From Landsat to leafhoppers: a multidisciplinary approach for sustainable stocking assessment and ecological monitoring in mountain grasslands.

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Abstract

We present a case study illustrating a multidisciplinary approach for characterizing, mapping and monitoring the bio-ecological properties of Mediterranean mountain grasslands in extensive grazing systems. The approach was developed to provide the basis for the management plan of a cluster of Natura 2000 special conservation areas in the Central Apennine mountains, Italy (with a total area of 79,500 ha, including 22,130 ha of grasslands). It includes a novel methodology for estimating sustainable stocking rates of different plant communities, at a detailed spatial scale over large areas, based on the integration of: *i)* a classification of grassland types, based on physical habitat stratification and vegetation sampling; *ii)* a forage-value assessment of each grassland type,

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obtained from field sampling of botanical composition and corrected with remote-sensing information on pasture microtopography; *iii)* an estimate of primary productivity at a detailed spatial scale, obtained from the remote-sensed Normalized Difference Vegetation Index (NDVI) calibrated with biomass field data. Additionally, to obtain a bioclimatic characterization of the grasslands and to determine the optimal grazing season for each grassland type, intra-annual phenological signatures were obtained from the Enhanced Vegetation Index (EVI). Given the inherent limitations in the sustainable stocking rates concept, and the particular susceptibility of dry grasslands to changes in grazing regimes, we tested two biological indicators, the Auchenorrhyncha quality index (AQI) and the Arthropod-based biological soil quality index (QBS-ar). These indicators take into account above- and below-ground arthropod diversity, respectively, and are applied here for the first time to the specific purpose of monitoring grazing load effects on ecological quality and biodiversity of Natura 2000 dry grasslands. We conclude that: *i)* it is possible to effectively integrate biomass estimates, obtained from publicly available satellite data, with a relatively simple field sampling of botanical composition, to achieve a detailed spatialization of sustainable stocking rates; *ii)* within the same Natura 2000 habitat type there can be a large spatial heterogeneity in both sustainable stocking rates and optimal stocking season: thus, grazing should be kept under careful human control to maintain the habitats in the desired conservation status; *iii)* while plant species richness was not correlated to grazing intensity, both AQI and QBS-ar had a significant negative correlation to grazing levels and can thus be useful for monitoring the actual "sustainability" of livestock loads on different aspects of grassland ecosystems.

Keywords: Auchenorrhyncha quality index, biological soil quality, Natura 2000, remote sensing, sustainable stocking rates, vegetation mapping

1. Introduction

In Europe and in the Mediterranean, wild species of conservation concern are often dependent on agro-ecosystems created by traditional, low-intensity farming (Benton et al., 2002; Kleijn et al., 2009; Lasanta et al., 2015; Maurer et al., 2006). In this respect, secondary grasslands are particularly important (Habel et al., 2013): they are semi-natural habitats originated and maintained by anthropogenic disturbance such as mowing or livestock grazing, in areas that would be potentially covered by forest vegetation (Dengler et al., 2014). Many types of secondary grasslands are listed in Annex 1 of the EU Habitats Directive (European Union, 1992) among the habitat types whose conservation requires the designation of special areas of conservation, forming the "Natura 2000" network. Within these conservation areas, EU Member States are required to adopt management plans meeting the ecological requirements of the protected habitats and maintaining them in a "Favourable Conservation Status", while accounting for economic, social and cultural issues (European Union, 1992). The conservation status of semi-natural grassland habitats is considered as threatened because of the abandonment of low-intensity agricultural practices such as extensive grazing (European Commission, 2014; Ostermann, 1998).

Grasslands have an inherently dynamic nature, and spatial-temporal heterogeneity plays a crucial role in their stability, productivity and response to grazing (Laca, 2009; Schwinning and Parsons, 1999): their properties are thus difficult to quantify, and research teams should be "as diverse as the pastures they hope to measure" (Kallenbach, 2015). In this paper, we discuss a multidisciplinary approach aimed at mapping, characterizing and monitoring the bio-ecological properties of grassland ecosystems, that was developed for the management plan for a cluster of Natura 2000 areas in the Central Apennine mountains, Italy (Fig. 1). Here, secondary dry grasslands occupy a large proportion of the landscape, and contribute to the habitat of two endangered large mammals: the endemic Apennine chamois (*Rupicapra pyrenaica* ssp. *ornata*) and the last surviving population of Marsican brown bear (*Ursus arctos* ssp. *marsicanus*). While in central Europe transhumant shepherding and the associated secondary dry grasslands are relatively recent phenomena (Ellenberg, 1988; Poschlod and Wallis DeVries, 2002), sheep grazing was shaping Apennine landscapes already in Roman times (Brown et al., 2013; Manzi, 2012): the floristic composition of present-day secondary grasslands of the Apennine mountains may thus be inherited from local xerothermic enclaves, that survived through the postglacial forest spread as relicts of the previously widely distributed steppe (Bredenkamp et al., 2002). However, the abandonment of traditional sheep grazing in the Apennines is now leading to grassland habitat loss because of scrub encroachment and forest expansion (Amici et al., 2013; Bracchetti et al., 2012).

A crucial issue in Natura 2000 habitat management concerns the identification and mapping of the different habitat types (Bunce et al., 2013) that are defined mostly on the basis of their floristic composition (Evans, 2006). However, the repeatability of traditional approaches to fine-scale vegetation and habitat mapping has been questioned (Ejrnæs et al., 2004; Hearn et al., 2011; Waterton, 2002). The use of remote-sensing methods in Natura 2000 habitat management has, up to now, been very limited (Vanden Borre et al., 2011), despite their strong potential to overcome some of the problems involved in large-scale field surveys (Nagendra et al., 2013) and to rapidly quantify important biotic characteristics of grasslands (Kallenbach, 2015). For instance, even though much higher-resolution private satellite data are now available (Pullanagary et al. 2013), the 30x30 m pixel resolution of the publicly available Landsat TM data (Nagendra et al., 2013) can allow relatively fast mapping, at an appropriate scale, of biomass patterns, through the use of well-known indexes such as NDVI (Normalized Difference Vegetation Index).

These issues have to be considered when dealing with large areas with a rugged topography, where traditional *in situ* measurements can take considerable time and effort (Aplin, 2005; Milton et al., 2009). For instance, for the secondary grasslands hosted in the Natura 2000 network, it is recommended that a careful assessment of specific stocking rates be made for each habitat type, taking into account local conditions such as grassland productivity, physical habitat and grazing intake by wild herbivores (European Commission, 2014), since both under- and over-grazing can lead to the loss of protected semi-natural habitats. However, determining sustainable stocking rates (or livestock carrying capacity: Allen et al., 2011), especially across wide expanses of land, is one of the most theoretically and practically difficult issues in natural grassland management (e.g. Jakoby et al., 2015; Laca, 2009; McKeon et al., 2009). The concept of sustainable stocking rates is a quite controversial one, when applied to Mediterranean vegetation, as it is often based on observations from pastures in summer-rain, cool climate types (e.g. the Alps), or from areas of the world where ungulates have become an important factor only during the last two centuries (e.g. Australia) (Grove and Rackham, 2001). Some studies suggested that, in the Mediterranean, heavy grazing levels that would elsewhere be defined as “overgrazing” are probably an essential management tool for the conservation of plant biodiversity (Perevolotsky and Seligman, 1998; Noy-Meir and Oron, 2001). In practice, methods for calculating sustainable stocking rates include approaches based on biomass or botanical composition (Pardini et al., 2000a), as well as farmer’s experience, land-unit attributes, historical climate data and simulation of forage production (McKeon et al., 2009). The approaches based solely on biomass lack information on the actual diversity, palatability and nutritional value of the pasture (Pardini et al., 2000a, 2000b); moreover, it is difficult to estimate spatial variations in biomass across large areas if one has to rely only on field samplings. Biomass methods, thus, have only rarely been used at the landscape scale (Argenti et al., 2002). On the other hand, methods considering only floristic composition can be unreliable too. For instance, the “pastoral method” (Daget and Poissonet, 1969, 1972), an approach frequently used in France and Italy, can oversimplify reality because of differences in productivity of very similar floristic assemblages. Moreover, the pastoral method, as originally developed, would require collecting data with the point-intercept method along transects, a precise but very intensive sampling method, that cannot be applied to large and heterogeneous areas (Bagella, 2001; Bagella and Roggero, 2004).

Given these difficulties, even if sustainable stocking rates have been assessed and grazing loads comply with them, it is advisable that protected grassland types be monitored using a set of indicators, suitable to objectively evaluate the impact of livestock on the ecosystem through time (Lebacq et al., 2012), and to describe how well the management regime meets the habitat conservation targets (Elzinga et al., 1998). In particular, biological indicators make use of relatively easily observed responses of various species as ecosystem status indicators (Zonneveld, 1983). Natura 2000 habitats are defined mostly on a phytosociological basis (Evans, 2006) and the assessment of their “Favourable Conservation Status” has to be based primarily on the floristic and structural properties of the vegetation (Petermann and Ssymank, 2007). However, the use of taxonomic groups other than plants can provide useful and complementary information in evaluating the effects of grazing management on grassland ecosystem services and biodiversity (Chillo and Ojeda, 2014). A still scarcely explored taxonomic group as a biological indicator in extensive grazing systems is Auchenorrhyncha (planthoppers and leafhoppers), a suborder of Hemiptera including exclusively phytophagous insects. Auchenorrhyncha can be viewed as ideal biological indicators (Achtziger and Nickel, 1997; Bornholdt, 2002; Bückle and Guglielmino, 2005, 2011), for many reasons: they include both conservative species (i.e., habitat specific and intolerant to degradation) and non-conservative species (i.e., generalist and tolerant to degradation, Wallner et al., 2013), they respond to grazing distinctly and rapidly (Nickel and Hildebrandt, 2003) and following a disturbance event they recover very slowly compared with their host plants (Achtziger et al., 1999). On this basis, Wallner et al. (2013) developed an Auchenorrhyncha Quality Index (AQI), originally proposed for measuring the quality of North American tallgrass prairie.

Another biological indicator, still unexplored within the context of sustainable grazing management, is the “Arthropod-based biological soil-quality index” (QBS-ar), proposed by Parisi et al. (2005) and found to perform very well when compared with other soil indicators (Ritz et al., 2009). QBS-ar is based on the below-ground functional diversity of arthropods, and assumes that soil quality is associated with the occurrence of groups that are well adapted to soil habitats. In recent years, QBS-ar was used for monitoring ecosystem responses to agriculture (Rüdisser et al., 2015) and forestry practices (Blasi et al., 2013), but to our knowledge it has not been applied to evaluate different grazing regimes within dry grasslands.

The aims of this paper are: first, to present a case-study on an original approach for estimating sustainable stocking rates and optimal grazing time of different grassland types at a detailed spatial scale over large areas, through an integration of remote sensing indices (calibrated with biomass field data), floristic field surveys and forage-value assessment. Second, to test, within the context of Natura 2000 dry grasslands, the responses to different grazing levels by both AQI and QBS-ar, in order to evaluate the potential of these indices for monitoring the effects of the assumed sustainable loads on the various components of pasture ecosystems. The results of this work can provide a more efficient way to map, quantify and monitor the bio-ecological properties of semi-natural grasslands in large and heterogeneous grazing landscapes of conservation interest, for maintaining both economic resources and native biodiversity.

2. Methods

2.1. Study area

2.1.1 Environmental features

The study area is located within the Central Apennine mountains (Italy) and includes Abruzzo Lazio e Molise National Park and the adjoining Natura 2000 sites (Fig. 1), with a total area of 79,500 hectares (lat. 42°00’37’’ to 41°35’25’’ N, long. 13°29’17’’ to 14°02’33’’ E). Elevation ranges between 500 and 2,249 m; most of the study area lies between 1,100-1,900 m. Prevailing bedrock types are Mesozoic limestones and dolomites; clayey and marly substrata also occur (Bigi et al., 1986). Geomorphology is characterized by widespread karstic landforms.

The area features steep climatic gradients (Filibeck et al., 2015), leading to a clear altitudinal sequence of vegetation belts (Bazzichelli and Furnari, 1979; Bruno and Bazzichelli, 1966). At low elevation (500-800 m), climate is sub-Mediterranean, with 1-2 dry months in summer, annual precipitation between 700-1,200 mm, mean annual temperature >10°C and only limited frost occurrence. Vegetation cover in this belt is dominated by *Quercus pubescens* and *Q. cerris* woods, along with secondary grasslands. Across the submontane (800-1,200 m) and montane belts (1,200-1,800 m), summer drought stress decreases with altitude (although the precipitation regime still features a minimum in summer and a maximum in autumn), and winter/spring frost increases: annual precipitation is between 1,100-1,600 mm, mean annual temperature between 9-6 °C. Most of the landscape within the montane belt is dominated by *Fagus sylvatica* forests and by secondary grasslands. Finally, the subalpine belt (>1,800 m) is characterized by prolonged snow cover and late-spring frost; this belt is covered mainly with grasslands, prostrated shrub vegetation, rocks and screes.

