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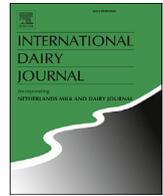
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# The effects of low-input grazing systems and milk pasteurisation on the chemical composition, microbial communities, and sensory properties of uncooked pressed cheeses



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## ABSTRACT

The objective of this work was to determine whether the effects of farming systems on the cheese sensory properties are reduced when the milk used to make cheese is pasteurised. The milk of cows reared under two grazing systems (extensive and semi-extensive) was processed into raw and pasteurised milk cheeses. These grazing systems were mainly differentiable by the milk fatty acid (FA) composition and, to a lesser extent, by their levels of lactic acid bacteria, which were higher in milk from the extensive grazing system. In cheese, the different FA composition persisted and affected the texture of the cheese regardless of the milk treatment. The effects of each grazing system were indistinguishable in several cheese characteristics (proteolysis, texture, and aroma) after pasteurisation. In contrast, among pasteurised milk cheeses, those resulting from an extensive system had greyer rinds, while no difference in rind colour was observed among raw milk cheeses.

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## 1. Introduction

The sensory quality of cheeses depends on many factors linked to cheese-making technology and to the chemical and microbial characteristics of raw milk. Farming practices that modify the characteristics of raw milk are important to consider, especially when milk is processed after minimal treatment, as in most farm-house and quality-labelled cheeses granted a geographical indication. Particular attention has been focused on animal feed, which varies widely according to geographic region and is therefore related to the concept of “terroir” (Coulon, Delacroix-Buchet, Martin, & Pirisi, 2004). The effects of the type of forage distributed to animals (e.g., pasture, hay, grass silage, or maize silage) on the sensory properties of cheese are well documented (Martin, Verdier-Metz, Buchin, Hurtaud, & Coulon, 2005). These effects are linked to changes in milk characteristics, and particular attention has been paid to milk fat, which is known to vary widely with different animal feeds (Chilliard et al., 2007; Collomb, Bütikofer, Siber, Jeangros,

& Bosset, 2002; Elgersma, Tamminga, & Ellen, 2006; Gómez-Cortés et al., 2009).

Grazing cows produce milk with a higher proportion of unsaturated fatty acids (FAs) and a lower melting point, which results in creamier cheeses, than that of cows receiving winter diets (Martin et al., 2005). The higher concentration of carotenoids in milk fat from grazing cows also explains the yellower colour of cheeses produced from this milk than those produced from the milk of cows fed hay-, grain- or maize silage-based diets (Martin et al., 2005; Nozière et al., 2006). The role of milk fat in the formation of volatile compounds by lipolysis and the oxidation of unsaturated FA has been proposed as a possible explanation for the more intense and diversified aromas of cheeses from grazing cows in comparison with those from cows fed winter diets (Bovolenta et al., 2014; Coppa et al., 2011a). On the pasture, grassland characteristics (phenological stage and botanical composition) and the supplementation of grazed grass with additional forages and concentrates modify the FA composition of milk (Bargo, Delahoy, Schroeder, & Muller, 2006; Coppa et al., 2015; Farruggia et al., 2014). The FA composition of milk has also been proposed to explain variations in the sensory properties of cheese attributed to grazing systems

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(Coppa et al., 2011a) or to the botanical composition of pastures (Coulon et al., 2004).

Milk microbiota has received little attention when explaining the influence of animal feeding on the sensory properties of cheese, although a great deal of research has been conducted on raw milk cheeses. In unpasteurised cheese production, the raw milk microbiota plays a key role on the cheese flavour development; for instance, experimental Salers-type cheeses manufactured from the same pasteurised milk and inoculated alternately with three different microbiota differed widely in their sensory attributes (Callon, Berdagué, Dufour, & Montel, 2005). The raw milk microbiota depends mainly on the microbiota composition of sources directly in contact with the milk, such as the cow's teat skin and dairy equipment (milking machines, milk lines, and tanks), and on indirect sources such as animal feeding and bedding, drinking and washing water, stable and milking parlour air, and the milker (Montel et al., 2014). Verdier-Metz et al. (2002) showed that the microbiota of teat skin varied according to animal feeding. Consequently, the raw milk microbiota could be modified, which may explain the influence of animal feeding on the sensory properties of cheeses. This hypothesis is reinforced because some observations suggest that the effects of animal feeding on the sensory properties of cheese are reduced when milk is pasteurised (Verdier-Metz et al., 2002). Indeed, the reduction of the microbial diversity and of the associated microbial activities in milk after undergoing thermal treatment is known to affect cheese flavour (Irlinger & Mounier, 2009).

Animal feeding in general, and grass grazing in particular, could influence milk microbiota, and we can hypothesise that these changes, associated with changes in milk composition, and especially with FA composition, could modify the sensory characteristics of cheeses. Based on the literature, milk pasteurisation may hide the putative differences between grazing systems due to an equalisation of milk microbiota level (Giaccone et al., 2016; Verdier-Metz et al., 2002). To test these hypotheses, we implemented an experiment using two herds reared separately for two years under two grazing systems. We evaluated the combined effect of grazing systems and milk pasteurisation on (i) chemical composition, including FA, (ii) levels of microbial groups in milks and cheeses, and (iii) the sensory properties of cheese.

