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Regenerative rotational grazing management of dairy sheep increases springtime grass production and topsoil carbon storage

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ABSTRACT

Increased traditional dairy sheep production in the Basque Country of northern Spain could substantially affect pasture soils. This type of agricultural land performs vital functions and provides essential ecosystem services. Regenerative farming practices such as rotational grazing with prolonged resting periods are designed to improve farmland soil health, while profitably delivering high-quality farm products. The aim of this study was to determine the mid-term effect of rotational grazing on soil ecosystem services and evaluate their synergies and trade-offs. A 4.5-ha experimental pasture was divided into two sections: one subjected to regenerative rotational grazing and the other to conventional rotational grazing. A flock of 135 Latxa breed dairy ewes was evenly distributed over the two areas during six consecutive years. On the conventional rotational grazing section, the sheep were allowed to feed for 6–10 d followed by a 15-d rest period. On the regenerative rotational grazing section, the sheep were allowed to feed for 1–2 d followed by a 24-d rest period. Vegetation and soil were then sampled according to a grid design. Springtime grass production was estimated by cutting the vegetation, topsoil carbon storage was determined through elemental analysis of soil organic carbon, nutrient cycling was calculated by measuring the activity of six enzymes (β-glucosidase, β-glucosaminidase, sulfatase, acid phosphatase, *L*-alanine aminopeptidase, and *L*-leucine aminopeptidase), water flow regulation was calculated using a simplified water retention index, and biodiversity was determined via 16S rRNA metabarcoding of soil prokaryotes. Regenerative rotational grazing achieved 30% higher springtime grass production and 3.6% higher topsoil carbon storage than conventional rotational grazing. The other parameters did not differ significantly between the grazing regimes. Regenerative rotational grazing reduced relative data dispersion for all ecosystem services, possibly because it supported comparatively homogeneous pasture use by livestock and avoided the negative consequences of overgrazing and undergrazing. Thus, regenerative rotational grazing might effectively improve certain soil ecosystem services without causing trade-offs to others.

1. Introduction

Agriculture and livestock provide various ecosystem services (ES) but also strongly depend upon them in order to function properly (Power, 2010). Soils perform various functions and ES are vital to the healthy and normal functioning of terrestrial ecosystems and society as a whole. These services include grass production, carbon sequestration, buffer for water, matter cycling, and habitat for biological activity (Vogel et al., 2019). In fact, most terrestrial biodiversity is hidden in the soil (Bardgett and van der Putten, 2014), of which prokaryotes account for the majority and are highly significant for soil functioning (Maron et al., 2018).

However, intensive agricultural and livestock management has severely affected service-producing ecosystems. For example, around 20% of the world's native grazing lands have been converted to cultivated crops, leading to a 60% loss of soil carbon stocks (Conant et al., 2017). Agriculture has also been identified as the major contributor to diffuse pollution of water resources (Lam et al., 2011). Moreover, intensive agriculture lowers soil biodiversity, making soil food webs less varied and composed of smaller bodied organisms (Tsiafouli et al.,

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2015). The magnitude of these effects might increase with global climate change, population growth, and food demand (Balbi et al., 2015).

Dairy sheep production in the Basque Country of northern Spain has traditionally relied on the local Latxa breed through pasture-based farming (Marijuán et al., 2004). Professional dairy sheep farms have 200–500 sheep, with an average milk production rate of 150–250 L per per ewe per lactation (MAPA, 2016). The sustainability of these sheep farming practices depends on their technical viability, profitability, environmental impact, and social acceptance. Land use and grazing management practices, for example, have changed over the past few decades due to the intensification of many flocks and the dependence on supplies purchased on international markets. Some of the consequences of these changes have a direct environmental impact. Under these circumstances, it is crucial to design grazing management practices suitable to cope with existing challenges (Ruiz et al., 2009).

The optimal forage theory postulates that when livestock graze large pastures, they optimize pasture intake and reduce energy costs by repeatedly feeding and grazing upon preferred plants in areas near water and shade (de Vries and Daleboudt, 1994). Consequently, the average stocking rates are far higher in preferred areas (overgrazing), whereas all other zones are only slightly used or not at all (undergrazing) (Wallisdevries et al., 1999; Witten et al., 2005; Teague et al., 2011). Heavy, continuous livestock grazing combined with insufficient resting time increases soil denudation, decreases aerial biomass productivity, reduces rooting depth and carbohydrate reserves in grazed plants, lowers soil aggregation and carbon content, and aggravates topsoil erosion (Frank et al., 1998; Peterson et al., 1998; Teague et al., 2011; Park et al., 2017; as cited in Wang et al., 2020).

The term “sustainable intensification” reflects the challenge confronting agricultural management to increase production without increasing environmental pressure (Garnett et al., 2013). Holistic management and regenerative farming practices are supposed to improve farmland soil quality while profitably generating high-quality crops, livestock, and poultry (LaCanne and Lundgren, 2018). In regenerative rotational grazing (RRG), pastures are divided into smaller areas, livestock are moved among them, and stocking rates are high over short time periods. In this manner, resting periods in the grazing areas are prolonged, defoliated grasses can recover and regenerate, productivity can be increased (Holechek et al., 1999), and land and resource homogeneity may be optimized (Bailey and Brown, 2011).

As soil management practices involve numerous complex processes, it is necessary to predict both their positive and negative impact on ecosystem function (Vogel et al., 2019). Evaluation of key ES, their trade-offs, and synergies could help identify systems that will ensure grazing sustainability. Previous studies have demonstrated the positive effect of RRG on milk production (Bryant et al., 1961), forage production (Holechek et al., 1999), forage quality (Jacobo et al., 2006), flower-visiting insects (Enri et al., 2017), soil carbon storage (Teague et al., 2011), and water conservation (Park et al., 2017). However, most of these studies failed to account for the entire range of ES, were short-term, or analyzed only a few trade-offs, synergies, or spatial aspects of ES (Balbi et al., 2015).

The main objective of the present study was to quantify and spatially represent the effect of two different rotational dairy sheep grazing regimes on grass production, carbon storage, water flow regulation, nutrient cycling and biodiversity, as well as evaluate the synergies and trade-offs among these ES. A mid-term controlled study was conducted by comparing RRG (with prolonged resting periods and a focus on restoring soil health) and conventional rotational grazing (CRG; with shorter resting periods) management on an experimental farm in the Basque Country. Our hypothesis was that RRG management would have a positive impact on certain ES provided by pasture soils. We also expected that subplots under CRG would be more heterogeneous than those under RRG.