The core section of the study area was designated as a National Park in 1923, for protecting the local endemic subspecies of bear (*Ursus arctos* ssp. *marsicanus*) and chamois (*Rupicapra pyrenaica* ssp. *ornata*). The area also hosts a large population of wolf (*Canis lupus*), while red deer (*Cervus elaphus*) was reintroduced in the 1970's (Tassi, 1976). The park's flora comprises >2,000 species, including >30 taxa endemic to the central Apennine mountains (Conti and Bartolucci, 2015).

2.1.2 Stocking systems and socio-economic framework

Transhumant sheep and goat grazing was the main stocking system in the Central Apennines for millennia (Manzi, 2012). Within the study area, it dates back to the 6th century BC or earlier (Brown et al., 2013), and was widely practiced until the 1950's, when ovine grazing started to dramatically decrease (Manzi, 2012) for the same socio-economic reasons as in other parts of Europe, such as mountain depopulation, lowland agriculture intensification, wool and meat price decrease, etc. (e.g. Caballero, 2015; Poschlod and Wallis DeVries, 2002). Most of the husbandry is now sedentary; only a few pastoralists still move to/from lowland regions. There are 223 registered livestock owners within the study area, with a total livestock population of c. 9,000 sheep, 1,800 goats, 3,400 cattle and 800 horses (Salvatori et al., 2012); average stocking rate across the Park grasslands can be estimated as <0.3 AU ha^{-1} (Animal Unit: Allen et al., 2011). Present-day stocking rate is drastically lower than in the early twentieth century, when c. 100,000-200,000 head of sheep were probably grazing within the study area (our estimate, from various local sources).

The most common stocking system now involves grazing from mid-June to mid-October in public pasturelands, leased by each municipality to individual farmers. However, also common pastures exist, where all local residents are entitled to introduce their animals upon payment of a fee. The shepherds lead the sheep or goat herd to the assigned pastures and remain with them. In the evening the herd is gathered for milking, and spends the night in a fenced area for protection against wolves.

In the last decades the abandonment of sheep husbandry has been followed by a steep increase in bovine and, above all, equine grazing. Since most of the cattle and horses belong to “part-time farmers” (i.e., people who have their main income from other professional activities), they are usually raised for meat production only and are left free-ranging in the wild without checking for many months – in spite of national and municipal regulations, requiring that the grazier stays with the animals at all times, and that the livestock are moved out of the pastures at dusk.

2.2. Vegetation and habitat mapping

Within the 79,500 ha study area, the total area of natural and semi-natural grassland patches, as extracted from a 1:10,000 land-cover map, was 22,130 ha. In order to achieve a repeatable approach to grassland type mapping, we performed a physical stratification (Bunce et al., 1996) of all the grassland areas at 1:50,000 scale (hereafter “land-units map”). This was obtained through overlaying the following GIS layers: geology (obtained reclassifying into 9 broader groups of ecological relevance the detailed stratigraphic map by Bigi et al., 1986); elevation [4 belts, following standard geobotanical subdivisions (Gerdol et al., 2008): “colline”, <800 m; “submontane”, 800-1,200 m; “montane”, 1,200-1,800 m; “subalpine”, > 1,800 m]; aspect (two classes: “warm”, i.e. from SE- to W-facing, and “cool”, i.e. from NW- to E-facing).

A database of all plant community types known in the phytosociological literature for the wider regional area was created, associating each vegetation type to the available information concerning its physical environment. A provisional vegetation type was then assigned to each polygon of the land-units map, building on the phytosociological database and on visual interpretation of digital aerial photographs.

The provisional vegetation map was checked in the field during spring-summer 2013 through 92 Ground Control Points (GCP), geo-referenced with a GPS. Because of the complexity and extension of the study area, and because of heavy time constraints, we decided not to follow a randomized sampling approach. The GCP were selected according to a preferential sampling scheme: approximately half of them were subjectively placed in sites that could be considered particularly representative of the various physical land-units; the remainder were placed in grassland patches whose vegetation attributes were particularly uncertain, e.g. because of unclear features in the aerial photographs. At each GCP, we recorded the dominant and most frequent plant species over a large area (1 ha), and estimated grazing disturbance level on a scale ranging from 1 to 4 (see Appendix A, tab. A1 for criteria).

The botanical composition of the mapped grassland types was then surveyed through 74 vegetation quadrats (squared plots of 2x2 m). Following the standard protocol for phytosociological sampling (Dengler et al., 2008), the quadrats were subjectively placed in sites that were considered as representative of each grassland type, within stands of visually homogeneous vegetation: all vascular plant species in the plot were recorded (we considered a species as “present” when the vertical projection of any above-ground part fell within the plot), and their percentage cover was estimated. Nomenclature was standardized following Conti et al. (2005). Additionally, grazing impact in the surrounding 1-ha area was assessed using the same criteria as for the GCP.

Given the complex topography of the study area, and in order to keep the mapping process as cost-effective as possible, we classified the grasslands into broad, informal types (hereafter “vegetation units”), based on dominant species and life-forms visible on a vegetation map at 1:50,000 , rather than to formalized syntaxa of the phytosociological system.

Finally, for each vegetation unit we assessed whether it corresponded to any of the habitat types listed by Annex 1 of the Habitats Directive, comparing its floristic composition and ecological features with the diagnosis provided by the Italian Interpretation Manual (Biondi et al., 2009), resulting in a 1:50,000 map of the Annex 1 habitat types .

2.3 Phenological analysis

To determine the optimal grazing period for each vegetation unit (cf. European Commission, 2014), phenological patterns were analysed using the Enhanced Vegetation Index (EVI) (Huete et al., 2002; Zhang et al., 2003). We acquired the Global MOD13Q1 2002-2012 EVI product (Solano et al., 2010), available every 16 days at 250 x 250 m spatial resolution from the NASA EOSDIS online database (http://modis-land.gsfc.nasa.gov/vi.html). For each pixel, we averaged data to obtain the mean EVI values over the 2002-2012 time series for every 16-day period, i.e. the pixel's EVI signature. This allowed us to describe the average intra-annual phenological cycle across the inter-annual climatic variability observed during the study period. To allow comparison of the phenological cycle between habitats with different total productivity, we standardized the values of each pixel signature according to the following formula:

$$EVI_{std} = (EVI - EVI_{min})/(EVI_{max} - EVI_{min}) \qquad \textit{(eq. 1)}$$

EVI_{std} values will thus range between 0 and 1.

We then defined as "sustained productivity period" the time of the year during which each pixel shows an EVI equal to or larger than 75% of its largest value (i.e., the period during which $EVI_{std} \geq 0.75$). To achieve an optimal subdivision of the study area in bio-climatic elevation belts relevant for pasture management, we searched for altitude intervals featuring consistent starting and ending dates of "sustained productivity" across different habitat types. Finally, for each vegetation type, and for each of the elevation belts, we calculated the average EVI and EVI_{std} signature.

2.4 Biomass and sustainable stocking rate model

To obtain the sustainable stocking rate (SSR) at detailed spatial scale across the whole area, we integrated a remote-sensed proxy for biomass with the botanical composition obtained from the vegetation quadrats.

As a proxy for biomass (Choler, 2015), we used the Normalized Difference Vegetation Index (NDVI) (Rouse et al., 1974). Because of the influence of grassland phenology on NDVI–biomass relationships, that can lead to unreliable results if data are collected much after the point of maximum greenness (Butterfield and Malmström, 2009), we identified the optimal period for both biomass field sampling and remote-sensed data collection for each vegetation type through the analysis described in section 2.3. Then, we sampled biomass in the field at 89 sites, selected in order to be representative of the various physical land-units. At each GPS geo-referenced sampling site, herbaceous biomass was collected on a plot of 1 x 5 m, and both fresh and dry weight measured (kg m^{-2}).

NDVI was obtained from panchromatic Landsat 7 images (acquired from http://glovis.usgs.gov), with 30x30m resolution, from the same dates as the biomass field sampling. NDVI was calculated for each pixel through the Image Analysis tool in ArcGis 8.3 (ESRI, 2002). To obtain a linear model correlating NDVI with pasture biomass, the biomass field measurements (fresh weight) were assigned to the respective Landsat pixel. After removing outliers (n=9), 50% of the field samples (n=40) and their associated NDVI values were used as a training dataset to calibrate a linear regression model. The other 50% of the samples (n=40) were used as a validation dataset. To obtain an estimated biomass value for each pixel of the study area, the validated NDVI-biomass model was applied, averaging the NDVI figures of ten years (Landsat 7 images from 2003 to 2012, chosen from the dates featuring the maximum greenness peak).

From the estimated biomass values, we calculated the SSR at the 30x30 m pixel scale, according to the method proposed by Pazzi (1980) and Pardini et al. (2000a), modified for use with remote-sensed data:

$$SSR = P\ F^{-1}\ D^{-1}\ K_a\ K_b\ K_c\ K_d \qquad \textit{(eq. 2)}$$

Where: P = dry biomass (expressed as Kg ha^{-1} and obtained multiplying the pixel's fresh biomass from the NDVI model by a conversion coefficient of 0.40, i.e the average proportion of dry matter measured in the field samples); F= average daily dry matter requirement of one animal unit (AU) (8.8 kg$_{\text{dry matter}}$ day^{-1}) (Allen et al., 2011); D= number of optimal grazing days, defined as the number of days featuring standardized EVI values higher than 0.75; K_a= forage value coefficient (ranging from 0 to 1), obtained weighing the forage values of the species according to their average cover value in the botanical composition of the relevant vegetation type (Pardini et al., 2000b); the specific forage values were derived from the "specific indexes" listed by Roggero et al. (2002), modified (F. Rossini, unpubl. data) in order to obtain an index of nutritional value only (as they originally included also a measure of the species' productivity potential); K_b = aspect coefficient (N, E, NE =1; SE, NW =0.95; S, W, SW=0.9), obtained from a Digital Elevation Model for each 30x30 m Landsat pixel; K_c = slope coefficient (S<9°= 1; 19<S>9°=0.9; S>19°=0.8), obtained from a Digital Elevation Model for each 30x30 m pixel; K_d = bare rock area coefficient (area<10%=1; 25%<area>10%=0.9; area>25%=0.8), obtained calculating, within each 30x30 m Landsat pixel, the proportion of bare rock area on a grey-scale digital aerial photograph (with a 1x1 m resolution). The resulting SSR was expressed (Allen et al., 2011) as AU ha^{-1} (over the specific grazing season for each vegetation type).

Note that the rationale for K_b, K_c and K_d, as modified in the present work, is to take into account the main physical factors affecting animal behaviour and its influence on soil stability: for instance, where there is a high percentage of bare rock, grazing animals will concentrate in the grassy patches, leading to soil deterioration.

Finally, we subtracted from SSR the proportion of carrying capacity already exploited by red deer (*Cervus elaphus*), because among the wild herbivores of the Park this is the only species with a very large population size and a feeding behaviour potentially competing with livestock. Density data, obtained from randomized transects and faecal pellet group-count method (faecal standing crop: Staines and Ratcliffe, 1987) performed by the Park Agency (Latini et al., 2012), were spatialized using the Inverse Distance Weighting tool in ArcGis 8.3 and expressed as AU according to a conversion factor based on metabolic weight (Allen et al., 2011) of red deer (on average 120 kg live weight = 0.34 AU).

2.5 *Biological indicators*

2.5.1 Vascular plants

For each vegetation unit we assessed the occurrence of species of conservation interest. These were defined as species with a narrow geographic range, or listed in the National Red List, in the Bern Convention, in the Washington Convention, or in Annexes II and IV of the Habitats Directive.

We also tested whether total plant richness at the plot-scale was correlated with the main environmental gradients (grazing load and elevation).

2.5.2 Auchenorrhyncha Quality Index (AQI)

The Auchenorrhyncha Quality Index (AQI: Wallner et al., 2013) ranks the quality of habitats by virtue of the abundance and diversity of Auchenorrhyncha taxa specifically bound to grassland ecosystems. Wallner et al. (2013) combined the species richness of a given habitat with a mean coefficient of conservatism which integrated different values (0-3) for six selected criteria: voltinism (number of generations per year), origin, overwintering microhabitat, wing length, habitat fidelity and host plant affinity.

However, this index was developed for an open and plain steppic region, while the grasslands studied in our research consist of patches of mountain pastures, often surrounded by forest. Thus, we developed some modifications in the score assignments concerning overwintering, wing length and habitat fidelity (see Appendix A, Table A.2). Of the two alternate formulas proposed by Wallner et al. (2013), only the one for qualitative data (i.e. presence/absence, without considering number of individuals) is used in the present work.