## 2. Materials and methods

### 2.1. Experimental design

The experiment was conducted at the experimental farm of Marcanat (INRA, UE1296 Monts d'Auvergne, France), which is located in an upland mountainous grassland area in central France (45°18'21" N, 2°50'13" E, altitude 1135–1215 m, annual rainfall 1100 mm). The study was conducted in 2012 during the 2nd year of a long-term research project assessing the interactions between management practices and animal performances at the farm level (Pires et al., 2015). A total of 24 Holstein and 24 Montbeliarde spring-calving dairy cows and their corresponding heifers were divided between two independent farmlets denominated extensive (EXT) and semi-extensive (SEMI). The EXT system consisted of 60.5 ha of highly diversified grasslands (61.1 species per paddock on average). The paddocks were grazed using a long rotational duration (4 grazing cycles of 51 days on average) managed at a low stocking rate (0.66 livestock unit (LU) ha<sup>-1</sup>; LU = 600 kg of body weight). No concentrate was added to the diet of cows. The SEMI system was composed of 29.2 ha of permanent and old temporary grasslands (+10 years; 38.6 species per paddock on average). The paddocks were grazed using a rapid rotational duration (5 grazing cycles of 41 days on average) exploited at a moderate stocking rate

(1.09 LU ha<sup>-1</sup>). Four kilogrammes per cow of a pelleted concentrate composed of 40% wheat, 30% barley, 25% maize, and 5% molasses were distributed daily to the SEMI cows in a collective feed trough, after the morning milking during the entire grazing period. The botanical composition of the two grazing systems is summarised in [Supplementary Table S1](#).

Two periods (P) of three dates each of bulk milk sampling and cheese-making were chosen to examine the effects of the two grazing systems at different phenological stages of grass and different growth cycles: three dates in July 2012 (the 5th, 10th, and 12th) corresponding to peak flowering in the EXT paddock, and three dates in September 2012 (the 6th, 11th, and 13th) when the flowering period had ended.

The milk used for cheese-making was produced by a total of 15 and 17 spring-calving cows selected from among the 24 cows grazed under the EXT and SEMI systems, respectively, so that the breed, parity and lactation stage of the EXT and SEMI animals were as similar as possible. The allotment of the dairy cows is given in [Supplementary Table S1](#). The animals were milked with the same milking machine at 6:30 am and 4:30 pm. All cows received a sterile 85% glycerol solution post-dipped on the teats following each milking. Animal and paddock characteristics are presented in [Supplementary Table S1](#).

### 2.2. Cheese-making

For each cheese-making date and for each grazing system, the bulk morning milk was pooled with the previous evening's milk and stored at 4 °C. Half of this milk was pasteurised (72 °C for 20 s; PierreRalisi, Italy) prior to cheese-making. During each cheese-making date, four small-size Cantal-type cheeses (10 kg) were manufactured concomitantly from 110 L of EXT or SEMI raw (R) or pasteurised (PA) full-fat milk in separate vats. Cheese-making was performed as described previously by [Martin, Pomiès, Pradel, Verdier-Metz, and Rémond \(2009\)](#), except that whole milk heated to 33 °C was inoculated with 0.05 g 100 kg<sup>-1</sup> of a mesophilic starter culture (Flora Danica Direct, Chr. Hansen, Arpajon, France, consisting of *Lactococcus lactis* ssp. *lactis*, *L. lactis* ssp. *cremoris*, *L. lactis* ssp. *lactis biovar diacetylactis*, and *Leuconostoc mesenteroides* ssp. *cremoris*) and a ripening starter (1.3 mL of Monilev consisting of *Debaryomyces hansenii* and *Sporendonema casei*, and 1.3 mL of Penbac consisting of *Brachy bacterium tyrofermentans*, *Brevibacterium linens*, and *Penicillium fuscoglaucum*, Laboratoire Interprofessionnel de Production (LIP), Aurillac, France) was inoculated on hessian cloths at the stage of moulding. Technological parameters (milk pH, visually assessed rennet clotting time, curd pH, and DM content) were monitored during each cheese-making assay. Overall, 24 cheeses were produced (2 grazing systems × 2 treatments of milk × 2 periods × 3 cheese-making dates). The cheeses were ripened for 20 weeks at 10 °C and 95% minimum relative humidity and then sampled for analyses.

