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Grazing in temporary paddocks with hardy breed horses (Konik polski) improved species-rich grasslands restoration in artificial embankments of the Rhône river (Southern France)

Cannelle MOINARDEAU[a*], François MESLEARD[a,b], Hervé RAMONE[a], Thierry DUTOIT[a]

[a] Institut Méditerranéen de Biodiversité et Ecologie, UMR CNRS-IRD, Avignon Université, Aix-Marseille Université, IUT d'Avignon, 337 chemin des Meinajariés, Site Agroparc BP 61207, 84911 Avignon, cedex 09, France.

[b] Institut de Recherche de la Tour du Valat, Le Sambuc, 13200 Arles, France

*Corresponding author: Cannelle Moinardeau, e-mail: cannelle.moinardeau[at]gmail.com

Abstract

Extensive horse grazing with hardy breeds is largely used in conservation management for restoring species-rich semi-natural grasslands in Europe. As an example, the Donzère-Mondragon Rhône river embankments (south-eastern France) where horse grazing with Konik polski succeeded to maintain high species richness in patches of open and short grassland, but failed for patches of tall grassland dominated by the grass tussock species *Brachypodium phoenicoides*. In response, an increase of herd density by the creation of temporary paddocks was applied in tall grassland patches. Grazing pressure over time between patches was estimated via GPS fixed to mares, biomass, plant species richness measurements and from multispectral photographs, the normalized Difference Vegetation

Index (NDVI) was calculated. Finally, telemetric data were collected. We wondered if the use of extensive horse grazing with temporary paddocks occupation, increase plant species richness. After three years, our results show that the increase in stocking rate enabled a significant increase of plant species richness in the tall grassland patches, with a significant decline of *B. phoenicoides* in all paddocks. The NDVI was strongly correlated with herbaceous biomass and plant species richness, while the number of GPS points indicating the position of the mares was higher in areas where the NDVI was high. These results are discussed for improving horse grazing conservation systems efficiency on grasslands established in anthropized sites such as embankments and the use of the NDVI as an appropriate tool to help decision-making for large-scale applications.

Keywords**:** extensive grazing – remote monitoring – vegetation index – livestock tracking – anthropized site – vegetation survey

1. Introduction

Extensive grazing, defined by Allen et al., (2011) as grazing management that uses relatively large land areas per animal, is commonly used in protected areas where the maintenance of open habitat is a major goal (Olff and Ritchie 1998; van Wieren and Bakker 1998; Rosenthal et al. 2012; Wallis De Vries et al. 2007; Török et al. 2016, for review). In these management systems moderate grazing through the consumption of dominant species and the input of propagules by endo- and epizoochory, promotes the coexistence of plant species as well as spatial heterogeneity (Adler et al. 2001; Kiss et al. 2021). Nevertheless, extensive grazing even with the use of hardy breeds of domestic herbivores does not always prevent plant succession and the dominance by one or a few perennial grass or shrub species (McNaughton, 1984; Adler et al., 2001; Mesléard et al., 2011; Rosenthal et al., 2012). The effectiveness of a stocking method depends mainly on grazing intensity and grazing period (Dumont et al. 2009). Then, the establishment of a paddock grazing system in order to control less palatable species if it allows to increase the stocking rate may be a response to a weak grazing pressure (Mesléard et al. 2017).

The grazing behaviour of hardy breed horses is known to induce a particular type of environmental heterogeneity (Marinier and Alexander 1991) that may promote biodiversity (McNaughton 1984). It fosters the opening up of the herbaceous canopy, the recycling of litter and the structuring of vegetation in a mosaic of herbaceous strata (Duncan 1992; Sharpe and Kenny 2019). Horses feed selectively, maintaining short grassland zones of good fodder quality within a matrix of zones of tall grass which they avoid and where faeces are often concentrated (Ménard et al. 2002). They create a heterogeneous mosaic of different vegetation patches, such as very short grassland dominated by annual and biennial herbaceous species and tall grassland dominated by perennial grasses. Thus, since several decades horse grazing especially with hardy breeds received a particular attention to manage protected areas (Köhler et al. 2016). Nevertheless, there are only a few studies that compared the effect of the animal breed, i.e. comparing grazing effects of breeds of the same species, but recent studies (Kovácsné Koncz et al. 2020, Pauler et al. 2019) showed that robust and hardy breeds can perform better than crossbred ones.

Many studies have examined the impact of horse grazing on plant communities and its pertinence in terms of achieving environmental management goals (e.g. Köhler et al. 2016). Horse grazing in paddocks has mainly been assessed in terms of its potential to enhance grassland productivity and quality (Kenny et al. 2019; Sharpe and Kenny 2019). Temporary occupations on small paddocks are considered powerful to ensure the maintenance of good forage quality grasslands and to avoid over-consumption of the most palatable species (Briske et al. 2008; Sharpe and Kenny 2019). However, grazing systems involving temporary occupancy of paddocks are not privileged to enhance species richness and heterogeneity of the environment although they can be effective ways to control plant succession (Danell et al. 2006; Allen et al. 2011; Mesléard et al. 2017).

Domestic herbivores in extensive grazing most often do not use rangelands homogeneously (Perevolotsky and Seligman 1998) and makes it difficult to assess the grazing pressure exerted on the different vegetation patches. The radio-tracking monitoring of herbivores can then be an effective way to record their movement (Manning al. 2017) and through the respective time spent in each part of the site, to assess the pressure exerted (Turner et al. 2000; Schieltz et al. 2017).

Above-ground biomass as it conditions the food available for livestock is an essential indicator in grazing management (Kong et al. 2019). As such, because of the light reflectance capacity of vegetation, the Normalized Difference Vegetation Index (NDVI) offers a basis for monitoring the photosynthesis of vegetation over time and enables both temporal and spatial comparison (Todd et al. 1998). Sensitive to variations in above-ground biomass (Todd et al. 1998) it reflects the vigour and the density of vegetation (Purevdorj et al. 1998). Thus multidisciplinary approaches combining field observation, aerial imagery and remote sensing are considered as particularly effective to consider different scales in conservation management (Skidmore and Ferwerda 2008).

In the present field experiment, we studied over a three-year period how changes in the stocking method of extensive grazing by hardy breed horses (Konik polski), in the form of paddocks temporary occupation modify the herbaceous vegetation of an anthropized area (artificial embankments). We used direct vegetation measurements to calculate diversity indices as well as vegetation biomass. Remote sensing made it possible to calculate NDVIs and link them to vegetation biomass, and to use radio monitoring to estimate spatial variations in grazing pressure. We addressed the two following questions:

- Does the use of extensive horse grazing by hardy breed horses (Konik polski) in the form of temporary paddocks occupation, increase (in the short time – 3 years) plant species richness especially in tall vegetation patches?
- Is there a differential use of space by horses corresponding to differences in NDVI index?

2. Materials and methods

2.1. Study site

Our study area is situated on the artificial dykes (elevation 60m, 200 m from the channel) and embankments of the dam and hydroelectric power station of Donzère-Mondragon, built between 1948 and 1952 in the lower course of the Rhône river (South-eastern France, N44°18′13.3″ E 4°44′32.9″, Fig. 1). The site became a protected area in 1954 (Moinardeau & Roux, 2013). The local climate is Mediterranean, with a mean yearly temperature of 15 °C and yearly total precipitation of 500 to 700 mm.

[Fig. 1]

The site is characterised by native herbaceous vegetation (53 % in 2014) identified as Phoenician torgrass grasslands which are closed dry perennial grasslands found on eutrophic soils within the meso- and thermo-Mediterranean zones. These grasslands are formed by relatively tall grasses and usually dominated by *Brachypodium phoenicoides*, a native tussock grass, numerous annual species, many orchids. Some shrubs (*Crataegus monogyna, Prunus spinosa, Rubus* spp., etc.) naturally colonised the area (47 % of the site in 2014) since 1952 in the absence of grazing or mowing management systems applied to conserve the grasslands until 2008.