2. Materials and methods

2.1. Experimental design

The field trial was conducted between 2014 and 2019 in the experimental pastures of NEIKER in Arkaute (42°51′11.41″N, 2°37′27.20″W; Basque Country, Spain). Mean annual temperature was 12 °C (mean maximum of 17.9 °C and mean minimum of 6.4 °C) and total precipitation was 855 mm (maximum of 145 mm in January and minimum of 17 mm in June). Mean elevation was 567 ± 4 m and slope was 6 ± 3%.

The grazing area consisted of 4.5 ha of permanent pasture divided into three plots. A completely randomized block experimental design was used. Each plot was further divided into two ~ 0.75-ha treatments. The first was subjected to CRG and the ewes therein could graze the entire plot for 6–10 d, followed by pasture rest for ~ 15 d. The second was allocated to RRG, wherein each plot was further subdivided into seven ~ 950-m^2 areas. The ewes thereupon grazed for 1–2 d, followed by pasture rest for ~ 24 d. The milking of dairy sheep took place during the annual spring grazing period from early April to late June. Grazing schedules of both treatments were adjusted according to grass availability and livestock requirements for milking. Grazing time ranged between 4 and 6 h in April, 6–8 h in May, and 12–16 h in June. The ewes were supplemented with fodder indoors after grazing. The field trial was run using an experimental flock of 135–140 Latxa breed dairy ewes split into two homogeneous groups of 65–70 ewes based on their age, daily milk yield, live weight, and body condition scores. Each group was randomly assigned daily to either CRG or RRG. A 3 m × 3 m exclusion area was established in each plot (n = 3) to study the effect of no grazing. The sheep had no access to these zones and biomass was not touched throughout the six-year trial.

Before the field trial, these pastures had no internal divisions and the entire flock was allowed to graze freely. The agro-climatic conditions of the study area allowed for two grazing seasons in spring and autumn; whereas pasture was limited by dry weather in summer and cold weather in winter. During both seasons and during the entire six-year trial period, the pastures were managed under the two grazing treatments (CRG and RRG).

2.2. Quantification of soil ecosystem services

2.2.1. Soil sample collection

Six years after implementing either CRG or RRG regimes, grid sampling was conducted in June 2019. Regular grid sampling enables the collection of spatial data from point locations (FAO, 2019). A total of 87 soil samples were collected. Forty-two were collected per grazing treatment, 28 per plot, and 14 per subplot. Three samples were taken from the exclusion areas (Supplementary Fig. 1). Topsoil samples at 0–10 cm of depth were randomly collected with a core soil sampler (25 mm in diameter) in each square of the grid and each sample comprised 15 pooled soil cores. A single soil depth of 0–10 cm (where biological activity is concentrated; Bardgett et al., 1997) was chosen to better relate the results obtained to the different soil properties or services measured. The samples were immediately taken to the laboratory in plastic bags. Once in the laboratory, fresh soil samples were sieved to < 2 mm. Subsamples used to determine nutrient cycling and biodiversity were stored at 4 °C and analyzed within a month. The remaining soil used to determine carbon storage and water flow regulation was air-dried at room temperature to a constant weight.

2.2.2. Grass production

Pasture grass was sampled in April and May of 2019, focusing on the spring grazing period because it corresponded to milking for the production of Idiazabal cheese. Because of technical difficulties, only blocks 2 and 3 could be sampled. Grass production was estimated by cutting the vegetation on a 0.5 m × 0.5 m surface with electric scissors, as in Pereira et al. (2020). CRG and RRG grass samples were collected every 15 d, because this coincided with the period sheep were returning to the first plots in CRG. Fourteen grass samples were taken per plot, as suggested by Brummer et al. (1994). For CRG, 0.5 m × 0.5 m cages were set in place to prevent the sheep from reaching the vegetation therein, the grass was sampled at the end of grazing in each plot, and the cages were moved after the grass was cut for the next sampling. For RRG, grass samples were collected in each subplot before the sheep were allowed to graze there. Grass sampling height was predetermined, and corresponded to 2.5 cm above ground level (Mosquera-Losada and González-Rodríguez, 1999). Samples were oven-dried at 60 °C for 48 h to a constant weight. Springtime grass production was estimated by adding the dry weights of all cuttings and extrapolating them to 1 ha (kg DM ha^{-1}).

2.2.3. Carbon storage

Soil carbon was determined by elemental analysis (LECO TruSpec CHN-S; Leco, Inc., St. Joseph, MI, USA) in conformance with ISO 10694:1995 [soil quality–determination of organic and total carbon after dry combustion (elementary analysis)]. Carbonates were subtracted and granulometry was estimated by laser diffractrometry. Apparent soil densities were estimated according to the pedotransference equations developed by Artetxe et al. (2014). The following formula was applied to determine the amount of carbon in the top 0–10 cm of soil:

$$SOC = C_{org} \times D_n \times D \times 100 \quad (1)$$

where SOC is the soil organic carbon content (t ha^{-1}), C_{org} is the percentage (%) of organic carbon in the sample, D_n is the density of the sample (t m^{-3}) derived from the aforementioned pedotransference equation, and D is the soil depth (m).

2.2.4. Water flow regulation

Water flow regulation was estimated by a simplified version of the water retention index (WRI) equation, which reflects the physical capacity of the soil to retain water (Vandecasteele et al., 2018). WRI determination accounts for the contributions of vegetation, water bodies, soil water, and groundwater properties, as well as terrain slope and sealing. Neither bedrock lithology, nor water retention by vegetation or groundwater markedly varied in the small study area and there were no surface water bodies or sealed terrain. Therefore, the simplified WRI (WRI′) model only considered soil water retention (R_s) derived from water-holding capacity (WHC) and slope (R_{sl}). WHC and R_{sl} were rescaled to a range of 1–10. A negative correlation was assumed between R_{sl} and potential water retention. WRI′ was computed after applying the optimized weighting proposed by Vandecasteele et al. (2018):

$$WRI' = 0.14 \times R_s - 0.10 \times R_{sl} \quad (2)$$

2.2.5. Nutrient cycling

The activity of six soil enzymes was measured to determine soil microbial activity and nutrient cycling processes (Sinsabaugh et al., 2008). Accordingly, β-glucosidase (EC 3.2.1.21), β-glucosaminidase (EC 3.2.1.30), sulfatase (E.C. 3.1.6.1), acid phosphatase (EC 3.1.3.2), *L*-alanine aminopeptidase (EC 3.4.11.12), and *L*-leucine aminopeptidase (EC 3.4.11.1) activities were determined in compliance with ISO/TS 22939:2010 (soil quality–measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates) with added fluorogenic substrates (4-methylumbelliferyl and 7-amino-4-methylcoumarin) in 96-well microplates as described by Anza et al. (2019). Enzymatic activity was normalized by dividing each value by the maximum value obtained for each specific activity and multiplying it by 10. Overall enzymatic activity (OEA) was calculated according to Epelde et al. (2012).