The sites for the Auchenorrhyncha sampling were chosen among the GCP (Ground Control Points of the vegetation mapping: see section 2.2). The choice of the GCP to be sampled for AQI was based on the criterion of covering different levels of grazing load and the main vegetation units (the following were sampled: Montane *Bromus*-grassl.; Montane *Brachypodium*-grassl.; Montane karstic mosaic; Acidophilous grassl.; Subalpine *Festuca*-grassl.; Subalpine karstic mosaic) across most of the elevation gradient (1200-1900 m). A total of 15 sites were sampled during June-July 2013: the chosen sampling period ensures that species which hibernate as adults, nymphs or eggs, respectively, are equally represented. Sampling was performed during dry-weather days, in order to avoid any negative impact of rain on the number and diversity of Auchenorrhyncha. Each sampling site had an area of c. 1 ha; the sampling took on average 3 hours (min. 2 – max. 4), and ended when no additional taxa could be found during half an hour. The insects were collected by entomological net and aspirator, and killed with ethyl acetate. Preparation and identification were conducted later in the laboratory. All specimens were identified up to the species level. In total, we collected c. 3,300 specimens of Auchenorrhyncha.

2.5.3 *Arthropod-based biological soil-quality index (QBS-ar)*

The QBS-ar index is based on the assumption that the higher the soil quality, the higher will be the number of microarthropod functional groups adapted to soil habitats; it adopts a life-form approach and hence does not require species-level identification (Parisi et al., 2005).

To control for the role of physical environment and soil types, we relied on some fenced areas in the Picinisco municipality that allowed us to sample both heavily-grazed and completely ungrazed grassland patches within the same physical land-unit. Fourteen sites (9 grazed and 5 ungrazed) were sampled in this area. Eighteen additional sites were sampled across the whole study area, in order to be representative of different habitat types and grazing regimes: the final dataset was thus constituted by 32 sites.

At each sampling site, three soil cores (10 x 10 x 10 cm), at least 10 m away from each other (but within the same vegetation type, slope and aspect), were collected. Cores were promptly transported to the laboratory in plastic bags. Microarthropods were extracted in the lab using a Berlese-Tüllgren funnel; the specimens were collected in a preserving solution and identified using a stereo-microscope to order level (except Myriapoda, that were determined to class level).

Microarthropods were classified into "biological forms" according to their morphological adaptation to soil environments; for each biological form, the "eco-morphological index" (EMI), ranging from 1 to 20, was obtained from Parisi et al. (2005). The QBS-ar value of a given site is obtained from the sum of the EMI of all collected groups (when a biological form showed different EMI values among the 3 soil cores from the same site, only the highest value was retained for subsequent calculations).

3 Results

3.1 Vegetation and habitat mapping

We identified 11 grassland "vegetation units" (broad vegetation types defined on a physiognomic-floristic basis) of sufficient extent to be mapped at 1:50,000 (Table 1). The vegetation type with the largest area was "Montane *Bromus*-grasslands" (dominated mainly by *Bromus erectus, Festuca circummediterranea, Koeleria lobata*), followed by the "Subalpine karstic mosaic" (dominated by *Festuca* sp.pl. or by chamaephytes such as *Globularia* sp.pl. and *Helianthemum* sp.pl.) and the "Subalpine *Festuca*-grasslands" (dominated by *Festuca* sp.pl. and *Avenula praetutiana*).

Three Habitat types of Community interest were identified (Table 1). Seven of the vegetation units could be referred to one of these Habitats (or to a mosaic between two Habitats). The Habitat type with the largest area was “6210 *Semi-natural dry grasslands and scrubland facies on calcareous substrates”* (including its priority variant “6210*-*Important orchid sites*”).

3.2 Pasture phenology

The analysis of the intra-annual distribution of the “sustained productivity” period (expressed as $EVI_{std} \geq 0.75$) showed that the study grasslands can be arranged into 5 bio-climatic elevation belts: 800-1200 m (sustained productivity: May 9 – July 12); 1200-1600 m (May 9 – July 28); 1600 – 1800 m (May 9 – August 13); 1800-2000 m (May 25 – August 29); 2000-2200 m (June 10 – September 14). The EVI and EVI_{std} signatures of the vegetation units for each bioclimatic belt are shown in Fig. 2. Across the first three bioclimatic belts, the length of the sustained productivity increases with altitude, because its end is delayed with increasing elevation. The two high-altitude belts, instead, have the same sustained productivity length (105 days), but the position of such season is shifted by 15 days between the two of them.

As shown by the intra-annual trend of raw EVI values (left column in Fig. 2), the vegetation types with the highest productivity are found within the low- and mid-elevation bioclimatic belts: the highest EVI absolute values (>5000) of the whole dataset are shown by the “Montane *Brachypodium*-grasslands in the 800-1200 and 1200-1600 m belts, and by the “Montane karstic mosaic” vegetation unit in the 1600-1800 m belt. The vegetation units with the lowest EVI absolute values (<3500) are found at the two altitudinal extremes, i.e. the “Subalpine *Sesleria*-grasslands” and the “Colline *Bromus*-grasslands”.

Standardized values (right column in Fig.2) showed that in the lower altitudinal belts most vegetation units peak in early June and feature a marked and sudden productivity drop in summer (dry season), often followed by a secondary peak in autumn (onset of the rainy season). The higher the altitude, the later the occurrence of the main productivity peak, until at high altitudes there is prolonged vegetation greenness over the whole summer, but without a productivity reprise in autumn.

3.3 NDVI-Biomass model and sustainable stocking rate assessment

Since the training dataset was not normally distributed, biomass weight was ln-transformed to obtain normality. The calibration linear model (training dataset: Fig. 3a) showed a good fit (r^2=0.784, adjusted r^2=0.778, p<0.001; standard error of the estimate for the training dataset was 0.54). When the model was tested on the validation dataset (Fig. 3b), it also showed a good fit (r^2=0.758, adjusted r^2=0.752, p<0.001, standard error of the estimate= 0.47), highly comparable with the calibration model. Residuals of calibration and validation models were randomly distributed (data not shown), suggesting independence of the prediction error from the prediction itself.

When the model was applied to the whole study area, the estimated biomass values at the pixel scale had a median of 2,335 kg ha^{-1} (fresh weight). The vegetation unit with highest median biomass was the “Mesophytic grasslands”, followed by the “Montane karstic basins mosaic” and by the “Montane *Brachypodium*-grasslands”; the unit with the lowest median biomass was the “Subalpine *Sesleria*-grasslands” (Table 2).

Forage value was largely independent from biomass (correlation was non-significant with both parametric and non-parametric tests), thus while some vegetation types had high levels of both parameters (“Mesophytic grasslands”) others had high nutritional value but low biomass (“Montane *Bromus*-grasslands”, “Subalpine *Festuca*-grasslands”).

Highest median SSR (Table 2 and Fig. 4a) was attained by the “Mesophytic grasslands” (2.6 AU ha^{-1}), followed by the “Montane *Brachypodium*-grasslands” (0.95 AU ha^{-1}) and by the “Mosaic between Montane *Bromus*-grasslands and Xerophytic communities” (0.84 AU ha^{-1}). The lowest value was that of “Subalpine *Sesleria*-grasslands” (0.05 AU ha^{-1}).

Red deer density as obtained through GIS spatialization was found to vary dramatically across the study area, probably because of a high heterogeneity in habitat suitability and food resources (median value 0.008 AU ha^{-1}, min 0, max 0.27). The estimated forage intake by red deer significantly affected the net SSR for domestic animals (i.e. red deer stocking rate was estimated $\geq$0.05 AU ha^{-1}) on 7.3% of total grassland area. However, 1.1% of total grassland area, i.e. 240 ha, yielded a negative value when subtracting estimated red deer load from SSR (Fig. 4b).

3.4 Biological indicators

3.4.1 Vascular plants

Species richness of vascular plants at the plot scale (4 m^2) had a median value of 28 (min 12, max 65). No correlation was detected between species richness and grazing intensity. However, species richness was (weakly) negatively correlated with altitude (Spearman's r_s=-0.29, p=0.01). When considering species richness by vegetation unit (Table 1), the highest diversity was found in the two most thermo-xerophytic vegetation units, namely the "Colline *Bromus*-grasslands" and the "Mosaic between montane *Bromus*-grasslands and Xerophytic communities", along with the most mesophytic unit, i.e. the "Mesophytic grasslands". The lowest richness was found in the "Acidophilous grasslands", a vegetation unit mostly dominated by *Nardus stricta* and found in the bottom of karstic depressions or on marley slopes with deep soils.

The most relevant species of conservation interest for each vegetation unit are listed in Table 1 (last column). The most important vegetation units for these species were the "Colline *Bromus*-grasslands" (as they were very rich in protected Orchidaceae such as *Himantoglossum adriaticum*, along with some endangered steppic relics such as *Androsace maxima*) and the high-altitude communities (e.g. the "Subalpine karstic mosaic", as they hosted narrow-range endemics such as *Geranium austroapenninum*, and arctic-alpine species at the southernmost edge of their range, e.g. *Sibbaldia procumbens, Juncus trifidus* ssp. *monanthos*).

3.4.2 Auchenorrhyncha quality index (AQI)

The c. 3,300 collected specimens belonged to 8 families, 91 genera and 132 species. Median species richness per sampling site (n=15) was 20 (max. 41, min. 2). Most delphacids and many Deltocephalinae taxa were species typically bound to grassland habitats, along with a few Typhlocybinae (genera *Chlorita*, *Emelyanoviana*, *Wagneriala*, *Zyginidia*) and other cicadellids (*Aphrodes*, *Megophthalmus*). The AQI values of the sampled sites ranged between 14.1 and 64.4 (median 47.4). AQI values had a strong negative relationship (Spearman's r_s=-0.78, p<0.001: Appendix A, Fig. A.1) with grazing load as estimated at the correspondent GCP during vegetation mapping (see section 2.2 and Appendix A, table A.1). Heavily grazed areas were characterized by taxa feeding on nitrophilous plants such as *Urtica* sp.pl. (*Eupteryx urticae*) or on plants which are avoided by livestock, e.g. many aromatic Lamiaceae (*Eupteryx* spp.) or *Verbascum* sp. pl. (*Micantulina stigmatipennis*).

3.4.3 Arthropod-based biological soil-quality index (QBS-ar)

Across the whole dataset (n=32), QBS-ar values ranged between 72 and 192, and had a significant negative correlation with grazing impact as estimated at the GCP correspondent to the QBS-ar sampling sites (Spearman's r_s=-0.66; p<0.001: Appendix A, Fig. A.2). Overgrazed sites almost always had QBS-ar values <100. Two sites behaved as outliers: one had a very low QBS-ar (94) despite being in a core area of the Park where grazing is prohibited; the other had a very high QBS-ar (174) despite being located within a grassland heavily impacted by cows and horses.

The analysis of the subset of samples from inside/outside the fenced exclosures, showed highly significant differences between grazed and ungrazed ground: median value for the grazed sites was 89 (n= 9), vs. 166 for the fenced areas (n=5) (p <0.001, exact permutation test).

The NMDS scatterplot of the taxa-by-sites matrix (Fig.5) showed a significant negative relationship between the degree of morpho-functional faunal complexity of arthropods and grazing intensity: the most disturbed sites featured an over-simplification of the faunal assemblages, because of the decrease or disappearance of those taxonomic and functional groups more specifically adapted to soil habitats, such as Diplura and Diplopoda.

4 Discussion

4.1 Vegetation and habitat mapping

The relatively simplified classification method adopted in this study yielded 11 broad vegetation units. From the results of phytosociological surveys of nearby areas (e.g. Biondi et al., 1999; Di Pietro et al., 2005; Lucchese et al., 1995), we estimate that in the study area the number of grassland communities at the association level in the Braun-Blanquet system (Dengler et al., 2008) could be >30. However, the spatial grain at which many of these associations are defined is very fine (Bazzichelli and Furnari, 1979; D'Angeli et al., 2011), and would require mapping vegetation at a scale >1:10,000, a formidable task in a large mountain area. Moreover, it has been suggested that for the purposes of mountain pasture management, even association-level phytosociological maps should be further subdivided into "pastoral variants" within each association (Bagella and Roggero, 2004). On the other hand, the approach adopted in this study, i.e. a land-unit classification based on physical features, followed by the assignment of broad physiognomic types obtained from field survey and mapped at 1:50,000 scale, should guarantee a reasonable level of repeatability and ecological significance (Bunce et al., 1996; Lawson et al., 2010). Fine-scale heterogeneity (i.e. within each vegetation unit) relevant for grazing land management was then assessed in an objective way through the analysis of the remote-sensed data (NDVI-biomass model at 30x30 m resolution).

The coarse vegetation types adopted in this study are still finer than the Natura 2000 Habitat classification, as many different vegetation units in our scheme corresponded clearly to the same Habitat type (e.g. all limestone grasslands from the colline to the upper-montane belt corresponded to only one Habitat, namely *6210 - Semi-natural dry grasslands and scrubland facies on calcareous substrates*).

4.2 Pasture phenology

The intra-annual productivity patterns across different elevation belts, as detected by the EVI analysis, are consistent with what is practiced by some cattle breeders, who in early autumn move the livestock back to the lowest grazing areas to exploit the October secondary peak. This can be explained as the Central Apennines are located within the Mediterranean basin, characterized by a precipitation minimum in summer: while at low altitudes this results in an actual drought period in summer, at mid- and high-altitude the decreasing temperature and the increasing amount of orographic rain (and perhaps of fog precipitation) (Filibeck et al., 2015; Gerdol et al., 2008) lead to a transitional climate between the Mediterranean and Temperate biomes ("zonoecotone" *sensu* Walter, 1985).