2.2. Management

Between 2008 and 2014, three mares of hardy breed horses (Konik polski) were introduced year-round to control natural shrub encroachment (Moinardeau et al., 2016). Thirty-one hectares were grazed at a very low stocking rate of 0.04 Livestock Units (LU) per ha per year. At that time, grazing intensity was based only on the possibility to have some horses on this site but not on grazing pressures which were formerly applied successfully in other sites of the same type. As a consequence for that very low stocking rate, no increase in plant species-richness was recorded at the different grassland patches studied during this period (Moinardeau et al., 2016).

In order to increase the annual grazing pressure, the area was divided in three paddocks separated by electric fences (Table 1). At the end of 2014, the 3 mares were placed in a 7-ha paddock in the northern part of the site, increasing the stocking rate in this paddock to 0.34 LU/ha/year until March 2016. In March 2016, four other mares were added year-round (Table 1, Figure 2) to the site. Then, the seven mares were distributed over two areas: four in a 19-ha paddock, which corresponds to a grazing pressure of 0.16 LU/ha/ from March 2016 to June 2018 for this paddock (Table 1, Figure 2). In March 2016, the three remaining mares were installed in a new 5-ha paddock for a grazing pressure of 0.35 LU/ha/year until July 2017 (Table 1, Figure 2). In July 2017, the fence separating the other two paddocks (7 ha and 5 ha) was opened to allow the animals to graze over 12 ha with, as a consequence, a strong decrease of the grazing pressure to 0.03 LU/ha/year from July 2017 to August 2017 (Table 1, Figure 2). In September 2017, the fence over these two paddocks (7 ha and 5 ha) was reinstalled and the 3 mares were placed again in the 7-ha paddock in the northern part of the site, increasing the stocking rate to 0.35 LU/ha/year until June 2018 (Table 1, Figure 2). They were no more additional cutting or clearing regimes inside the whole area and the different paddocks during the study period (2008-2018). The control sites were closed to the three paddocks with the same soil conditions and with the same initial vegetation of Phoenician torgrass grasslands at the beginning of the experiment (Moinardeau et al., 2016).

[Table 1]

[Fig. 2]

[Photographs 1 & 2]

2.3. Vegetation sampling

Sampling measurements were performed between spring 2016 and spring 2018 in permanent quadrats, which size was determined by calculating a minimum area of plant community (2 x 2 m, Guinochet 1973). Plant inventories were realized in May when species-richness reach its maximum and when all the species were present in flower or in vegetative forms that can be identified for earlier or later flowering species. For each species, abundance and cover were measured using the Braun-Blanquet coefficients (Guinochet, 1973): 1 = species covering less than 5% of the quadrat, 2 = between 5 and 25 %, 3 = between 26 and 50 %, 4 = between 51 and 75 %, 5 = more than 75 %, and 0.2 = species represented by a single individual in the quadrat. Cover (%) and height (cm) of vegetation were also recorded. Nomenclature of vascular plants followed Tison et al. (2014).

To accurately reflect the diversity of vegetation stages encountered before the re-distribution of the horses in 2014, we placed randomly five quadrats (2 x 2 m) in each of the three enclosures grazed by the horses and for each herbaceous vegetation patches previously identified via aerial photographs and field observations (Moinardeau et al. 2016; Moinardeau et al. 2019): open grassland (OG) composed by annual plant species with bare zones representing areas with silt and clay deposits; short grassland (SG) covered by 10–20 cm high herbaceous perennial vegetation and annual species ; tall grassland (TG) dominated by the species *Brachypodium phoenicoides* (L.) Roem. & Schult. 20–30 cm in height; and non-grazed tall grassland (> 30 cm) outside the grazed areas, used as controls (C). Yearly, a total of 15 quadrats in each paddock (15 x 3 paddocks = 45 quadrats) and 5 in the control without grazing were surveyed (45 quadrats + 5 control = 50 quadrats). We estimated alpha-diversity (species richness/4m²) and beta-diversity using Bray–Curtis distance between each treatment (Borcard et al. 2011).

2.4. Phytomass and NDVI

Normalized Difference Vegetation Index (NDVI), based on the reflectance properties of vegetation in the visible spectrum and infrared is sensitive to variations in above-ground biomass (Purevdorj et al. 1998). Multispectral photographs (Red, Green, Blue and Near-Infrared) were then taken at 1000 m altitude by overflight of the reserve during the second fortnight in May 2016 and 2017. They were orthorectified using field reference points calibrated by means of differential GPS to a precision of one cm. We chose a high resolution (10 cm) in order to match with the observation scale on the ground (Mouat et al. 1993). The images were analysed via the Geographic Information System (GIS) using the software ArcGis 10.2 (ESRI).

The above-ground dry biomass of grasses was measured in May 2017 immediately after the photographic overflight. The vegetation was cut to a height of 2 cm in a total of 50 biomass quadrats distributed randomly outside vegetation quadrats (50 x 50 cm) and then oven-dried at 50 °C to constant weight. The geographical coordinates of all the biomass quadrats were recorded using a GPS with a precision of one cm (Trimble Geographic Positioning System receiver) and the NDVI values for the same biomass quadrats were calculated and extracted using ArcGis 10.2. The NDVI values for each vegetation quadrat were also extracted by GIS between 2017 and 2018.

2.5. Use of space by the horses

Telemetric data were collected during the year 2017 using a GPS (Global Positioning System) Data Logger (CatTrack^{TM}) fixed to the collar of the dominant mare in each herd. The gregarious behaviour of horses allowed to put a data logger on a single mare. The GPS points were recorded every 15 min and were imported via the software ArcGis 10.2. We estimated density using the non-parametric method of Kernel. Kernel density calculates density on the basis of GPS points and their surroundings (interpolation), enabling the acquisition of a heatmap showing the horses' locations for each of the paddocks studied.

2.6. Statistical analyses

2.6.1. Vegetation

We used ANOVAs for multiple comparisons between species richness, vegetation height and Bray-Curtis distance between paddocks, when the data followed a normal distribution pattern (Lilliefors or Shapiro-Wilk tests). When we detected a significant difference between treatments, this first analysis was followed by a Tukey post-hoc test. When the data did not follow a normal distribution pattern, we used a Kruskal-Wallis test, followed by a Mann-Whitney-Wilcoxon non-parametric test where the *P* value was adjusted (Benjamini and Hochberg 1995) for two-by-two comparison.

2.6.2. NDVI (Normalized Difference Vegetation Index)

We carried out Spearman rank correlation test on correlations between plant index values and the biomass measured. We also performed a Spearman rank correlation tests between plant index values obtained by GIS and species richness measurements for each quadrat.

2.6.3. Use of space by the horses

Kernel density was extracted using ArcGis software. We then used a Wilcoxon test (software R) to compare mean densities for each type of vegetation because the data did not follow a Normal law (Lilliefors test). The correlation between the mares' location in the paddocks and the NDVI was also calculated.

All statistical analyses were performed with R software version R 3.2.2 (R Core team), using its native packages for univariate analyses.

3. Results

3.1. Horse grazing impact on vegetation

The increase of grazing pressure in the three paddocks between 2016 and 2018 resulted in significantly higher species richness in the tall grasslands (TG) compared to the non-grazed control, as measured in 2018 (2016 : F = 4.38, $P<0.05$; 2017 : F =0.15, NS ; 2018 : F = 9.57, $P<0.001$; Fig. 3A). Average vegetation height followed the opposite trend, with lower heights in the three paddocks compared to the non-grazed control (2016: F = 10.25, $P<0.001$; 2017: F =8.87, $P<0.01$; 2018: F = 9.57, $P<0.001$; Fig. 3B).

