. 2006), plant species composition (He et al. 2009) and plant health (Boiarskii & Hasegawa 2019), we expect the remote sensing patterns to follow field pattern.

2 Material and Methods

2.1 Study area

The study area is situated in the upper Altmühl valley (Bavaria, Germany), a wide valley frequently subjected to flooding events in late winter. For 30 years, water management measures linked to water transfer to the Main catchment reduced the occurrence and intensities of those floodings. The soils of the study sites consist of loamy to clayey Gleyic Fluvisols (Auengley and Vega-Gley) (LfU 2021b). The large-scale nature conservation project "Lebensraum Altmühltal" was established in 2020. All grasslands studied are protected under the European Habitats 2000 directive and are labelled as Natura2000 sites (Fig. 1). In EUNIS, these sites are classified as "Moist or wet eutrophic and mesotrophic grassland". Regional conservation measures like the Bavarian VNP (Vertragsnaturschutzprogramm = contract-based conservation program) apply. All surveyed grasslands are part of the same VNP category (H22), with management restriction (no mowing before the 15th of June) and complete prohibition of fertilization (LfU 2021a). Sampling sites cover a spatial gradient of 3 km. As only marginal differences in altitude, climate and soil properties are present as well as regulations for management are identical, we can assume that the sampling sites are comparable regarding environmental conditions. The analysed grasslands (moist lowland hay meadows) phytosociologically belong to the order *Arrhenatheretalia*. They comprise more species than the *Ranunculus repens-Alopecurus-pratensis* communities, but less herb species compared to well-developed *Arrhenatheretum* stands. The nutrient supply is moderate to fair, and the area is periodically flooded. Therefore, we find species characteristic of *the Lolio-Potentillion* alliance as well.

2.2 Methods

The grasslands were sampled in August 2020. At each site a 60 m x 60 m square was placed, its edges marked with a handheld GPS device (Garmin GPSMap 64s) and divided into 36 10 m x 10 m squares using a tape measure. Vegetation was recorded in a systematic grid within the 10 m x 10 m cells. As negative impact on the grasslands by trampling had to be limited, a point-line-method rather than the classical square plot approach was applied diagonally within the 10 m² cells (see Fig. 2). Data were recorded along 10 m length by recording species touching a vertically placed stick every 50 cm (Daget & Poissonnet 1971, Bartha et al. 1995). Additionally, all species not yet recorded along the 10 m line but growing within a buffer of 1 m on both sides of the 10 m line were recorded with a cover value smaller than 5%. Frequency values of point observations reflect the probability to observe a certain species, which equals the cover value of this species (Daget & Poissonet 1971).

The workflow for the data analyses is visualized in Fig. 3. After sampling the vegetation, a matching Sentinel-2 scene from 9 Aug 2020 was preprocessed

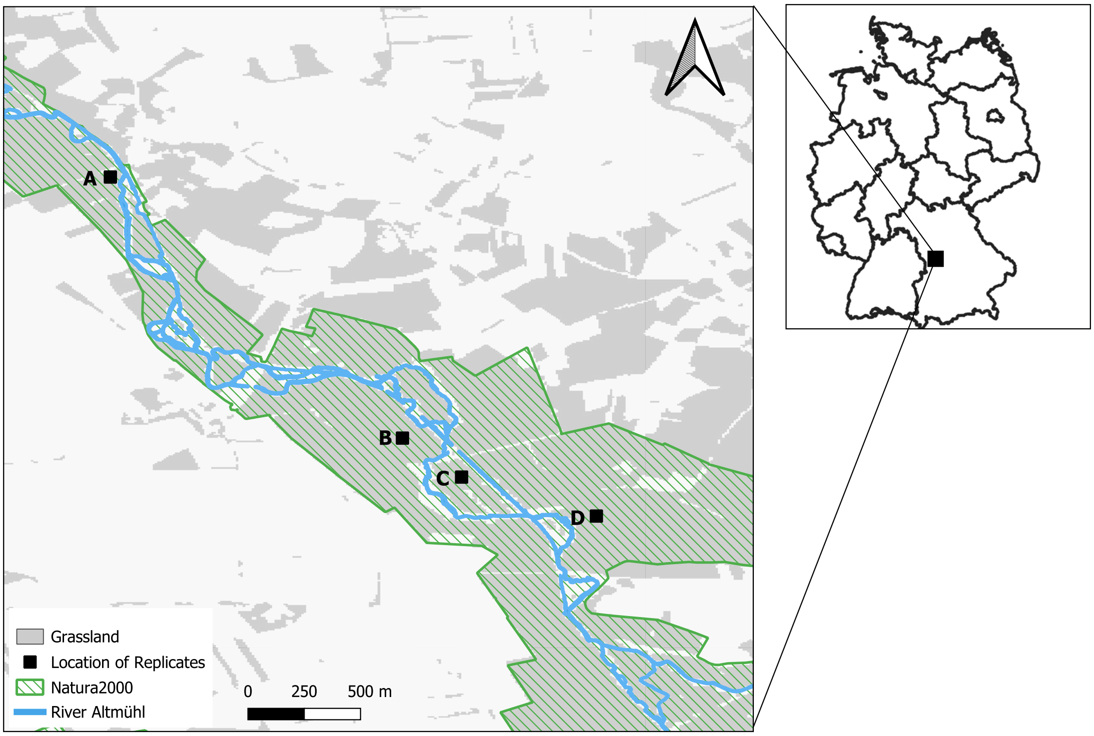

Fig. 1: Field sampling sites located in the Altmühl Valley, Germany. "Grasslands" are mapped via the 2018 Copernicus Land Cover Classification (Copernicus 2020). Research sites (A to E) with 20 vegetation samples follow the gradient of the valley (coordinate reference system: UTM 32, ETRS1989, Natura2000-sites (LfU n.d.).

