Comparing the effects of horse grazing alone or with cattle on horse parasitism and vegetation use in a mesophile pasture

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Abstract
Mixing different herbivore species is assumed to increase vegetation use because of the complementarity of their feeding choices and to reduce animal parasite burden through the dilution effect. Here, we compare the effects of mixed horse-cattle grazing and monospecific horse grazing (1.4 LU/ha) on animal foraging behaviour, sward characteristics and horse parasitism in a mesophile grassland of central France. In both treatments, animals were stocked alternately on two subplots with rotation lengths between 15 and 21 days according to season. The horses quickly acclimated to the cattle, and very few agonistic interactions were observed between them. All the horses selected short (≤4 cm) and intermediate (5–8 cm) high-quality regrowths and avoided reproductive and dead herbage areas contaminated by their faeces as a consequence of their latrine behaviour. Cattle, which are more constrained by plant height, selected intermediate and tall vegetative swards. However, the alternate stocking of animals also provided them with high-quality regrowth on the shortest patches. Consequently, the cattle used these short patches proportionally to their availability while avoiding reproductive and dead herbage areas. We assume this limited their consumption of infective cyathostome larvae, which are concentrated close to horse dung. Moreover, co-grazing horses and cattle did not reduce sward structural heterogeneity and thus did not enhance overall herbage quality. We conclude that rather than considering mixed grazing as a turn-key solution, its management needs to be adapted to support the complementarity of horse and cattle dietary choices and thus provide the expected benefits of multi-species grazing.

KEYWORDS
agroecology, diet selection, dilution effect, herbage quality, mixed grazing, strongyle

INTRODUCTION
The incorporation of diversity into animal production systems, which has been assumed to increase their multiperformance and strengthen their resilience, is a key principle of agroecology (Dumont et al., 2013; Martin et al., 2020). Among ruminants, several studies have demonstrated that mixing different species in pastures can increase vegetation use as a result of the complementarity of dietary choices...
(Animut & Goetsch, 2008; Cucullo-Hilario et al., 2018; Nolan & Connolly, 1989). Under mixed grazing, residual biomass is thus reduced, and the feeding value of the grazed forage is enhanced (Nolan et al., 1999; Sehested et al., 2004). Moreover, because of the high specificity of gastrointestinal nematodes for their host, a decrease in the egg excretion by small ruminants has been reported when they graze with cattle as the result of a dilution effect (sheep: [Marley et al., 2006; Rocha et al., 2008], goats: [Mahieu, 2013]). A meta-analysis quantified the benefits of mixed grazing between sheep and cattle on the individual performances of sheep, which grew 14.5 g/d faster than under monospecific grazing, and on the meat production per hectare (d’Alexis et al., 2014). Consistently, lamb liveweight gain increased by 17% in another mixed versus monospecific grazing comparison (Jerrentrup et al., 2020).

Among large domestic herbivores, horses, with their two sets of incisors, create the most stable short grass patches within a matrix of tall sward areas, and this occurs even at high stocking densities (Fleurance et al., 2001; Fleurance et al., 2016; Ödberg & Francis-Smith, 1977). This selection for short patches has long been interpreted as an anti-parasite strategy (Taylor, 1954), as horses usually perform latrine behaviour, that is, they deposit their faeces in tall sward areas that they then avoid. More recent studies have suggested that horse selection of short grass patches could also have a nutritional basis, as their digestible protein intake rate was the highest in short vegetative patches (Edouard et al., 2010; Fleurance et al., 2005). Cattle are more constrained than horses by sward surface height (SSH) (Mandaluniz et al., 2011; Ménard et al., 2002) and are thus unable to graze on the shortest patches. Conversely, cattle can use dicotyledons to a greater extent because they are better able than horses to detoxify forb secondary metabolites (Ménard et al., 2002; Nolte et al., 2017). This complementarity in the dietary choices of cattle and horses is assumed to enhance the overall use of pastures and as a consequence to improve herbage quality.

Most studies on mixed grazing between horses and cattle were conducted in semi-natural habitats (e.g., wetlands, heathlands, and dune grasslands), especially with regard to the ability of animals to limit their woody encroachment (Cornelissen & Vulink, 2015; Ménard et al., 2002; Rupprecht et al., 2016) and preserve biodiversity (Henning et al., 2017; Loucougaray et al., 2004; Rupprecht et al., 2016). Similar information would be particularly useful on mesophile grasslands, as these more productive grasslands provide most of the feed of saddle horses used for sport and leisure. Securing animal performances while maximizing the use of grazed forages is a challenge for horse breeders (Morhain, 2011). So far, no study has analysed if the complementarity of feeding choices between horses and cattle grazing mesophile pastures may improve the use and quality of herbage compared to situations where horses graze alone. Moreover, grazing horses are infected by gastro-intestinal nematodes of the Strongylidae family that encompasses members of the Cyathostominae and Strongylinae subfamilies (Lichtenfels et al., 2008). This latter subfamily includes Strongylus spp. that have the highest mortality rate but whose prevalence has dropped thanks to the use of modern anthelmintic drugs (Herd, 1990). Patent infection by cyathostomin leads to milder symptoms including unthriftness and growth retardation in younger animals, but the massive emergence of infective larvae from the caeco-colic mucosa can cause to a so-called ‘larval cyathostomosis’ leading to sudden diarrhoea, oedema and weight loss (Giles et al., 1985). This seasonal syndrome is the main cause of parasite-mediated death in young horses (Salle et al., 2020).