2.2.6. Biodiversity

Soil sample DNA extraction and 16S rRNA amplicon library preparation were conducted as described in Lanzén et al. (2016). PCR analyses were performed in triplicate on single DNA extracts and the PCR products were pooled for sequencing. A DNeasy PowerSoil Pro kit was used to extract the DNA (Qiagen, Hilden, Germany). The adapter-linked primer pairs 519F (CAGCMGCCGCGGTAA; Øvreås et al., 1997) and 806R (GGACTACHVGGGTWTCTAAT; D'Amore et al., 2016) were used to target the prokaryotic 16S rRNA hypervariable region V4. Paired-end sequencing was run in an Illumina MiSeq (Illumina, San Diego, CA, USA) with a V2 kit (Tecnalia Corporation, Miñano, Spain).

Amplicon sequence read-pairs were quality-filtered and overlapped using vsearch (Rognes et al., 2016). The 16S rRNA sequences were trimmed at both ends with cutadapt (Martin, 2011) to remove N5 and primer sequences. Low-quality trimmed sequences (fastq_maxee = 0.5) were truncated to 253 nt and those below this length were discarded. All quality-filtered overlapping sequences were clustered into operational taxonomic units (OTUs) with Swarm v. 2 (Mahé et al., 2015). The SWARM_OTUs were subjected to *de novo* and reference-based (rdp_gold.fa) chimera filtering in UCHIME (Edgar, 2013). The OTUs that were not removed as potential chimeras were clustered by vsearch into other OTUs with a 3% maximum sequence divergence threshold. OTU abundance was established by mapping the reads back to their representative OTU sequences. The OTUs were taxonomically classified by aligning their sequences to the SilvaMod database with blastn and reclassifying them in CREST using its default parameters (Lanzén et al., 2012). Unclassified OTUs below the alignment threshold were excluded from any further analysis. The order rank of relative taxon abundances derived by CREST was used in subsequent analyses. Richness, Shannon, Simpson and Pielou diversity indices (Magurran, 2004) were calculated in the vegan package of R (Oksanen et al., 2018). Compensation for variations in read number was made by using rarefied richness estimates interpolating the expected richness at the lowest sample-specific sequencing depth (Lanzén et al., 2016).

2.3. Spatial representation and statistical analyses

Data were spatially represented using the inverse distance weighting interpolation tool in QGIS (QGIS Development Team, 2019). A default value of two for the interpolation index P was applied.

The MIXED procedure in SAS (SAS, 2007) (SAS Institute, Cary, NC, USA) was used to establish significant differences between CRG and RRG in terms of the measured parameters. The following model was applied:

$$Y_{ijk} = \mu + Treat_i + B_j + \left(Treat_i x B_j\right)_K + \varepsilon_{ijk} \quad (3)$$

where Y represents the different observations of the response variable within the replicated $_i$ levels of *Treat*, the $_j$ levels of *B*, and the $_k$ levels on the interactions between *Treat* and *B*; μ represents the overall mean value of the response variable; *Treat* is the fixed effect of grazing regime (CRG or RRG); *B* is the random effect of block (n = 3), *Treat xB* is the effect of the interaction between the aforementioned parameters; and ε is the residual (Galwey, 2014). Least-square means were calculated for each grazing treatment and model, and were adjusted with Tukey's test.

Data dispersion for each parameter was calculated as the percentage associated with the standard deviation (SD) of the mean as follows:

$$\sigma_{over\ the\ mean}(\%) = \frac{SD \times 100}{\mu} \quad (4)$$

where σ is the deviation (%) over the mean (μ) considering the SD.

Synergies and trade-offs between ES were analyzed via Pearson correlation coefficients in the rcorr () module of R (R Core Team, 2012). The correlations were graphically represented with the corrplot package in R (Wei and Simko, 2017) and its chart.

3. Results

3.1. Grass production

The average, CRG, and RRG springtime grass production rates were 1,155 ± 241, 1,005 ± 233 and 1,306 ± 250 kg DM ha^{-1}, respectively (Table 1). Springtime grass production was 30% higher with RRG than with CRG. In 2014, average pasture production for the same plots during spring was 4,712 kg DM ha^{-1} for RRG and 4,062 kg ha^{-1} for CRG (Mandaluniz et al., 2015). Dispersion for CRG and RRG was 23% SD and 19% SD, respectively (Table 1), suggesting relatively more heterogeneity for the former. Spatial distribution of springtime grass production data showed a trend towards higher rates in the northeastern areas, which had the steepest slopes; the only exception was the CRG subplot in block 2 (Fig. 1).

3.2. Carbon storage

Six years after the onset of the study, the average, CRG, and RRG topsoil SOC values were 57 ± 3.1, 56 ± 3.6 and 58 ± 2.6 t C ha^{-1}, respectively (Table 1). This translates into an average 3.6% higher ES for RRG than CRG. Relatively lower SOC values were measured in the exclusion zones (average 52 ± 1.4 t C ha^{-1}) (Supplementary Table 1). Once again, data dispersion was wider for CRG (6.4% SD) than RRG (4.6% SD), implying comparatively greater heterogeneity under CRG (Table 1). The spatial distribution of topsoil SOC data is shown in Fig. 2. The southeastern plots had relatively lower SOC content.

3.3. Water flow regulation

The average, CRG, and RRG WRI′ values were 0.75 ± 0.25, 0.70 ± 0.28, and 0.80 ± 0.22, respectively (Table 1). WRI′ did not differ significantly between grazing treatments. Moderate average WRI′ was measured also for the exclusion zones (0.78 ± 0.14) (Supplementary Table 1). Once again, data dispersion was wider for the CRG (40% SD) than RRG (28% SD) treatment (Table 1). Spatial distribution of WRI′ revealed that water retention was comparatively lower in the steeper areas (Fig. 3).