### 2.3. Chemical analyses

#### 2.3.1. Milk

Samples from vat milk used for cheese-making were collected just before the addition of rennet and starter. Immediately, milk pH was determined at 20 °C using an Ingold needle (Ingold France, Paris, France). Fat, protein and lactose contents were measured (LIAL, Aurillac, France) by infrared spectrometry (Milkoscan 4000, Foss System, Hillerød, Denmark) and, in the same samples, somatic cells (SCC) were automatically counted (Fossomatic 5000, Foss System). The free fatty acid (FFA) content was measured by the copper soap method ([Jellema et al., 1991](#)) on fresh samples. Sub-samples of milk were stored at -20 °C until analysis of lipoprotein

lipase (LPL) activity and FA composition. The milk LPL activity was measured using an artificial emulsion containing  $^3\text{H}$  triolein emulsion (Faulconnier, Thévenet, Fléchet, & Chilliard, 1994). Fatty acid methyl esters in 100 mg samples of lyophilised milk were prepared by direct methylation according to Lerch et al. (2015).

### 2.3.2. Cheese

After ripening, cheeses were cut and divided into triangular pieces of approximately  $7 \times 12 \times 3.5$  cm for chemical analyses. Those sub-samples were packed under vacuum in aluminium foil and kept in a plastic bag to preserve antioxidants from oxidation by oxygen or light. Cheese core pH, dry matter (DM) content, and colour were determined as described by Verdier-Metz et al. (2000); fat, calcium, and phosphorus contents were determined as described by Lucas et al. (2006) (Galilait, Theix, France), and total nitrogen (N), water-soluble nitrogen (WSN), and phosphotungstic acid-soluble nitrogen (PTSN) were measured using the methods described by Ardö (1999). The rheological method used was uniaxial compression at a constant displacement rate, as described by Lerch et al. (2015). Resistance to penetration at 10%, 30%, and 50% of the height of the cheese core piece was recorded on five points uniformly distributed over the surface of the test sample. Cheese core and rind samples intended for an FA analysis were stored at  $-20^\circ\text{C}$ . The FA compositions of cheese core and rind were determined in 100 mg samples of lyophilised and ground cheeses as described by Lerch et al. (2015), with one modification compared with milk: methanol/HCl (95:5, v/v) was substituted for methanol/boron trifluoride (95:5, v/v). Volatile fatty acids (VFA) were solvent extracted and saponified from 15 g of cheese core, then separated and quantified by gas chromatography coupled to a Flame Ionization Detector (FID) detector (Trace GC Ultra, Thermo Scientific, Gometz le Châtel, France) as described by Bugaud, Buchin, Hauwuy, and Coulon (2001).

### 2.4. Microbial analyses

Milk and cheese samples were stored at  $-20^\circ\text{C}$  until an analysis was performed. Ten grams of ripened cheese rind or core samples were suspended in 90 mL phosphate buffer pH 7.5 (Gomri, 1946). Milk and cheese samples were homogenised for 2 min (milk) or 4 min (cheese) in a Stomacher Laboratory Blender (Interscience, St. Nom la Bretèche, France). This suspension was used for all microbial analyses and appropriate dilutions were plated on the following media (two replicates per sample): heterofermentative facultative *Lactobacillus* were enumerated on Facultative Heterofermentative (FH) agar medium (Isolini, Grand, & Glättli, 1990) incubated at  $30^\circ\text{C}$  for 3 days under anaerobic conditions (Anaerocult A, VWR International, Fontenay-sous-Bois, France), total mesophilic bacteria on Plate Count Agar with Milk (PCAM) medium (IDF, 1990) with milk, Gram-negative bacteria on Plate Count Agar with Inhibitors (PCAI) medium with crystal violet (1%) and vancomycin (0.5%) as inhibitors of the Gram-positive bacteria, lactic acid bacteria on Man Rogosa Sharpe (MRS) (De Man, Rogosa, & Sharpe, 1960), and dextran-producing *Leuconostoc* on Mayeux Sandine Elliker (MSE) (Mayeux, Sandine, & Elliker, 1962) agar medium at  $30^\circ\text{C}$  for 3 days, yeasts and moulds on Oxytetracycline Glucose Agar (OGA) (Mossel, Visser, & Merringerink, 1962) at  $25^\circ\text{C}$  for 3 days, *Enterococcus* on Slanetz Bartley (SB) agar medium (Slanetz & Bartley, 1957) at  $42^\circ\text{C}$  for 48 h, *Enterobacteriaceae* on Violet Red Bile Glucose (VRBG) agar at  $42^\circ\text{C}$  for 24 h (Mossel, Mengerink, & Scholts, 1962), Gram-positive catalase-positive bacteria on Cheese Ripening Bacteria Medium (CRBM) agar (Denis, Gueguen, Henry, & Levert, 2001) at  $25^\circ\text{C}$  for 5 days in the dark then for 5 days under daylight. Ready-to-use media were purchased from Biokar Diagnostics (Biokar Diagnostics, Beauvais, France).

### 2.5. Sensory analyses

The sensory properties of the cheeses were assessed by a panel of 11 expert assessors who performed routine sensory analyses of Cantal-type cheeses (ENILV, Aurillac, France). The selection and training of the panellists were performed as described by Martin et al. (2009). During testing, the panellists gave an intensity score of between 0 and 10 for 35 attributes (4 for rind cheese appearance, 3 for core cheese appearance, 2 for touch texture, 3 for mouth texture, 9 for odour, 9 for aroma, and 5 for taste). A score of 0 for a given attribute was defined as not perceptible, while a score of 10 was defined as maximal expression of the attribute in a Cantal-type cheese. During one session, each of the 4 cheeses made on the same day were coded with 3-digit random numbers and presented at  $20^\circ\text{C}$  to the panellists in a varying order using a monadic sequential method. In all, 6 sessions were necessary to analyse all the cheeses (three sessions on December 2012 for July 2012 cheeses, and three on February 2013 for September 2012 cheeses).