[Fig. 3]

In the 7-ha paddock, the species richness of the three patches did not significantly differ over the three years of the survey (Table 2). In the 5-ha paddock, species richness was higher in 2018 for the short grasslands (SG) and higher in 2017 and 2018 for the tall grasslands (TG), (Table 2). In the 19-ha paddock, the species richness was higher in open grasslands (OG) and short grasslands (SG) in 2017-2018, and in tall grasslands (TG) one in 2018. In the 5- and 19-ha paddocks, species richness tended to increase from year to year in short grasslands (SG) and tall grasslands (TG). Overall, the species richness of tall grasslands (TG) was lower than that of the other two types of grassland. The average height of the vegetation in the 7-ha paddock for all three types of grassland was greatest in 2017 (Table 2), and lowest in 2018. We measured no significant differences in average height for open grasslands (OG) and short grasslands (SG) in the 5-ha paddock, while tall grasslands average height was distinctly lower in 2017 and 2018. Open grasslands (OG) in the 19-ha paddock was taller in 2018 and tall grasslands (TG) in 2016, but no significant difference between years was recorded for short grasslands (SG).

[Table 2]

The abundance in annual species (e.g. *Lysimachia arvensis, Filago germanica, Sherardia arvensis,* etc.) for the three types of grassland and for the three paddocks taken together was significantly higher in 2018 than in 2016 and 2017 (2016: $F = 11.55$, $P<0.001$; 2017: $F = 11.86$, $P<0.001$; 2018: $X^2 = 9.95$, $P<0.01$, Fig. 4A, see Appendix S1 for the frequency of all species contacted). The abundance of *B. phoenicoides,* the dominant species, followed the opposite trend; the cover of this species was significantly lower in 2018 (2016: $X^2 = 16.75$, *P<0.001*; 2017: $X^2 = 15.47$, *P<0.001*; 2018: $X^2 = 14.62$, *P<0.001;* Fig. 4B).

[Fig. 4]

3.2. NDVI index and spatial distribution of horses

The NDVI and herbaceous biomass were strongly correlated (rho = 0.70, P<0.001, n = 50). For the two years of the survey, taking all the paddocks together, we found the NDVI negatively correlated with species richness (2016: rho = - 0.45, *P<0.01*; 2017: rho = - 0.69, *P<0.001*, Fig. 5).

[Fig. 5]

The GPS points recorded during the movements of the mares over the year 2017 were distributed in a heterogeneous manner within the study site (5-, 7- and 19-ha paddocks). In the 5-ha paddock, the density of the mares around the point of entry to the site and the junction with the 19-ha paddock was higher than elsewhere (Fig. 6). Conversely, in the 19-ha paddock, the mares would appear to favour the part lying next to the other parks.

The mean pressure exercised by the mares on the ligneous vegetation was significantly lower than that exercised on the open vegetation (23.47 points GPS per m² and 47.89 points GPS per m², W = 17469, respectively, *P<0.001*).

[Fig. 6]

4. Discussion

Horse grazing's ability to create and maintain heterogeneous patches of vegetation has already been widely demonstrated (Beever et al. 2008), in particular for semi-natural environments (Köhler et al. 2016) and with the use of hardy breed horses such as Konik polski (Doboszewski et al. 2017). In our study, before paddock division, weak grazing pressure limited the impact of grazing by Konik polski horses to the more open grassland patches, whose species richness and heterogeneity were significantly higher (Moinardeau et al. 2016; Moinardeau et al. 2019). The augmentation of grazing pressure in temporary paddocks led to a significant increase in species richness, particularly in annual species (*Lysimachia arvensis*, *Filago germanica, Sherardia arvensis),* some of which are considered rare. This is notably the case for *Limonium echioides, Aphanes arvensis, Polypogon maritimus* and *Ranunculus parviflorus,* for which occur only at the study site in the region (Tison et al. 2014). In all three paddocks, the increasing in species richness also corresponded to a parallel reduction in the height of the vegetation of tall grasslands, in particular of its dominant perennial tussock grass species, *B. phoenicoides.* This result has been already shown in other studied for *Brachypodium pinnatum* but with different cutting regimes of grazing involving sheep livestock (Bobbink and Willems 1987, Dutoit & Alard, 1997, Catorci et al., 2014). Concerning the specific effects of Koniks, Köhler et al. (2016) have also shown that after five years of horse grazing, the mean number of typical dry calcareous grassland species increased by one third. They also conclude that in large-scale calcareous grasslands low-intensity year-round grazing with Konik horses will be suitable for maintaining and even improving species diversity.

Attaching a GPS device to the horses made it possible to quantify their spatial use of the site and to explain our results. Horses preferentially used some parts of the paddocks, in particular near enclosure doors where the horses have daily visits from their guard as well as around to the water troughs. In addition, the two herds regularly sought visual contact, although they could not group together in a single herd. Various factors common to grazing systems are known to influence the NDVI, including the plant's physiology, growth rate and senescence (Manning et al. 2017). On the whole site, horses showed a preference for patches with high NDVI corresponding to high photosynthetic activity. We found a strong correlation between the NDVI and the aboveground biomass which reflects its reliability to estimate the food available for domestic herbivores and *B. phoenicoides* cover. We also found highly significant correlations between the NDVI and the species richness recorded into the field for both years of aerial imaging (2016-2017). The increase in the NDVI, negatively correlated with plant species richness, thus coincides with the results of the vegetation monitoring. Indeed the increasing of leaf cover, in particular of *B. phoenicoides*, corresponds to a significant decline in number of species. This suggests a possible extrapolation of the results obtained at the scale of the quadrats (5 x $4m^2$ = 20 m^2 for each patch) to the whole of the site via the significant relationships recorded between the NDVI, biomass and species richness. It confirms the NDVI's potential to provide accurate information on the dynamics and patterns of distribution of vegetation at large scale (Pettorelli et al. 2011, Burai et al. 2015). NDVI can be considered here as a good proxy for vegetation heterogeneity also in a mosaic grassland vegetation. It also confirms the relevance of the NDVI as an operational tool for spatial and temporal comparisons of vegetation at larger scales with direct measurements in the field, like those using quadrats (Pettorelli et al. 2011). Forage production measurements, whether carried out directly in the field or using NDVI, require specialized assistance (Moinardeau et al., 2020). When the vegetation is complex and mosaic-like which is most often the case in protected areas composed of numerous plant patches of varying size, the time saved in the field compensates for the additional costs, which argues for its use (Pettorelli et al. 2011). In the context of conservation management, its potential for large-scale application makes NDVI an appropriate tool to help decision-making (Kong et al. 2019).

The increase of the stocking rate in each of the three paddocks modified the plant biodiversity in the desired direction. However, these changes were made without being based on previous measures, in particular that concerning the availability of fodder. The grazing management applied was better than before (Moinardeau et al. 2016) but could probably be further improved. Adopting an optimal level of grazing pressure or being able to readjust it rapidly is key to successful grazing management at both small and large scale (Dengler et al. 2014). It requires having previously assessed the availability of fodder, which can be time-consuming if only direct measurements in the field have to be made (Moinardeau et al., 2020). In our study, we find the NDVI powerful to estimate the available biomass as the respective richness of the different vegetation patches. Its earlier use would have made it possible to adjust the pastoral load when installing the enclosures.

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Period	**Mares**	**Paddock size**	**Calculated Stocking rate (LU)**
2008 – 2013	4	31 ha	0.04
December 2014 – March 2016	3	7 ha	0.34
March 2016 – June 2017	3	5 ha	0.35
	4	19 ha	0.16
July 2017 – August 2017	3	12 ha	0.03
	4	19 ha	0.16
September 2017 – June 2018	3	7 ha	0.34
	4	19 ha	0.16

Table 1. Number of Konik polski mares, period of presence and stocking rate in each paddock (7, 5 and 19 ha) between December 2014 and June 2018 on an embankment of the Rhône canal (Vaucluse, southern France).