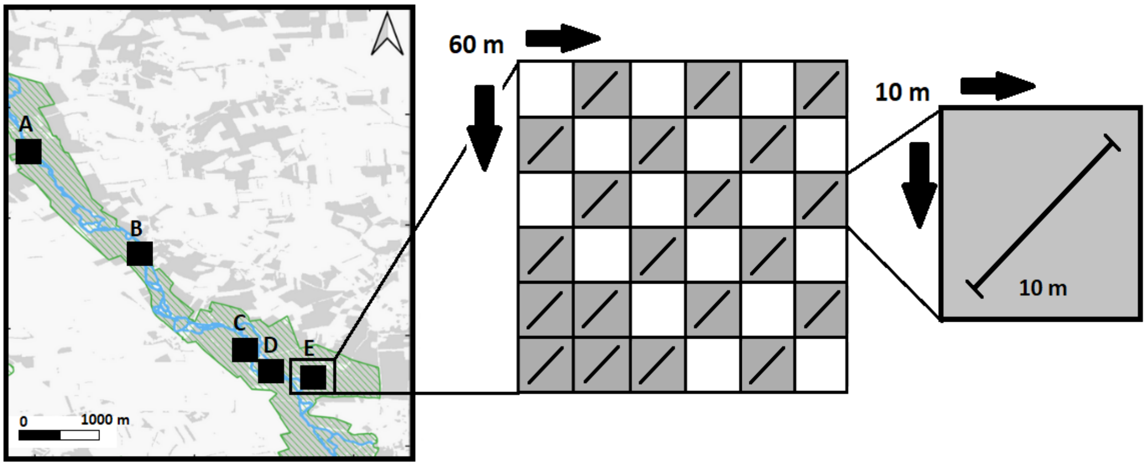

Fig. 2: Sampling design. In a), the general setup of the five sites along the river Altmühl (blue line) is depicted schematically. One grid as depicted in b) is located in each one of the five sites visible in a). The square is segmented in 36 10 m x 10 m squares and in each gray square vegetation sampling is conducted (c). The sampling is done along a 10 m line, including the surrounding 1 m on both sides along the sampling line.

to Level-2 analysis-ready data (ARD) using FORCE version 3.5.2 (Frantz 2019). This study used the bands of 10 m and 20 m resolution (Copernicus 2021). A resolution merge was performed improving the spatial resolution of the 20 m bands to 10 m using the ImproPhe method (Frantz et al. 2016). In R (version 4.0.3, R Core Team 2020) the spectral information was extracted for the GPS locations.

To account for deviating GPS precision, pixels were selected that fit best within the margins marked in the field. Within each grid cell, all pixel values were extracted. The grain of the pixels was 10 m x