However, absolute EVI values in the study area decreased with increasing altitude, so that the "Subalpine *Sesleria*-grasslands", although featuring a prolonged primary production through summer, were found to have only a very small SSR. On the other hand, within the lowest elevation belt we found a large degree of variation between different vegetation units: the "Montane *Brachypodium*-grasslands", bound to deep clayey soils, featured very high EVI, but the "Colline *Bromus*-grasslands", usually found on shallow soils on limestones, showed even lower EVI values than the "Subalpine *Sesleria*-grasslands". Thus, while the differences in EVI values across different altitude belts highlighted the role of climate parameters (such as growing season temperature, precipitation regime and snow-cover duration: e.g. Choler, 2015; Filibeck et al., 2015) as productivity drivers, the within-belt differences underlined the role played by soil moisture factors (e.g. Ellenberg, 1988), showing that they can be of an order of magnitude as large as that of macroclimate.

Finally, we note that the 5 bio-climatic elevation belts obtained from the phenological analysis are partly consistent with the traditionally accepted geobotanical belts for the Apennines (also used in this study for the preliminary physical stratification: see Section 2.2), but highlight the need of further subdividing them: for instance, the usually recognized “montane belt” was divided by EVI analysis into two phenologically different parts, below and above 1600 m, respectively.

4.3. Biomass and sustainable stocking rate estimates

Biomass distribution by vegetation unit showed very high values for the community types bound to deep (“Mesophytic grasslands”) or clayey (“Montane *Brachypodium*-grasslands”) soils, allowing for forage production through the drought period. High-elevation vegetation units (“Subalpine *Sesleria*-grasslands”, “Subalpine *Festuca*-grasslands”) featured very low biomass values, that can be explained with the shallow soils, the short growing season and the prolonged snow-cover (Choler, 2015). However, within each vegetation unit, estimated biomass variance was extremely large. This is because of the physical heterogeneity at fine spatial scale, which is typical of limestone mountain landscapes (e.g. Catorci and Gatti, 2010), and is effectively detected by the Landsat 30x30m resolution but is far beyond the resolution of the 1:50,000 vegetation mapping.

Remote-sensed biomass maps are not directly related to a measure of livestock production, because the primary production data have to be corrected using field information on botanical composition patterns and nutritional value of the different species (Santos et al. 2013; Swain et al. 2013). The analysis of our results confirmed that this is essential in order to correctly estimate SSR: some vegetation units with very high biomass production had poor nutritional value, thus lowering their actual SSR compared to what would result from biomass only.

However, since in the Mediterranean mountains primary production is influenced by the inter-annual variability of summer drought, future developments of the approach presented here could aim at more flexible, adaptive livestock strategies (Jakoby et al., 2015), taking into account real-time primary productivity oscillations, rather than estimating averages over a multi-year time series.

Since in our study area both domestic grazers and wild large herbivores coexist, it was necessary to consider also the forage intake by wild animals (European Commission, 2014; Marchiori et al., 2012). Only a very small fraction of pasture area was significantly affected by red deer biomass intake, although the seasonal migration behaviour of red deer (Heurich et al., 2015; Mysterud et al., 2011) may have affected density estimates.

Two crucial points may have affected our SSR estimates: botanical composition sampling design, and specific forage-value data sources. The first issue is very difficult to deal with in a cost-effective way for very large and heterogeneous areas, given the multiple, nested spatial scales at which floristic assemblages can vary in mountain areas (Catorci and Gatti, 2010; D'Angeli et al., 2011). In this study, subjective sampling, and a low sampling density, had to be adopted because of financial and temporal constraints. This could explain why our SSR values for some vegetation units seem to be overestimated (*Brachypodium*-grasslands) or underestimated (Montane kartsic mosaic), compared with what could be expected based on previous experience on forage values of Apennines grasslands (cf. Catorci et al., 2014; D'Ottavio et al., 2005). Stratified randomized sampling could be considered the best trade-off, although for large complex mountain areas it is not trivial to determine the ecologically sound spatial scale at which to define the strata (Lepš and Šmilauer, 2007). Moreover, the resulting number of sampling plots for a thorough assessment could be cost prohibitive. For instance, in a methodological test in another area of the Apennines, aimed at SSR estimation through the "pastoral value" method, D'Ottavio et al. (2005) had to sample 81 quadrats for a pasture of only 300 hectares.

Databases of nutritional value of wild plant species are scarce and incomplete (see references in Pardini et al., 2000a), or merge in a single value multiple features of a plant species (including e.g. specific productivity: Roggero et al., 2002). Thus, in this study the forage value for many species had to be inferred from that of other plants within the same genus, or estimated, correcting a comprehensive "pastoral" value assigned by the available sources.

Finally, as summarized in the introduction, the very concept of SSR is somewhat controversial within the specific context of the Mediterranean. However, as discussed in section 4.2, at mid- and (especially) high-altitudes, our study area lies within a climate that can be likened to the Temperate biome, and some vegetation units show a reasonable degree of compositional similarity with grassland communities of the Alps and Central Europe (Apostolova et al., 2014; Gerdol et al., 2008). Thus, we deemed it acceptable to consider the "classical" SSR concept; we are conscious that, at lower altitudes within the study area, our approach could have yielded a very conservative estimate of the SSR, because of the resilient response of Mediterranean plant communities to high grazing loads (Perevolotsky and Seligman, 1998).

4.4 Biological indicators

4.4.1 Vascular plants

Total species richness at $4m^2$ plot-scale weakly decreased with altitude: although this is in agreement with some previous studies on grassland diversity (e.g. Austrheim, 2002), recent studies have found contrasting results, perhaps depending on plot size and gradient length (see references in Dengler et al., 2014).

Interestingly however, the highest diversity of protected/endangered species was found at the two extremes of the altitudinal gradient: both the sub-Mediterranean grasslands and the high-altitude communities were found to be hotspots for protected species, although the latter had a much lower total species richness than the former. On the other hand, the "Mesophytic grasslands", although featuring both a high total species richness and an extremely high productivity, did not show a significant contingent of species of conservation interest.

Vascular plant richness at $4m^2$ scale was not correlated with grazing disturbance. A number of studies found a higher plot-scale richness in moderately grazed than un-grazed European or Mediterranean grasslands (e.g. Noy-Meir and Oron, 2001; Pierce et al., 2007; Turtureanu et al., 2014), although the relationship was found to be affected by herbivore species (Bakker et al., 2006), plot size (de Bello et al., 2007; Dupré and Diekmann, 2001), and grassland productivity (Bakker et al., 2006; de Bello et al., 2007; Osem et al., 2002). Kruess and Tsharntke (2002) didn't find any effect of different cattle loads on plant richness at the plot scale ($25m^2$), and a meta-analysis by Scohier and Dumont (2012) did not reveal any significant trend for plant richness along a wide gradient of sheep grazing loads. In a complex landscape, fine-scale physical heterogeneity can have a much larger explanatory power on grassland species richness at the plot scale than grazing load (e.g. Cingolani et al., 2010; Moeslund et al., 2013). Moreover, the positive effects of grazing on plant diversity in Mediterranean grasslands might be more evident at landscape scale (whole floras: Filibeck et al., 2016; see also Perevolotsky and Seligman, 1998) than at plot scale. However, our sampling plots were not specifically stratified on the basis of grazing intensity, as they were distributed to be representative of vegetation units (see Methods): for instance, only four quadrats corresponded to un-grazed vegetation (disturbance level 1), and only one was placed in "degraded" pasture (disturbance level 4).

4.4.2 Auchenorrhyncha Quality Index (AQI)

The use of AQI in the present work is very promising, given the strong negative correlation with grazing intensity. This agrees with Nickel and Hildebrandt (2003), who demonstrated that in floodplain grasslands in Germany, high-intensity grazing seriously reduces Auchenorrhyncha diversity and, in particular, the richness of specialists.

To our knowledge, this study is the first application of AQI for evaluating Mediterranean mountain pastures, and some methodological adjustments were necessary from what Wallner et al. (2013) originally proposed. The main differences between our pastures and the ecosystems where the AQI index was developed (prairie habitats in North America) lie in the rugged orography, the complex micro-topography (originated by karst geomorphology) and the presence of surrounding forests. For these reasons, on the one hand it was necessary to modify the criteria for assessing the coefficient of conservatism (Appendix A, Table A.2); on the other hand, we deemed it more reliable and more cost-effective to apply only the qualitative (presence/absence) version of the AQI. Taking into account abundance values would require a statistically formalized sampling (e.g. with transects of fixed length and fixed number of sweeps), but the fine-scale complexity of the Apennine secondary grasslands would make it very difficult to appropriately stratify the sampling without either missing many micro-habitats and their associated biodiversity, or ending up with an extremely high effort (especially for laboratory sorting, preparation and identification of specimens).

Within a secondary grassland ecosystem, Auchenorrhyncha are usually clustered in small micro-habitats (e.g. single grass tussocks) with large uncolonized interspaces (this holds especially for the brachypterous taxa, which are particularly important ecological indicators). Thus, it was necessary to sample a rather large area at each site to get comparable data. It is important to note that this kind of sampling might include larger environmental gradients, leading to higher species diversity and higher AQI values than with transect sampling. One component of this environmental heterogeneity is created by grazing itself (heterogeneity of the vegetation structure) or by the inherent micro-topographical heterogeneity of the habitat, and has to be represented in the sampling: we suggest that in Mediterranean mountains the sampled area at each site for presence/absence analysis should not be smaller than 1 ha. However, the heterogeneity caused by larger-scale abiotic factors may bias the results: e.g. water streams and patches of wet soil will have a very high species richness and should either be excluded from sampling or evaluated separately and compared only with analogous habitats.

An alternate option could be a semi-quantitative approach (Holzinger et al., 2003; cf. also Palmer et al., 2002), integrating a qualitative sampling strategy with several transect lines, perpendicular to each other. To enhance comparability of abundance data gathered in this way, a reduced number of abundance classes should be used. Species should then be weighted by a factor proportional to these abundance classes when calculating AQI.

Wallner et al. (2013) recommended the use of the vacuum sampler in the North American tallgrass prairie. For the European secondary grasslands the sweeping net is more appropriate, especially for qualitative sampling, because the grass height is lower and the vegetation is sparser; for semi-quantitative sampling, however, the vacuum sampler could be used to provide more standardized abundance data.

Finally, some specific problems were encountered in applying the index to Italian taxa. While morphological data (wing length) are easily available for almost all Auchenorrhyncha taxa, the other types of data needed for calculating AQI (i.e., the species' ecology and biology) are to some degree incomplete for the Mediterranean, as most research has been conducted in Central Europe (Nickel, 2003). Moreover, a taxon may present different feeding habits or life cycles in different geographic zones. Thus, traits for some species had to be inferred, using similar taxa (same genus and similar ecology) as a reference.

More research is needed to further explore the potential and limits of applying the AQI in Mediterranean habitats, and to refine the methodological adjustments. However, despite all the methodological limitations, the index was very well correlated with grazing impact, demonstrating that the AQI is a robust ecological indicator.

4.4.3 Arthropod-based biological soil-quality index (QBS-ar)

The faunal assemblages within the QBS-ar samples featured both microarthropod taxa specifically bound to grassland ecosystems (Hemiptera, Hymenoptera, Coleoptera) and taxa adapted to forest habitats as well (e.g. Protura); these latter are probably connected to shrub nuclei (Menta et al., 2011). Pauropoda were surprisingly scarce, considering they are usually well-represented in grasslands (cf. Menta et al., 2011) – a finding worth further study.

The QBS-ar index showed a very large variation range (72-193), consistent with that observed by Rüdisser et al. (2015) in South Tyrol grasslands (where values ranged between 57-179). However, the land-cover class defined as "grasslands" by Rüdisser et al. (2015) actually included very different management regimes, including ploughed and re-seeded hay meadows; instead, all our data were from (semi-)natural dry grasslands, where the only human intervention is livestock grazing.

Our QBS-ar values showed a good (negative) correlation with the levels of grazing disturbance estimated at the corresponding GCP during the vegetation mapping; moreover, since there was a significant difference between fenced and grazed patches within the same land-unit, we conclude that most of the observed variation in our sample is explained by the different degree of grazing disturbance rather than by physical habitat heterogeneity.

Probably, below-ground microarthropod communities are negatively affected by grazing because of the effect of soil compaction, in turn due to livestock trampling, rather than by biomass removal per se. Blasi et al. (2013) showed that, in forest ecosystems, soil compaction leads to the disappearance of specialized groups: this is consistent with what resulted by the NMDS ordination of our samples (Fig. 5), where Diplura, Diplopoda and Chilopoda had a significant negative correlation with grazing load. These taxa are already well known for their sensitivity to environmental stresses, and were found in previous studies to be negatively correlated with soil disturbance in cropland (e.g. Menta et al., 2011); our results confirm that they can be used as biological indicators in extensive grazing systems also. On the other hand, (adult) Diptera showed an increase in the most grazed sites that can be explained by the accumulation of cattle dung. Finally, we note that Isopoda (found to be connected with grasslands in previous studies: Menta et al., 2011) were found only in samples from inside the fenced areas, and could thus be worth further attention as potential indicators of very low-disturbance grasslands.