3.4. Nutrient cycling

Neither OEA nor any of the six enzyme activities measured differed significantly between treatments. CRG and RRG OEA were 5.1 ± 1.1 and 5.0 ± 1.0, respectively (Table 1). The data were slightly more dispersed under CRG (21% SD) than RRG (20% SD). Average β-glucosidase, β-glucosamidase, sulfatase, acid phosphatase, *L*-leucine aminopeptidase, and *L*-alanine aminopeptidase activities (in mg $kg^{-1}h^{-1}$) were 3,597 ± 1,125, 1,416 ± 447, 385 ± 159, 2,073 ± 657, 3,702 ± 639, and 2,955 ± 562, respectively. Below-average OEA and enzymatic activity levels were measured in the exclusion zones (Supplementary Table 1). Spatial distribution of nutrient cycling had a patchy pattern (Fig. 4).

3.5. Biodiversity

There were no significant differences between treatments in terms of soil prokaryotic diversity indices. Average richness, Shannon, Simpson, and Pielou indices were 1,551 ± 139, 6.3 ± 0.35, 0.98 ± 0.011, and 0.75 ± 0.12, respectively. Further, similar values were measured for both grazed and ungrazed soils. In the exclusion zones, the average richness, Shannon, Simpson, and Pielou indices were 1,537 ± 154, 6.1 ± 0.52, 0.98 ± 0.52, and 0.75 ± 0.058, respectively (Supplementary Table 1). Except for richness, data for all other indices were more dispersed under CRG than RRG (Table 1). Fig. 5 shows the spatial distribution of the Shannon index and reveals that the northwestern plots displayed the lowest values.

Eleven orders of prokaryotes were significantly more abundant under CRG, whereas an equal number were significantly more abundant under RRG (Table 2).

3.6. Trade-offs and synergies among ES

Three of the five soil ES displayed moderate, yet significant interaction. Under CRG, carbon storage and nutrient cycling were positively correlated ($r = 0.32$; $p = 0.0368$), as were also water flow regulation and nutrient cycling ($r = 0.33$; $p = 0.0328$) (Fig. 6a). In contrast, grass production and water flow regulation were negatively correlated under RRG ($r = -0.45$; $p = 0.0152$) (Fig. 6b).

a)

4. Discussion

Intensive grazing practices, short grazing periods, and high stocking rates have been proposed to improve ES provided by grazing areas (Eaton et al., 2011). Here, relatively higher springtime pasture production on RRG subplots may be explained by comparatively longer defoliated grass recovery periods after livestock disturbance. The increase in springtime grass production observed under RRG management has direct environmental and socioeconomic effects. Farms increase self-

Table 1
Mean, standard deviation (SD), maximum (MAX), minimum (MIN), standard error of the mean (SEM), and *p*-values of MIXED procedure between conventional rotational grazing (CRG) and regenerative rotational grazing (RRG) for measured parameters.

	CRG					RRG						
	Mean	SD	% SD	MAX	MIN	Mean	SD	% SD	MAX	MIN	SEM	*p*-value
Springtime grass production (kg ha^{-1})	1,005	233	23	1,348	598	1,306	250	19	1,825	1,312	76	<0.0001
Topsoil carbon storage (t C ha^{-1})	56	3.6	6.4	64	49	58	2.6	4.6	64	53	0.47	0.0162
Water flow regulation (WRI′)	0.70	0.28	40	1.2	0.1	0.80	0.22	28	1.2	0.4	0.043	0.2578
Nutrient cycling (OEA)	5.1	1.1	21	7.7	3.7	5.0	1.0	21	8.3	3.1	0.16	0.3857
β-Glucosidase (mg $kg^{-1}h^{-1}$)	3,774	1,055	28	6,577	1,515	3,421	1189	35	8,123	1,561	169	0.1443
β-Glucosamidase (mg $kg^{-1}h^{-1}$)	1,447	388	27	2,486	759	1,386	508	37	3,588	597	70	0.4512
Sulfatase (mg $kg^{-1}h^{-1}$)	397	172	43	916	124	374	148	40	819	144	25	0.5155
Acid phosphatase (mg $kg^{-1}h^{-1}$)	2,122	561	26	3,659	1,221	2,024	752	37	4,361	674	103	0.5054
L-leucine aminopeptidase (mg $kg^{-1}h^{-1}$)	3,674	684	19	5,213	2,353	3,729	606	16	5,008	2,536	99	0.6939
L-alanine aminopeptidase (mg $kg^{-1}h^{-1}$)	3,038	614	20	4,916	2,131	2,871	505	18	3,952	3,952	77	0.1300
Biodiversity												
Richness	1,549	135	8.7	1,750	1,113	1,552	144	9.3	1,825	1,312	23	0.7878
Shannon index	6.2	0.43	6.9	6.7	4.9	6.3	0.27	4.3	6.6	5.5	0.060	0.3409
Simpson "	0.98	0.016	1.6	0.99	0.92	0.99	0.006	0.61	0.99	0.96	0.002	0.1107
Pielou "	0.70	0.14	20	0.85	0.60	0.80	0.060	7.5	0.85	0.71	0.008	0.1428

WRI′: Simplified water retention index.
OEA: Overall enzymatic activity.

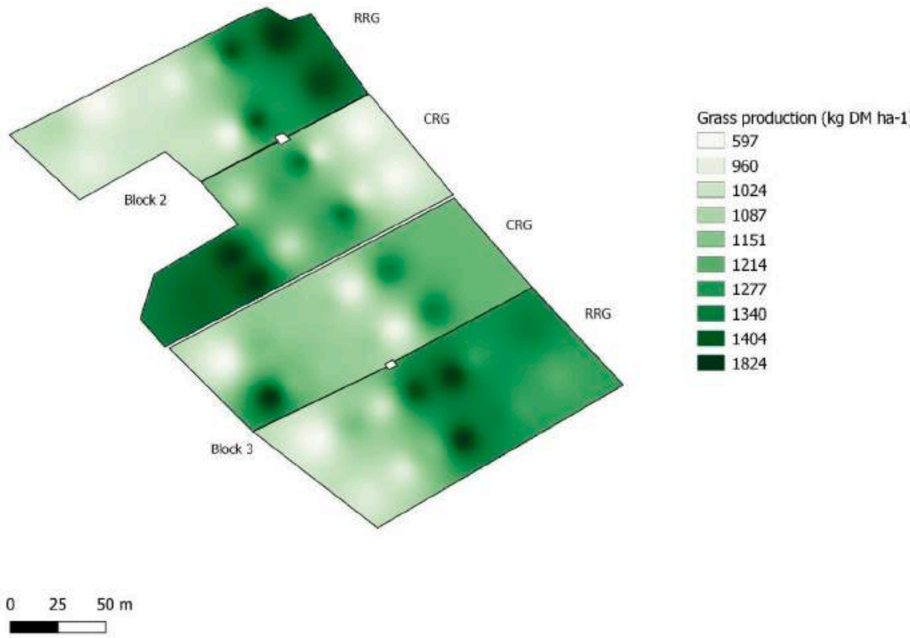

Fig. 1. Spatial representation of springtime grass production (kg DM ha^{-1}). CRG: conventional rotational grazing; RRG: regenerative rotational grazing.