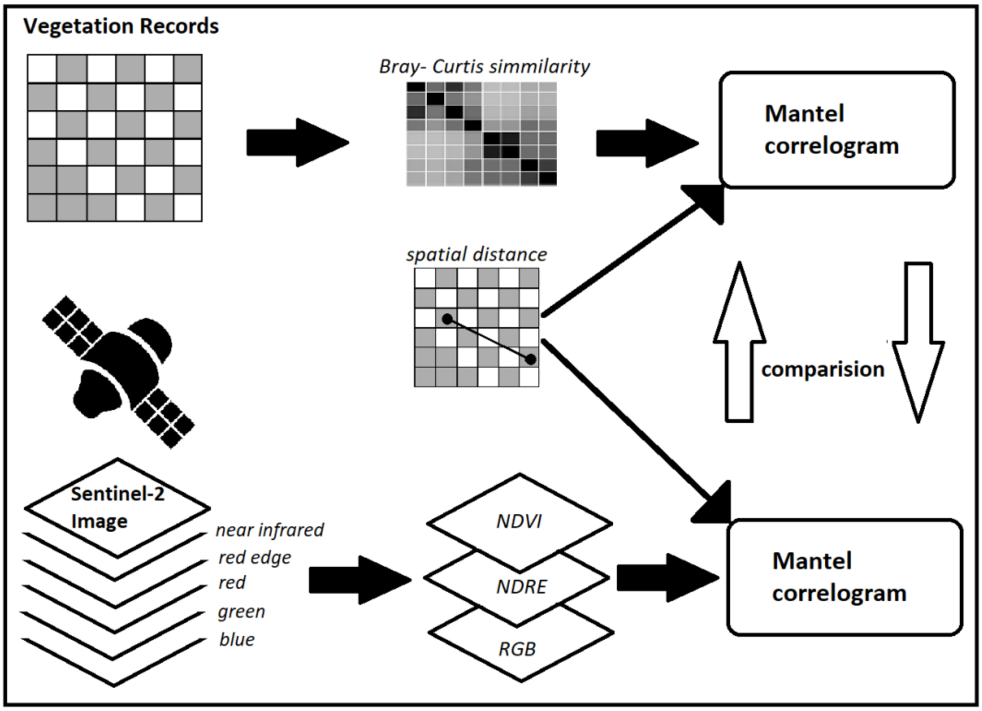

Fig. 3: Methodical approach and workflow for the assessment of spatial autocorrelation in vegetation and remote sensing data. Vegetation records and data form the downloaded Sentinel-2 image were separately analysed with the same approach and the results compared via linear models.

10 m, corresponding with the cell sizes of vegetation records. NDVI was calculated with 10 m resolution according to Equation 1.

$$NDVI = \frac{NIR - RED}{NIR + RED} = \frac{Band\ 8 - Band\ 4}{Band\ 8 + Band\ 4} \quad 1)$$

NDVI: Normalized Difference vegetation Index, NIR: Near Infrared (Sentinel-2 band 8), RED: Red (Sentinel-2 band 4)

The normalized difference red edge index (NDRE) is calculated similar to the NDVI but uses the red edge instead of the red channel. The NDRE was calculated with 10 m resolution according to Equation 2.

$$NDRE = \frac{NIR - RED_{EDGE}}{NIR + RED_{EDGE}} = \frac{Band\ 8 - Band\ 5}{Band\ 8 + Band\ 5} \quad 2)$$

NDRE: Normalized Difference Red Edge Index, NIR: Near Infrared (Sentinel-2 band 8), RED_{EDGE}: Red Edge (Sentinel-2 band 5)

Additionally, we used the combination of the red, green and blue bands (Sentinel-2 bands 4, 3, 2) at a spatial resolution of 10 m. We calculated the Euclidean distance in a three-dimensional colourspace spanned by the standardized red, green and blue bands to analyse the spectral variability instead of deriving an RGB-vegetation index (Biró et al. 2024, Ludwig et al. 2024). Given a grassland sampling site of 6 x 6 neighbouring pixels we assumed uniform brightness and the Euclidean distances are sufficient.

Mantel correlograms were applied for spatial autocorrelation using floristic and spatial distances. Floristic distances were calculated using Bray-Curtis distances (D). The complementary Bray-Curtis similarities (S) are calculated by subtracting the Bray-Curtis distances from 1 (S=1-D). The following breakpoints were chosen: 5, 15, 25, 35, 45, 55, 65, 75 [m] for the analysis using the R-package 'dave' (Wildi 2017). The spatial autocorrelation analysis of the spectral data was done in analogy to the floristic data using Euclidean distances. The distances up to which data are spatially autocorrelated are compared for the floristic data and the spectral indices. Using linear models without intercept, the dependence of the Mantel correlation coefficients of the spectral indices in dependence on the Mantel correlation coefficients of vegetation across all distance classes is analysed. The distribution of Bray-Curtis similarities for the vegetation data by step width was visualized using boxplots. The vegetation samples were assigned to grassland types following Sturm et al. (2018).

The general species assemblage for the five sampling sites was investigated via NMDS with the R package 'vegan' (Oksanen et al. 2020). The species frequencies were square root transformed and subjected to a Wisconsin double standardization before calculating Bray-Curtis distances. Only species occurring in at least three samples were included in the ordination. The NMDS-result is rotated so that the variance of points is maximized on the first dimension. The resulting plot was rescaled following a half-change scaling approach (Oksanen et al. 2020). Ellenberg indicator values were used to describe moisture conditions, as the indicator values have been proven suitable for describing local environmental conditions (Schaffers & Sýkora 2000). The arithmetic mean of the Ellenberg indicator value moisture was calculated for every inventory (Ellenberg & Leuschner 2010; Wesche et al. 2012). A surface representing the mean moisture values of the inventories was interpolated using a GAM-approach. A linear fit of mean moisture indicator values was applied and reported.

3 Results

The floristic Bray-Curtis similarity showed that the smaller the distance between plots, the higher the similarity of the species composition (Fig. 4). Up until 50 m distance the median Bray-Curtis similarity value was higher than 0.5. Between the samples of the different sites the median similarity value was 0.42. The inter-quartile-range of the similarity values comprised 0.2 across spatial distance classes. Even in the shortest distance classes, a quarter of the sample pairs showed a species composition turnover of 50% (Bray-Curtis similarity < 0.5).

To quantify to which extent spatial autocorrelation is responsible for this pattern, the vegetation data was analysed via Mantel correlograms. These indicated spatial autocorrelation in the two shortest distance classes 5-15 m and 15-25 m (Fig. 5). At none of the studied sites, the spatial autocorrelation reached the distance class 25-35 m. At larger distance classes (35-65 m) weak negative correlations can be observed. Additionally, vegetation and spatial data are significantly correlated as shown by Mantel test (r < 0.5) (Table 1).