Strongyle control in horses has long been reliant on the systematic and frequent use of anthelmintic drugs but the rising occurrence of anthelmintic resistance in many countries (Nielsen et al., 2018; Salle et al., 2017) and concerns about the environmental side effects of medications (Verdu et al., 2018) urge the development of alternative options. As gastro-intestinal nematodes exhibit a high specificity for either horses or cattle, mixed grazing is assumed to reduce horse parasite burden. Consistently, saddle horses grazing with cattle in mixed farms had 50% fewer strongyle eggs excreted in their faeces than horses grazing alone in specialized systems (Forteauf et al., 2020). This first evidence of the dilution effect in horses grazing with cattle is an encouraging perspective, but there is a need to further analyse how pasture and herd management can modulate horse contamination.

In this three-year study, we investigated the benefits of simultaneous grazing of saddle horses and beef cattle relative to horse grazing alone at the same stocking density in a hill-range mesophile grassland of central France. We simultaneously analysed the influence of grazing management (i.e., mixed grazing vs. monospecific horse grazing) on animal foraging behaviour, sward characteristics, and horse parasite infection and performance. We also recorded potential agonistic interactions between species and analysed the influence of co-grazing with cattle on the frequency of vigilant postures in the horses. We hypothesized that the strong selection of short high-quality patches by horses would constrain cattle to switch onto areas with taller swards, which horses avoid. Mixed grazing between the two species is therefore expected to homogenize sward structure and enhance sward quality compared to horses grazing alone. We also tested the prediction that cattle would reduce the level of horse infection by strongyle larvae by grazing close to horse latrine areas. Consequently, horse daily liveweight gain was expected to be higher under mixed grazing.

2 | MATERIALS AND METHODS

The study was conducted at the French Horse and Riding Institute experimental farm (Chamberet, 01°43′14″–45′35″03″, 440 m a.s.l.) in accordance with the European Union Directive 2010/63/EU. The experimental procedure was reviewed by the local animal care and use committee (Comité des Utilisateurs de la Station Expérimentale de Chamberet). It received approval from the local ethics committee (Comité Régional d’Ethique pour l’Expérimentation Animale du Limousin – C2EA-33) and the French Ministry of Research under protocol number #18070-2018121416354890v3.
2.1 Experimental design

Our study was carried out in a mesophile grassland over three grazing seasons, from 2015 to 2017. The climate of the experimental site is oceanic, and the soil is an episkeletic podzol (http://eusoils.jrc.ec.europa.eu/). The average annual precipitation ranged from 1023 mm in 2015 to 1293 mm in 2016. Large variation between years was observed in June (66–108 mm), July (13–88 mm) and August (18–180 mm). This grassland had been intensively managed under rotational grazing by horses for many years, which explains its relatively low botanical diversity; 47 plant species were recorded over the whole area (16.2 ha) in 2015. Two modalities of grazing were compared at the same stocking density during each grazing season (1.4 LU/ha, with 1 LU = 600 kg liveweight): monospecific grazing with four saddle horses and mixed grazing with two saddle horses and three dehorned heifers grazing simultaneously. Six 2.7-ha plots were created within the grassland so that each treatment could be replicated three times in a randomized block design. In each plot, all animals (i.e., horses or horses and cattle) were stocked alternately on two subplots of 1.35 ha (A then B). Animals were turned out to the grassland in mid-April and first grazed subplot A for 1 week. Then, they switched between subplots A and B every 21 days from the end of April to mid-July and every 15 days from mid-July to the end of the grazing season. All the animals were removed from the plots in mid-October, except in 2016, when drought led us to take them out 2 weeks earlier (i.e., after grazing subplot A for the last time).

Anglo-Arab and French saddle horses (24 months old; 435.5 ± 5.8 kg at the start of the grazing season) (mean ± SE) and Limousin heifers (17 months old; 407.1 ± 9.1 at the start of the grazing season) were used each year. The six groups of animals were balanced in April for body size by species (measured with a portable livestock scale) and for breed and sex in horses. The horses were drenched with 6.6 mg per kg bodyweight pyrantel (Strongid®, Zoetis, France) before pasture turn-out to reset strongyle egg excretion in every group. In 2017, two individuals in the horse-only group were diagnosed with a patent cestode infection and were administered an ivermectin/praziquantel combination (Eqvalan Duo®, 1.29 g per 100 kg bodyweight, Boehringer Ingelheim Animal Health, France). Reinfection and the associated egg reappearance period therefore took longer for these two individuals than for their pyrantel-treated counterparts. Nevertheless, the observations from these individuals were kept to limit the reduction in statistical power and because this egg excretion reduction in the horse-only group was conservative relative to our hypothesis, that is, parasite dilution in the mixed group.

2.2 Animal measurements

Observations of horse and cattle foraging behaviours were carried out in each subplot A at three periods during each grazing season: spring (first half of May), before the major plant components flowered, summer (second half of July), when sward heterogeneity is expected to be at a maximum, and early autumn (end of September) to account for the cumulative effects of the grazing treatments.

