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# How does stocking rate influence horse behaviour, performances and pasture biodiversity in mesophile grasslands?

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ABSTRACT

Reducing grazing intensity is widely recommended as a strategy to increase grassland biodiversity through greater sward heterogeneity. Horses are playing an increasing role in the management of permanent grasslands across Europe, but the effects of horse grazing under contrasting stocking rates have been poorly studied. Here we describe the effects of two contrasted stocking rates ("High" 1.8 LU $ha^{-1}$ *vs.* "Moderate" 1.1 LU $ha^{-1}$) on sward structure, horse foraging behaviour and performances, and pasture biodiversity in a mesophile grassland of central France. Horses selectively grazed vegetative patches of high nutritive value, especially at the moderate stocking rate ($p < 0.01$). This enabled them to maintain diet quality (diet dry matter digestibility: 59% DM) and performances (daily liveweight gain > 270 g $animal^{-1}$ $d^{-1}$) at the same level in highly and moderately grazed plots despite contrasted herbage biomass and quality. Horses, with their two sets of incisors, created and maintained patches of short grass in a matrix of tall vegetation in both treatments. Consequently, sward structural heterogeneity did not significantly differ between highly and moderately grazed plots, and there was no detectable effect of grazing intensity on floristic and arthropod diversity over the four-year study. The two stocking rates did however result in a divergent evolution of legumes for which abundance increased at the high stocking rate ($p < 0.05$). Abundance of Carabidae and grasshoppers from tall grasslands was higher at the moderate stocking rate ($p < 0.05$). We conclude that decreasing stocking rate would only have a marginal effect on grassland biodiversity, while farm performance will be strongly affected by the decrease in the number of horses per unit area.



## 1. Introduction

Permanent grasslands are recognized as an important source of biodiversity across Europe, making it essential to develop and promote grazing management that prioritizes ecosystem integrity as much as livestock production. Several studies report how reducing grazing intensity in semi-natural grasslands can increase plant diversity as a result of reduced disturbance and higher sward heterogeneity (Dumont et al., 2009; Klimek et al., 2007; Marriott et al., 2004). Moderate grazing intensity also benefits butterflies, grasshoppers and ground-dwelling arthropods by providing a greater number of ecological niches (Dumont et al., 2009; Kruess and Tscharntke, 2002; Wallis de Vries et al., 2007). Grazing intensity further determines vegetation biomass and herbage quality along the grazing season. Applying a high stocking rate maintains herbage quality in homogeneous swards by limiting reproductive stem growth and herbage senescence (Garcia et al., 2003; Parsons and Chapman, 1999). In contrast, when stocking rate is decreased, the imbalance between herbage offer and animal intake leads to a coexistence of short vegetative patches in grazed areas and under-grazed vegetation with high biomass accumulation including dead leaves and low-digestibility stems (Isselstein et al., 2003; Marriott et al., 2005; White et al., 2004).

Of all the large domestic herbivores, horses are the most likely to create and maintain patches of short grass that they preferentially graze within a matrix of tall vegetation in which they concentrate their faeces (Loucougaray et al., 2004; Ménard et al., 2002; Ödberg and Francis-Smith, 1977). This behaviour has long been interpreted as an anti-parasite strategy (Taylor, 1954) but recent studies suggest that the selection of short high-quality

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patches by horses could also be explained as a strategy designed to maximize digestible protein intake (Edouard et al., 2010; Fleurance et al., 2005). Despite the increasing numbers of horses grazing permanent grasslands across Europe (European Horse Network, 2010), few studies have investigated the effects of horse grazing under contrasting stocking rates on diet selection and sward structure. Moreover, these studies have focused on grazeland of high nature value (Cornelissen and Vulink, 2015; Magnússon and Magnússon, 1990; Nolte et al., 2013, 2014) and not on the mesophile grasslands most commonly used in horse production systems. In addition, general trends in the impact of horses on grassland diversity have mainly come from comparisons between grazed and ungrazed areas (Duncan, 1992; Holmquist et al., 2010, 2013; Hoste-Danylow et al., 2010; Ten Harkel and Van der Meulen, 1995) or with other herbivore species (Catorci et al., 2012; Loucougaray et al., 2004; Öckinger et al., 2006; Rosa Garcia et al., 2013; Vulink et al., 2000).

In the present study, we simultaneously analyze the effects of two contrasting stocking rates (“High” *vs.* “Moderate”) on sward structure, horse foraging behaviour and performances, and pasture biodiversity in a hill-range mesophile grassland of central France. We analyzed how horses adapt their foraging behaviour in response to variations in sward availability and quality and investigated whether they can maintain diet quality and performance as grazing intensity decreases. We hypothesize that the strong selection of short high quality patches by horses will create strong sward structural heterogeneity at both stocking rates, and therefore buffer the benefits of reducing grazing intensity on grassland biodiversity.

## 2. Materials and methods

### 2.1. Experimental design

The study was carried out in a mesophile grassland of central France (French Horse and Riding Institute experimental farm station in Chamberet, 01°43′14′ – 45°35′03′′, 440 m a.s.l.) over four grazing seasons from 2006 to 2009. Climate is oceanic and soil is episkeletic podzol (http://eusoils.jrc.ec.europa.eu/). Precipitation fluctuated strongly along the study from 1104 mm in 2009–1437 mm in 2008. Plots had been intensively managed for rotational grazing by horses for many years, which explains their relatively low botanical diversity with a total of 48 plant species recorded over the whole area (16.2 ha) in 2006. Two stocking rates were compared: a high stocking rate (1.8 LU $ha^{-1}$; 1 LU = 600 kg liveweight) designed to have most of the edible biomass consumed at the end of the grazing season, and a moderate stocking rate (1.1 LU $ha^{-1}$). Six 2.7-ha plots were created within the pasture, so that each treatment was replicated three times in a randomized block design. At the high stocking rate, five horses were continuously grazed in each plot from mid-April to end of July then from early September to early November, while at the moderate stocking rate, three horses grazed each plot during the same periods. Horses were removed from experimental pastures from the end of July to early September to avoid pasture degradation at the high stocking rate. They were thus kept together in another permanent grassland, or indoors in severe summer drought.