### 2.6. Statistical analyses

Statistical analyses were performed with R for Windows (version 3.1.0). Milk data were analysed by a multi-way analysis of variance (ANOVA), including variables corresponding to the grazing system used (S), the period of cheese-making (P), and their interactions as fixed factors. Cheese were analysed by ANOVA where variables corresponding to the grazing system used (S), the period (P), the treatment of milk (T), and their interactions were the fixed factors. Sensory data were analysed using a mixed model where the grazing system, the period, the treatment of milk, and their interactions were the fixed factors, and the assessor was the random factor. Significance was declared at  $P < 0.05$ .  $P$  values between 0.05 and 0.10 were considered a tendency, while  $P > 0.10$  was considered not significant. When interactions were not significant,  $P$  values were not reported. If significant differences were found by an ANOVA or a mixed-model analysis, the Benjamini–Hochberg method was used to compare differences between multiple groups. A principal component analysis (PCA) was performed on biochemical and microbial data using cheese parameters with significant differences among grazing systems, period, and milk treatment in the ANOVA as active variables and the highest discriminant sensorial attributes as non-active variables.

## 3. Results

### 3.1. Milk biochemical characteristics and microbial composition

Results for milk composition are given in Table 1. For every studied period of cheese-making, the milk fat content and the fat to protein ratio were significantly higher in EXT than in SEMI milks ( $P < 0.001$ ). The SEMI milks had higher pH and SCC values than EXT milks.

The FFA content of milk was not affected by the grazing system, contrary to the FA profiles. The EXT milks were higher in C18:0 ( $+4.1 \text{ g } 100 \text{ g}^{-1}$ ,  $P < 0.001$ ), *cis*9-C18:1 ( $+5.4 \text{ g } 100 \text{ g}^{-1}$ ,  $P < 0.01$ ), long-chain saturated FA (C20:0 + C22:0 + C24:0;  $+0.16 \text{ g } 100 \text{ g}^{-1}$ ,  $P < 0.001$ ), *cis* monounsaturated FA (*cis*-MUFA;  $+5.0 \text{ g } 100 \text{ g}^{-1}$ ,  $P < 0.01$ ), and lower in short- and medium-chain FA (C6:0 + C8:0 + C10:0 and C12:0 + C14:0;  $-1.31$  and  $-3.35 \text{ g } 100 \text{ g}^{-1}$ , respectively), C16:0 ( $-3.25 \text{ g } 100 \text{ g}^{-1}$ ,  $P < 0.01$ ), *trans*11-C18:1 ( $-1.07 \text{ g } 100 \text{ g}^{-1}$ ,  $P < 0.01$ ), *trans*-MUFA ( $-1.31 \text{ g } 100 \text{ g}^{-1}$ ,  $P < 0.01$ ), and poly-unsaturated FA (PUFA;  $-0.48 \text{ g } 100 \text{ g}^{-1}$ ,  $P < 0.05$ ) than SEMI milks. The *cis*9-C18:1 to C16:0 ratio was significantly higher ( $P < 0.001$ ) in EXT than SEMI milks. The fatty acid composition of milks is detailed in Supplementary Table S2. Regarding microbial

**Table 1**  
Effects of grazing system and period of cheese-making on bulk milk gross composition, fatty acid concentrations and the level of the major microbial groups.<sup>a</sup>