Our hypothesis that the main driver of QBS-ar values in dry grasslands is soil trampling is confirmed by the two outliers that emerged when correlating QBS-ar to grazing load (see Results and Appendix A, Fig. A.2): one sample featured a very low value in an area where livestock grazing is prohibited, but soil surface appeared to be heavily trampled by red deer; the other sample had a high QBS-ar value in a pasture heavily grazed by cattle, but was taken from a relatively steep slope, where livestock cannot remain for prolonged periods.

The majority of very heavily grazed sites had QBS-ar <100, while most sites without (or with very light) livestock grazing had QBS-ar > 150. Thus, our results suggest that QBS-ar monitoring can contribute to guide decisions for sustainable grazing management: the grazing resource planning should aim at maintaining a mosaic of areas with very little soil disturbance (QBS-ar > 150) and areas with sustainable grazing (QBS-ar > 100), while QBS-ar values <80-90 should be considered a warning signal of habitat degradation and lead to reconsidering the allowed grazing load or duration.

Although most of the study area is presently understocked, leading to shrub advancement or tall-grass encroachment, below-ground arthropod communities showed that in the areas where livestock are particularly concentrated there is a major disruption of soil biota. This can be explained because the abandonment of traditional sheep husbandry has brought new forms of grazing resources exploitation: “part-time farmers” breed horses or cattle that are left in public pastureland without surveillance. Instead, in order to prevent both woody vegetation development and soil degradation, we recommend that the grasslands of the study area be managed on a rotational basis, through short but intense grazing (e.g. Teague et al., 2011), preferably by sheep in order to avoid soil compaction and the consequent loss of important ecosystem services. The effectiveness of such a management strategy should be monitored through repeated QBS-ar sampling. This could be based on a sampling scheme stratified according to grazing levels and to grassland types. Due to inter-annual variability of primary production and related livestock behavior, monitoring should be repeated at intervals of 3-5 years.

5. Conclusions

In this work we applied a novel approach to assessing sustainable stocking rates to Mediterranean mountain grasslands, and in particular to Natura 2000 habitat types, through an integration of remote-sensed biomass data, phenological analysis and botanical composition. Our analysis showed that, since Natura 2000 habitats have very coarse definitions, within the same habitat type there can be a large spatial heterogeneity in the sustainable stocking rates and in optimal stocking season. Thus, grazing load, distribution and timing should be kept under careful human control to maintain grassland habitats. Unfortunately, in most of Italy, regulations issued by town councils are inadequate: for instance, they usually set a fixed grazing season across large areas without taking into account bio-climatic heterogeneity. Multidisciplinary scientific evidence is essential to help inform policy decisions, and remote sensing can provide data on both the background potential and real-time variability of primary productivity.

However, we argue that, even after sustainable stocking rates have been estimated for each habitat type, it is necessary to monitor over time the actual impact of livestock on the whole ecosystem. For this reason, two arthropod-based biological indicators were tested: we found that both of them are negatively correlated with grazing levels. Since different groups of organisms respond differently to changes in grassland quality (references in Wallner et al., 2013), we suggest monitoring the ecological effects of grazing through an integrated suite of indicators, including both AQI and QBS-ar, as they will provide information on both above- and below-ground invertebrate diversity.

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Authors’ contribution: the overall study was planned and supervised by G.P., B.R., G.F., R.P and C.S.; vegetation and habitat survey was designed by G.F. and performed by G.F., L.C., L.D.M. and A.S; critical vascular plants were revised by L.C. and A.S.; photo-interpretation, GIS analysis and NDVI elaboration were performed by C.M.R.; biomass field data were collected by C.G. and R.P.; EVI analysis was done by A.D.F.; SSR assessment was performed by R.P. (NDVI-biomass estimates) and F.R. (forage value assessment); AQI survey was designed and performed by A.G. and C.B.; QBS survey was performed by G.P. and R.V.; red-deer sampling was coordinated by R.L. and its outputs were analysed by A.A. and R.P.; stocking systems were analysed by R.P. and B.R. The manuscript was written by G.F., with major contributions by G.P. and R.P. and specific sections contributed by A.G., C.B., A.D.F., F.R.; S.A.M. revised it linguistically. All authors discussed the results and contributed to the final editing.

Appendices A – B. Supplementary data

Supplementary material related to this article can be found in the online version.

Appendix A: supplementary tables and figures

Appendix B: photographs of the vegetation units

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Table 1. Physical and botanical features of the vegetation units (ecological/physiognomic grassland types) identified in the study area. The vegetation units are listed in approximate order of increasing elevation. For each unit, one or more photographs can be found in Appendix B.

Vegetation unit	Corresponding Natura2000 Habitat	Land units	Area (%)	Dominant and frequent species	Mean richness (of the 4 m^2 plots)	Main species of conservation interest
Colline *Bromus*-grasslands	*6210* - Semi-natural dry grasslands and scrubland facies on calcareous substrates(Festuco-Brometalia) (* important orchid sites)*	Limestones - colline belt; Limestones - submontane belt	6.5	Dominant: *Festuca circummediterranea, Bromus erectus, Phleum hirsutum* ssp. *ambiguum, Satureja montana, Sideritis italica, Stipa dasyvaginata* ssp. *apenninicola, Helianthemum* sp.pl. Frequent: *Arenaria serpyllifolia, Medicago minima, Anthyllis vulneraria, Petrorhagia prolifera, Sanguisorba minor, Triticum ovatum*	*39.8 (n=4)*	*Androsace maxima, Crepis lacera, Epipactis atrorubens, Himantoglossum adriaticum, Iris marsica, Ophrys apifera, Orchis pauciflora, O. provincialis, O. tridentata, O. ustulata,*
Mesophytic grasslands	no corresponding habitats (does not fit diagnostic features of *6510-Lowland hay meadows*)	Terra rossa - submontane belt; Terra rossa - montane belt; Alluvial plains - submontane belt	1.0	Dominant: *Cynosurus cristatus, Dactylis glomerata, Lotus corniculatus, Phleum bertolonii, Poa pratensis* Frequent: *Medicago lupulina, Trifolium repens, Leucanthemum vulgare*	*32.8 (n=6)*	*Euphorbia gasparrinii*
Montane *Bromus*-grasslands	*6210 - Semi-natural dry grasslands and scrubland facies on calcareous substrates(Festuco-Brometalia)*	Limestones & dolomites - montane belt	30.1	Dominant: *Bromus erectus, Festuca circummediterranea, Koeleria lobata, Phleum hirsutum* ssp. *ambiguum, Avenula praetutiana* Frequent: *Hieracium pilosella, Helianthemum* sp.pl.*, Minuartia verna, Poa bulbosa, Anthyllis vulneraria, Arenaria serpyllifolia, Cerastium tomentosum, Sedum rupestre, Thymus longicaulis*	*30.6 (n=15)*	*Crepis lacera, Cynoglossum magellense, Geranium austroapenninum, Iris marsica, Orchis pauciflora, O. provincialis, O. tridentata, O. ustulata, Viola eugeniae* ssp. *eugeniae*

Vegetation unit	**Corresponding Natura2000 Habitat**	**Land units**	**Area (%)**	**Dominant and frequent species**	**Mean richness (of the 4 m^2 plots)**	**Main species of conservation interest**
Mosaic between Montane *Bromus*-grasslands and Xerophytic communities	Xerophytic communities: *6110*- Rupicolous calcareous or basophilic grasslands of the Alysso-Sedion albi.* Grassland matrix: *6210* - Semi-natural dry grasslands and scrubland facies on calcareous substrates(Festuco-Brometalia)* *(* important orchid sites)*	Conglomerates - montane belt (lower part)	3.3	Dominant: *Festuca circummediterranea, Bromus erectus, Cerastium tomentosum, Koeleria lobata, Phleum hirsutum* ssp. *ambiguum, Anthyllis vulneraria, Sedum* sp.pl. Frequent: *Alyssum alyssoides, Saxifraga tridactylites, Hieracium pilosella, Erophila verna, Hornungia petraea*	*42.5 (n=2)*	*Ophrys apifera, Orchis pauciflora, O. provincialis, O. tridentata, O. ustulata,*
Montane *Brachypodium*-grasslands	no corresponding habitats	Clay - submontane belt; clay - montane belt (lower part)	7.7	Dominant: *Brachypodium rupestre* Frequent: *Dorycnium pentaphyllum, Polygala nicaeensis,Trifolium ochroleucum*	*30.5 (n=4)*	
Montane karstic mosaic	Some communities correspond to *6210 - Semi-natural dry grasslands and scrubland facies on calcareous substrates (Festuco-Brometalia)*	Terra rossa - montane belt; Limestones - montane belt	3.1	Dominant: *Agrostis capillaris, Bromus erectus, Festuca circummediterranea, Festuca* sect. *Aulaxyper, Koeleria lobata, Nardus stricta, Poa alpina* Frequent: *Arenaria serpyllifolia, Cerastium tomentosum, Potentilla rigoana, Sedum acre, Thymus longicaulis, Veronica arvensis*	*23.7 (n=10)*	*Viola eugeniae* ssp. *eugeniae*

Vegetation unit	**Corresponding Natura2000 Habitat**	**Land units**	**Area (%)**	**Dominant and frequent species**	**Mean richness (of the 4 m^2 plots)**	**Main species of conservation interest**
Acidophilous grasslands	no corresponding habitats (does not fit diagnostic features of *6230 * Species-rich Nardus grasslands, on siliceous substrates in mountain areas)*	Terra rossa - montane belt; marls - montane belt; marls - subalpine belt	2.3	Dominant: *Nardus stricta, Festuca* sect. *Aulaxyper, Agrostis capillaris, Plantago atrata, Trifolium repens* Frequent: *Potentilla rigoana, Achillea millefolium, Galium verum, Ranunculus pollinensis*	*20.8 (n=12)*	*Ajuga tenorei, Taraxacum glaciale*
Subalpine *Brachypodium*-grasslands	no corresponding habitats	Limestones - subalpine belt; Marls - subalpine belt	2.0	Dominant: *Brachypodium genuense* Frequent: *Bunium bulbocastanum, Galium lucidum*	*25.7 (n=3)*	
Subalpine *Festuca* - grasslands	Mostly corresponding to *6210 - Semi-natural dry grasslands and scrubland facies on calcareous substrates (Festuco-Brometalia)*. At higher elevations, corresponding to *6170-Alpine and subalpine calcareous grasslands*	Limestones & dolomites - montane belt (upper part); Limestones & dolomites - subalpine belt	15.5	Dominant: *Avenula praetutiana, Festuca circummediterranea, F. laevigata* ssp. *laevigata, F.* sect. *Aulaxyper, Koeleria lobata, Poa alpina* Frequent: *Hieracium pilosella, Armeria canescens, Thymus longicaulis*	*26.2 (n=12)*	*Botrychium lunaria, Cynoglossum magellense Erodium alpinum, Geranium austroapenninum, Leucanthemum tridactylites, Viola eugeniae* ssp. *eugeniae,*
Subalpine karstic mosaic	Some communities correspond to *6170-Alpine and subalpine calcareous grasslands*	Terra rossa - subalpine belt; Limestones - subalpine belt	17.3	Dominant: *Festuca circummediterranea, F.* sect. *Aulaxyper, F. violacea, Globularia meridionalis, Helianthemum* sp.pl.*, Nardus stricta, Thymus longicaulis* Frequent: *Alchemilla colorata, Cerastium tomentosum, Hieracium pilosella, Plantago atrata*	*29.3 (n=3)*	*Ajuga tenorei, Botrychium lunaria, Cynoglossum magellense, Dryas octopetala, Juncus trifidus* ssp. *monanthos, Sibbaldia procumbens, Taraxacum glaciale, Viola eugeniae* ssp. *eugeniae,*

Vegetation unit	Corresponding Natura2000 Habitat	Land units	Area (%)	Dominant and frequent species	Mean richness (of the 4 m^2 plots)	Main species of conservation interest
Subalpine *Sesleria*-grasslands	*6170-Alpine and subalpine calcareous grasslands*	Limestones & dolomites - subalpine belt	11.1	Dominant: *Sesleria juncifolia* ssp. *juncifolia* Frequent: *Carex kitaibeliana, Koeleria lobata, Festuca* sp.pl.*, Trinia dalechampii*	*22.7 (n=3)*	*Dryas octopetala, Oxytropis pilosa* ssp. *caputoi, Pedicularis elegans*

Table 2. Estimates of biomass production (left: fresh weight, kg ha^{-1} $year^{-1}$) and sustainable stocking rate (right: AU ha^{-1} over grazing season) at the pixel scale for each vegetation unit, in order of decreasing median biomass (*n*= number of 30x30 m pixels).