Anglo-Arab and French saddle breeds (3.2 ± 0.1 years old, 521.7 ± 6.5 kg) were used each year. At the start of each grazing season, the six groups of horses were balanced for body size, body condition score, breed, sex, age, and previous feeding experience.

Sward measurements and behavioural observations were carried out in each plot from 2006 to 2008 at three periods along the grazing season: spring (early May) before flowering of major sward components, summer (end of June) when sward heterogeneity is expected to be at a maximum and autumn (October) to account for cumulative effects of treatments. Sward botanical diversity was measured in June 2006 and 2009, and insect diversity was recorded each summer, in June for ground-dwelling arthropods and in July for grasshoppers.

### 2.2. Sward measurements

Sward height was measured on parallel transects covering the entire plot (about 500 sample points per plot) at the first place where a stick contacted the undisturbed sward surface. The coefficient of variation (CV) was then used as an indicator of sward heterogeneity (Eschen et al., 2012). At each sample point, we also recorded vegetation stage (vegetative, reproductive or dead), dominant botanical family (grass, legumes or forbs), distinguished between pure and mixed potential bites, and recorded bare ground gaps and the presence of faeces.

Herbage biomass was determined for four sward types, i.e. vegetative short patches (VS, ≤4 cm), vegetative intermediate (VI, 5–8 cm), vegetative tall (VT, ≥9 cm), reproductive and dead vegetation (Repro/Dead), by randomly cutting six 0.5 $m^2$ (10 cm × 5 m-long) strips to ground level in each sward type and plot. Quality of herbage offered was assessed for each sward type (VS, VI, VT, Repro/Dead) from three biomass samples randomly selected among these six. Samples were dried at 60 °C to constant weight and analyzed for crude protein (CP; Kjeldahl method N × 6.25) and fibre content (NDF: neutral detergent fiber according to the method of Van Soest et al., 1991). Mean biomass per plot and mean herbage quality were then estimated from the proportion of each sward type in the plot (i.e. number of times that sward type i was encountered in the plot/total number of sample points in the plot) and the herbage biomass and quality of each sward type. The Shannon index was used to calculate the evenness of the distribution of highly grazed patches (in line with Ménard et al., 2002, VS and VI patches ≤8 cm were combined), tall vegetative patches (VT) and reproductive/dead patches (Repro/Dead) at plot scale.

### 2.3. Animal measurements

Dietary choices were measured by scan sampling at 5-min intervals of the activity of three animals within each group, with one 24-h observation per plot (i.e. three per treatment) and per period. Horses were identified by a number painted on both sides. A horse was considered to be grazing when it was biting, chewing or swallowing grass, or when it was walking with its muzzle close to the sward. For each individual recorded as grazing, the observer moved as close to the animal as possible without disturbing it in order to record one selected bite. Observers decided beforehand which bite to record once they were close enough to the animal (i.e. the fifth one) in order to avoid bias due to picking bites that were most clearly visible. This required animals to be trained for a week before measurements so that they would readily accept close proximity of the observer during grazing.

Bite types were recorded according to sward height type, i.e. vegetative short (VS, ≤4 cm), vegetative intermediate (VI, 5–8 cm), vegetative tall (VT, ≥9 cm), reproductive/dead. A lamp was used for nighttime observations but the procedure was the same. Daytime observations also recorded dominant botanical family (grass, legumes or forbs) and whether the bite was pure (one botanical family only) or mixed. Ingestive behaviour measurements (bite rate and step rate during 1 min of uninterrupted grazing) were taken between scans throughout the 24 h to obtain further information on selective behaviour. A step was defined as a movement of one front leg. Four to eight recordings a day per sward type (i.e. VS, VI, VT, Repro/Dead) were targeted for each individual. Recordings were interrupted if the animal took at least three consecutive steps with its head up, stood head up without chewing grass for more than 3 s, or started grazing another vegetation type. Mean bite rate and mean step rate were then estimated at plot level from the measurements on each sward type and the proportion of time spent grazing it. Mean bite rate-to-mean step rate ratio informs on animal selectivity; the lower this ratio, the more selective the animal (Lazo and Soriguer, 1993).

Moreover, diet selection, defined as the proportion of a sward (bite) type in the diet relative to its proportion in the plot, was quantified by calculating selectivity indices ($S_i$) for each sward type using Jacobs' (1974) modification of Ivlev's electivity index:

$$S_i = (c_i - a_i)/(c_i + a_i - 2c_i a_i),$$

where $c_i$ is proportion (between 0 and 1) of component $i$ in the diet and $a_i$ is proportion (between 0 and 1) of component $i$ in the plot. For each bite type ($c_i$), data for individual animals were first aggregated per day and per plot and then linked to the relative abundance of this bite type in the plot. $Si$ varies from −1 (never used) to +1 (exclusively used), with negative and positive values indicating avoidance and preference, respectively, and 0 indicating that a sward component is used in proportion to its availability. Jacobs' index was chosen for its low sensitivity to variations in the relative abundance of plant components, thus making it possible to rank both abundant and rare plant components according to their acceptability to the animals.