Vegetation type	Statistical parameters (Df = 2)	*P value*	2016	2017	2018
		Species richness (/4 m²)			
		7 ha paddock			
OG	F = 0.67	NS	30.6 ± 1.57	32 ± 2.38	34 ± 2.19
SG	F = 0.79	NS	35.8 ± 2.37	33.8 ± 3.15	38.4 ± 2.11
TG	F = 0.55	NS	27.8 ± 1.8	21.8 ± 5.75	24 ± 3.64
		5 ha paddock			
OG	F = 0.15	NS	33.8 ± 0.97	33.8 ± 1.02	34.6 ± 1.50
SG	F = 9.46	**	22.4 ± 1.57 A	26 ± 1.97 A	32 ± 1.04 B
TG	F = 6.78	*	18 ± 3.77 a	28 ± 2.47 ab	31.6 ± 1.28 b
		19 ha paddock			
OG	F = 11.24	**	25.8 ± 0.97 A	32.6 ± 0.68 B	36 ± 2.41 B
SG	F = 8.03	*	23.2 ± 0.91 a	29.6 ± 0.51 b	33.6 ± 2.76 b
TG	F = 11.37	**	19.6 ± 1.03 A	23.4 ± 2.04 A	30.8 ± 1.82 B
		Mean vegetation height (cm)			
		7 ha paddock			
OG	χ^2 = 10.04	*	3 ± 0.31 A	12 ± 1.22 B	4.4 ± 1.07 A
SG	F = 17.31	***	7.4 ± 1.25 A	11.8 ± 1.11 B	3.4 ± 0.51 C
TG	F = 4.64	*	18 ± 2.55 ab	31 ± 6.20 a	14 ± 2.45 b
		5 ha paddock			
OG	F = 3.13	NS	3.6 ± 1.03	3.6 ± 0.6	8.2 ± 2.31
SG	χ^2 = 2.09	NS	9.6 ± 2.76	4.4 ± 0.75	4.4 ± 1.43
TG	F = 11.91	**	25.6 ± 2.97 a	9.4 ± 0.6 ab	13 ± 3 b
		19 ha paddock			
OG	χ^2 = 8.15	*	4.4 ± 0.4 a	5.6 ± 1.32 ab	10.6 ± 1.16 b
SG	χ^2 = 5.26	NS	12.8 ± 1.85	6.2 ± 2.2	10.6 ± 2.52
TG	F = 10.79	**	30 ± 3.16 a	13 ± 3 b	15 ± 2.23 b

Table 2. Mean and standard error for species richness and mean vegetation height of the grasslands studied (open- OG, short-SG and tall-TG grasslands) for each year studied (2016-2018) and for each paddock (7. 5 and 19 ha) (N = 5 for each grassland in each paddock each year) on the site grazed by the mares. For each paddock and each vegetation type, common letters show no significant difference between treatments for a given year according to Tukey's post-hoc test. a. b for *P < 0.05*; A. B for *P < 0.01*; *P value*: **<0.05*. ***<0.01*. ****<0.001*.

Figures

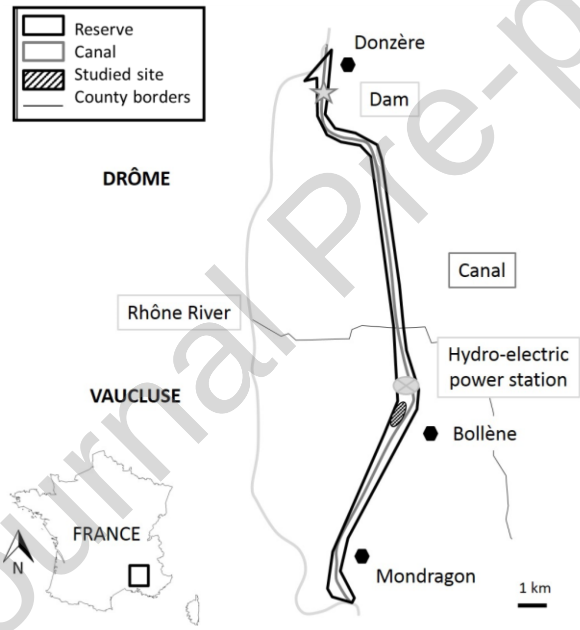

Fig.1. Location of the Donzère-Mondragon study site in south-eastern France, in the lower Rhone valley. Location of the study site (dashed), boundaries of the reserve (black lines), hydroelectric plant (light grey dots), canal (dark grey lines), the Rhone (light grey lines). 'The Plateau' is the local name for the embankment where the horses were introduced in 2008.

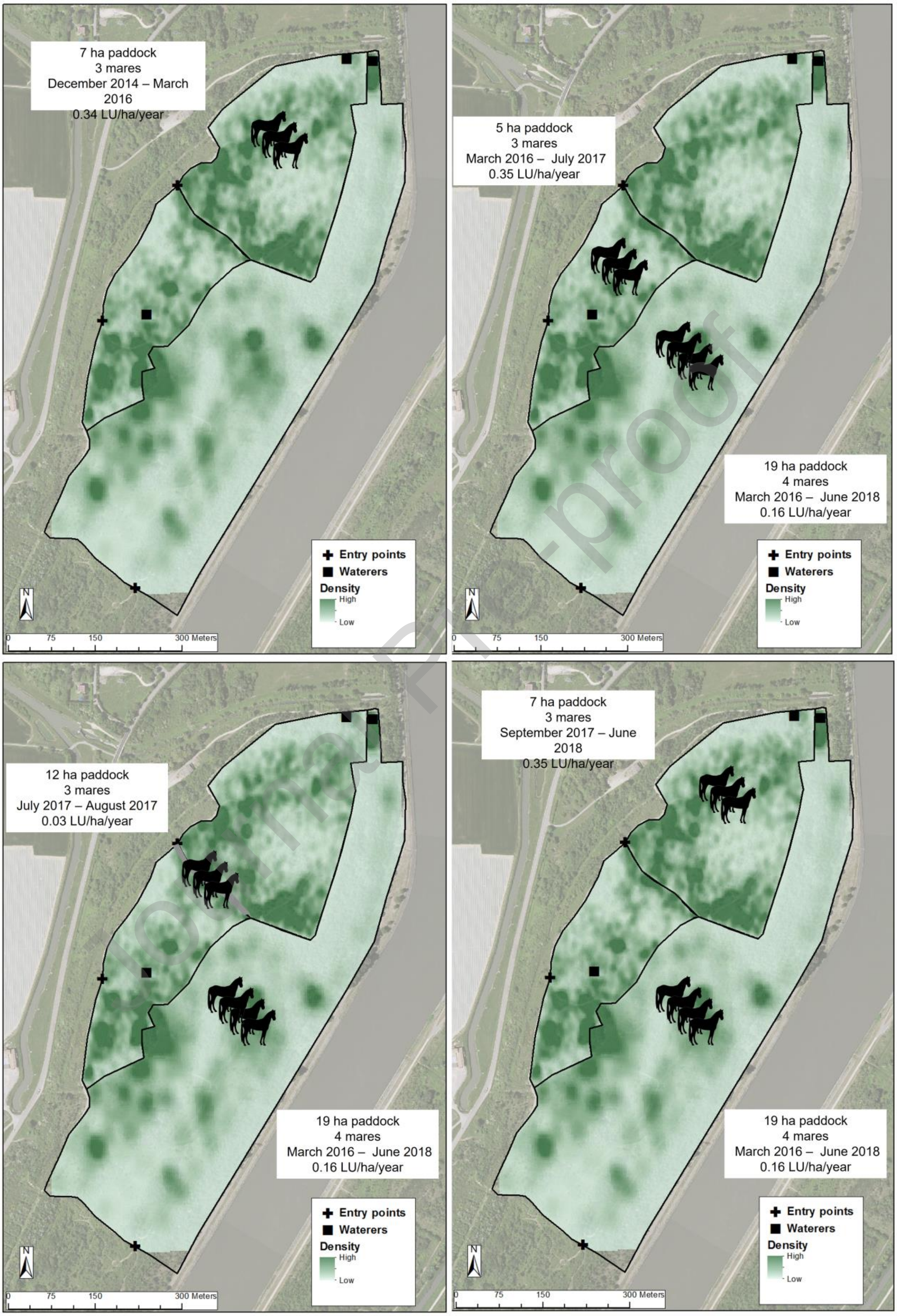
7 ha paddock
3 mares
December 2014 – March 2016
0.34 LU/ha/year
5 ha paddock
3 mares
March 2016 – July 2017
0.35 LU/ha/year
19 ha paddock
4 mares
March 2016 – June 2018
0.16 LU/ha/year
12 ha paddock
3 mares
July 2017 – August 2017
0.03 LU/ha/year
7 ha paddock
3 mares
September 2017 – June 2018
0.35 LU/ha/year
Entry points
Waterers
Density
High
Low
0 75 150 300 Meters
N