The pattern of spatial autocorrelation of the spectral indices behaved comparable to the one for the vegetation. In most replicates autocorrelation reached up to 10-25 m. At the distance class 25-35 m the indices were not autocorrelated. At larger distances, weak negative autocorrelation can be observed. The correlograms of the different indices ran almost parallel. For two sites, the RGB curve deviated slightly from the NDVI and NDRE-curves (Fig. 5). The correlation

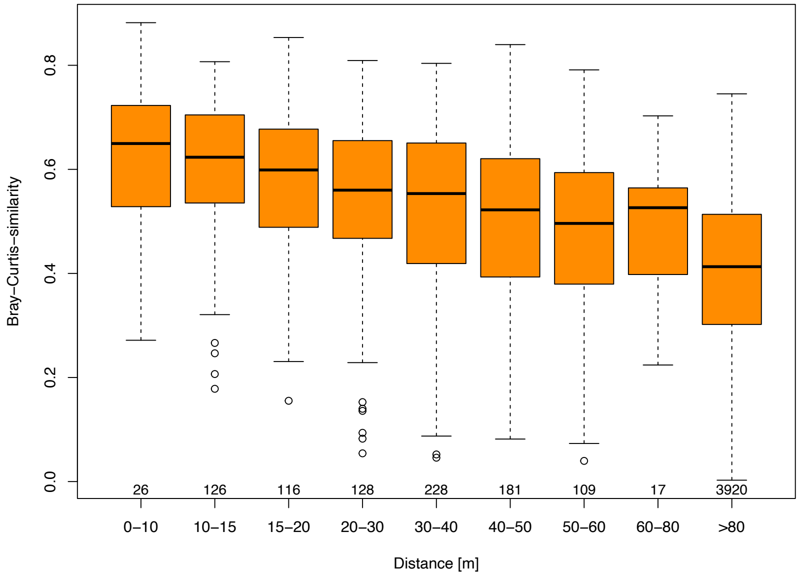

Fig. 4: Bray-Curtis-similarities along a gradient of increasing spatial distance based on a pooled vegetation dataset of all sites. Distance from 0-10 m up to 60-80 m show similarities within sites. Distance over >80 m represents similarities between sites.