In each period, the daily grazing time of the horses was recorded over 24 h using Ethosys collars (Greenway System GmbH, Frankfurt, Germany; [Scheibe et al., 1998]). The horses were acclimated to the collars for 1 week before the experiment began.

Dietary choices were measured by scan sampling the activity of all the animals within each group at 10-min intervals, with one observation day (from dawn to dusk) per subplot A and per season. Each day, two persons simultaneously observed the animals while they were grazing in subplot A in each treatment; therefore, 3 days per season were required to carry out the observations in the three blocks. The horses were identified by a number painted on both sides, and heifers were identified by their ear marks. An animal 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 to record one selected bite. The observer decided beforehand which bite to record once he was close enough to the animal (i.e., the fifth one) to avoid bias due to picking bites that were more clearly visible (Fleurance et al., 2016). This required animals to be trained for 1 week before measurements so that they would readily accept observers in close proximity during grazing.

The bite types were recorded according to (i) sward height type, taking into account previous observations of horses and cattle in less fertile swards (Ménard et al., 2002), that is, vegetative short (VS, ≤4 cm), vegetative intermediate (VI, 5–8 cm), vegetative tall (VT, ≥9 cm), reproductive (repro) and dead herbage; (ii) dominant botanical family (grasses, legumes or forbs). We also recorded the presence of horse dung within 1 m around the bite location, as most of the small strongyle infective larvae move less than 1 m horizontally from horse dung (Fleurance et al., 2007). Diet selection, defined as the proportion of a sward type in the diet relative to its proportion in the plot, was quantified by calculating selectivity indexes (Si) for each sward type using (Jacobs, 1974) modification of Ivlev’s selectivity index:

\[ S_i = \frac{(c_i - a_i)}{(c_i + a_i - 2ca_i)} \]

where \( c_i \) is the proportion (between 0 and 1) of component \( i \) in the diet and \( a_i \) is the proportion of component \( i \) in the subplot. The Jacobs’ index was chosen for its low sensitivity to variations in the relative abundance of plant components; moreover, it was calculated for bite types whose proportion in the subplot was higher than 0.02 to limit these variations. For each bite type \( c_i \), data for individual animals within each species were first aggregated per day and per subplot A as their foraging behaviour was not independent. They were then linked to the relative abundance of this bite type in subplot A. \( S_i \) varies from -1 (never used) to +1 (exclusively used), with negative and positive values indicating avoidance and selection, respectively, and 0 indicating that a sward component is used in proportion to its availability.
Digestibility of the horses’ diet (% dry matter, DM) in monospecific or mixed plots was estimated from faecal crude protein (CP) content according to the equation of (Mesochina et al., 1998):

\[
\text{diet digestibility} = 73.4 - (178.72/\text{faecal CP content})
\]

where faecal CP content is expressed in %DM by the Kjeldahl method (N × 6.25). This equation is appropriate when herbage CP content is higher than 7 g/kg DM, which limits nitrogen recycling by horses (Mesochina et al., 1998); this was always the case in the present experiment. Three samples of fresh faeces from each horse were collected on the ground immediately after their emission in the morning, at midday and in the evening of the observation day in each subplot A. The samples were then mixed by individual and dried at 60°C to a constant weight. The faecal nitrogen content was determined by the Dumas method and then estimated with the Kjeldahl method using the following equation: N Kjeldahl (% DM) = 0.9509 × N Dumas (%DM) – 0.0156 (\(r^2 = 0.9983, N = 29\), range N Dumas: 1.14–3.55).

The parasite infection level in horses was estimated from individual faecal egg counts (FECs) measured 1 day a month from pasture turn-out to housing. The FECs were carried out on faeces taken from the horse rectums at the same time of the day for all animals. The FECs were determined from 5 g of faecal material diluted in 70 ml of a dense NaCl solution (\(d = 1.2\)). Eggs were counted across two grids of a McMaster slide (detection limit of 50 eggs/g) under light microscopy at 150× magnification.

Observations of social behaviour between the horses and cattle were conducted daily between 8:30 AM and 4:30 PM for 3 weeks in each month and each year. The vigilant postures of horses towards heifers were observed using 20 scan samplings distributed throughout the day. Agonistic behaviours between the two species (biting, kicking, head threatening, kick threatening, rearing and pursuits) were recorded using a focal sampling method, with 20 timeslots of 1 minute divided equally throughout the day. These behavioural observation methods have previously been used in horses (Lansade et al., 2018). Moreover, the horses and heifers were observed throughout the experiment to monitor for the presence of potential injuries.

The animals were weighed (on two successive days) at the beginning and at the end of the grazing season to calculate daily liveweight gains.

### 2.3 Sward measurements

SSH was measured each time the animals entered and were turned out from a subplot. The measurement levels were taken on parallel transects covering the entire subplot (approximately 200 sample points per subplot) at the first place where a stick contacted the sward surface. The SSH coefficient of variation (CV) was used as an indicator of sward heterogeneity (Eschen et al., 2012; Fleurance et al., 2016).