Fig.2. Location of the paddocks, movement of the mares and stocking rates between 2016 and 2018 on an embankment (31 ha) at the Donzère-Mondragon reserve, in the lower Rhône valley. Mares are represented by black silhouettes. The location of the water troughs and two points of entry to the study site are also shown. Kernel density tool (ArcGis) is used to represent vegetation density. © BD Ortho 50 cm 2015 IGN.

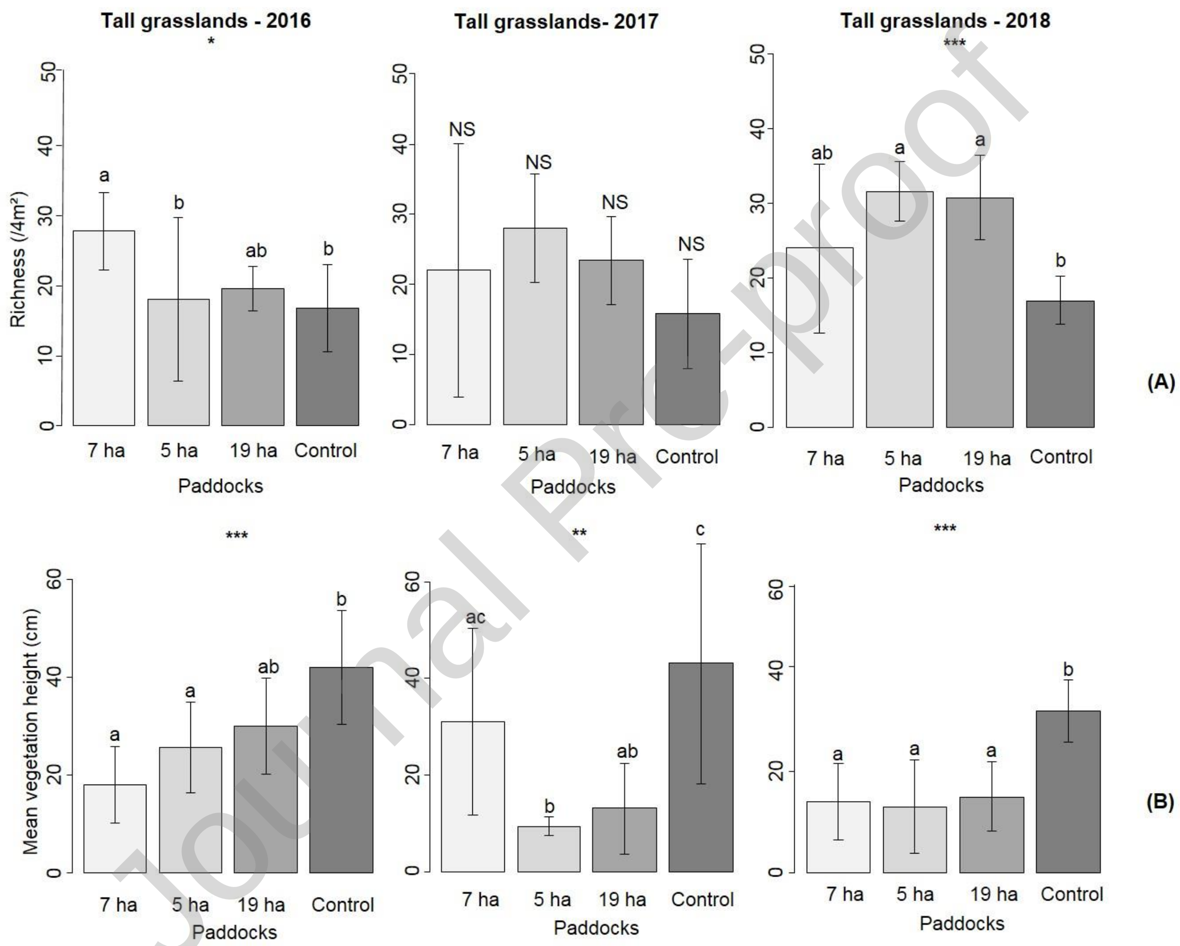

Fig.3. A. Mean and standard error for species richness of tall grasslands (TG) of the three paddocks and control (N = 5 for each paddock each year) for the three years of the study (2016-2018) at the site grazed by the mares. Different superscript letters between groups indicate significant differences between treatments for a given year according to Tukey's post-hoc test. *P value: * <0.05. ** <0.01. *** <0.001*. B. Mean and standard error in mean tall grassland (TG) vegetation height of the three paddocks and control (N = 5 for each paddock each year) for the three years studied (2016-2018) at the site grazed by the mares. Different

superscript letters between groups indicate significant differences between treatments for a given year according to Tukey's post-hoc test. *P value: * <0.05. ** <0.01. *** <0.001.*

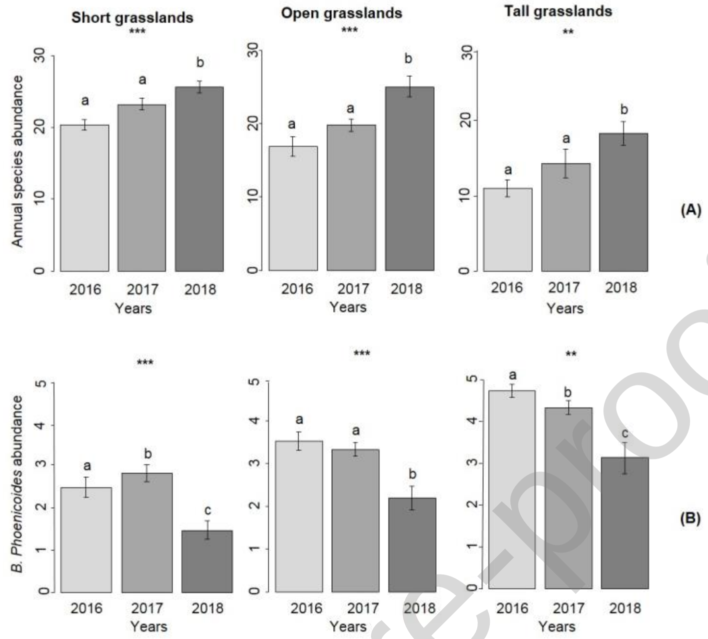

Fig.4. A. Mean and standard error for annual species abundance of the three types of grassland studied (open- OG, short-SG and tall-TG grasslands) for each year of monitoring (N = 15 for each grassland each year) at the site grazed by the mares. Different superscript letters between groups indicate significant differences between treatments for the same year according to Tukey's post-hoc test. *P value: * <0.05. ** <0.01. *** <0.001*. B. Mean and standard error in *B. phoenicoides* abundance of the three types of grassland studied (open-OG, short-SG and tall-TG grasslands) for each year of monitoring (N = 15 for each grassland each year) at the site grazed by the mares. Different superscript letters between groups indicate significant differences between treatments for a given year according to Tukey's post-hoc test. *P value: * <0.05. ** <0.01. *** <0.001.*