Item	July		September		SEM	Effects and significance		
	EXT	SEMI	EXT	SEMI		S	P	S*P
Milk pH	6.71	6.73	6.70	6.72	0.003	**	ns	ns
Fat content (g kg <sup>-1</sup> )	40.1	34.4	42.1	37.3	0.910	***	**	ns
Protein content (g kg <sup>-1</sup> )	29.1 <sup>b</sup>	29.8 <sup>b</sup>	29.9 <sup>b</sup>	32.9 <sup>a</sup>	0.466	***	***	**
Fat/protein ratio	1.37	1.17	1.37	1.10	0.038	***	ns	ns
Lactose content (g kg <sup>-1</sup> )	50.3 <sup>a</sup>	49.3 <sup>a</sup>	47.8 <sup>b</sup>	49.2 <sup>ab</sup>	0.317	ns	*	*
SCC (10 <sup>3</sup> cfu mL <sup>-1</sup> )	184	262	213	365	23.80	**	*	ns
FFA (mEq 100 g <sup>-1</sup> of fat)	0.19	0.20	0.37	0.29	0.068	ns	ns	ns
LPL activity (nmol min <sup>-1</sup> mL <sup>-1</sup> )	634	643	641	596	13.41	ns	ns	ns
FA (g 100 g <sup>-1</sup> of FA)								
C4:0	3.29	3.02	3.17	3.10	0.045	•	ns	ns
C6:0 + C8:0 + C10:0	4.72	6.34	4.65	5.65	0.248	**	ns	ns
C12:0 + C14:0	10.9	14.7	11.8	14.7	0.583	***	ns	ns
C16:0	24.3	26.4	24.7	29.1	0.654	**	•	ns
C18:0	13.2	9.42	12.5	8.08	0.687	***	ns	ns
C20:0 + C22:0 + C24:0	0.30	0.18	0.36	0.17	0.026	***	ns	ns
<i>cis</i> 9-C18:1	24.9	19.8	23.5	17.8	0.997	**	ns	ns
Σ other <i>cis</i> -C18:1	1.17	1.07	1.06	0.86	0.037	**	**	ns
<i>trans</i> 11-C18:1	2.60	3.25	2.33	3.82	0.215	**	ns	ns
Σ other <i>trans</i> -C18:1	4.06	5.04	3.77	5.41	0.265	*	ns	ns
C18:3 <i>n</i> -3	0.86	0.77	0.94	0.66	0.033	***	ns	**
Σ CLA	1.10 <sup>b</sup>	1.45 <sup>b</sup>	1.14 <sup>b</sup>	2.26 <sup>a</sup>	0.152	***	*	*
Σ <i>n</i> -3 LCPUFA	1.11 <sup>b</sup>	0.99 <sup>c</sup>	1.24 <sup>a</sup>	0.91 <sup>d</sup>	0.038	***	ns	**
Σ <i>n</i> -6 LCPUFA	1.45 <sup>a</sup>	1.49 <sup>a</sup>	1.38 <sup>a</sup>	1.20 <sup>b</sup>	0.037	ns	**	*
Σ SFA <sup>7</sup>	60.9	64.3	62.0	65.0	0.675	*	ns	ns
Σ <i>cis</i> -MUFA	29.0	24.2	27.7	22.5	0.939	**	ns	ns
Σ <i>trans</i> -MUFA	5.06	6.00	4.79	6.47	0.268	**	ns	ns
Σ PUFA	4.16	4.51	4.33	4.94	0.117	*	ns	ns
<i>cis</i> 9-C18:1/C16:0	1.03	0.75	0.95	0.62	0.054	***	ns	ns
Presumed microbial group (log cfu mL <sup>-1</sup> )								
Total mesophilic bacteria	3.22	2.96	3.46	3.11	0.070	*	•	ns
Lactic acid bacteria	3.16	2.76	3.31	3.00	0.089	*	ns	ns
Heterofermentative facultative <i>Lactobacillus</i>	2.83	2.32	2.95	2.60	0.097	*	ns	ns
Dextran-producing <i>Leuconostoc</i>	1.71	1.54	0.40	0.52	0.232	ns	*	ns
Gram-positive catalase-positive bacteria	1.97	1.75	1.53	1.53	0.096	ns	ns	ns
Gram-negative bacteria	2.06	1.93	1.43	1.49	0.221	ns	ns	ns
<i>Enterococcus</i>	0.60	0.45	0.73	0.94	0.141	ns	ns	ns
Yeast	1.42	1.30	1.38	1.02	0.138	ns	ns	ns
Mould	0.77	0.48	0.91	0.92	0.092	ns	ns	ns

<sup>a</sup> Values reported are the mean from triplicate milk samples (n = 3); values with different superscript letters differ at P < 0.05 by statistical Benjamini–Hochberg test (• P < 0.10; \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; ns, not significant). The detailed description of the sums of fatty acids are reported in the supplementary information. Abbreviations are: EXT, extensive grazing system; SEMI, semi-intensive grazing system; S, grazing system; P, period; SCC, somatic cell counts; FA, fatty acids; FFA, free FA; CLA, conjugated linoleic acid; SFA, saturated FA; PUFA, poly-unsaturated FA; LCPUFA, long-chain PUFA; MUFA, mono-unsaturated FA; SEM, standard error of the mean.

levels, three interrelated microbial groups were affected (P < 0.05) by the grazing system regardless of the period studied. The levels of total mesophilic bacteria, lactic acid bacteria, and heterofermentative facultative *Lactobacillus* were higher in EXT than in SEMI milks (+0.31, +0.36 and + 0.43 log cfu mL<sup>-1</sup>, respectively).

With regard to the period, the milk fat content and SCC increased from July to September. The period had a limited effect on milk FA composition and microbial levels regardless of the grazing system used. The concentration of *cis*-C18:1 other than oleic acid and the level of dextran-producing *Leuconostoc* decreased from July to September. In September, milk protein and lactose contents were higher in SEMI milks than in EXT milks (+3.0 and 1.4 g kg<sup>-1</sup>, respectively) while no difference was observed in milks from July. The proportion of *n*-3 FA was higher in EXT than in SEMI milks. This difference was greater in September (+0.33 g 100 g<sup>-1</sup>). During this period, SEMI milks had a higher proportion of conjugated linoleic acids (CLA) but a lower proportion of *n*-6 PUFA than EXT milks.