		Biomass			SSR		
Vegetation unit	***n***	**min**	**max**	**median**	**min**	**max**	**median**
Mesophytic grassl.	*1918*	1386	18169	9584	0	6.34	2.64
Montane karstic mosaic	*5801*	199	15784	7677	0	4.35	0.57
Montane *Brachypodium*-grassl.	*11032*	0	16738	7200	0	4.14	0.95
Mosaic Montane *Bromus*/Xeroph.	*5863*	0	14353	5292	0	2.64	0.84
Acidophilous grassl.	*5143*	0	15784	4815	0	2.03	0.48
Montane *Bromus*-grassl.	*51087*	0	17215	2907	0	4.41	0.33
Subalpine *Brachypodium*-grassl.	*4212*	0	15784	2691	0	1.43	0.13
Colline *Bromus*-grassl.	*9580*	0	13877	1979	0	3.08	0.35
Subalpine *Festuca*-grassl.	*34284*	0	14830	1979	0	2.58	0.26
Subalpine karstic mosaic	*39669*	0	16261	1979	0	1.64	0.16
Subalpine *Sesleria*-grassl.	*24205*	0	10538	436	0	1.44	0.05

Fig.1 Study area position (inset) and physiography (main map). Dashed line: study area boundary (Abruzzo Lazio e Molise National Park, and adjoining Natura 2000 sites).

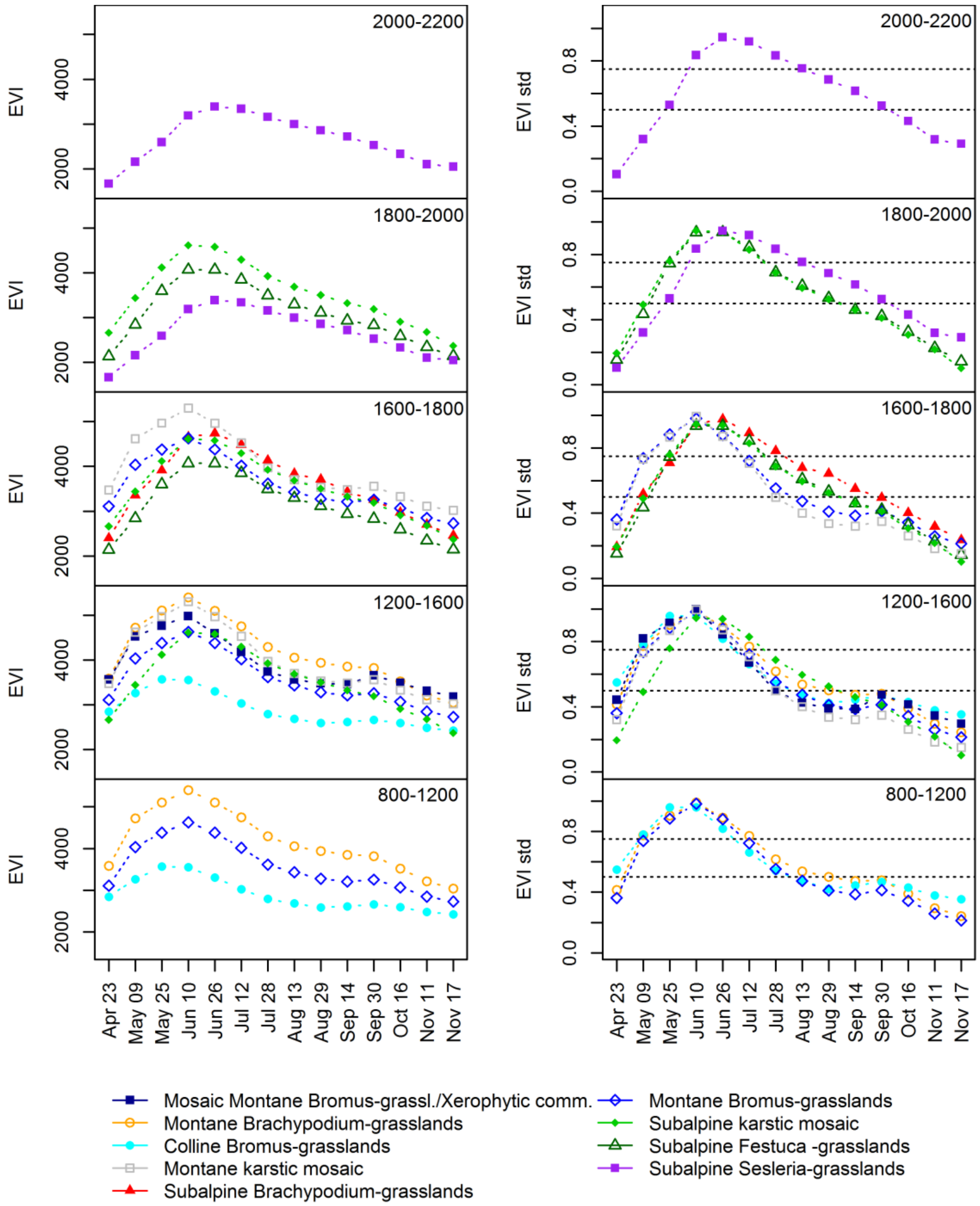

Fig. 2 Averaged EVI intra-annual signatures for each vegetation unit and within each altitudinal belt. Horizontal axis: date (at 16-day intervals). Vertical axis, left column: raw EVI values. Vertical axis, right column: standardized EVI values (EVI_{std}). Dotted lines represent 50 and 75% of the maximum EVI_{std}. Only the vegetation units featuring at least 10 pixels in at least one vegetation belt are shown.

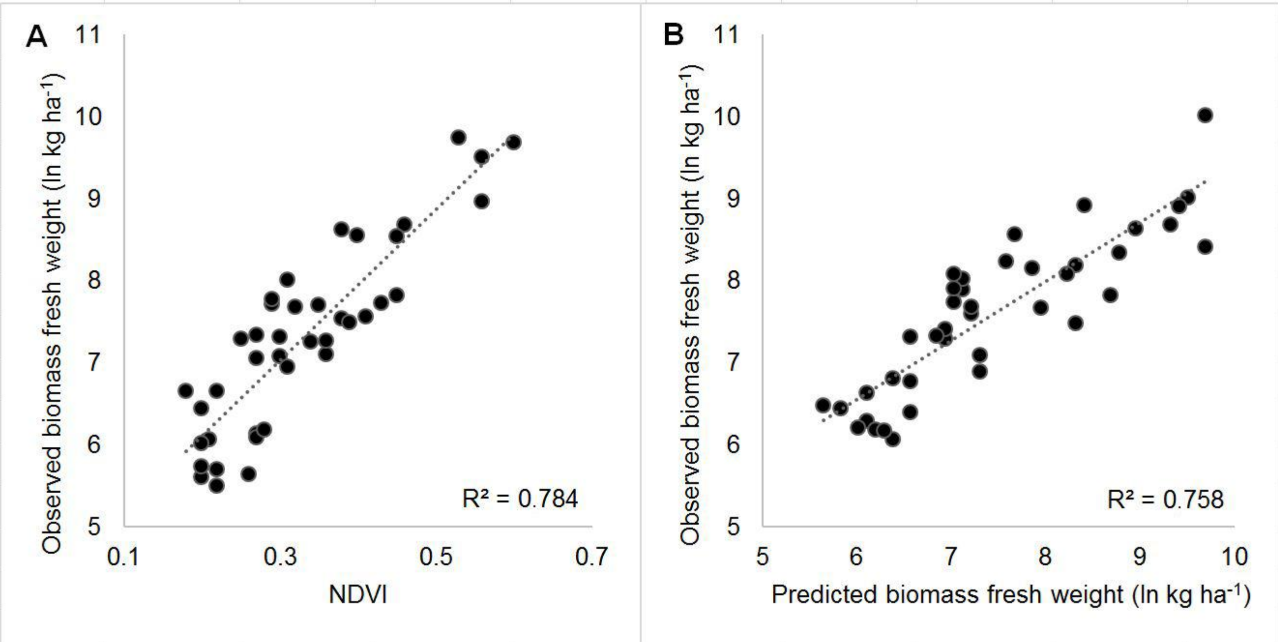

Fig. 3 Regression model correlating NDVI with pasture biomass. a) (left) Linear regression model of training dataset for biomass, fitting observed (ln-transformed) biomass fresh weight vs. NDVI. b) (right) Linear regression model fitting observed values of the validation dataset vs. predicted values of the model.

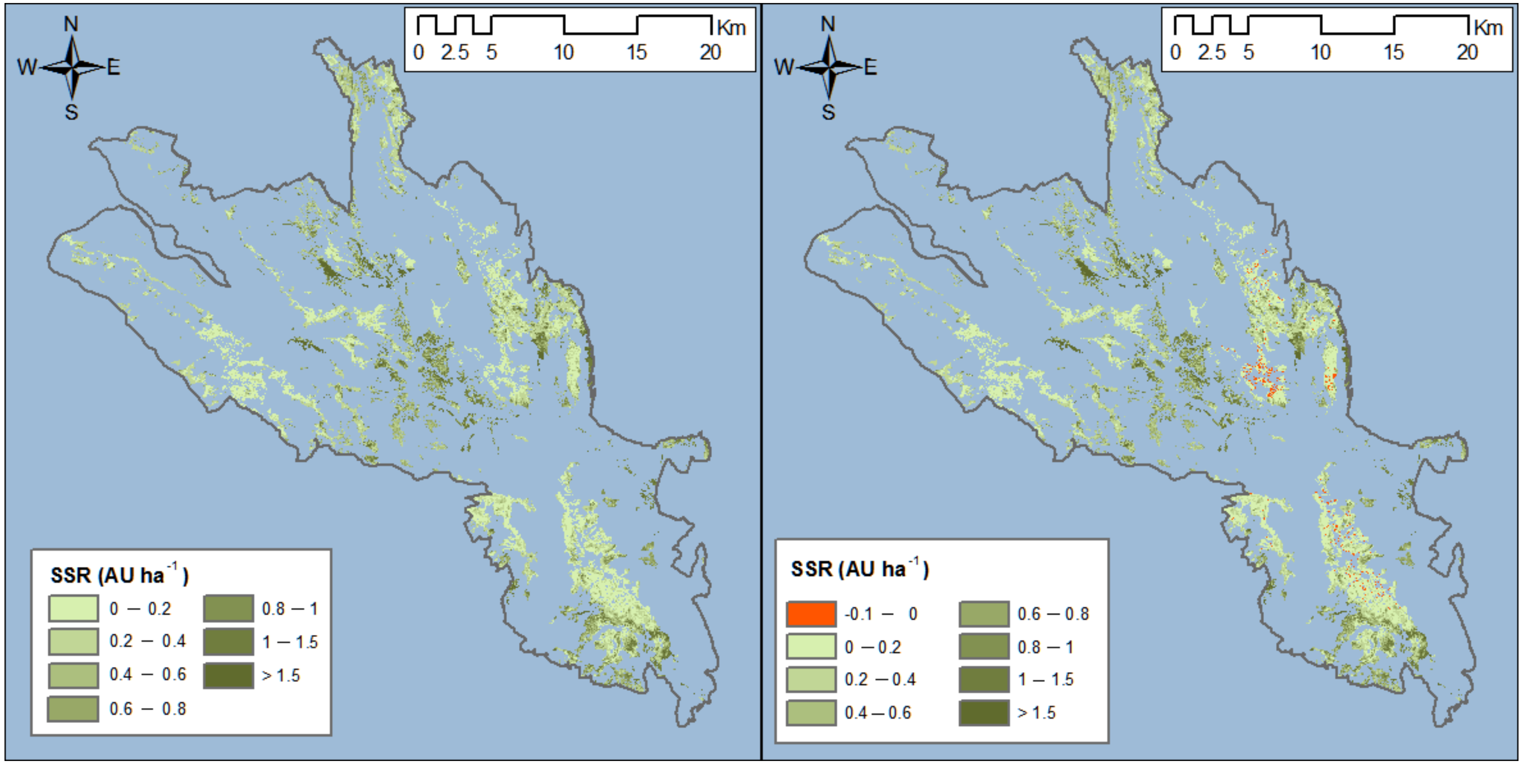

Fig. 4. Map of estimated Sustainable Stocking Rate (SSR), at 30 m x 30 m resolution (Landsat pixels), for the study area. a) (Left) Estimated total SSR . b) (Right) Estimated SSR available for domestic livestock, after having subtracted the red deer stocking rate. AU= animal units.