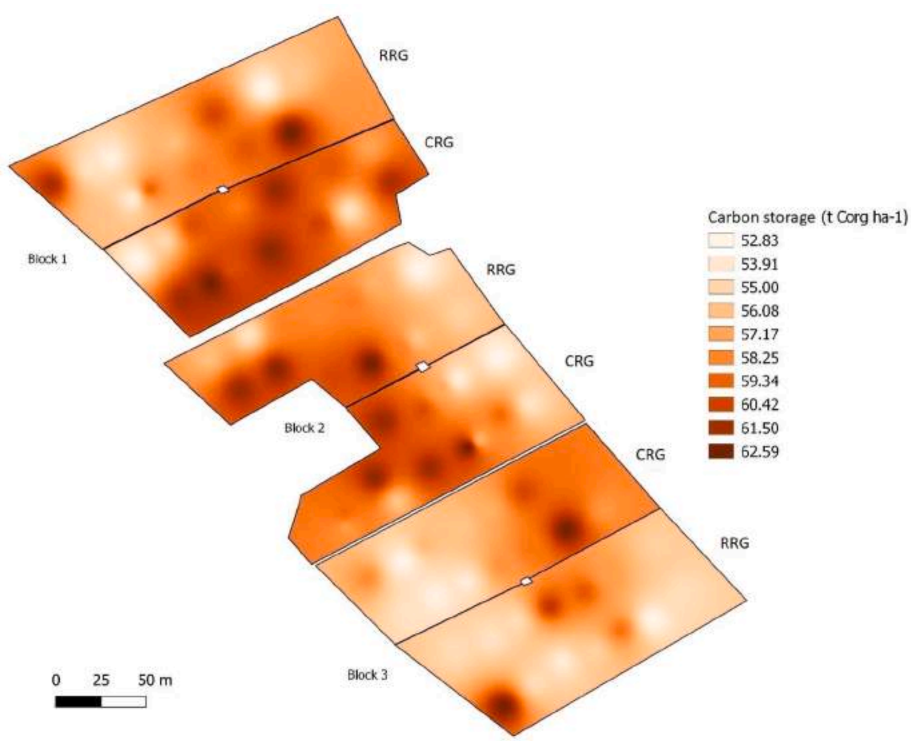

Fig. 2. Spatial representation of topsoil carbon storage (t C ha^{-1}). CRG: conventional rotational grazing; RRG: regenerative rotational grazing.

supply and decrease external market dependence for the production of cheese by using local resources. The present grass production data corresponded only to April and May 2019 and represented 35–40% of the total annual grass yield, which is normally estimated to average ~ 7,000 kg ha^{-1} y^{-1}. The low overall grass production of springtime 2019 might have resulted from low average temperatures (9.5 °C in April and 11.3 °C in May), late frost days (3 d in April and 2 d in May), and low springtime rainfall (69.3 L m^{-2} in April and 46.8 L m^{-2} in May). Therefore, the augmented springtime pasture production observed for RRG might help maintain productivity under projected climate change conditions (Su et al., 2020). However, Holechek et al. (1999) compared 15 studies of RRG systems on native rangeland vegetation and livestock production in North America and concluded that RRG was better than continuous grazing at conserving desirable forage species in humid range areas but not in semi-arid or arid regions.

Here, RRG management increased topsoil SOC content at a depth of 0–10 cm, corroborating previous reports (Teague et al., 2011; Waters et al., 2017). A 3.6% increase in topsoil SOC was achieved under RRG

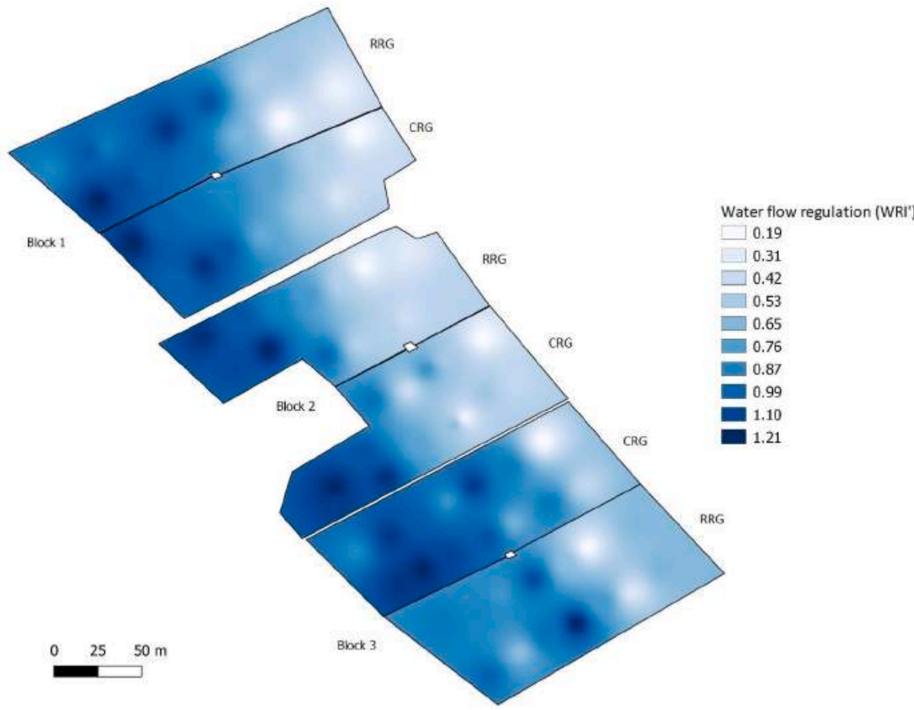

Fig. 3. Spatial representation of water flow regulation (simplified water retention index, WRI′). CRG: conventional rotational grazing; RRG: regenerative rotational grazing.