### 3.2. Cheese chemical characteristics and microbial composition

The rennet clotting time was lower for EXT than SEMI milks (–2 min 58 s, P < 0.001; Supplementary Table S3). The acidification

of the curd was similar, but the draining was faster in EXT than SEMI cheeses, resulting in a significantly higher DM content in EXT cheeses placed in the cellar (P < 0.001). Milk pasteurisation resulted in slightly reduced curd draining, while R and PA cheeses placed in the cellar had similar pH values. From July to September, the rennet clotting time decreased (–1 min 26 s, P < 0.05) and curd draining was slower, even if the cheeses placed in the cellar had similar pH and dry matter content (DM) values.

For every grazing system and period of cheese-making tested, the fat in DM content was significantly higher in R than in PA cheeses (+1.68%, P < 0.001; Table 2). R cheeses had higher pH values than PA cheese (P < 0.001). The maximal deformation strength was lower for R than for PA cheeses (–82 N cm<sup>-2</sup>, P < 0.001). The R cheeses were brighter than the PA cheeses (P < 0.01). The milk treatment did not affect the total FA profile of the cheeses, but it significantly reduced the concentration of the major VFA released through lipolysis (Table 3). In cheese rind, the levels of most microbial groups were lower in PA than in R cheeses (Table 4). However, the levels of Gram-positive catalase-positive bacteria tended to be higher in PA cheese rinds. In cheese cores, the levels of Gram-positive catalase-positive bacteria and of *Enterobacteriaceae* were slightly lower (P < 0.05) in PA than in R cheeses. Furthermore, primary (WSN/N) and secondary (PTSN/N)

**Table 2**  
Effects of grazing system, period and treatment of milk on cheese gross composition, proteolysis, rheology, and colour.<sup>a</sup>

Parameters	Raw				Pasteurised				SEM	Effects and significance				
	July		September		July		September			S	T	S*T	P	P*T
	EXT	SEMI	EXT	SEMI	EXT	SEMI	EXT	SEMI						
DM (%)	63.6 <sup>abc</sup>	63.6 <sup>abc</sup>	65.0 <sup>a</sup>	62.8 <sup>c</sup>	63.6 <sup>abc</sup>	64.4 <sup>ab</sup>	63.4 <sup>bc</sup>	63.8 <sup>abc</sup>	0.199	ns	ns	*	ns	ns
Fat (%)	35.4 <sup>ab</sup>	32.4 <sup>de</sup>	36.1 <sup>a</sup>	31.8 <sup>e</sup>	34.3 <sup>bc</sup>	32.2 <sup>de</sup>	33.5 <sup>cd</sup>	31.5 <sup>e</sup>	0.360	***	**	*	ns	ns
Fat in DM (%)	55.7	51.0	55.5	50.7	54.0	50.0	52.8	49.4	0.506	***	***	ns	ns	ns
pH	5.56	5.60	5.67	5.57	5.36	5.47	5.44	5.42	0.025	ns	***	ns	ns	ns
Calcium (%)	0.66	0.76	0.67	0.76	0.69	0.74	0.72	0.73	0.011	**	ns	•	ns	ns
Phosphorus (%)	0.46	0.53	0.47	0.53	0.47	0.53	0.48	0.51	0.008	***	ns	ns	ns	ns
Proteolysis														
WSN/N (%)	26.6 <sup>a</sup>	23.0 <sup>b</sup>	21.3 <sup>bc</sup>	19.8 <sup>c</sup>	20.1 <sup>c</sup>	20.8 <sup>bc</sup>	20.9 <sup>bc</sup>	21.0 <sup>bc</sup>	0.510	ns	**	*	*	**
PTSN/WSN (%)	17.7	15.7	16.2	15.1	12.6	11.2	13.4	12.4	0.528	•	***	ns	ns	ns
PTSN/N (%)	4.70 <sup>a</sup>	3.60 <sup>b</sup>	3.42 <sup>bc</sup>	2.99 <sup>bcd</sup>	2.55 <sup>de</sup>	2.33 <sup>e</sup>	2.79 <sup>cde</sup>	2.61 <sup>de</sup>	0.163	**	***	•	*	**
Rheology														
Strength to 10% of deformation (N cm <sup>-2</sup> )	1.41	1.48	1.28	1.53	1.71	1.92	1.70	1.97	0.071	ns	**	ns	ns	ns
Strength to 30% of deformation (N cm <sup>-2</sup> )	3.59	4.27	3.40	3.83	5.38	5.88	4.88	5.60	0.218	*	***	ns	ns	ns
Strength to 50% of deformation (N cm <sup>-2</sup> )	5.36	6.43	5.11	6.17	8.14	9.46	7.56	8.71	0.353	**	***	ns	ns	ns
Strength to max deformation (N cm <sup>-2</sup> )	206	250	209	259	290	353	276	332	11.50	***	***	ns	ns	ns
Colour (cheese core)														
L* (black–white)	76.9	75.3	78.4	78.7	75.9	74.6	78.1	77.6	0.315	**	**	ns	***	ns
a* (green–red)	1.76	2.18	1.90	2.08	1.90	1.98	1.91	2.09	0.039	**	ns	ns	ns	ns
b* (blue–yellow)	20.0 <sup>bc</sup>	21.1 <sup>a</sup>	19.6 <sup>c</sup>	20.4 <sup>ab</sup>	20.5 <sup>ab</sup>	21.0 <sup>a</sup>	21.0 <sup>a</sup>	20.9 <sup>a</sup>	0.124	**	**	*	ns	•