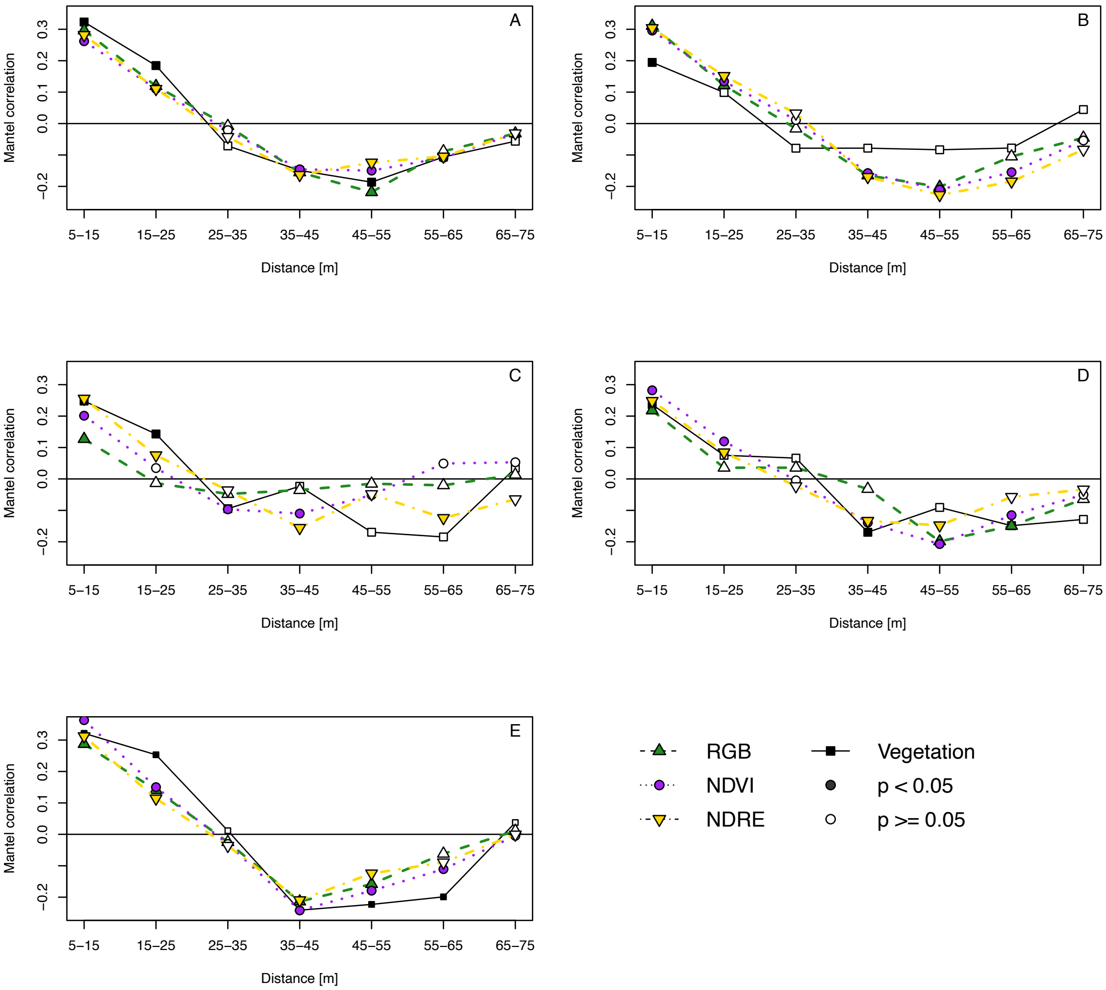

Fig. 5: Mantel-correlogram for each site (indicated in capital letters within each graph composition) for vegetation data and spectral indices. Filled symbols indicate significant values, no-fill symbols not significant values. In black the vegetation data is displayed, while the spectral data is indicated with different colours.

values were all similar, only site C showed higher variation in the comparison regarding the mantel correlation for greater distances.

Linear models of the Mantel correlations of spectral indices depending on the Mantel correlations of the vegetation had a high explanatory value (approx. 75%) (Fig. 6). All three selected measures from the spectral data showed almost identical explanatory power in their linear models.

The 2-dimensional NMDS provides an overview of the floristic similarity of the sites and the individual records (Fig. 7). The sites are heterogeneous to varying degrees. While sites C and D appeared quite compact, the point clouds of the records from sites A, B and E were more widely distributed along the first dimension. Since one unit on the axes corresponds to a half change, i.e., a species turnover of 50%, it can be concluded that the grassland sites were relatively similar to each other, as the centroids of the sites were less than one unit apart. The point clouds were mainly extended along the first axis. This axis corresponded to a soil moisture gradient from fresh to moist (linear fit of moisture indicator values: $r^2 = 0.61$, $p < 0.01$).

The sites were arranged along the second axis with varying degrees of overlap. This gradient corresponded to a management gradient, as the sites are managed uniformly. The beta diversity within a site was usually about 25-50% species turnover (0.5- 1.0 units). Site A had a maximum turnover of about 60% (1.5 units).

Tab. 1: Mantel test and Mantel correlations of vegetation data and spatial distances

SITE	Mantel Test		Distance class [m]												
			5-15		15-25		25-35	35-45		45-55		55-65		65-75	
A	0.389	***	0.323	***	0.185	**	-0.071	-0.149	*	-0.187	*	-0.106		-0.056	
B	0.203	*	0.195	**	0.099		-0.078	-0.078		-0.083		-0.078		0.044	
C	0.339	***	0.248	***	0.143	*	-0.095	-0.023		-0.017		-0.185		0.035	
D	0.336	***	0.237	**	0.075		0.066	-0.169	*	-0.091		-0.149		-0.129	
E	0.487	***	0.321	***	0.253	**	0.012	-0.241	**	-0.223	**	-0.199	*	0.038	

(* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

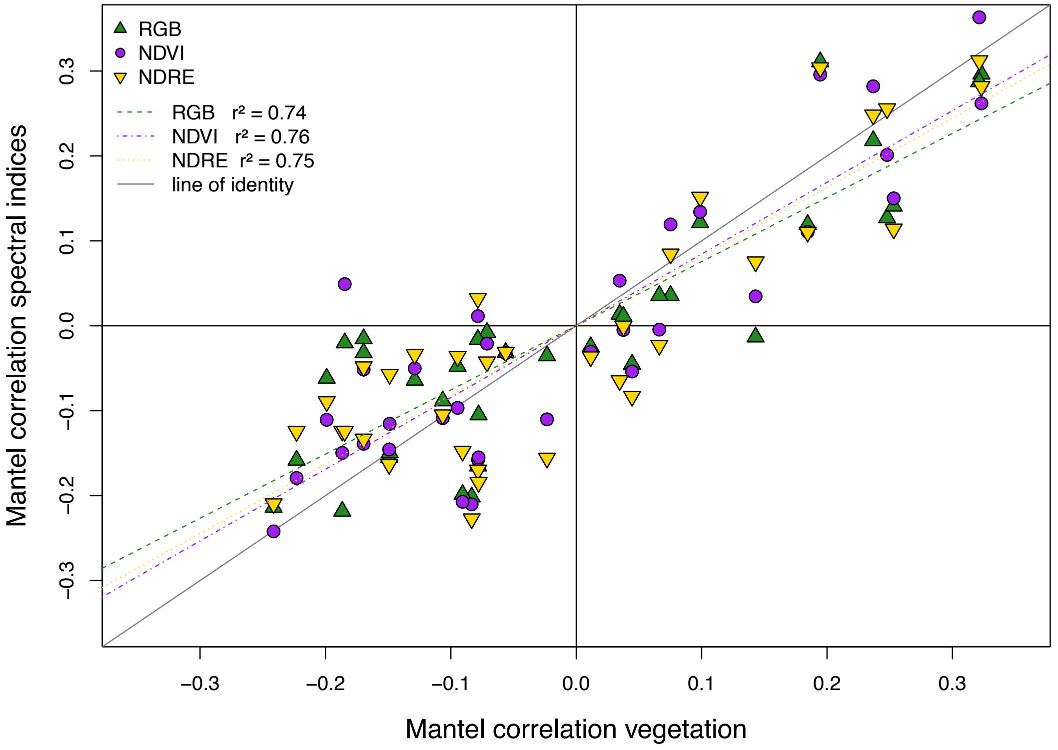

Fig. 6: Mantel correlation of spectral indices and vegetation data. The symbols represent the spectral indices (RGB, NDVI and NDRE). The lines indicate linear regression between spectral measures and vegetation data.

4 Discussion

All grasslands investigated in this study represent the habitat type "6510 Lowland hay meadows (*Alopecurus pratensis, Sanguisorba officinalis*), following the EU habitats directive. This habitat type is characterised by a high diversity of flowering plants and extensive management. Its conservation status in Germany is classified as "unfavourable-bad". In consequence, there is an urgent need for monitoring and restoration. The large areas covered by this habitat and the liability for reporting makes remote sensing indispensable. The analysed landscape is heavily dominated by grasslands of varying management regimes and typical for central European managed grassland vegetation. The communities in the moist valley bottom of the inves-

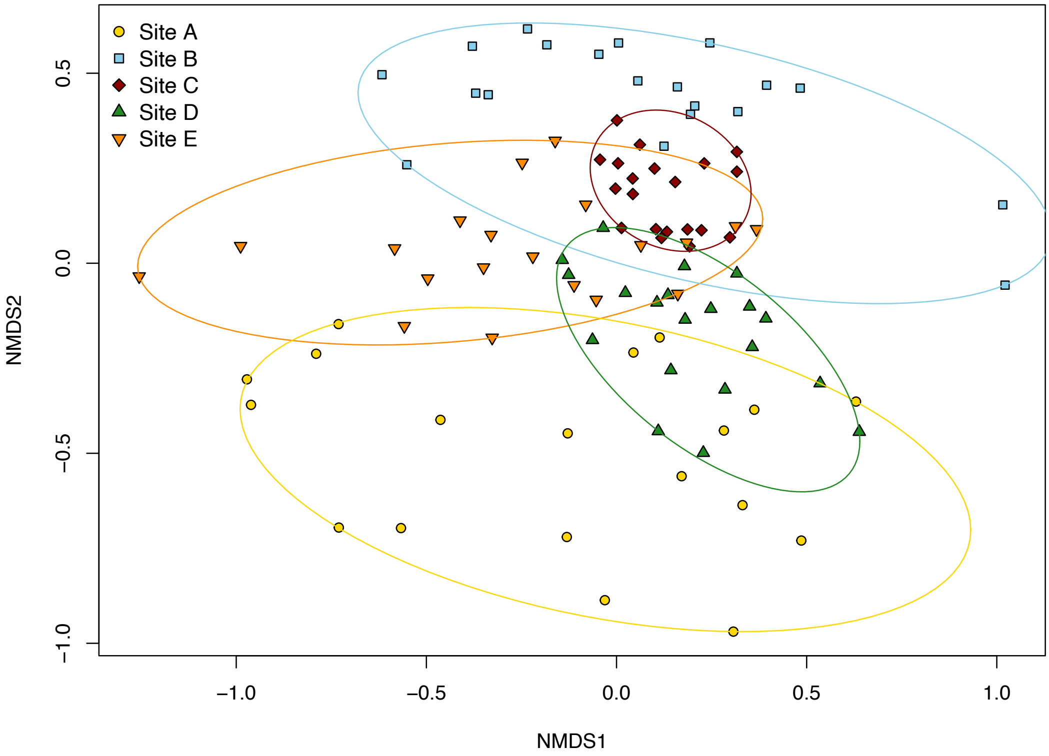

Fig. 7: Ordination of vegetation (two-dimensional NDMS, stress 0.19). Different colours and shapes represent the five 60 m x 60 m squares, drawn ellipses enclosing all recorded line transects.

tigation area are attributed to the phytosociological order *Arrhenatheretalia*. While management within the overall landscape differs, the grasslands investigated are all managed uniformly. Additionally, all sites exhibit a similar species pool. This is a prerequisite for our study testing the potential of satellite imagery in detecting spatial autocorrelation.

All sites are contracted by the same conservation category (no fertilizer application, no mowing before the 15th of June). They are also comparable in terms of climate, elevation and soil types. Differences in species assemblages can be assigned to either spatial autocorrelation or other spatial dependencies, such as small-scale changes in relief (e.g., drainage ditches).