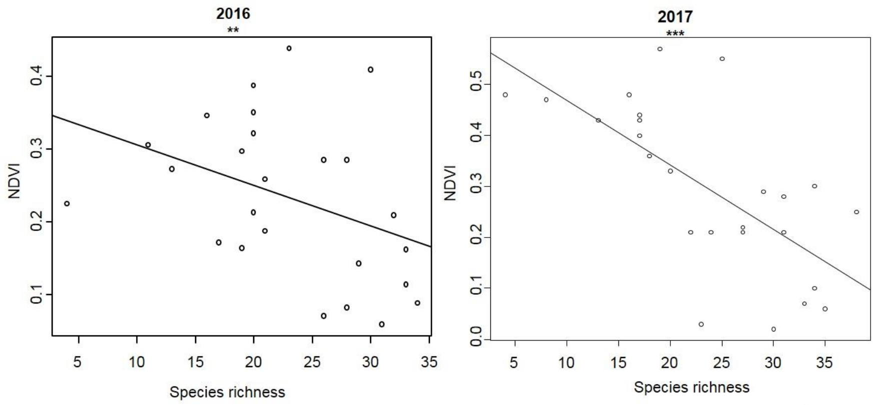

Fig.5. Linear regressions between the mean species richness of plant species derived from vegetation surveys (4-m2-sized quadrats) of the site grazed by mares and the vegetation index (NDVI) for each year of monitoring (2016, 2017). N = 30 for each year. *P value: * <0.05. ** <0.01. *** <0.001.*

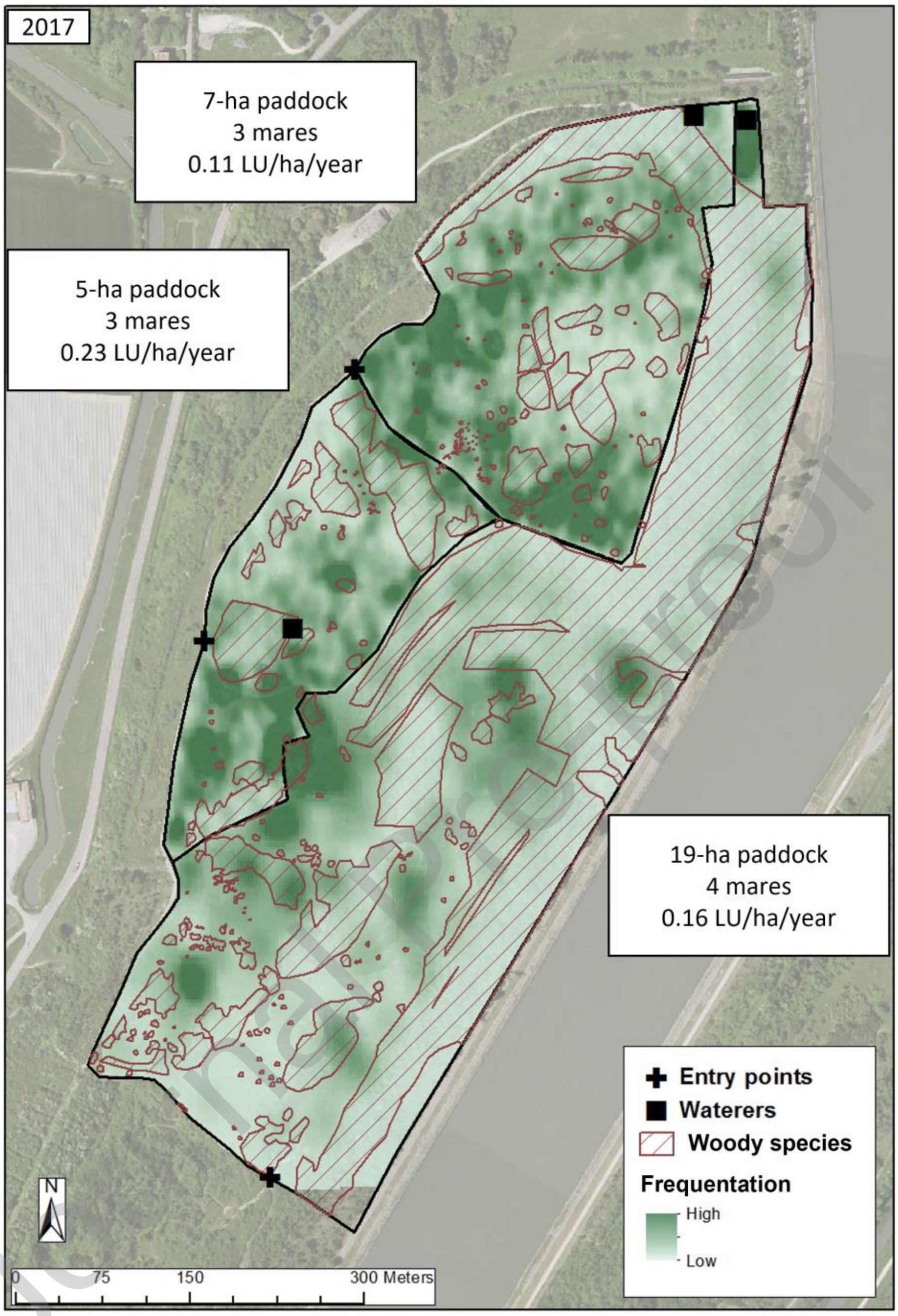

Fig.6. Frequentation of mares on each of the studied paddocks, showing grazing pressure and period of mares' presence in the paddocks, during the year 2017. Grazing pressure is calculated here for the year 2017. The location of the water troughs and two points of entry to the study site are also shown. The cover of woody vegetation is indicated by hatching. © BD Ortho 50 cm 2015 IGN.

Photograph 1: Konik polski horses grazing short grassland in Donzère-Mondragon reserve (South-eastern France) © Julie Chenot/IMBE.

Photograph 2: Konik polski horses grazing woody vegetation regrowth in Donzère-Mondragon reserve (South-eastern France) © Cannelle Moinardeau/IMBE.

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.