As subplots B were not grazed the same number of times each year due to climatic conditions (see section 2.1), the influence of grazing management and date on sward structure was studied from measurements performed in subplots A only. We also estimated the sward regrowth below 4 cm in VS patches (in mm/day) between two consecutive stocking periods of horses and cattle in subplots A of the mixed treatment as:

\[
\text{Sward regrowth below 4cm} = \frac{\text{VS mean SSH at the new entrance in A} - \text{VS mean SSH at the previous removal of A}}{\text{number of days between the removal of animals and their new entrance}}
\]

Sward regrowth ≤4 cm = (VS mean SSH at the new entrance in A – VS mean SSH at the previous removal of A)/number of days between the removal of animals and their new entrance with VS mean SSH calculated from the sward heights ≤4 cm in the subplot A.

The day before or the day after dietary choice measurements, we recorded at 400 sample points in each subplot A: sward height type, dominant botanical family and the presence of horse dung within a radius of 1 m. We also determined herbage biomass separately for VS, VI, VT and reproductive swards by randomly cutting six 0.5 m² (10 cm × 5 m-long) strips to ground level in each of these four sward height types. The quality of the herbage offered was assessed for each sward height type from three biomass samples that were selected randomly. The samples were dried at 60°C to a constant weight and analysed for CP (Dumas method N × 6.25) and neutral detergent fibre content (NDF, according to the method of Van Soest et al. [1991]). The mean biomass and herbage quality were then estimated from the proportion of each sward height type (i.e., the number of times that sward height type i was encountered in subplot A/the total number of sample points in subplot A), which was multiplied by the herbage biomass and quality of each sward height type.

The botanical diversity was measured in June 2015 and 2017 on three 25-m-long fixed transects per subplot A and B (i.e., six transects per plot), with one sample location every metre. At each sample location, six points (from zero, absence; to six, exclusive presence) were distributed among the different plant species. The specific contribution of each plant species i at the transect scale was therefore (\(C_{Oi}\) in %):

\[
C_{Oi} = \frac{\text{total number of points awarded to species } i}{\text{total number of presences of species } i} \times 100
\]

The occurrence of each plant species i at the transect scale (\(O_{Oi}\) in %) was estimated based on its presence at each sample point.

\[
O_{Oi} = \frac{\text{total number of presences of species } i}{\text{total number of presences of all species}} \times 100
\]

Plant identification followed (Tutin et al., 1964) and, for Festuca species, (Kerguélen & Plonka, 1989). The Shannon index was used to calculate the evenness of the plant species distribution on each transect. The plant species were then split into grasses, legumes and forbs.

Pasture contamination by strongyle infective larvae was determined on three occasions (April, June and September) annually by randomly sampling four handfuls of grass in 100 locations in every subplot B (Gruner & Sauve, 1982). The grass material (600 g) was subsequently put in water with soap for 18 h overnight. The grass fibres were then filtered with 1-cm mesh before sequential filtering was performed with 125 and 20 μm mesh sieves. Infective larvae were collected in four 15-ml glass tubes and pelleted by centrifugation at
### TABLE 1

Daily grazing time (GT), dry matter digestibility (DMD), daily liveweight gain (LWG) and diet selection by horses (mean ± SE) according to grazing management and season

<table>
<thead>
<tr>
<th>Grazing management</th>
<th>Season</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sp</td>
<td>Sum</td>
</tr>
<tr>
<td>Horse grazing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GT (min)</td>
<td>901 ± 31</td>
<td>944 ± 31</td>
</tr>
<tr>
<td>DMD (%)</td>
<td>57.5 ± 0.4</td>
<td>57.7 ± 0.4</td>
</tr>
<tr>
<td>LWG (g/d)</td>
<td>365 ± 22</td>
<td>390 ± 25</td>
</tr>
<tr>
<td>Jacobs' indexes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VS</td>
<td>+0.14† ± 0.07</td>
<td>+0.33 ± 0.10</td>
</tr>
<tr>
<td>VI</td>
<td>+0.15 ± 0.06</td>
<td>+0.25 ± 0.06</td>
</tr>
<tr>
<td>VT</td>
<td>+0.03 ± 0.05</td>
<td>-0.03 ± 0.07</td>
</tr>
<tr>
<td>Repro</td>
<td>-0.25 ± 0.09</td>
<td>-0.51 ± 0.11</td>
</tr>
<tr>
<td>Grasses</td>
<td>+0.23 ± 0.04</td>
<td>+0.30 ± 0.04</td>
</tr>
<tr>
<td>Legumes</td>
<td>-0.02 ± 0.08</td>
<td>+0.00 ± 0.07</td>
</tr>
<tr>
<td>Forbs</td>
<td>-0.15 ± 0.06</td>
<td>-0.21 ± 0.06</td>
</tr>
<tr>
<td>Dead herbage</td>
<td>-0.55 ± 0.06</td>
<td>-0.50 ± 0.08</td>
</tr>
<tr>
<td>Horse faeces &lt;1 m</td>
<td>-0.30 ± 0.04</td>
<td>-0.31 ± 0.05</td>
</tr>
</tbody>
</table>

Note: The grazing management × season interaction, the block × year interaction and the block effect were never significant. Details for significant year effects are given in Appendix SA1. †‡Values in the same lines with different superscripts are significantly different at p < .05. Bold characters indicate items selected for and italics indicate items avoided at p < .05. Abbreviations: Aut, autumn; Repro, reproductive; Sp, Spring; Sum, summer; VI, vegetative intermediate (5–8 cm); VS, vegetative short (≤4 cm); VT, vegetative tall (≥9 cm). †Indicate a trend for selection (positive value) or avoidance (negative value) (p < .1).
### TABLE 2  Diet selection by cattle and horses in mixed plots according to season (mean ± SE)