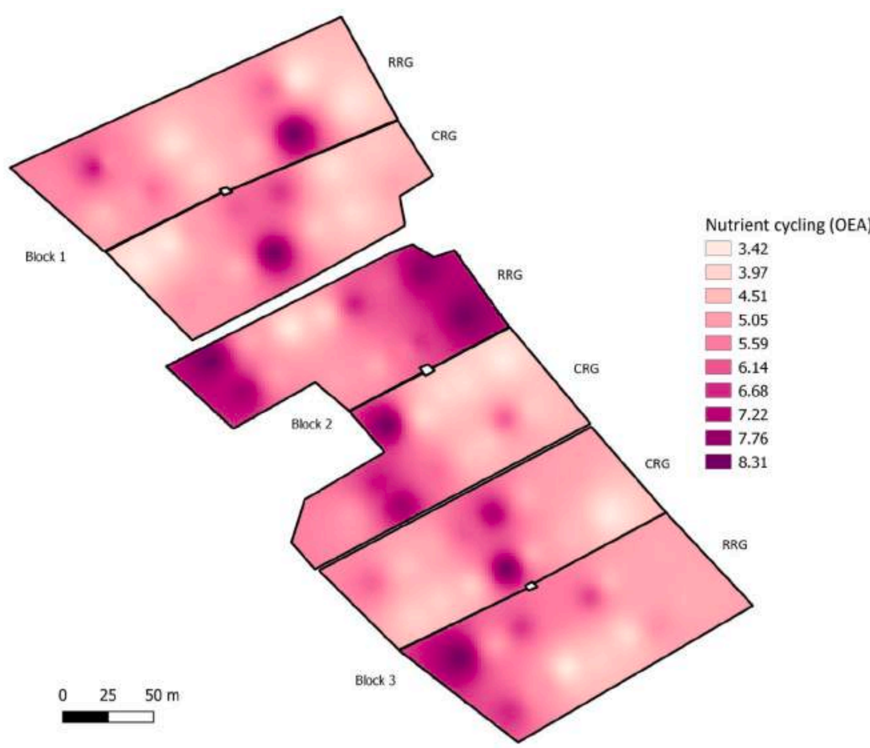

Fig. 4. Spatial representation of nutrient cycling (overall enzymatic activity, OEA). CRG: conventional rotational grazing; RRG: regenerative rotational grazing.

management over six years, corresponding to a 0.6% increase per annum. This value was higher than that proposed by the "4 per Thousand" initiative launched by COP21 (Lal, 2016). However, it has to be taken into account that most research on SOC sequestration has focused on sampling depths within 0–30 cm, which is also the default soil depth for carbon studies by the IPCC (FAO, 2019). Mid-term changes in SOC stocks such as the one observed here are more likely to occur in the upper soil profile (Badgery et al., 2014), while it might be necessary to wait several years following such a change in management to see variations in deeper soil layers (Knops and Bradley, 2009; Stahl et al., 2016).

A *meta*-analysis by Abdalla et al. (2018) disclosed that a higher grazing intensity was generally associated with a decrease in SOC stocks.

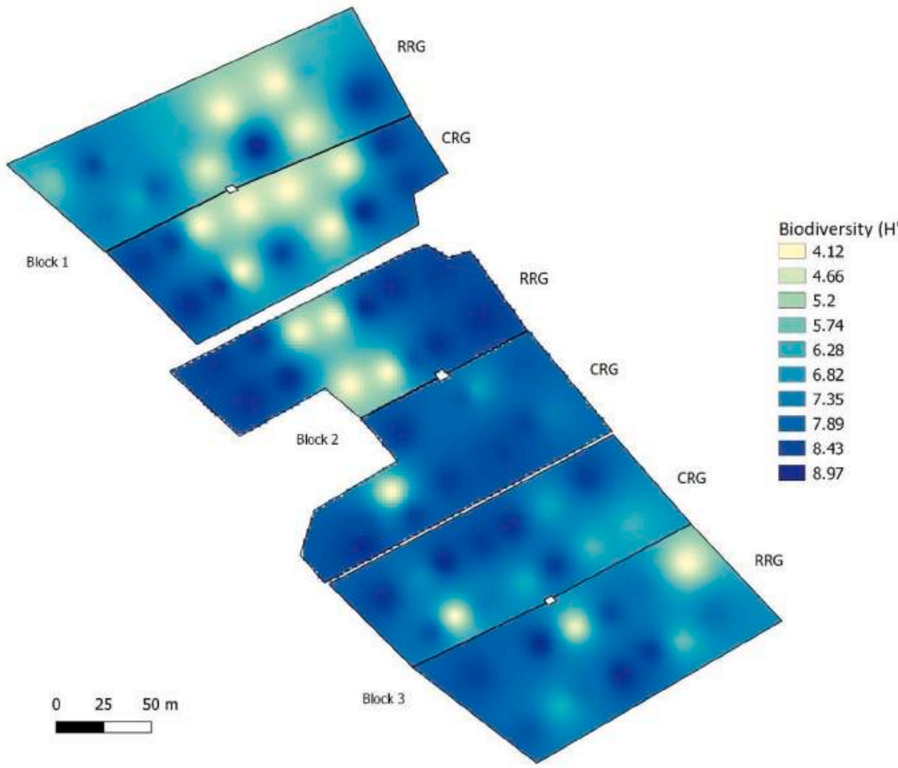

Fig. 5. Spatial representation of biodiversity (Shannon index of soil prokaryotes, H'). CRG: conventional rotational grazing; RRG: regenerative rotational grazing.

Table 2
Significant differences in MIXED procedure between conventional rotational grazing (CRG) and regenerative rotational grazing (RRG) in terms of relative abundance of soil prokaryotes at order level.

Higher in CRG	*p*-value	Higher in RRG	*p*-value
Armatimonadales	0.0009	Ardenticatenales	0.0344
Corynebacteriales	0.0114	Chitinophagales	0.0112
Kineosporiales	0.0252	Cytophagales	0.0243
Streptomycetales	0.0271	JG30.KF.CM45	0.0248
Unknown. Alphaproteobacteria. order.14	0.0405	MSBL9	0.0525
Unknown.Planctomycetacia. order.2	0.0448	Nitrososphaerales	0.0316
Unknown.Spartobacteria. order.4	0.0129	Opitutales	0.0242
Unknown.Thermoplasmata. order.1	0.0188	Saprospirales	0.0012
Unknown.Thermoplasmata. order.3	0.0209	TRA3.20	0.0047
Unknown.Verrucomicrobiae. order.2	0.0050	Unknown.Chloroflexia. order.1	0.0237
X35	0.0064	Xanthomonadales	0.0279

Nevertheless, the impact of grazing on SOC content is climate-dependent. In this study, RRG management counteracted the drop in topsoil SOC content caused by intensive grazing. Enhanced grass production could explain the relatively elevated SOC observed under RRG management. Increased root and shoot exudate production might be associated with compensatory growth (Bardgett and Wardle, 2003). The lack of grazing may have reduced SOC in the exclusion zones; as sheep could not defecate in such areas, the availability of labile carbon decreased (Bardgett and Wardle, 2003).