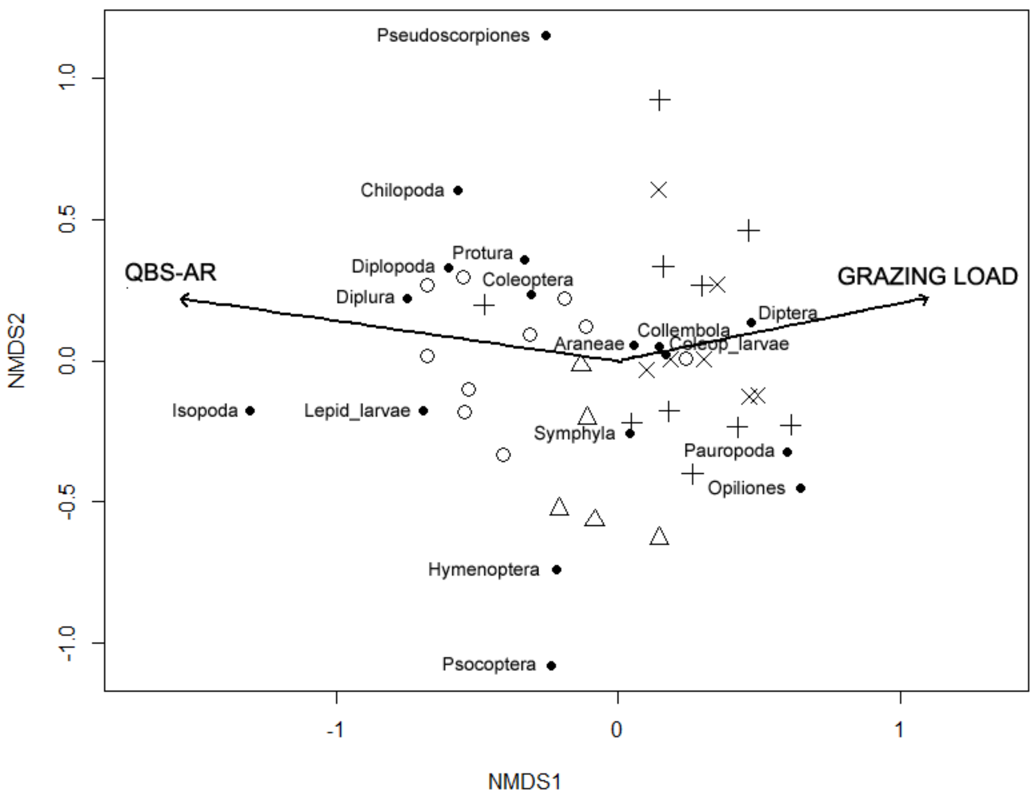

Fig. 5 Non-Metric Multidimensional Scaling (Bray-Curtis dissimilarity, 2 dimensions; stress value = 0.19) triplot of the taxa-by-sites matrix of the QBS-ar samples (taxa are weighted according to their eco-morphological index, see section 2.5.3). Sample site symbols refer to grazing load levels as estimated in the field, following the scale in App. A, table A.1 (empty circles = ungrazed/undergrazed; empty triangles = intermediate; crosses = heavily grazed; multiplication sign = degraded pasture). Black dots represent the taxonomic groups (taxa with frequency <10%, as well as those occurring at all sites, were excluded from the analysis). QBS-ar values and grazing load did not contribute to the ordination and are plotted as an aid to interpretation. Correlation between grazing load and ordination scores is highly significant (p<0.001, based on 999 permutations).

Fig. 1
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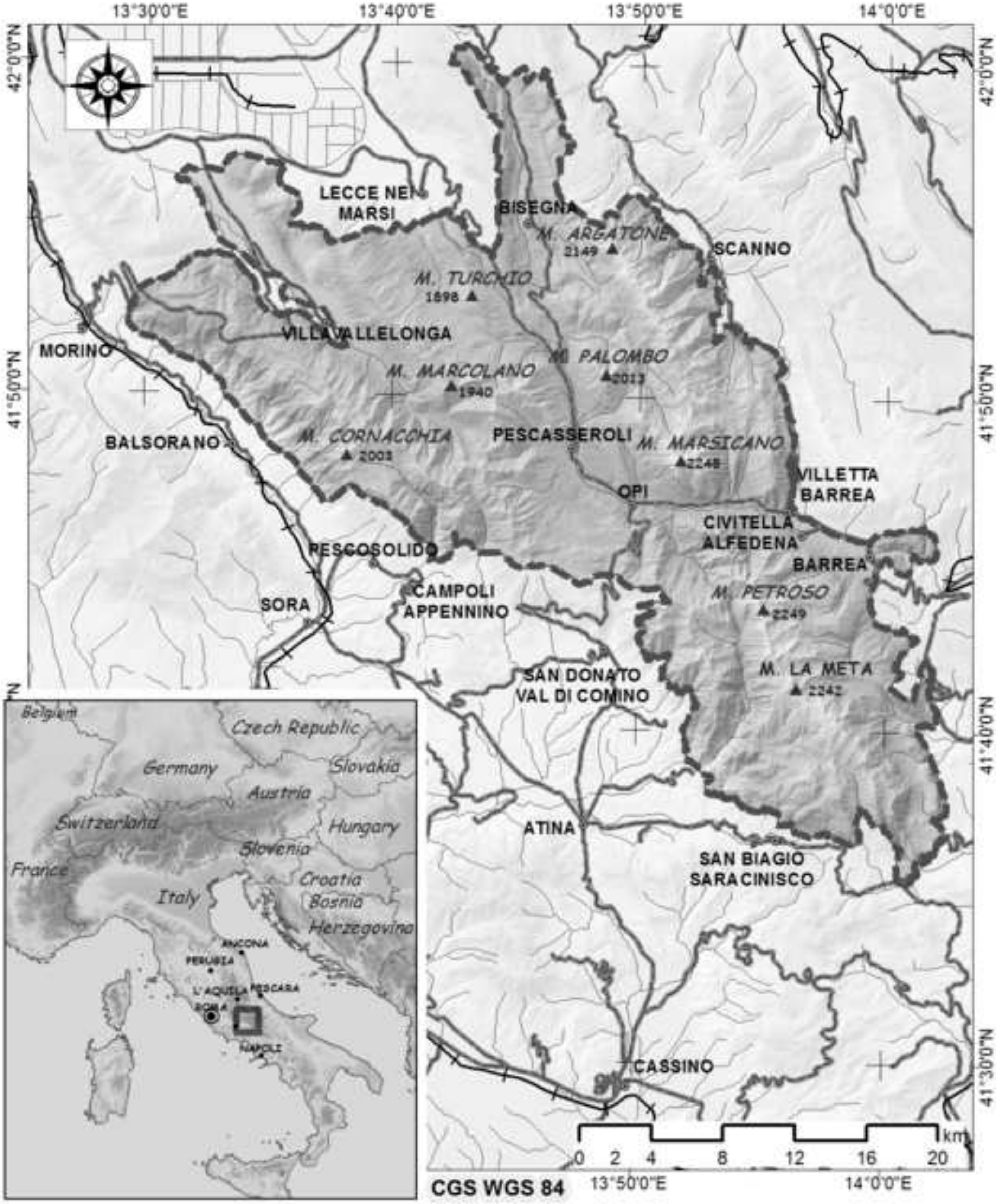

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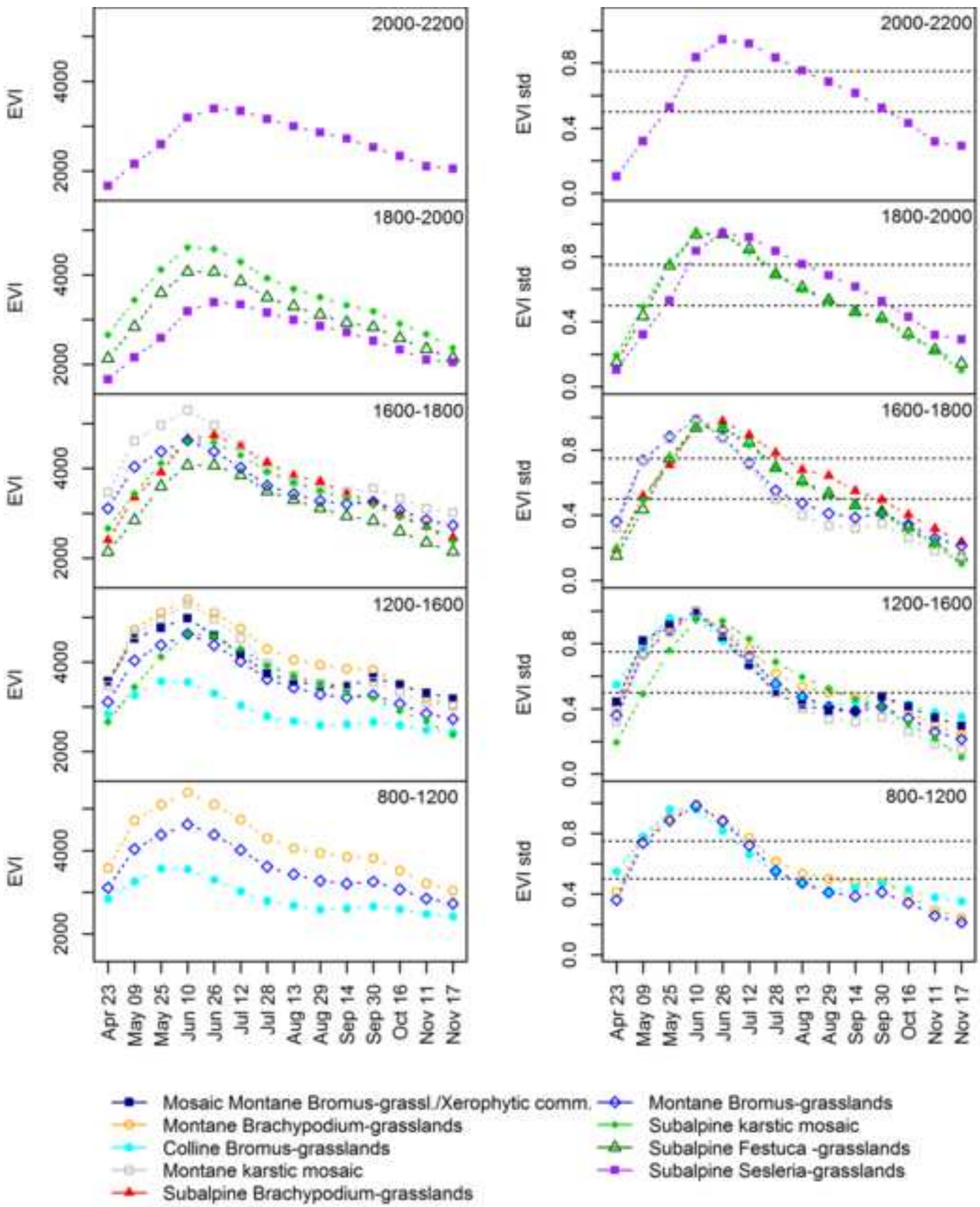

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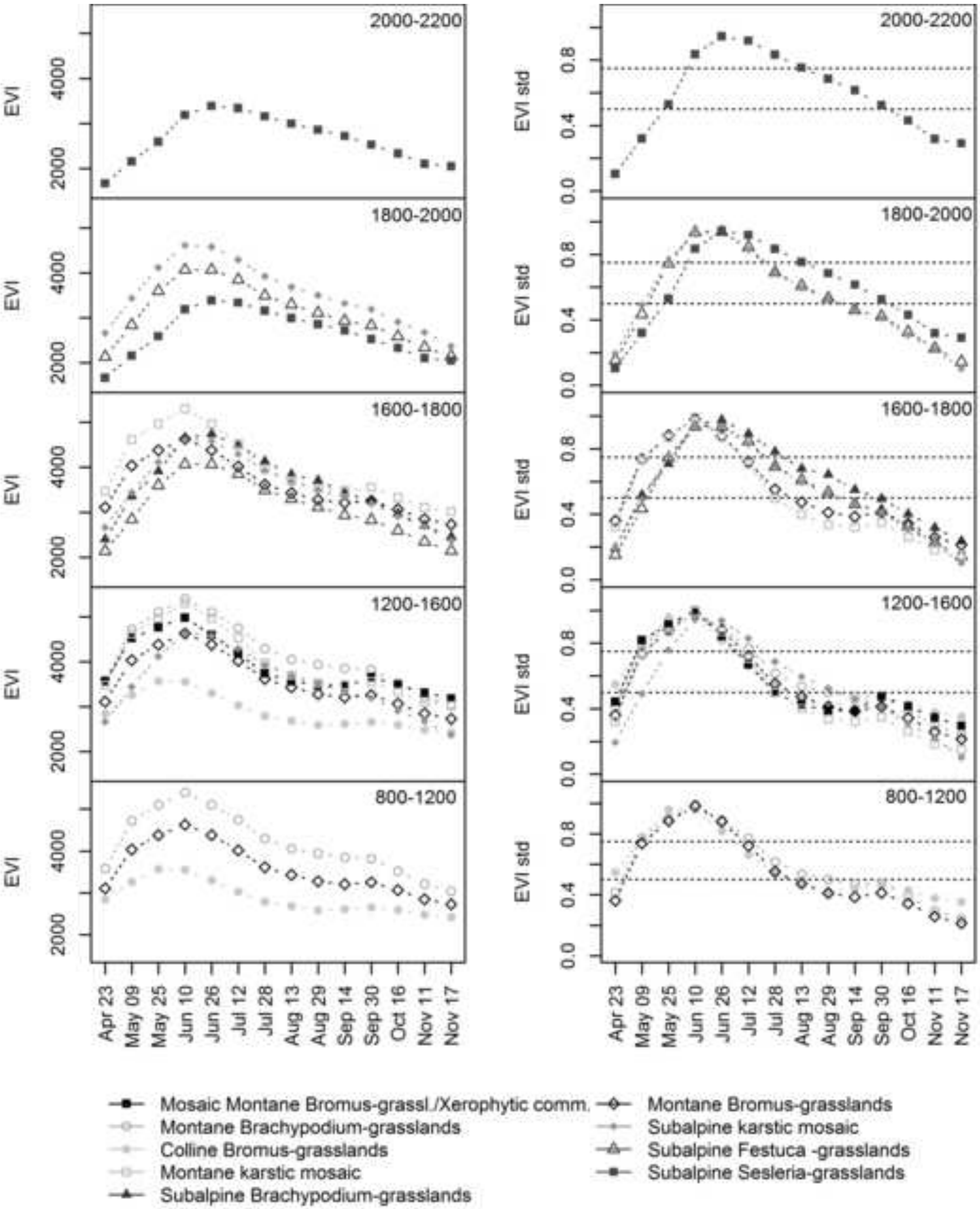