<sup>a</sup> Values reported are the mean from triplicate cheese samples (n = 3); values with different superscript letters differ at P < 0.05 by statistical Benjamini–Hochberg test (• P < 0.10; \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; ns, not significant). The non-significant interaction (S\*T) is not reported in the table. Abbreviations are: EXT, extensive grazing system; SEMI, semi-intensive grazing system; S, grazing system; T, treatment of milk; P, period; DM, dry matter; N, nitrogen; WSN, water-soluble nitrogen; PTSN, phototungstic acid soluble nitrogen; SEM, standard error of the means.

**Table 3**  
Effects of grazing system, period of cheese-making and treatment of milk on concentrations of fatty acids and volatile fatty acids in cheeses.<sup>a</sup>

Fatty acids (g 100 g <sup>-1</sup> of FA)	Raw				Pasteurised				SEM	Effects and significance			
	July		September		July		September			S	T	P	S*P
	EXT	SEMI	EXT	SEMI	EXT	SEMI	EXT	SEMI					
C4:0	3.77	3.42	3.35	3.56	3.68	3.60	3.32	3.41	0.051	ns	ns	*	•
C6:0 + C8:0 + C10:0	4.91	6.35	4.61	6.01	4.85	6.54	4.68	5.78	0.178	***	ns	•	ns
C12:0 + C14:0	10.9	14.7	11.7	14.9	11.0	14.5	11.8	14.8	0.393	***	ns	ns	ns
C16:0	24.0 <sup>c</sup>	26.2 <sup>b</sup>	24.5 <sup>bc</sup>	28.7 <sup>a</sup>	23.9 <sup>c</sup>	25.8 <sup>bc</sup>	24.5 <sup>bc</sup>	28.9 <sup>a</sup>	0.444	***	ns	**	*
C18:0	13.2	9.45	12.5	7.98	13.0	9.38	12.4	7.99	0.468	***	ns	*	ns
C20:0 + C22:0 + C24:0	0.31 <sup>b</sup>	0.20 <sup>c</sup>	0.38 <sup>a</sup>	0.16 <sup>c</sup>	0.31 <sup>b</sup>	0.19 <sup>c</sup>	0.38 <sup>ab</sup>	0.17 <sup>c</sup>	0.019	***	ns	ns	*
cis9-C18:1	24.6	19.5	23.2	17.4	25.0	19.8	23.2	17.6	0.680	***	ns	*	ns
Σ cis-C18:1 isomers	1.50 <sup>a</sup>	1.40 <sup>ab</sup>	1.40 <sup>ab</sup>	1.11 <sup>c</sup>	1.48 <sup>ab</sup>	1.39 <sup>b</sup>	1.41 <sup>ab</sup>	1.13 <sup>c</sup>	0.031	***	ns	***	**
trans11-C18:1	2.61	3.34	2.37	3.82	2.62	3.33	2.45	3.85	0.149	***	ns	ns	ns
Σ trans-C18:1 isomers	3.64	4.61	3.44	5.09	3.57	4.63	3.52	5.14	0.180	***	ns	ns	ns
C18:3 n-3	0.88	0.78	0.95	0.65	0.89	0.80	0.94	0.66	0.024	***	ns	•	***
Σ CLA	1.06 <sup>b</sup>	1.44 <sup>b</sup>	1.13 <sup>b</sup>	2.15 <sup>a</sup>	1.06 <sup>b</sup>	1.45 <sup>b</sup>	1.19 <sup>b</sup>	2.21 <sup>a</sup>	0.099	***	ns	***	**
Σ n-3 LCPUFA	1.15 <sup>b</sup>	1.03 <sup>c</sup>	1.26 <sup>a</sup>	0.89 <sup>d</sup>	1.15 <sup>b</sup>	1.05 <sup>c</sup>	1.26 <sup>a</sup>	0.90 <sup>d</sup>	0.029	***	ns	ns	1.26 <sup>a</sup>
Σ n-6 LCPUFA	1.47 <sup>a</sup>	1.52 <sup>a</sup>	1.43 <sup>a</sup>	1.16 <sup>b</sup>	1.48 <sup>a</sup>	1.53 <sup>a</sup>	1.44 <sup>a</sup>	1.20 <sup>b</sup>	0.031	**	ns	***	***
Σ SFA	61.3	64.6	62.1	65.6	60.9	64.2	62.0	65.3	0.454	***	ns	ns	ns
Σ cis-MUFA	28.7	23.9	27.5	22.1	29.1	24.2	27.5	22.3	0.637	***	ns	*	ns
Σ trans-MUFA	4.95	5.89	4.81	6.40	4.88	5.90	4.88	6.44	0.179	***	ns	ns	ns
Σ PUFA	4.19	4.62	4.42	4.77	4.20	4.67	4.49	4.88	0.072	**	ns	•	ns
cis9-C18:1/C16:0	1.02	0.75	0.95	0.61	1.04	0.77	0.95	0.61	0.038	***	ns	**	ns
Volatile fatty acids													
Acetic acid	61.1	72.1	56.6	64.5	46.7	43.1	33.5	43.9	3.393	ns	**	ns	ns
Propionic acid	0.35	0.70	1.58	0.32	0.14	0.10	0.09	0.08	0.137	ns	*	ns	•
Isobutyric acid	0.16 <sup>abc</sup>	0.23 <sup>a</sup>	0.19 <sup>ab</sup>	0.08 <sup>de</sup>	0.12 <sup>bcd</sup>	0.10 <sup>cde</sup>	0.10 <sup>cde</sup>	0.05 <sup>e</sup>	0.013	•	***	**	**
Butyric acid	15.7 <sup>b</sup>	13.8 <sup>bc</sup>	20.5 <sup>a</sup>	10.3 <sup>cde</sup>	9.88 <sup>def</sup>	7.50 <sup>ef</sup>	11.4 <sup>cd</sup>	6.44 <sup>f</sup>	0.963	***	***	ns	**
Isovaleric acid	0.38 <sup>abc</sup>	0.42 <sup>abc</sup>	0.45 <sup>ab</sup>	0.21 <sup>bc</sup>	0.32 <sup>abc</sup>	0.50 <sup>a</sup>	0.36 <sup>abc</sup>	0.20 <sup>c</sup>	0.031	ns	ns	•	*
Isocaproic acid	0.06	0.06	0.04	0.05	0.05	0.06	0.05	0.05	0.004	ns	ns	ns	ns
Caproic acid	6.04 <sup>b</sup>	6.07 <sup>b</sup>	7.76 <sup>a</sup>	5.06 <sup>bc</sup>	3.94 <sup>cd</sup>	3.66 <sup>cd</sup>	4.74 <sup>bc</sup>	2.62 <sup>d</sup>	0.348	**	***	ns	**