We hypothesized that the spatial autocorrelation present in the collected field data can also be detected by Sentinel 2 satellite imagery. This was confirmed for a specific scale in the studied grasslands. Plots sampled more than 30 m apart from each other but still within the same piece of land are already considered spatially independent. This coincides with Meyers et al. (2014) who found spatial autocorrelation in grasslands up to 40-80 m distance. Including individual species' cover and applying Bray-Curtis-similarity yields a shorter range of spatial autocorrelation than expected from presence-absence data (see Podani & Csontos 2006) with values between 80 and 100 m range for autocorrelation) because cover is non-stationary. Negative Mantel correlations are likely to relate to moisture gradients within sites. The compact grouping of the site data in the ordination diagram indicates that one randomly selected vegetation record would be sufficient for the managed piece of land. However, moisture gradients do not hamper the comparability of the different sites, as spatial autocorrelation was found to be uniform along moisture and management gradients (see also Meyers et al. 2014). The remaining scattering and variation within sites is due to inherent noise in vegetation samples.

We detected small variation in species assemblage in grasslands, that have the same environmental, climatic and management conditions. In this study area, the observed heterogeneity in the vegetation data is related to moisture gradients within the sites (NMDS

Dimension 1). This is confirmed by the species present and their Ellenberg indicator values (found in the supplemental material) for moisture, respectively (Wesche et al. 2012).

Additionally, we observed spatial heterogeneity within sites despite the visually and agriculturally homogeneous vegetation lending further weight to criticism regarding preferential sampling based on visual homogeneity of vegetation stands (Chiarruci 2007, Lájer 2007). This internal heterogeneity in our sampled vegetation is illustrated by Bray-Curtis distance values of 0.5 and is in line with Pouget et al. (2021) who found that even seemingly homogeneous mown grasslands have a level of high spatial heterogeneity.

Moreover, small-scale environmental differences can influence species richness patterns and assemblages in grasslands (Gazol et al. 2012), therefore, small distances can be significant for diversity patterns within grasslands (Zhou et al. 2008). Additionally, micro- topographic heterogeneity is a known influence on plant species diversity (Deák et al. 2015). Furthermore, spatial autocorrelation can be genetically driven and was for example identified as the reason behind in synchronisation of flowering events (e.g. Almeida-Neto & Lewinsohn 2004), which in turn may significantly influence the calculation of spectral vegetation indices (Shen et al. 2009). By detecting and excluding spatial autocorrelation, additional spatial gradients and patterns can be identified.

Non- explained variance in our study can result from distance to the river and occasional flooding, groundwater depth, or micro-topography. Those specific site conditions can be additionally relevant because of regular flooding of the Altmühl river in the winter months. Small depressions modify dwelling times for the water increasing the importance of micro-topography changes for variance explanations. Even though most space borne remote sensing is used for large scale analyses (e.g., Gould 2000, Coops et al. 2018, Recuero et al. 2019), we show the applicability for the analysis of the grassland composition within one valley . We found that spectral indices as well as spectral variability (in relation to the combination of red, green and blue bands) exhibit a similar pattern of spatial autocorrelation compared to that of the in- situ sampled grassland vegetation. The correlation of the field data with the used spectral indices is strong. The regression of the Mantel correlation values on vegetation data explains around 75% of variation of the variance of the spectral indices.

Deviations between both could stem from sampling design. The grids in the field were oriented along a north to south and east to west axis, while the pixels of the Sentinel-2 images are slightly rotated along the east-west axis. Field samples are not depicting exactly the same location as the satellite sensors. However, the aim was not to directly compare single pixel data from the Sentinel- image to information of an individual 10 m² field sampling unit. Rather we aimed at comparing the differences with increasing spatial distances within the same management unit.

The NDRE uses the red edge which is highly correlated with chlorophyll content (Pinar & Curran 1996) and is focused on the stress level of the vegetation (Dawson & Curran 1998, Barnes et al. 2000). The NDRE is known to outperform the NDVI in mid-to-late stages of plant developments as it is more sensitive to densities past the canopy cover, which is a well-known NDVI saturation problem (Moriondo et al. 2007). However, in our study NDVI and NDRE perform equally. As these grasslands are mowed at the end of June and data was collected in August, the canopy density and the developmental age were still in a range, where the NDVI performs well and the advantages of the NDRE do not apply.

The almost interchangeable performance of NDVI and NDRE is an indicator for overall relatively homogeneous environmental conditions, as the NDRE is more sensitive to small areas with different textures (Jorge et al. 2019). One major advantage of using the NDVI when relying on Sentinel-2 images is, that all the needed bands are in the same spatial resolution (10 m), while for the NDRE one band must be resampled from 20 m to 10 m resolution, which potentially decreases the accuracy and increases the margin for errors.

Generally, the spatial structures of grasslands are better depicted in NDVI and NDRE than in the RGB, representing the visible light spectrum. The suitability of spectral variability of the visible spectrum for the differentiation of the grassland composition is limited (Biró et al. 2024, Ludwig et al. 2024).