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

Species	Annual species	OG			SG			TG		
		2016	2017	2018	2016	2017	2018	2016	2017	2018
Agrimonia eupatoria		0.06	0.22	0.1	0.6	0.08	0.2	0.52	0.54	0.44
Ajuga chamaepitys	x	0.2	0.15	0.14	0.1	0.04	0.12	0.1	0.12	0.1
Ajuga iva		-	-	0.02	-	-	0.1	-	-	-
Anacamptis pyramidalis		-	-	-	-	0.02	-	-	-	-
Anisantha madritensis	x	-	-	0.2	-	-	0.32	-	0.1	0.1
Anisantha rubens	x	0.1	-	-	-	0.1	-	-	0.1	0.1
Aphanes arvensis	x	-	-	-	-	-	0.12	-	-	-
Arctium minus	x	-	0.02	0.1	-	0.06	0.24	-	-	0.22
Arenaria leptoclados		-	-	-	-	-	0.04	-	-	-
Arenaria serpyllifolia	x	-	-	-	0.12	-	-	-	-	-
Artemisia annua	x	-	-	-	-	-	0.02	-	-	-
Asparagus sp		-	-	-	-	-	-	-	0.02	0.02
Avena sterilis	x	-	-	-	-	-	-	-	-	0.02
Bituminaria bituminosa		0.32	0.33	0.3	0.34	0.36	0.34	1.12	1.1	0.7
Blackstonia perfoliata	x	0.84	0.71	0.1	0.9	0.5	0.12	0.5	0.48	0.4
Bombycilaena erecta	x	-	0.11	-	-	-	-	-	-	-
Brachypodium distachyon	x	0.2	-	0.3	-	-	-	-	-	0.4
Brachypodium phoenicoides		2.5	3.11	1.5	3.2	3.4	2.3	4.7	4.3	2.9
Bromus erectus		-	0.02	-	0.1	0.02	-	-	-	-
Bromus hordeaceus	x	-	0.13	0.12	-	-	0.02	-	-	0.12
Campanula erinus	x	0.06	0.22	0.16	0.2	0.14	0.3	0.04	-	0.2
Capsella bursa.pastoris		-	-	0.02	-	-	-	-	-	-
Lepidium draba		0.14	0.02	-	0.1	0.1	-	0.1	-	-
Carduus pycnocephalus		-	0.11	0.06	0.02	-	0.3	-	0.1	0.12
Carduus tenuiflorus		-	-	-	-	0.06	-	-	-	-

Carex otrubae		-	-	-	0.1	0.2	-	-	-	-
Carlina hispanica		0.44	0.35	0.5	0.12	0.24	0.06	0.74	0.68	0.74
Carlina lanata	x	-	0.24	-	-	0.1	0.16	-	0.1	-
Catapodium rigidum	x	0.36	0.82	1	1	0.52	1.02	0.06	0.66	0.92
Centaurea paniculata	x	-	-	0.1	-	-	-	-	-	-
Centaurium erythraea	x	1.04	0.28	0.12	0.9	0.5	0.22	0.72	0.34	0.04
Centaurium tenuiflorus	x	0.02	0.24	-	0.02	-	-	-	0.02	-
Centranthus calcitrapae	x	-	-	0.12	-	0.04	0.04	-	-	-
Cerastium glomeratum	x	0.02	0.02	0.1	0.2	0.04	-	0.1	-	0.02
Cerastium pumilum	x	-	-	0.02	-	-	0.12	-	-	-
Chenopodium album	x	-	-	0.2	-	-	0.1	-	-	-
Chondrilla juncea	x	0.02	0.04	0.02	-	0.02	-	-	0.06	0.02
Cirsium arvense		-	-	0.02	-	-	-	-	-	-
Cirsium vulgare	x	-	-	0.1	0.06	-	0.26	-	0.1	0.06
Clinopodium nepeta		0.7	1.13	1	0.5	0.64	0.72	0.82	0.82	0.9
Convolvulus arvensis		-	-	-	0.04	0.02	-	0.02	-	-
Cornus sanguinea		-	-	0.02	0.12	0.02	-	0.12	0.02	0.2
Crataegus monogyna		0.2	0.55	0.52	0.72	0.28	0.44	0.28	0.36	0.46
Crepis bursifolia	x	-	-	0.02	-	-	-	-	-	-
Crepis foetida	x	0.06	0.46	0.54	0.02	0.2	0.3	0.02	0.3	0.36
Crepis pulchra	x	-	-	0.02	-	0.04	0.22	-	-	0.1
Crepis sancta	x	0.46	0.71	1	0.1	0.48	1	0.14	0.3	0.82
Crepis vesicaria .taraxacifolia.	x	-	0.68	0.8	0.12	0.58	0.8	0.04	0.44	0.8
Cynodon dactylon		-	-	-	0.1	-	-	-	-	-
Cynoglossum creticum	x	-	-	-	-	0.02	0.02	-	-	-
Dactylis glomerata		0.54	1	0.72	0.9	1.22	1.12	1	1.7	1.5
Dipsacus fullonum	x	-	-	-	0.1	0.04	0.02	0.02	0.02	0.1
Dittrichia graveolens	x	0.04	0.13	0.12	-	-	0.14	-	-	0.1
Echium asperrimum		-	-	-	0.02	-	0.06	-	-	-
Echium vulgare	x	0.34	0.13	0.2	-	-	0.02	0.1	0.04	0.02
Erigeron sp	x	0.18	0.46	0.02	0.22	0.2	0.24	0.06	0.24	0.04
Erodium cicutarium	x	-	0.06	-	-	0.02	0.1	-	-	-
Erodium cyconium	x	-	-	0.4	-	-	0.1	-	-	0.1
Eryngium campestre		0.12	0.24	-	0.5	0.16	0.2	0.22	0.3	0.2
Euphorbia cyparissias		-	-	-	0.1	0.04	-	-	-	0.02
Euphorbia peplus	x	0.18	0.46	0.5	-	-	0.1	0.02	0.2	0.12
Euphorbia segetalis	x	0.14	0.13	0.22	0.1	0.02	0.02	-	0.1	-
Euphorbia serrata		-	0.22	-	-	-	0.02	-	-	-
Falcaria vulgaris	x	-	-	-	-	0.1	-	-	-	-
Filago germanica	x	0.58	0.8	0.9	0.62	0.4	0.9	0.1	0.36	0.7
Filago pyramidata	x	-	-	0.8	-	-	0.46	-	-	0.1
Galium aparine	x	-	-	-	-	-	-	-	0.02	0.02
Galium parisiense	x	0.44	0.82	0.8	0.9	0.5	0.8	0.28	0.38	0.6
Gastridium ventricosum	x	-	-	0.2	0.3	-	0.1	0.1	-	-
Geranium columbinum	x	-	0.13	-	0.1	0.18	0.1	0.02	0.34	0.12
Geranium dissectum	x	0.04	0.13	0.32	0.34	-	0.3	0.18	0.22	0.52
Geranium molle	x	-	-	-	0.12	0.02	0.04	0.04	-	0.1
Geranium rotundifolium	x	-	-	-	0.04	-	-	0.1	-	-