No significant differences were found among treatments regarding WRI′. High stocking rates without sufficient recovery time could increase soil bulk density and compaction (Epelde et al., 2017) and, by extension, decrease water-holding capacity. In a 44-year study, Dormaar and Willms (1998) found that heavy grazing pressure reduced soil water-holding capacity. Park et al. (2017) reported that RRG was the best grazing management in terms of water conservation and flood risk reduction. In the present study, similar trends might have been observed and confirmed over the long term. Somewhat unexpectedly, a relatively lower WRI′ in the steeper areas did not reduce springtime grass productivity, probably because water was not limiting to plant growth. In fact, these two ES were negatively correlated under RRG.

Soil microbial activity is positively associated with soil aggregate stability, plant nutrient maintenance and availability, favorable plant growth conditions, and increased organic matter incorporation in the soil (Teague et al., 2011). At the same time, other studies revealed that heavy grazing had a negative impact on soil enzymatic activity (Holt, 1997; Prieto et al., 2011) and prokaryotic diversity (Beneduzi et al., 2019). In this study, no differences were observed between CRG and RRG in terms of nutrient cycling or biodiversity. One possible explanation is that the impact of grazing (e.g., due to physical disturbance; Epelde et al., 2017) persists despite a longer resting time for the recovery of soil microbial communities under RRG.

Under CRG, OEA correlated positively with topsoil C storage and WRI′. Soil organic matter mineralization is catalyzed by soil enzymes. However, SOC increases also the water-holding capacity (Teague et al., 2011). CRG and RRG differed in terms of relative abundance of certain prokaryotic orders. RRG led to comparatively more Nitrososphaerales, which can oxidize ammonia (Kerou et al., 2018). Ammonia oxidation is a rate-limiting step in nitrification and is essential for nutrient turnover (Lehtovirta-Morley, 2018). The relatively higher abundance of Nitrososphaerales under RRG could account for the comparatively higher grass production and carbon storage in this form of soil management.

RRG management narrowed data dispersion for three (springtime grass production, topsoil carbon storage, and WRI′) of the five ES analyzed; the other two ES (nutrient cycling and biodiversity) showed no difference between treatments. This was possibly because livestock pasture use was relatively more homogeneous and the negative

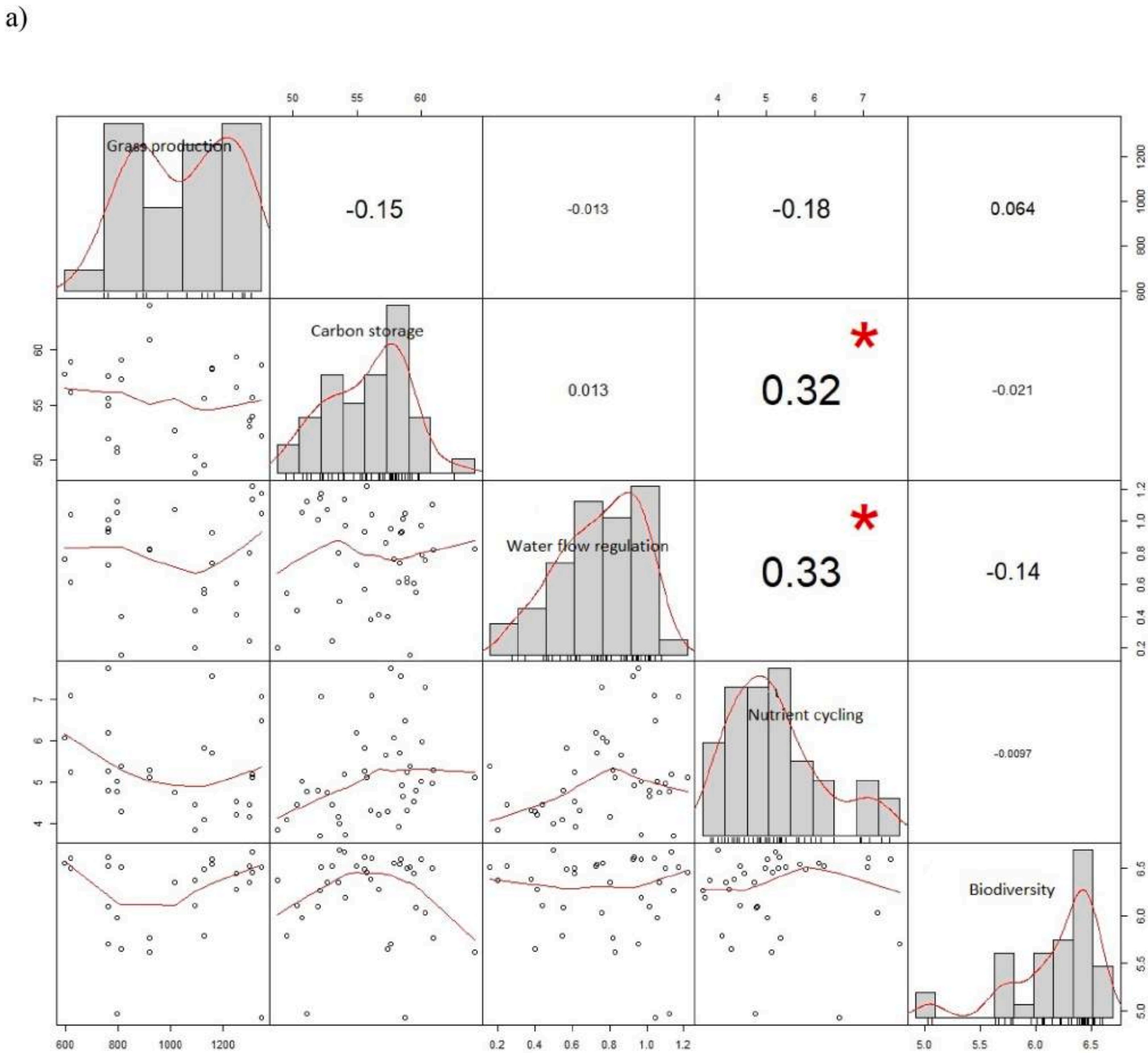

Fig. 6. Pearson's pair-wise correlation coefficients among springtime grass production (kg DM ha^{-1}), topsoil carbon storage (t C ha^{-1}), water flow regulation (simplified water retention index, WRI′), nutrient cycling (overall enzymatic activity, OEA), and biodiversity (Shannon index of soil prokaryotes, H') for (a) conventional rotational grazing (CRG) and (b) regenerative rotational grazing (RRG). * $p < 0.05$.

consequences of overgrazing and undergrazing were avoided. To the best of our knowledge, this major advantage of RRG management has not been studied in depth so far. Here, topsoil carbon storage and biodiversity followed a spatial gradient under both CRG and RRG not because of the treatment but possibly because of the intrinsic natural properties of the soil. Soil is a highly compact three-dimensionally structured habitat featuring fine-scale gradients reflecting physico-chemical characteristics, resource availability, and gas concentrations (Young and Crawford, 2004).