<table>
<thead>
<tr>
<th>Species</th>
<th>Cattle</th>
<th>Horses</th>
<th>F-value</th>
<th>p</th>
<th>Sp</th>
<th>Sum</th>
<th>Aut</th>
<th>F-value</th>
<th>p</th>
<th>Year</th>
<th>F-value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS</td>
<td>-0.05 ± 0.14</td>
<td>+0.33 ± 0.10</td>
<td>6.92</td>
<td>.014</td>
<td>.03 ± 0.12</td>
<td>+0.24† ± 0.13</td>
<td>1.68</td>
<td>.205</td>
<td>3.69</td>
<td>.038</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>+0.24 ± 0.06</td>
<td>+0.25 ± 0.06</td>
<td>0.02</td>
<td>.894</td>
<td></td>
<td>+0.37 ± 0.07</td>
<td>+0.19 ± 0.09</td>
<td>-0.18 ± 0.05</td>
<td>1.89</td>
<td>.163</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VT</td>
<td>+0.17 ± 0.07</td>
<td>-0.03 ± 0.07</td>
<td>5.95</td>
<td>.019</td>
<td>-0.00 ± 0.07</td>
<td>+0.31 ± 0.05</td>
<td>-0.10 ± 0.11</td>
<td>8.85</td>
<td>&lt;.001</td>
<td>0.99</td>
<td>.379</td>
<td></td>
</tr>
<tr>
<td>Repro</td>
<td>-0.56 ± 0.09</td>
<td>-0.51 ± 0.11</td>
<td>0.10</td>
<td>.758</td>
<td>-0.45 ± 0.10</td>
<td>-0.80 ± 0.08</td>
<td>-</td>
<td>6.19</td>
<td>.024</td>
<td>0.89</td>
<td>.431</td>
<td></td>
</tr>
<tr>
<td>Grasses</td>
<td>+0.32 ± 0.05</td>
<td>+0.30 ± 0.04</td>
<td>0.20</td>
<td>.661</td>
<td>+0.25 ± 0.05</td>
<td>+0.28 ± 0.05</td>
<td>+0.40 ± 0.06</td>
<td>2.70</td>
<td>.079</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Legumes</td>
<td>-0.13 ± 0.10</td>
<td>-0.00 ± 0.07</td>
<td>1.20</td>
<td>.284</td>
<td>-0.06 ± 0.08</td>
<td>+0.05 ± 0.10</td>
<td>-0.20 ± 0.13</td>
<td>1.63</td>
<td>.217</td>
<td>0.02</td>
<td>.982</td>
<td></td>
</tr>
<tr>
<td>Forbs</td>
<td>-0.18 ± 0.06</td>
<td>-0.21 ± 0.06</td>
<td>0.15</td>
<td>.700</td>
<td>-0.29 ± 0.07</td>
<td>-0.08 ± 0.06</td>
<td>-0.21 ± 0.07</td>
<td>2.29</td>
<td>.113</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead herbage</td>
<td>-0.64 ± 0.05</td>
<td>-0.50 ± 0.08</td>
<td>2.44</td>
<td>.132</td>
<td>-</td>
<td>-0.50 ± 0.09</td>
<td>-0.68 ± 0.05</td>
<td>1.27</td>
<td>.271</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horse faeces &lt;1 m</td>
<td>-0.17 ± 0.06</td>
<td>-0.31 ± 0.05</td>
<td>4.06</td>
<td>.049</td>
<td>-0.38° ± 0.07</td>
<td>-0.16° ± 0.06</td>
<td>-0.19ab ± 0.07</td>
<td>4.34</td>
<td>.019</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The species × season interaction was never significant. Details for significant year effects are given in Appendix S1. a,b Values in the same lines with different superscripts are significantly different at p < .05. Bold characters indicate items selected for and italics indicate items avoided at p < .05.
Abbreviations: Aut, autumn; Repro, reproductive; Sp, spring; Sum, summer; VI, vegetative intermediate (5–8 cm); VS, vegetative short (<4 cm); VT, vegetative tall (<9 cm).
†Indicate a trend for selection (positive value) or avoidance (negative value) (p < .1).

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As the foraging behaviour (i.e., daily grazing time, Jacobs’ index) varies with grazing management (mixed vs. monospecific grazing), season (or date) while taking into account the effects of block, year and season × year interaction rather than the one of grazing management × year interaction since the above approach was used to test for the interaction two grazing treatments and because this allowed us to test for the interaction grazing treatments. Subplot was used as the statistical unit.

We compared the daily grazing time of horses, Jacobs’ indexes and herbage digestibility, FEC and daily liveweight gain, individual values were arcsine transformed prior to statistical analysis (Sokal & Rohlf, 1995). We tested for the effects of grazing management (mixed vs. monospecific grazing), season (or date) while taking into account the effects of block, year and season × year interaction rather than the one of grazing management × year interaction since the above approach was used to test for the interaction two grazing treatments and because this allowed us to test for the interaction grazing treatments.