Diet digestibility (%DM) was estimated from faecal CP content (%DM) according to the equation of Mésochina et al. (1998):

$$\text{Diet digestibility} = 73.4 - (178.72/\text{faecal CP content}).$$

The conditions of application of this equation were for herbage CP content higher than 7 g/kg DM, which limits nitrogen recycling by horses (Mésochina et al., 1998); this was always the case in the present experiment. Faecal nitrogen has been used to estimate diet digestibility in a large number of horse studies (e.g. Edouard et al., 2009, 2010; Fleurance et al., 2010) as well as in other herbivores species (e.g. zebra: Barnier et al., 2014; sheep & goats: Boval et al., 2003; cattle: Lukas et al., 2005). Five samples of fresh faeces from each of the three identified horses per plot were collected on the ground immediately after their emission throughout the 24 h behavioural observations. Samples were then mixed by individual and dried at 80 °C to constant weight. Nitrogen was determined by the Kjeldahl method.

Liveweight gain data were obtained by weighing the three individuals observed in each plot each time the animals were turned in and turned out from pastures (mid-April, end of July, early September, early November).

### 2.4. Biodiversity measurements

Botanical diversity, i.e. percentage cover of all plant species in each plot, was estimated in June 2006 and 2009 in 25 quadrats (50 cm × 50 cm) randomly placed along the parallel transects that were used for sward height measurements. Plant identification followed Tutin et al. (1964–1980) and, for *Festuca* species, Kerguélen and Plonka (1989). Shannon index was used to calculate the evenness of plant species distribution at the plot scale. Plant species were split into grasses, legumes and forbs, and were also classified according to the C-S-R model of primary strategies proposed by Grime et al. (1988).

Ground-dwellling arthropods were sampled for one month (June) by pitfall trapping from 2006 to 2009. Eighteen traps were placed in each paddock: 6 traps in vegetative short or intermediate patches (VS-VI), 6 in vegetative tall patches (VT), and 6 in reproductive patches (Repro). Each trap was filled with a mixture of 70% alcohol and 30% water. Traps were 10 cm in diameter, and protected against horse trampling by a cage-like construction. Carabidae were counted and identified at species level while adults of other arthropod groups were counted and identified at higher taxonomic levels. Abundance of the different groups was calculated from the number of individuals captured per sward type, and from the proportion of each sward type in the plot. Adult grasshoppers were counted at the end of July 2006–2009, along three permanent transects (50 m × 2 m) in each plot, and identified at species level from either visual observations or acoustic signature.

### 2.5. Statistical analyses

Data were analyzed using the SAS PROC Mixed procedure (version 9.2; SAS Institute Inc., Cary, NC, USA) for repeated measurements (Littell et al., 1998).

Herbage structure, biomass, quality, foraging behaviour, Jacobs' indices, and dry matter digestibility were analyzed, after arcsine transformation for proportions, in a model including main effects of stocking rate, year and season, and stocking rate × year, stocking rate × season, year × season, stocking rate × year × season interactions. Plot was used as statistical unit. Block was considered as a random effect, which generates a more powerful analysis by ensuring that variance due to block is taken into account and not just included in the error term. Differences between treatments were investigated using the Tukey correction for multiple comparisons. Significance of selection for ($Si > 0$) or against ($Si < 0$) each vegetation item was determined by comparing Jacobs' indices to zero using a Student's $t$-test.

As horses were removed from their pastures from the end of July to early September, livestock performance was analyzed separately from April to July and from September to November. The model included the main effects of stocking rate and year and stocking rate × year interaction. Livestock performance measured when animals were removed from their experimental plots was taken as a covariate when analyzing the influence of stocking rate between September and November. Block was considered as a random effect.

The influence of stocking rate on abundance and number of plant species recorded in 2009 was tested with 2006 data taken as a covariate. Insect abundance and diversity data were analyzed in a model including the main effects of stocking rate and year and stocking rate × year interaction. Abundance data for ground beetles and grasshoppers were further analyzed according to habitat affinity, i.e. short or tall grassland (Bellmann and Luquet, 1995; Coulon et al., 2000; Jeannel, 1941, 1942). Plot was used as statistical unit and block was considered as a random effect.

## 3. Results

### 3.1. Herbage structure, biomass and quality

Mean sward surface height (SSH) decreased from the beginning (15.5 ± 0.5 cm s.e.) to the end of the grazing season (10.7 ± 0.7 cm s.e.) in highly-grazed plots but peaked in summer (30.6 ± 2.1 cm s.e.) in moderately grazed plots, resulting in a significant stocking rate × season interaction ($p < 0.0001$; Table 1). Mean SSH was higher at moderate stocking rate whatever the season ($p < 0.01$). Sward height CV did not significantly differ between treatments and was higher during summer and autumn ($p < 0.0001$; Table 1).