The integrated system tested here supported direct comparisons of the trade-offs and synergies among various soil ES associated with CRG and RRG. Other studies investigated the trade-offs and synergies between ES and livestock grazing intensity (Petz et al., 2014; Austrheim et al., 2016; Fan et al., 2019). In the present study, only a few moderate correlations were detected between the five tested ES under both CRG and RRG. This discovery is promising as it means that trade-offs between ES could be minimal and livestock production under RRG could help achieve sustainable global food production without significant drawbacks.

5. Conclusion

A regenerative rotational dairy sheep grazing management system implemented for six years showed significantly higher springtime grass production (30%) and topsoil carbon storage (3.6%) than conventional rotational grazing. In contrast, there were no significant differences between CRG and RRG in terms of water flow regulation, nutrient cycling, or biodiversity. ES data for RRG subplots showed relatively narrower dispersion than ES data for CRG subplots, possibly because the former supported more homogeneous pasture use by the ewes and avoided the negative consequences of overgrazing and undergrazing.

Under the edaphoclimatic conditions of the present study, RRG

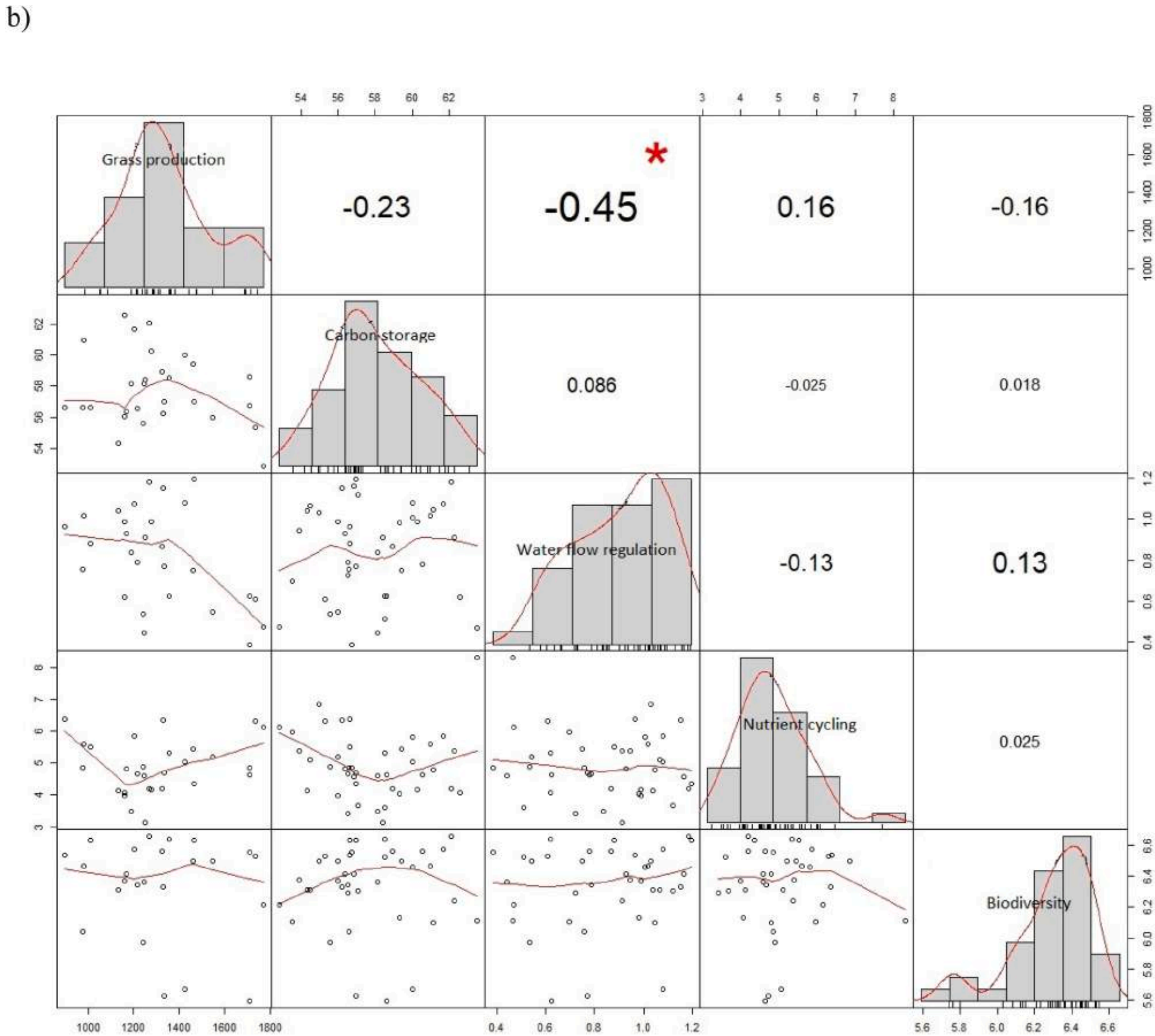

Fig. 6. (*continued*).

substantially improved certain soil ES without negatively affecting others. Global climate change presents growing socioeconomic and environmental challenges to the livestock sector. Agricultural practices such as RRG may be appropriate in this context as they could contribute to the sustainability of this agricultural market. It would be interesting to carry out more studies in other soil and climate conditions and to evaluate more ES.

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CRediT authorship contribution statement

Xabier Díaz Otálora: Formal analysis, Investigation, Writing - original draft. **Lur Epelde:** Conceptualization, Investigation, Writing - original draft. **Josune Arranz:** Investigation. **Carlos Garbisu:** Conceptualization, Writing - review & editing. **Roberto Ruiz:** Conceptualization, Writing - review & editing, Funding acquisition. **Nerea Mandaluniz:** Conceptualization, Investigation, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

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

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Data Availability Statement

The sequences generated for this study are available at the European Nucleotide Archive under Accession No. PRJEB35935.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.107484.

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