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As the foraging behaviour (i.e., daily grazing time, Jacobs’ index) varies with grazing management (mixed vs. monospecific grazing), season (or date) while taking into account the effects of block, year and season × year interaction rather than the one of grazing management × year interaction since the above approach was used to test for the interaction two grazing treatments and because this allowed us to test for the interaction grazing treatments. Subplot was used as the statistical unit.
The horse daily liveweight gain during the grazing season was analysed in a model including the main effects of grazing management, block, year and the block × year interaction. Individual was considered a random effect. The influence of grazing management and block on the abundance and number of plant species recorded in 2017 was tested with data from 2015 as a covariate. Transect was used as the statistical unit.

Finally, for the mixed plots, the Jacobs’ indexes of horses and cattle were analysed using a model testing for the effects of species, season, species × season and year. Jacobs’ indexes were compared to zero using Student’s t-test. Moreover, the short sward regrowth (i.e., below 4 cm in VS patches expressed in mm/day) between two stocking periods was analysed in a model including the effects of stocking period and year. This short sward regrowth was compared to zero using Student’s t-test. For these analyses, subplot was used as the statistical unit and the differences between the treatments were investigated using a Tukey correction for multiple comparisons. The frequency of vigilance behaviour of the horses towards the cattle was calculated at the plot scale from the number of observations of states of vigilance over the total number of observations. Due to the small number of plots and the distribution of the data, non-parametric statistics were used (Wilcoxon test, Xlstats software, Addinsoft, Paris, France). The data are presented as the median (first quartile and third quartile) for each month, and two-tailed tests were performed. Due to the low frequency of agonistic behaviours, no statistical analyses were conducted on these data.

3 | RESULTS

3.1 | Influence of co-grazing with cattle on horse behaviour

Over the 3 years of observations, only seven agonistic interactions were reported in horses towards heifers (one head threatening and six pursuits), and none were reported in heifers towards horses. No injury was observed. Horses expressed vigilant postures towards cattle during the first month of mixed grazing (proportion of scans during which a vigilant posture was observed: 0.036 [0.014, 0.077]), but this vigilance significantly decreased to almost zero from the second month (0.002 [0.002, 0.011], V = 45, p = .004).

Co-grazing with cattle did not affect horse daily grazing time or their feeding choices (Table 1). The horses selected VS and VI patches and preferred bites dominated by grasses (Table 1). They used VT swards and legumes in proportion to their availability and rejected reproductive swards and forbs (Table 1). Horses in both treatments strongly rejected dead herbage and avoided feeding within 1 m of their faeces (Table 1).

Significant seasonal variations were observed for the daily grazing time that increased in summer and autumn (Table 1). In summer, horses avoided reproductive swards more strongly while they used forbs proportionally to their availability (Table 1). Horses selected grasses whatever the season, but less strongly in summer (Table 1).
3.2 Comparison of feeding choices between horses and cattle in mixed plots

Cattle, unlike horses, used VS patches proportionally to their abundance and selected VT swards, especially in summer (Table 2). No significant differences in selection between the two species were found for the other bite types defined according to sward height type and dominant botanical family. Both the horses and cattle selected VI patches and avoided dead herbage areas and reproductive swards, even more so in summer for the latter (Table 2). Horses and cattle selected the different botanical families similarly (Table 2). The cattle avoided feeding near horse dung even though this avoidance was slightly less pronounced than in the horses (Table 2). The avoidance of horse dung by the two species was higher in spring compared to summer (Table 2).

3.3 Herbage structure, biomass and quality

The mean SSH and its CV when the animals both entered and were turned out from each subplot A were not affected by grazing management (Tables 3 and 4). Mean SSH was the lowest from mid-August (Tables 3 and 4). The CV of SSH was greatest from July and at the end of August, when the animals entered and were turned out from each subplot A, respectively (Tables 3 and 4). Grazing management did not affect the abundance of the different bite types available to animals, except for the reproductive swards, which were slightly more abundant in the cattle-horse pastures, and for the bites close to horse faeces, which were more abundant in plots grazed by horses only (Table 5). Total herbage biomass and quality (CP and NDF contents) were similar between the two treatments (Table 5). Significant seasonal variations were observed for the availability of VS and VI bites, which increased from spring to autumn while the abundance of VT sward decreased (Table 5). Reproductive swards no longer existed in autumn, and the availability of dead herbage and bites close to horse faeces were maximal in summer and autumn. Abundance of bite types dominated by grasses decreased significantly during the grazing season and forb abundance was minimal in summer. Finally, total herbage biomass decreased in autumn while herbage quality was minimal in summer (Table 5).

In the mixed plots, the short patch regrowth (below 4 cm) between two stocking periods was 0.6 ± 0.2 mm/day (±SE) (Student’s t-test, \( p = .001 \)) on average, with regrowth tending to be slower in August (\( F_{4,8} = 2.94, p = .091 \), independently of year (\( F_{2,4} = 0.52, p = 0.628 \)). The mean SSH in the VS patches was therefore 3.9 ± 0.2 cm (±SE) when the animals entered a new subplot. The mean CP and NDF contents of these short patches were 125.5 ± 4.8 g/kg DM and 574.6 ± 9.1 g/kg DM (±SE), respectively.