Higher abundance of VS and VI patches was found at the high stocking rate, with the highest values recorded during autumn for both stocking rate treatments (Table 1). Conversely, VT patches were more abundant at the moderate stocking rate and values were the highest in spring and the lowest in summer for both

**Table 1**
Average sward characteristics in the two stocking rate treatments (High, 1.8 LU $ha^{-1}$ and Moderate, 1.1 LU $ha^{-1}$) over the grazing season (Spring, Sp; Summer, Sum; Autumn, Aut) (mean ± s.e.).

| | Stocking rate (SR) | | | Season (S) | | | | SR × S |
|---|---|---|---|---|---|---|---|---|
| | H | M | p | Sp | Sum | Aut | p | p |
| Mean SSH[1] (cm) | 13.2 ±0.7 | 23.2 ±1.6 | | 18.7 ±1.4 | 22 ±2.5 | 13.9 ±1.1 | | *** |
| CV SSH[2] (%) | 65.8 ±2.4 | 60.7 ±2.6 | ns | 48.7[b] ±1.5 | 71.8[a] ±9.3 | 69.2[a] ±2.0 | *** | ns |
| % VS[3] | 14.2 ±1.9 | 4.8 ±0.7 | *** | 2.9[c] ±0.6 | 10.3[b] ±2.0 | 15.3[a] ±2.1 | *** | ns |
| % VI[3] | 22.2 ±1.6 | 10.1 ±1.1 | *** | 12.8[b] ±2.3 | 15.0[b] ±2.2 | 20.6[a] ±1.9 | *** | ns |
| % VT[3] | 54.9 ±3.2 | 66.3 ±3.1 | ** | 74.2[a] ±3.2 | 48.1[c] ±2.8 | 59.5[b] ±3.7 | *** | ns |
| % R/D[3] | 8.7 ±1.7 | 18.8 ±3.4 | | 10.1 ±2.7 | 26.6 ±3.9 | 4.5 ±0.5 | | ** |
| Evenness[4] | 0.914 ±0.031 | 0.756 ±0.041 | ** | 0.690[b] ±0.063 | 0.917[a] ±0.024 | 0.898[a] ±0.030 | *** | ns |
| Biomass (gDM $m^{-2}$) | 131.4 ±6.4 | 188.4 ±9.3 | *** | 171.3[a] ±9.2 | 175.9[a] ±15.3 | 132.5[b] ±7.2 | ** | ns |
| CP[5] (%DM) | 13.3 ±0.3 | 12.2 ±0.4 | ** | 13.4[a] ±0.3 | 11.6[b] ±0.4 | 13.3[a] ±0.4 | *** | ns |
| NDF[6] (%DM) | 56.1 ±1.1 | 57.8 ±1.1 | † | 51.1[b] ±1.0 | 60.1[a] ±1.0 | 59.7[a] ±0.7 | *** | ns |

[a,b,c]Same-line values with different superscripts are significantly different at $p < 0.05$.
†$p < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
[1] Sward surface height (SSH).
[2] coefficient of variation (CV).
[3] Vegetative Short (VS, ≤4 cm), Vegetative Intermediate (VI, 5–8 cm), Vegetative Tall (VT, ≥9 cm), Reproductive and Dead sward (R/D).
[4] evenness of the distribution of highly grazed patches (VS + VI), vegetative tall patches (VT) and reproductive/dead patches (R/D) at plot level.
[5] crude protein (CP).
[6] neutral detergent fiber (NDF), dry matter (DM).

treatments (Table 1). Abundance of R/D patches was the highest at the moderate stocking rate during summer (39.2 ± 2.8% s.e *vs.* 14.0 ± 4.0% s.e. in highly grazed plots) (stocking rate × season, $p < 0.001$; Table 1); lower values were found in spring and autumn for both treatments ($p < 0.01$). Evenness of the distribution of highly-grazed patches (VS + VI), VT patches and R/D patches at plot scale was higher at high stocking rate than moderate stocking rate and higher in summer and autumn than in spring, but without any significant stocking rate x season interaction (Table 1).

Total herbage biomass was higher at the moderate stocking rate, with the lowest values found in autumn for both stocking rates (Table 1). Conversely, herbage quality was higher in highly grazed plots (Table 1). Herbage CP content was the lowest in summer whereas NDF content was higher during summer and autumn (Table 1). At both stocking rates, herbage biomass increased with patch-type height (VS: 46.0 ± 2.9, VI: 89.8 ± 3.8, VT: 174.6 ± 5.1, R/D: 226.5 ± 8.0 gDM $m^{-2}$s.e. on average), but the difference between VT and R/D patches was only significant for

**Table 2**
Daily foraging behaviour and diet selection (bite types defined according to vegetation stage and height) by horses according to stocking rate (High, 1.8 LU $ha^{-1}$ and Moderate, 1.1 LU $ha^{-1}$) and season (mean ± s.e.).

| | Stocking Rate (SR) | | | Season (S) | | | | SR × S |
|---|---|---|---|---|---|---|---|---|
| | H | M | p | spring | summer | autumn | p | p |
| Foraging behaviour | | | | | | | | |
| GT[1] (min) | 793 ±17 | 780 ±19 | ns | 725[c] ±12 | 796[b] ±24 | 838[a] ±20 | *** | ns |
| [2]B/S | 9.2 ±0.4 | 7.8 ±0.4 | * | 7.6[b] ±0.5 | 8.7[a] ±0.6 | 9.2[a] ±0.4 | * | ns |
| Diet digestibility (%DM) | 59.4 ±0.4 | 58.6 ±0.5 | ns | 61.1[a] ±0.4 | 57.7[c] ±0.5 | 58.9[b] ±0.5 | ** | ns |
| Jacobs' indices | | | | | | | | |
| VS[3] | +0.01 ±0.07 | **+0.26 ±0.07** | ** | −0.15[b] ±0.11 | **+0.38**[a] **±0.07** | **+0.18**[a] **±0.06** | *** | ns |
| VI[3] | **+0.16 ±0.05** | **+0.39 ±0.04** | *** | **+0.19**[b] **±0.08** | **+0.36**[a] **±0.05** | **+0.27**[a,b] **±0.05** | * | ns |
| VT[3] | *−0.11 ±0.05* | −0.09† ±0.05 | ns | +0.03[a] ±0.06 | −0.08[a] ±0.06 | *−0.25*[b] *±0.04* | ** | ns |
| Repro/Dead[3] | *−0.35 ±0.08* | *−0.52 ±0.07* | † | *−0.27*[a] *±0.12* | *−0.35*[a] *±0.07* | *−0.67*[b] *±0.05* | ** | ns |