Field data and the satellite data for this study were acquired during the same time (sampling in August 2020, Satellite image from 9 Aug 2020). Therefore, effects of a time delay between field data and acquisition date of the Sentinel-2 image do not have to be considered. Our results suggest that spectral data can be used for assessing spatial autocorrelation in grassland. The application of remote sensing in detecting autocorrelation is only restricted by its resolution. In our study, the plot size of the field data matches the resolution of the Sentinel-2 images. This must be considered in future studies as well. In theory spatial autocorrelation can be continuously mapped but this can be biased by pixel size and the consequences for data smoothing by coarse image resolution. This confirms theoretical considerations that remote sensing data can be taken as a proxy for the in-situ heterogeneity and species diversity of vegetation (Spectral Variation Hypothesis (SVH), Palmer et al. 2002). Habitat heterogeneity as a proxy for species diversity has been recognized as a valuable tool in biodiversity research (Rocchini et al., 2010). However, a recent study (Ludwig et al. 2024) critically tested the potential of the SVH for grasslands and concluded while it works for some it does not hold true for all grasslands. Other studies found a strong positive correlation between NDVI and soil moisture levels indicating the potential of spectral indices depicting environmental gradients of habitat heterogeneity (e.g., Chen et al. 2014, Ahmed et al. 2017).

The results in this study confirm that two very well-known and established spectral indices (NDVI and NDRE) are suitable for the estimation of the spatial autocorrelation in grassland concerning the species combination even at a rather local scale and demonstrates that freely available data is sufficient for the analyses of spatial autocorrelation on a small scale. This applies even to grasslands that are managed in small pieces of land (1 - 2 ha). Sentinel- 2 data with its resolution of 10 m were successful in reflecting small scale differences in spatial autocorrelation, equally to cost and time intensive field work.

5 Conclusion

Vegetation samples are a common source for analyses of species-environment relationships. They are the basis for decision making in conservation and management plans for endangered species and other environmental risk assessment. The statistical soundness of those datasets is crucial and avoiding spatial autocorrelation within the dataset is necessary.

We show that this important factor disrupting sound statistical analyses can be reliably detected via freely available satellite data. Fieldwork can be adjusted a priori, avoiding issues in the analyses later. In this study, the spatial autocorrelation in the in-situ dataset showed an effect for not exceeding 30 m distances. This is true for all sites in the study area; its effects can therefore be neglected when analysing the data in similar landscapes if a minimum distance of 30 m between each sampling site is granted.

Employing satellite data for analysing the effects of spatial autocorrelation produced similar patterns as the in-situ data. The detected patterns showed the same trends as observed in the field data. This implies that a priori testing of necessary distances to avoid spatial autocorrelation issues in the analyses later can be provided via space-borne satellite imagery if resolution size (remote sensing) and sampling size (field data) are in the same range. In this study, the freely available Sentinel-2 images with a resolution of 10 m proved to be sufficient.

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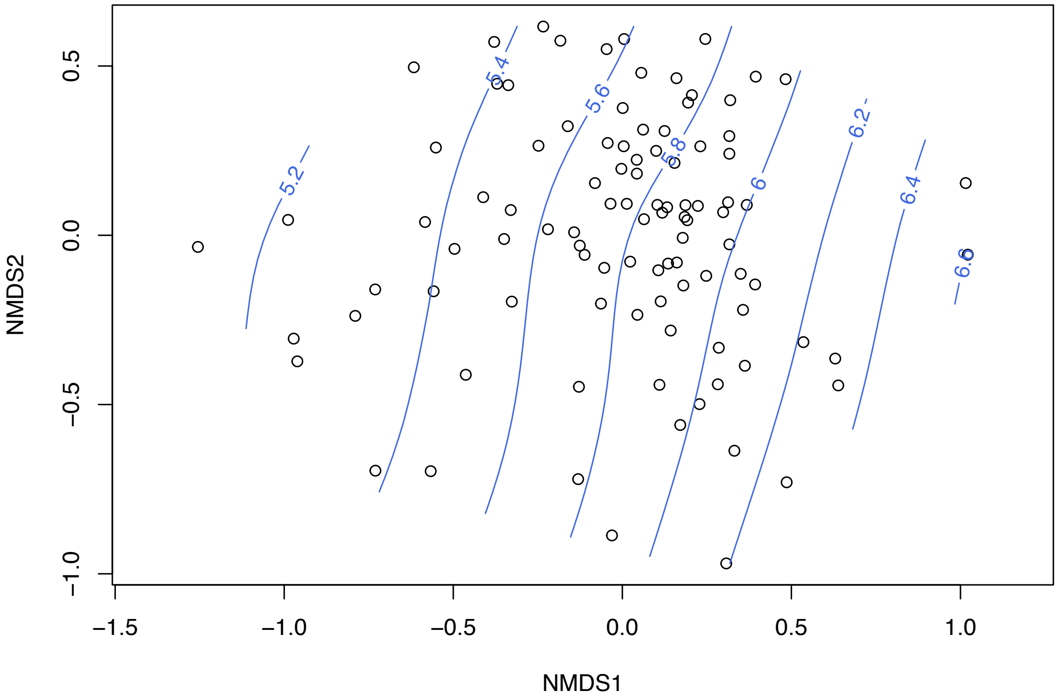
Appendix



A1: Ordination (NMDS) with an overlay of mean Ellenberg indicator values moisture (blue lines)