Hedera helix		-	-	-	-	-	-	0.02	-	0.02
Himantoglossum hircinum		-	-	-	-	0.02	-	-	-	0.02
Himantoglossum robertianum		-	-	-	-	-	-	-	0.02	-
Himantoglossum sp.		-	-	-	0.02	-	-	-	-	-
Hordeum murinum	x	-	-	-	-	-	0.1	-	-	-
Hypericum perforatum		0.6	0.82	0.72	1	0.42	0.56	0.64	0.38	0.6
Hypochaeris glabra	x	-	-	0.02	-	-	-	-	-	-
Hypochaeris radicata		-	-	0.02	-	-	-	0.02	-	0.02
Lactuca serriola	x	-	-	0.06	-	-	0.08	-	-	0.02
Lathyrus cicera	x	-	-	-	-	-	-	-	-	0.02
Lathyrus hirsutus		-	-	-	-	-	-	0.3	-	-
Lathyrus pratensis		-	-	0.02	-	0.02	-	-	0.04	0.32
Lathyrus sphaericus	x	-	-	-	-	-	-	-	-	0.02
Leontodon tuberosus		-	-	0.04	-	-	0.08	-	-	0.14
Linum strictum	x	0.32	0.71	0.9	0.42	0.36	1.1	0.12	0.16	0.62
Lolium rigidum	x	0.02	0.13	-	-	0.1	-	-	0.1	-
Lotus corniculatus		0.46	0.35	0.2	0.7	0.22	0.16	0.3	0.42	0.22
Lysimachia arvensis	x	0.16	0.11	0.52	0.14	0.08	0.42	-	0.02	0.32
Lysimachia foemina	x	0.14	0.22	0.9	0.1	0.1	0.8	0.02	0.2	0.32
Medicago lupulina	x	0.1	0.33	-	0.1	0.12	-	0.12	0.02	-
Medicago minima	x	0.62	0.68	0.9	0.7	0.54	0.7	0.34	0.56	1.3
Medicago monspeliaca	x	-	-	0.1	-	-	-	-	-	-
Medicago polymorpha		-	-	-	0.2	0.2	-	-	-	-
Medicago rigidula	x	-	-	0.1	-	-	-	-	0.02	-
Melica ciliata		-	-	-	-	0.02	-	-	-	-
Melilotus sp	x	-	0.11	-	-	0.1	-	-	0.02	-
Minuartia hybrida		-	-	-	0.12	-	-	-	-	-
Myosotis arvensis	x	-	-	0.1	0.22	0.02	0.04	0.3	0.26	0.44
Myosotis ramosissima	x	-	0.11	-	-	-	0.1	-	0.12	-
Odontites rubra	x	0.12	0.13	0.02	-	-	-	-	-	-
Ophrys apifera		-	-	-	-	-	-	0.1	0.02	-
Orchis sp		-	-	-	0.02	-	-	-	-	-
Parapholis incurva	x	0.24	0.24	0.52	-	0.22	0.2	-	-	0.1
Pardoglossum cheirifolium	x	-	0.02	-	-	-	-	-	-	-
Phleum pratense		-	-	0.02	-	-	0.1	-	0.02	0.1
Phragmites australis		-	-	-	-	-	-	0.1	0.2	0.1
Picris hieracioides	x	-	-	-	0.02	0.02	0.02	0.04	-	-
Pilosella officinarum		0.72	0.88	0.9	0.4	0.02	0.2	0.06	0.02	0.2
Plantago lanceolata		0.02	-	0.02	-	-	-	0.02	0.12	0.04
Plantago sempervirens		-	0.02	-	-	-	-	-	-	-
Poa annua	x	0.1	0.11	0.2	0.1	0.12	0.14	-	0.02	0.1
Poa bulbosa		0.16	0.11	0.5	-	-	0.2	-	-	0.1
Poa pratensis		-	0.11	-	-	0.12	-	0.02	0.1	-
Poa trivialis		-	0.02	-	-	-	-	-	0.04	-
Podospermum lacinatum		-	-	0.04	0.2	0.02	-	-	-	-
Populus alba		-	-	0.1	-	-	-	-	-	-
Populus nigra		-	-	-	0.12	0.02	-	-	0.1	-
Potentilla reptans		-	0.11	0.02	-	-	-	-	-	-

Potentilla verna		0.02	-	-	-	-	-	-	-	-
Poterium sanguisorba		0.64	0.8	0.62	0.8	0.46	0.54	0.62	0.62	0.52
Prunella vulgaris		0.02	-	-	-	-	-	-	-	-
Prunus avium		-	-	-	-	-	-	-	0.1	-
Prunus spinosa		-	-	-	-	-	-	0.02	-	-
Ranunculus parviflorus	x	-	-	0.04	-	-	0.22	0.02	0.02	0.1
Robinia pseudoacacia		-	-	-	-	-	0.02	-	-	-
Rosa agrestis		-	0.11	-	0.1	0.04	-	-	0.02	0.02
Rosa canina		0.2	0.15	0.1	0.1	-	0.04	0.22	0.02	0.22
Rostraria cristata	x	0.68	0.82	1	0.8	0.52	0.76	0.3	0.36	0.42
Rubia peregrina		-	-	-	-	-	-	-	0.02	-
Rubus ulmifolius		0.04	-	-	0.12	0.04	0.02	0.02	-	0.02
Rumex crispus		-	-	-	-	-	-	0.12	-	0.12
Rumex pulcher	x	-	-	0.04	-	-	-	-	-	0.02
Sagina apetala	x	0.14	0.02	0.34	0.12	0.04	0.32	-	-	0.1
Sambucus ebulus		-	-	-	0.1	-	-	-	-	-
Scabiosa atropurpurea maritima	x	0.02	0.02	-	-	-	-	-	-	-
Schedonorus arundinaceus		-	-	-	0.02	-	-	0.04	0.02	0.02
Senecio inaequidens		-	-	-	-	-	0.02	-	-	-
Sherardia arvensis	x	0.56	0.82	0.8	0.9	0.5	0.92	0.28	0.36	0.76
Sonchus asper	x	0.02	0.22	0.02	0.1	0.2	0.46	-	0.12	0.14
Sonchus oleraceus	x	0.02	0.02	-	0.1	0.08	0.02	-	-	-
Spiranthes spiralis		-	-	0.04	0.02	-	-	-	0.02	0.02
Taraxacum erythrospermum		-	-	0.32	-	-	0.54	-	-	0.36
Taraxacum sp		0.06	0.13	-	-	0.04	-	0.28	0.14	-
Torilis arvensis	x	-	-	-	-	0.02	0.02	0.12	0.02	0.02
Torilis nodosa	x	-	-	0.12	0.12	0.06	0.2	-	0.12	-
Tragopogon porrifolius	x	-	0.02	-	-	-	-	-	-	-
Trifolium angustifolium	x	-	-	-	-	-	-	-	-	0.02
Trifolium campestre	x	0.86	1.02	1.7	0.72	0.54	1.6	0.56	0.7	1.62
Trifolium glomeratum	x	-	-	-	0.1	-	0.02	-	-	-
Trifolium scabrum	x	-	0.22	0.3	0.2	0.2	0.24	-	0.02	0.2
Trifolium sp		-	-	-	-	-	-	-	-	-
Trifolium suffocatum	x	0.44	0.13	0.3	-	0.1	0.1	-	-	-
Trigonella smalii	x	-	-	0.1	-	-	-	-	-	-
Trisetum flavescens		0.42	0.35	0.4	0.1	0.1	0.1	-	-	0.02
Ulmus minor		-	-	-	0.1	0.1	0.2	-	-	-
Urospermum picroides	x	-	0.02	-	-	0.04	-	-	-	0.1
Verbascum sinuatum	x	-	-	-	-	-	0.04	-	-	-
Verbascum thapsus	x	0.04	-	-	-	-	-	0.02	-	-
Verbena officinalis		0.16	0.17	0.12	0.32	0.28	0.2	0.04	0.02	0.02
Veronica arvensis	x	0.04	0.26	0.54	0.74	0.2	0.32	0.2	0.26	0.56
Veronica persica	x	-	-	-	-	-	-	-	-	0.02
Veronica polita	x	-	-	0.04	-	-	-	-	-	-
Vicia hybrida	x	-	-	0.02	-	-	-	-	-	-
Vicia lathyroides	x	-	0.15	-	-	0.44	-	-	0.42	-
Vicia parviflora	x	0.44	0.35	0.44	0.5	0.16	0.28	0.4	0.16	0.16
Vicia sativa	x	0.56	0.22	0.62	0.72	0.1	0.62	0.64	0.3	0.72

Vulpia ciliata	x	0.76	0.46	0.4	0.8	0.32	0.2	0.02	0.1	0.5
Vulpia myuros	x	0.12	0.91	0.7	0.1	0.66	0.9	-	0.32	0.5
Vulpia unilateralis	x	0.1	0.44	0.8	-	0.16	0.4	0.1	-	0.3

Appendix A : Mean frequency of taxa recorded in the studied embankment in Donzère-Mondragon in the Lower Rhône valley, Southeastern France. Values correspond to the mean frequency of each taxa for each treatment over 3 years (2016-2018). OG = open grasslands with the presence of bare soils grazed by horses, SG = short grasslands grazed by horses, TG = tall grasslands grazed by horses.