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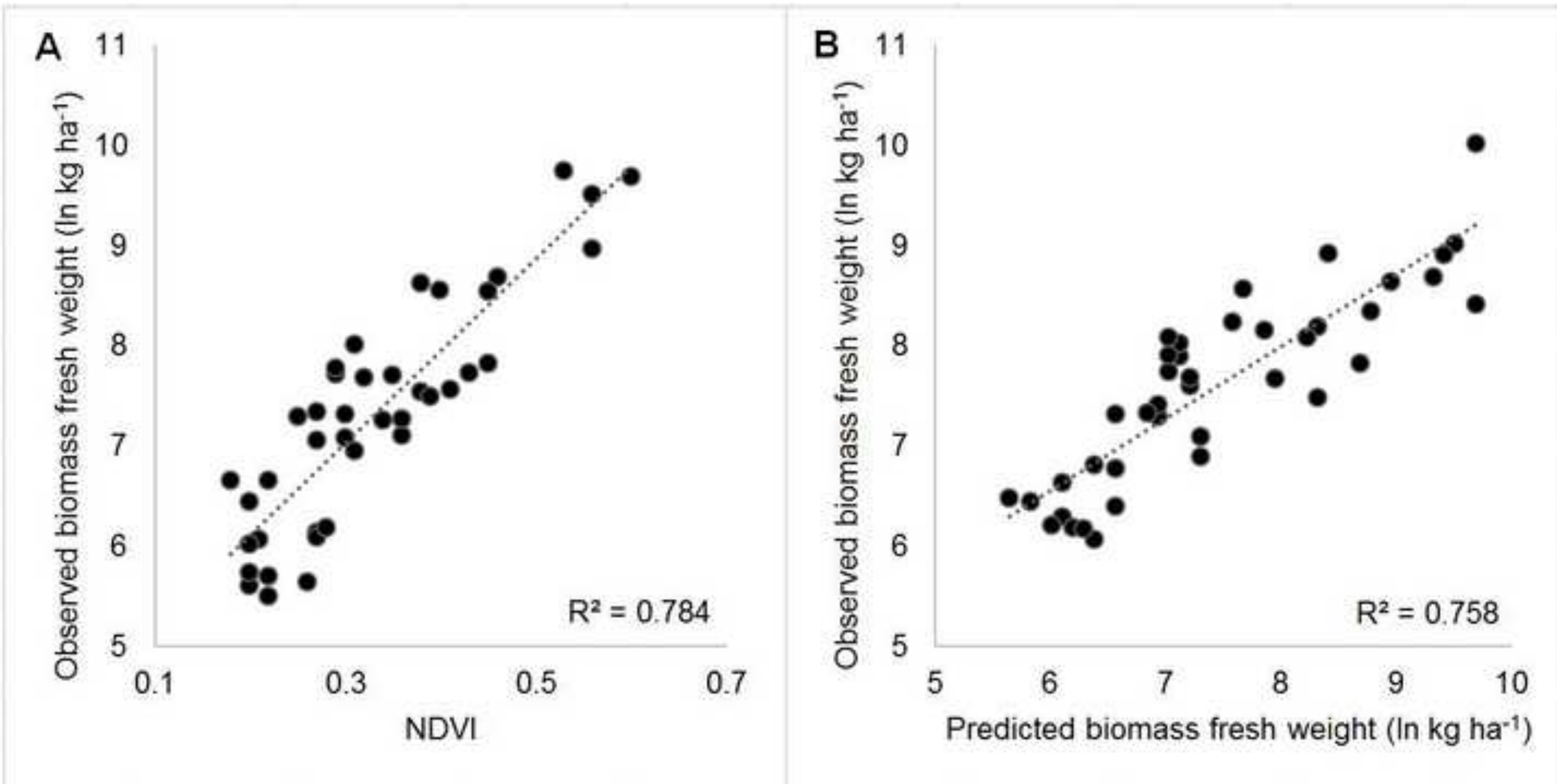

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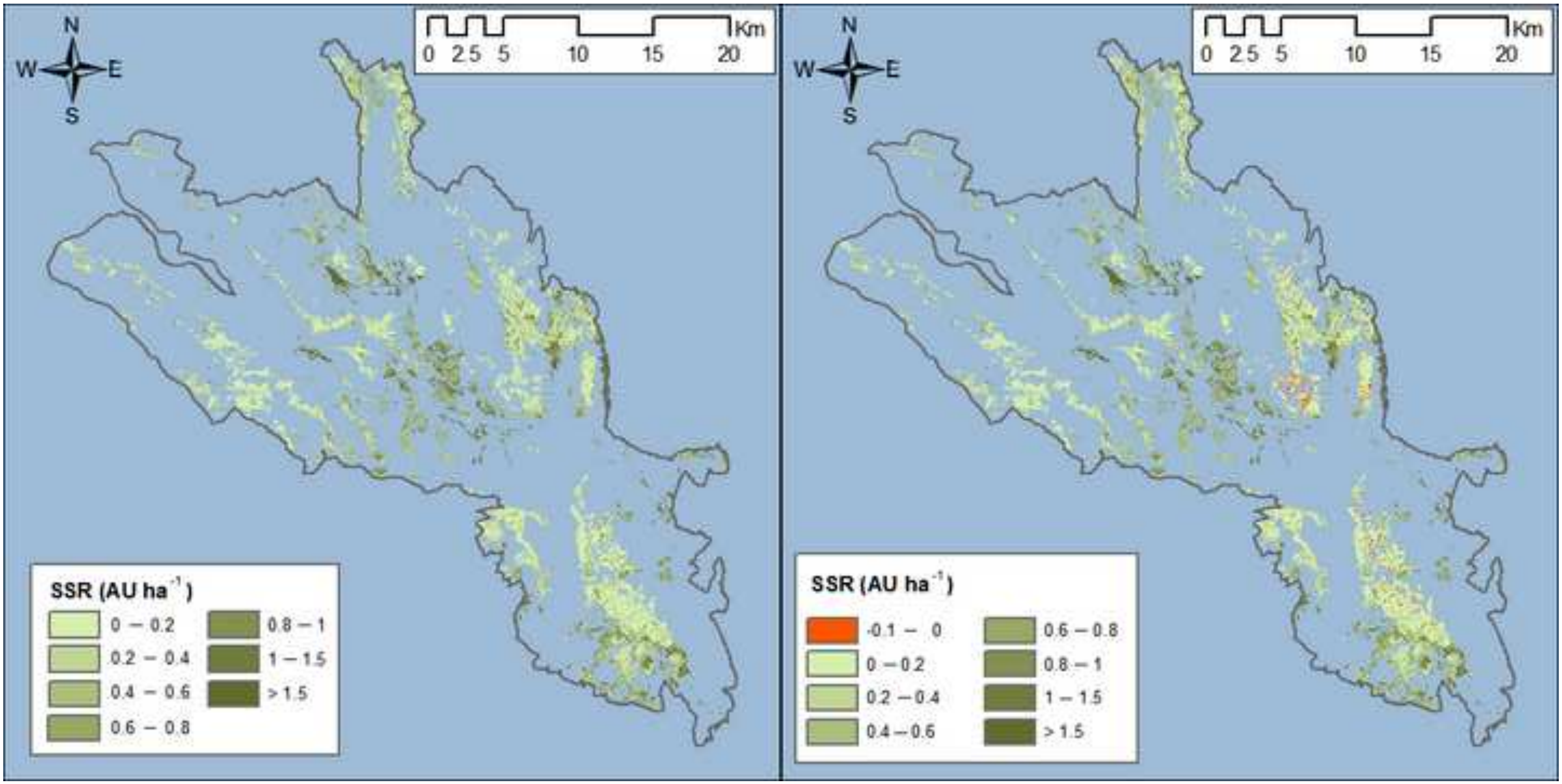

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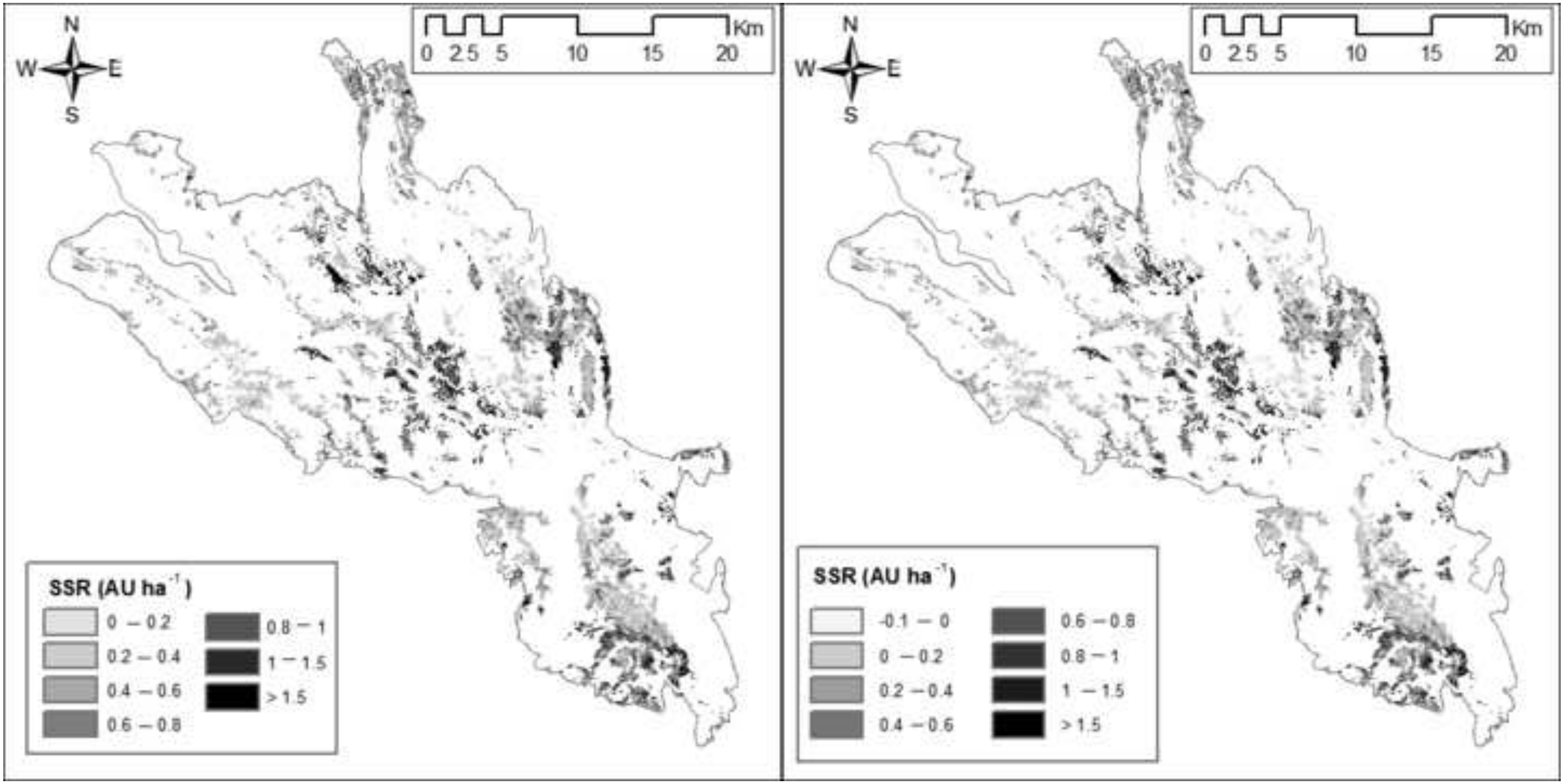

Fig.5

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