3.4 Botanical composition

The abundance of the main plant species in the third year of contrasted pasture management (June 2017) is given in Appendice SB.


**TABLE 5** Proportions of bite types available to animals (defined according to sward height type or dominant botanical family), herbage biomass and quality in subplots A according to grazing management and season (mean ± SE)

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<th>Grazing management</th>
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Note: The grazing management × season interaction, the block × year interaction and the block effect were never significant. Details for significant year effects are given in Appendix SA4. Values in the same lines with different superscripts are significantly different at \( p < .05 \).

Abbreviations: Aut, autumn; CP, crude protein; DM, dry matter; NDF, neutral detergent fibre; Repro, reproductive; Sp, spring; Sum, summer; VI, vegetative intermediate (5–8 cm); VS, vegetative short (≤4 cm); VT, vegetative tall (≥9 cm).
We did not find any effect of grazing management on plant species richness at the transect scale (17.4 ± 0.5 SE, $F_{1,31} = 0.42$, $p = .521$) or on the evenness of plant species distribution (0.818 ± 0.008 SE, $F_{1,31} = 0.31$, $p = .580$) in 2017. The evenness of plant species distribution was however affected by block ($F_{2,31} = 7.62$, $p = .002$, details in Appendix SA5). The abundance of the different botanical families (in percentage) was similar among grazing management treatments when considering the specific contributions of species (Cs) (grasses: 63.5 ± 1.8 SE, $F_{1,31} = 0.00$, $p = .981$; legumes: 5.5 ± 0.7 SE, $F_{1,31} = 0.01$, $p = .938$; forbs: 30.9 ± 1.5 SE, $F_{1,31} = 2.38$, $p = .133$). However, we found a slightly higher occurrence of forbs in the plots grazed by horses alone (forbs were recorded at 35.9% ± 1.1 SE of sampling points in horse-grazed plots vs. 33.0% ± 1.3 SE in mixed grazing treatment, $F_{1,31} = 8.06$, $p = .008$). The abundance of grasses and forbs were also affected by block ($F_{2,31} = 4.75$, $p = .016$ for grasses and $F_{2,31} = 4.79$, $p = .015$ for forbs; details in Appendix SA5).

3.5 | Parasitology

Faecal egg excretion did not significantly differ between the two grazing management treatments, with a difference of 11 eggs/g only throughout the study period ($F_{1,265} = 0.005$, $p = .945$). Similarly, we did not find any significant effect of grazing treatment on the number of individuals in need of deworming (odds ratio = 1.25 ± 3.24; $p = .850$). No significant difference in larval density was found between the two grazing treatments when total larvae counts were divided by the number of horses grazing in each plot ($\chi^2 = 0.25, p = .617$).

FEC showed an increasing excretion pattern throughout the grazing season ($F_{2,265} = 45.4, p < 10^{-4}$, Figure 1), reaching its highest levels in autumn (877 ± 97 more eggs/g than in spring on average). This pattern matched the levels of pasture larval contamination that was also the highest in autumn (3516 ± 3865 in autumn compared with 581 ± 1274 L3/kg of DM in spring–summer, $\chi^2 = 6.3, p = .024$). Significant variation in the prevalence of horses in need of deworming and in pasture contamination occurred between years ($p = .003$ and $p = .043$, respectively; details in Appendix SA6). However, the grazing management × season interaction, the block × year interaction and the block effect were never significant.

3.6 | Animal performance

Co-grazing with cattle did not modify the digestibility of the horses’ diet, which was maximal in spring (Table 1). The average horse daily liveweight gain (in g per day) was similar between the horses grazed alone or with cattle (Table 1). The daily liveweight gain of the heifers averaged 779 g/d ± 34 SE.

4 | DISCUSSION

Horses exhibited long daily grazing duration and typical patterns of their diet selection (Duncan, 1980; Edouard et al., 2010; Lamoot et al., 2005b) whether they grazed alone or with cattle. Our results suggest that young saddle horses quickly acclimated to dehorned
heifers and that their co-grazing presents little risk of agonistic interactions or injury. These findings extend what was observed in other interspecific combinations with only few interspecific tensions observed as long as space was not limiting, for example, between red deer and sheep (Blanc et al., 1999), or heifers and sows (Sehested et al., 2004).

In line with the observations made in previous comparative studies, horses and cattle strongly selected grasses (Fleurance et al., 2016; Karmiris et al., 2011; Ménard et al., 2002), and grazed legumes (i.e., *Trifolium repens*) in proportion to their availability. Although ruminants are better able to detoxify plant secondary metabolites than horses (Duncan, 1992), cattle did not consume more forbs than did horses. This result is in line with most previous observations made in semi-natural habitats (Cromsigt et al., 2018; Lamoot et al., 2005a; Vulink et al., 2000), although (Ménard et al., 2002) reported a stronger use of forbs by cattle compared to horses. Discrepancies between studies probably result from variations in forb availability and palatability. Overall, both species avoided bites dominated by forbs in our study (as in Karmiris et al. [2011]), although the main forb species, *P. lanceolata* and *Taraxacum gr. vulgare* (accounting for 10% and 6% of sward cover, respectively) are highly palatable to cattle (e.g., [Dumont et al., 2011; Stewart, 1996]). In summer, the horses increased forb consumption and used them proportionally to their availability in the sward. This may result from a low palatability of grasses that remained green; for example, of *Dactylis glomerata*, a drought-tolerant species that accounted for 18% of sward cover (Allen et al., 2013).