[a,b]Same-line values within line with different superscripts are significantly different at $p < 0.05$.
Bold characters indicate items selected for and italics indicate items avoided at $p < 0.05$.
†$p < 0.1$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
[1] GT: grazing time per day.
[2] B/S: bite per step.
[3] Vegetative Short (VS, ≤4 cm), Vegetative Intermediate (VI, 5–8 cm), Vegetative Tall (VT, ≥9 cm), Reproductive and Dead sward (Repro/Dead).

summer season (VT: 176.6 ± 10.3 *vs.* R/D: 243.1 ± 16.0 gDM $m^{-2}$ s.e.; sward type × season, $p < 0.01$). Patch CP content decreased as sward height increased (VS: 14.5 ± 0.3, VI: 13.6 ± 0.3, VT: 12.6 ± 0.2, R/D: 11.6 ± 0.3%DM s.e. on average) ($p < 0.001$); in highly grazed plots, there were no significant differences between CP content of short (14.4 ± 0.4% s.e.) and intermediate patches (14.1 ± 0.4% s.e.). Herbage NDF content was higher in VT and R/D patches (58.1 ± 0.6% s.e. on average) than in shorter patches (55.3 ± 0.5% s.e.) ($p < 0.01$).

### 3.2. Foraging behaviour and animal performance

Horse daily grazing time did not differ between stocking rates and increased along the grazing season (Table 2). Horses spent a large part of their feeding time on VT patches, especially at the moderate stocking rate. The use of these patches was highest in spring at both stocking rates (proportion in the diet: 0.815 ± 0.025 *vs.* 0.719 ± 0.020 s.e. at the moderate and high stocking rate, respectively, $p < 0.001$) but at the moderate stocking rate it was lowest in summer (0.481 ± 0.028 s.e) whereas at the high stocking rate it was lower in summer and autumn (0.382 ± 0.022 s.e.) (stocking rate × season, $p < 0.01$). Horses made very little use of R/D patches (0.081 ± 0.002 and 0.050 ± 0.010 s.e. at the moderate and high stocking rate, $p < 0.05$) with the highest values found in summer for both treatments (0.132 ± 0.020 s.e) ($p < 0.01$).

Horses strongly selected VI patches with an even more pronounced selection at the moderate stocking rate ($p < 0.001$) and during summer ($p < 0.05$) (Table 2). Horses grazed at moderate stocking rate also selected for VS patches in summer and autumn (Table 2). Analysis of bite botanical composition revealed that horses selected VI and VS patches when they were dominated by grasses (SIG) or legumes (L, mainly clover; Fig. 1) whereas patches dominated by forbs (SIF) were avoided at the high stocking rate and used proportionally to their availability at the moderate stocking rate (Fig. 1). VT patches were avoided in autumn at both stocking rates, ($p < 0.01$; Table 2). This was mainly due to a strong rejection of tall forbs (TF) by horses while tall grass patches (TPG) were selected (Fig. 1). Overall, horses avoided forbs (F, Fig. 1) and R/D patches (Table 2).

**Table 3**
Mean percentage cover of dominant plant species at plot scale in 2009 after four years of contrasted stocking rates (High, 1.8 LU $ha^{-1}$ *vs.* Moderate, 1.1 LU $ha^{-1}$).

| High stocking rate | | Moderate stocking rate | |
|---|---|---|---|
| *Poa trivialis* | 18.5 | *Dactylis glomerata* | 16.6 |
| *Trifolium repens* | 15.2 | *Poa trivialis* | 15.6 |
| *Agrostis tenuis* | 10.8 | *Holcus lanatus* | 11.9 |
| *Holcus lanatus* | 9.1 | *Ranunculus repens* | 8.6 |
| *Taraxacum officinalis* | 7.2 | *Trifolium repens* | 6.0 |
| *Ranunculus repens* | 6.7 | *Festuca arundinacea* | 4.8 |
| *Dactylis glomerata* | 6.6 | *Agrostis tenuis* | 3.7 |
| *Lolium perenne* | 4.5 | *Plantago lanceolata* | 3.4 |
| *Stellaria graminaea* | 4.0 | *Lolium perenne* | 3.3 |
| *Poa pratensis* | 2.7 | *Poa pratensis* | 3.0 |

The lower bite-to-step ratio ($p < 0.05$, Table 2) found at moderate compared to high stocking rate confirmed that horses grazed more selectively as grazing intensity decreased. Horse selectivity also decreased along the grazing season (Table 2). Reduction in grazing intensity did not significantly affect the diet digestibility (Table 2), which was highest in spring, lowest in summer and intermediate in autumn (Table 2).

Average horse daily liveweight gain was significantly higher at moderate stocking rate than high stocking rate between April and the end of July (441 ± 45 g $animal^{-1}$ $d^{-1}$ *vs.* 317 ± 43 g $animal^{-1}$ $d^{-1}$ s.e. at the high stocking rate; stocking rate, $p < 0.05$) but was not significantly different between treatments during autumn (343 ± 65 g $animal^{-1}$ $d^{-1}$ and 271 ± 62 g $animal^{-1}$ $d^{-1}$ s.e. at the moderate and high stocking rate, respectively; stocking rate, $p = 0.378$).

### 3.3. Plant diversity

The abundance of dominant plant species after four years of contrasted stocking rates is reported in Table 3. There was not stocking rate effect on plant species richness or evenness of their

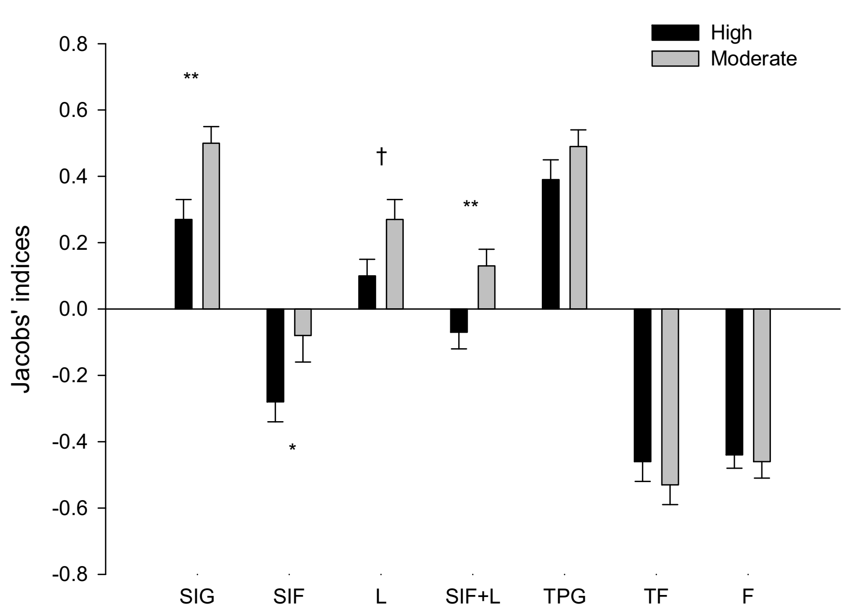


**Fig. 1.** Diet selection by horses in daytime according to stocking rate (High, 1.8 LU $ha^{-1}$ and Moderate, 1.1 LU $ha^{-1}$) (mean ± s.e.) (stocking rate × season was not significant). Bites composed of Tall Pure Grass (TPG, ≥9 cm) and bites dominated by Short and Intermediate Grass (SIG, ≤8 cm), Short and Intermediate Forbs (SIF, ≤8 cm), Tall Forbs (TF, ≥9 cm), Legumes (L) or Forbs (F) irrespective of sward surface height. Bites dominated by Short and Intermediate Forbs were also pooled together with bites dominated by Legumes in a synthetic bite type SIF + L.
†p < 0.1, *p < 0.05, **p < 0.01
Jacobs' indices were significantly different from zero (Student's *t*-test, $p < 0.05$) apart for SIF at Moderate stocking rate and for SIF + L at High stocking rate.

**Table 4**
Plant species richness (mean ± s.e.), evenness of plant species distribution, and abundance of functional types after four years of contrasted stocking rates (High, 1.8 LU $ha^{-1}$ *vs.* Moderate, 1.1 LU $ha^{-1}$).[1]

| | Stocking rate | | |
|---|---|---|---|
| | High | Moderate | p |
| Species richness | 27.3 ±0.3 | 28.3 ±2.9 | ns |
| Evenness | 0.749 ±0.019 | 0.782 ±0.021 | ns |
| % Grasses | 55.3 ±6.1 | 68.7 ±1.2 | ns |
| % Legumes | 15.7 ±1.9 | 6.8 ±1.9 | ns |
| Incl. competitive types (C-S-R and C)[2] | 15.2 ±2.1 | 6.0 ±1.2 | * |
| % Forbs | 29.3 ±4.8 | 24.3 ±1.3 | ns |
| Incl. ruderal types (R and C-R)[2] | 15.2 ±1.2 | 13.3 ±3.1 | † |

† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
[1] For each variable, data registered in 2006 was considered as a covariate in the model.
[2] In the model by Grime et al. (1988), competitive perennial species (C-S-R and C types) are characterized by a rapid leaf turnover. Ruderal species (the annual R type and the most competitive C-R type) are fast-growing and capable of colonizing gaps resulting from trampling and summer drought.

**Table 5**
Overall abundance of ground-dwelling arthropods (log (N + 1) individuals per plot) and abundance of selected groups under the two stocking rate treatments (High, 1.8 LU $ha^{-1}$ and Moderate, 1.1 LU $ha^{-1}$).

| | Stocking rate (SR) | | | Year (Y) | SR × Y |
|---|---|---|---|---|---|
| | High | Moderate | p | p | p |
| TOTAL | 2.74 ±0.07 | 2.76 ±0.06 | ns | *** | ns |
| Aranaeae | 2.29 ±0.09 | 2.32 ±0.11 | ns | *** | ns |
| Coleoptera | | | | | |
| Carabidae | 2.03 ±0.06 | 2.13 ±0.05 | † | ** | ns |
| Chrysomelidae | 0.62 ±0.10 | 0.53 ±0.07 | ns | ns | ns |
| Coccinellidae | 0.45 ±0.13 | 0.46 ±0.12 | ns | *** | ns |
| Curculionidae | 0.76 ±0.07 | 0.54 ±0.06 | * | † | ns |
| Elateridae | 0.83 ±0.06 | 1.00 ±0.06 | ** | ns | ns |
| Staphylinidae | 1.60 ±0.10 | 1.67 ±0.06 | ns | ** | ns |
| Diplopoda | 0.01 ±0.01 | 0.15 ±0.07 | * | ns | ns |

† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

distribution at plot scale (Table 4). Competitive *Trifolium repens* was 2.5-fold more abundant after four years at the higher stocking rate ($p < 0.05$). Ruderal forb species that can colonize gaps (among which *Taraxacum officinalis*) tended to be more abundant in highly grazed plots ($p = 0.085$, Tables 3 and 4). We found no significant differences for abundance of overall grasses or grass species classified according to Grime et al. (1988) (Table 4).