The horses also exhibited a typical patch grazing pattern (Adler et al., 2001), selecting the highly nutritive short (<4 cm) and intermediate (5–8 cm) patches where they could maximize the intake rate of digestible proteins (Edouard et al., 2010). They used tall vegetative swards (>9 cm) in proportion to their availability and avoided reproductive and dead herbage despite their ability to consume roughage (Duncan et al., 1990; Edouard et al., 2008). Horses increased their grazing time from 12 to 17 h over the grazing season, probably to compensate for lower herbage availability and digestibility (Arnold, 1984; Fleurance et al., 2016). The cattle used high-quality VS patches in proportion to their availability. This result differs from a number of previous studies in which cattle were excluded from the lawns (1–4 cm) selected by horses, where they could not meet their daily requirements (Cornelissen & Vulink, 2015; Edwards & Hollis, 1982; Lopez Lopez et al., 2019; Ménard et al., 2002). Here, the strong use of the VS patches by cattle probably resulted from the alternate stocking of animals between two subplots, which let short swards regrow to an average of 3.9 cm before animals entered the plots again. Further research comparing this alternate stocking management to continuous stocking at the same stocking density is needed to confirm that alternate stocking could have limited the use of tall mature and dead grass material by cattle. An additional explanation can be found in the high selectivity of Limousin cattle (D’Hour et al., 1995).

A consequence of the unexpected similarity between horses and cattle choices is that the sward height variability did not differ between the monospecific and mixed grazing treatments. We thus did not validate our hypothesis that mixed grazing between horses and cattle would homogenize sward structure compared to horses grazing alone. Cattle, by avoiding the reproductive and dead herbage areas as did horses, also did not improve herbage quality nor the digestibility of the horses’ diet. We could also not evidence any reduction of parasite egg excretion in horses grazing with cattle although recorded excretion levels were in a higher range of values - thereby ensuring a fair comparison between the two management systems - and matched the expected seasonal variation under temperate area (Salle et al., 2021; Wood et al., 2012). In this study, 40% of the horse faeces were recorded in the reproductive and dead herbage areas which were avoided by the cattle. By selecting vegetative regrowths, the cattle avoided grazing close to horse dungs and thus ingested few parasitic larvae, which limited their ability to remove parasites from the environment. While (Forteau et al., 2020) have recently reported that mixed grazing with cattle could partly control strongyle infection in horses thanks to parasite dilution, our findings suggest that mixed grazing would require an appropriate management of herds and plots to decrease horse parasite burden rather than being applied as a turn-key solution. It is a core principle of any agroecological management to account for local and seasonal conditions (Bland & Bell, 2007; Ravetto Enri et al., 2017) so that ecological processes, such as the interactions between system components, can provide the expected benefits.

Another logical consequence of the similarity of horse daily grazing time, diet digestibility and faecal excretion between grazing treatments is that horse liveweight gains were similar whether horses grazed alone or in presence of cattle. Liveweight gains of horses and heifers along the grazing season met growth recommendations (INRA, 2015, 2018), suggesting that neither species was limited in its intake by this herd management. While mesophile grasslands are commonly used in horse production systems in Europe, very few references report how grazing horses impact their biodiversity (Fleurance et al., 2016; Mangels et al., 2017; Schmitz & Isselstein, 2020). This is unfortunate as mesophile grasslands play a significant role for the preservation of biodiversity in temperate ecosystems and because a number of ecological functions and ecosystem services (e.g., recycling of nutrients, pollination) are closely linked with sward functional diversity (Dumont et al., 2013). Here, biodiversity effects were very limited due to the short time-course of the survey that is unlikely to induce drastic shifts in vegetation communities, and to the lack of impacts of grazing management on sward structure. In wet grasslands of high nature value of western France (Loucougaray et al., 2004), 6 years of continuous grazing by horses and cattle produced swards with higher species richness than monospecific horse grazing, and in this case changes in biodiversity reflected changes in sward structure in the mixed-grazing treatment.

### Conclusion

This is the first study to simultaneously assess the effects of mixing horses with cattle on vegetation use, herbage structure and quality, and horse parasitism. Alternate stocking of co-grazing horses and
cattle between two subplots in this mesophile grassland provided high-quality sward regrowth on the shortest patches, which remained accessible to cattle. Consequently, cattle used these short patches in proportion to their availability and avoided reproductive and dead herbage where horse faeces were concentrated. This could have limited their potential for removing parasites from the environment. Moreover, co-grazing horses and cattle did not reduce sward structural heterogeneity and therefore did not improve herbage quality. We conclude that for improving horses’ performances through mixed grazing with cattle, one must constrain cattle to graze on horse refusals. This could be achieved with cattle breeds having low requirements and a high ability to feed on roughages. Continuous stocking could also limit the regrowth of short patches, leading cattle to switch to horse refusals. Further research should therefore search for the combinations of pasture management and ratio between the two species that could provide the best equilibrium between horses performances and ecosystem integrity.

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DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES


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