### 3.4. Insect diversity

Insect abundance and species richness were highly variable between experimental years due to fluctuating climatic conditions, especially precipitations (Tables 5, 6 and 7). Total number of ground-dwelling arthropods per plot (626 individuals per year on average) was unaffected by stocking rate (Table 5). However, Diplopoda ($p < 0.05$) and among the Coleoptera, Elateridae ($p < 0.01$) and Carabidae ($p = 0.06$) were more abundant at moderate stocking rate whereas Curculionidae were more abundant at high stocking rate ($p < 0.05$) (Table 5). Stocking rate did not significantly affect Carabidae species richness (22 species per plot and per year on average; Table 6). However, a reduction of stocking rate benefited Carabidae from tall grasslands (including *Ophonus rufipes* and *Poecilus versicolor*), which significantly increased abundance ($p < 0.05$, Table 6). For grasshoppers, neither species richness (8 species per plot and per year) nor abundance (95 individuals) was significantly affected by stocking rate (Table 7). However, grasshoppers from tall grasslands (including *Chortippus parallellus, Chortippus albomarginatus, Stethophyma grossum, Conocephalus fuscus, Metrioptera roeselli*) were more abundant in plots grazed at a moderate stocking rate ($p < 0.05$, Table 7).

## 4. Discussion

Horses exhibited a typical patch grazing pattern (Adler et al., 2001), selectively grazing highly-nutritive patches below 8 cm in summer and autumn. They avoided tall vegetative patches during autumn and reproductive/dead patches along the whole grazing season. Edouard et al. (2010) showed that digestible protein is the

**Table 6**
Species richness and abundance of Carabidae (log N individuals per plot) under the two stocking rate treatments (High, 1.8 LU $ha^{-1}$ and Moderate, 1.1 LU $ha^{-1}$).

| | Stocking rate (SR) | | | Year (Y) | SR × Y |
|---|---|---|---|---|---|
| | High | Moderate | p | p | p |
| Species richness | 21.2 ±1.6 | 22.4 ±1.6 | ns | *** | ns |
| Abundance[1] | | | | | |
| short grassland | 0.84 ±0.09 | 0.81 ±0.14 | ns | * | ns |
| tall grassland | 1.72 ±0.05 | 1.83 ±0.03 | * | ns | ns |
| generalists and undetermined | 1.62 ±0.10 | 1.72 ±0.08 | ns | ** | ns |

† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
[1] Abundance data were analyzed in relation to habitat affinity: i.e. short grassland (<10 cm) or tall grassland (>10 cm). Abundance of wide-ranging generalist ground beetles and species for which habitat affinity is undetermined represented 44.3% of the total number of individuals.

**Table 7**
Species richness and abundance of grasshoppers (log (N + 1) individuals per plot) under the two stocking rate treatments (High, 1.8 LU $ha^{-1}$ and Moderate, 1.1 LU $ha^{-1}$).

| | Stocking rate (SR) | | | Year (Y) | SR × Y |
|---|---|---|---|---|---|
| | High | Moderate | p | p | p |
| Species richness | 7.7 ±0.7 | 8.6 ±0.8 | ns | *** | ns |
| Abundance[1] | 1.75 ±0.15 | 1.79 ±0.17 | ns | *** | ns |
| short grassland | 1.09 ±0.15 | 0.86 ±0.16 | ns | *** | ns |
| tall grassland | 1.56 ±0.19 | 1.73 ±0.17 | * | *** | ns |

† $p < 0.1$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
[1] Abundance data were analyzed in relation to habitat affinity: i.e. short grassland (<10 cm) or tall grassland (>10 cm).

best predictor of horse grazing selection pattern. Our results are in line with this conclusion since crude protein content was the highest in short and intermediate patches. Horses selected grasses, which confirms previous observations in semi-natural grasslands (Cornelissen and Vulink, 2015; Duncan, 1983; Gordon, 1989) and, to a lesser extent, legumes. Horses avoided forbs, especially in tall patches, which could be explained by their inability to detoxify forb secondary metabolites (Duncan, 1992).

Horse selection for short and intermediate vegetative patches was even more pronounced in plots grazed at a moderate stocking rate where sward height and herbage biomass were higher. In these plots, horses also had a lower bite-to-step ratio that confirms they grazed more selectively. This allowed them to maintain diet digestibility at the same level as in highly grazed plots while average herbage quality was lower. Similarly, cattle decreased bite-to-step ratio as stocking rate decreased (Dumont et al., 2007a), which allowed them to maintain diet quality in semi-natural grasslands (Dumont et al., 2007a; Schlegel et al., 2000). In mesophile grasslands, selection for short patches by cattle was also more pronounced at a lenient stocking rate, which represents a key mechanism in the creation of pasture heterogeneity *via* the maintenance of leaves in an early phenological state and a reduction of senescent material (Dumont et al., 2007b).

At both stocking rates, horses increased their grazing time from 12 to 14 h over the grazing season, probably to compensate for lower herbage availability and digestibility (Arnold, 1984). Liveweight gains per animal matched horse growth recommendations in both treatments (INRA, 2015) and were significantly higher during spring and early summer in plots grazed at a moderate stocking rate than at a high stocking rate. As daily grazing time and diet digestibility did not differ between treatments, the higher performance by horses under moderate grazing intensity probably resulted from higher herbage biomass (+64 gDM $m^{-2}$ on average in spring and summer). In autumn, the difference in herbage biomass was lower (+43 gDM $m^{-2}$ at the moderate stocking rate) and maybe not enough to affect nutrient intake rate; liveweight gains per animal no longer differed between treatments.

The limited use of flowering plants by horses may benefit grassland diversity (Marion et al., 2010; Nolte et al., 2013; Stewart and Pullin, 2008) as well as the diversity of flower-visiting insects (Öckinger et al., 2006). Despite their more pronounced selection of short and intermediate vegetative patches at the moderate stocking rate, horses created and maintained a strong sward structural heterogeneity in both treatments. Hence, the coefficient of variation of sward surface height did not significantly differ between stocking rate treatments, and the evenness of patch distribution was even higher at the high stocking rate. In line with our hypothesis, decreasing stocking rate did not increase sward structural heterogeneity in horse-grazed pastures, which is also consistent with recent observations by Nolte et al. (2014) in grasslands of high nature value. Stability of short grazed patches within a mosaic of ungrazed tall vegetation patches has previously been evidenced in the same experimental plots with a stable pattern from the summer peak of biomass to the autumn, and also between two successive years at both stocking rates (Dumont et al., 2012). Patch stability was related to local abundance of legumes and forbs, which is consistent with our observations that horses selected legumes at both stocking rates and selected short and intermediate patches dominated by forbs and legumes under lenient grazing. Patch stability can favour functional diversity in pastures grazed by cattle at a low stocking rate (Bloor and Pottier, 2014; Dumont et al., 2011). Very few studies have analyzed the consequences of contrasted grazing management on vegetation diversity in horse-grazed pastures (Nolte et al., 2013Magnusson and Magnusson, 1990; Nolte et al., 2013). Here, the two stocking rates resulted in divergent dynamics of legumes, which increased from 4 to 16% of plot area at the high stocking rate but remained constant at around 7% in taller swards grazed at the moderate stocking rate. Plant species richness was unaffected by stocking rate after 4 years. A similar conclusion was drawn in moist grasslands of the Poitevin Marsh (Mid-West France, close to the Atlantic coast), where 5 years of horse grazing under contrasting stocking rates (0.5–1.5LU $ha^{-1}$) left plant species richness unchanged (Amiaud, 1998). These results should, however be confirmed in the longer term, as shifts in species richness are known to be slower than shifts in functional type abundance. In Iceland, Magnússon and Magnússon (1990) reported shifts in species abundance and species richness in horse-grazed pastures under contrasted stocking rates after 8 years. However, these shifts were driven by overgrazing at the highest stocking rate (average height < 5 cm, 7% of bare soil) resulting in the development of new species, mainly bryophytes.

Consistent with the lack of effect of stocking rate on sward heterogeneity, we did not record any benefit of decreasing grazing intensity on insect species richness. The higher abundance of Carabidae and grasshoppers from tall grasslands in moderate-stocking-rate plots can be explained by a higher vegetation biomass. In the case of predatory Carabidae, it could also reflect increased abundance of prey in the tall vegetation of moderately-grazed plots as well as a more favourable microclimate (Dennis et al., 1998, 2004; Tscharntke and Greiler, 1995). Horse faeces in tall grass areas (Loucougaray et al., 2004; Ödberg and Francis-Smith, 1977) may also have attracted dung beetles which, in turn, would attract their predators. For grasshoppers, our results are in line with numerous studies that demonstrate the benefit of lesser disturbance in plots grazed at a moderate stocking rate (Cherril and Brown, 1992; Dumont et al., 2009; Hutchinson and King, 1980; Kati et al., 2012; Kruess and Tscharntke, 2002; Marini et al., 2009; O'Neill et al., 2003; Tscharntke and Greiler, 1995; Wallis de Vries et al., 2007). Furthermore, the stability of tall patches in successive years (Dumont et al., 2012) could limit the risk of oviposition sites being destroyed, and provides forage and shelter for the next generations (Jerrentrup et al., 2014). At the patch scale, Holmquist et al. (2014) reported a lower diversity of arthropods in lawns that are repeatedly grazed by horses compared to ungrazed patches. Two more groups that benefited from a decrease in grazing intensity by horses were Elateridae, whose adults are phytophagous, and Diplopoda that rely on dead biomass (Hutchinson and King, 1980). A high stocking rate did however benefit Curculionidae that largely depend on clover (Dennis et al., 2004). No effects of grazing intensity were found on spider abundance, which confirms previous observations in semi-natural grasslands (Dennis et al., 2001, 2008; Scohier and Dumont, 2012), though spider abundance sometimes declined in intensively-grazed pastures (Hutchinson and King, 1980).

This is the first study to simultaneously assess the influence of grazing management on horse foraging behaviour, horse performances and pasture biodiversity in mesophile grasslands. Horses were able to maintain performances in plots grazed at a lenient stocking rate by selectively grazing on short vegetative patches of high nutritive value. In line with our hypothesis, decreasing grazing intensity did not increase sward heterogeneity as the horses created stable vegetation patches in both treatments. Floristic and arthropod diversity remained unaffected by stocking rate over the course of this four-year study. Extensive management only increased the abundance of some insect taxa that rely on tall vegetation, and did not benefit all species from the local pool. Conversely, decreasing stocking rate will have a strong direct effect on number of horses per unit area that needs to be trade-off relative to its relatively weak benefits on biodiversity. Based on the evidence of this study, a more sustainable way of preserving floristic and arthropod diversity in horse farming systems would be to preserve a mosaic of grasslands under contrasted management regimes at farm level.

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