<sup>a</sup> Values reported are the mean from triplicate cheese samples (n = 3); values with different superscript letters differ at P < 0.05 by statistical Benjamini–Hochberg test (• P < 0.10; \*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; ns, not significant). The non-significant interactions (P\*T and S\*T) are not reported in the table. The detailed description of the sums of fatty acids are reported in the supplementary information. Abbreviations are: EXT, extensive grazing system; SEMI, semi-intensive grazing system; S, grazing system; T, treatment of milk; P, period; FA, fatty acids; CLA, conjugated linoleic acid; SFA, saturated FA; PUFA, poly-unsaturated FA; LCPUFA, long-chain PUFA; MUFA, mono-unsaturated FA; SEM, standard error of the means.

**Table 4**  
Effects of grazing system, period of cheese-making and treatment of milk on the level of the major microbial groups in rind and core of cheeses.<sup>a</sup>

Presumed microbial group (log cfu g <sup>-1</sup> )	Raw				Pasteurised				SEM	Effects and significance			
	July		September		July		September			S	T	P	P*T
	EXT	SEMI	EXT	SEMI	EXT	SEMI	EXT	SEMI					
<b>Cheese rind</b>													
Total mesophilic bacteria	9.20	9.42	9.00	9.11	9.47	9.17	9.22	9.16	0.049	ns	ns	•	ns
Lactic acid bacteria	7.27	6.93	7.32	7.23	6.43	6.08	6.31	6.36	0.108	•	***	ns	ns
Heterofermentative facultative <i>Lactobacillus</i>	7.24 <sup>a</sup>	6.82 <sup>a</sup>	7.34 <sup>a</sup>	7.04 <sup>a</sup>	4.92 <sup>c</sup>	4.52 <sup>c</sup>	5.98 <sup>b</sup>	5.99 <sup>b</sup>	0.223	ns	***	**	*
Gram-positive catalase-positive bacteria	9.71	9.98	9.70	9.47	10.0	10.1	9.94	9.86	0.072	ns	•	ns	ns
<i>Enterococcus</i>	5.45	5.20	5.33	5.07	nd	nd	1.94	1.70	0.417	ns	***	ns	•
<i>Enterobacteriaceae</i>	2.74	2.35	2.75	2.45	nd	nd	2.15	nd	0.190	•	***	ns	ns
Yeast	6.81	6.49	6.26	5.95	4.73	4.18	5.38	3.74	0.253	*	***	ns	ns
Mould	6.50	6.73	6.66	6.71	6.05	6.13	5.93	6.16	0.091	ns	**	ns	ns
<b>Cheese core</b>													
Total mesophilic bacteria	7.14	6.93	7.15	7.27	7.12	6.75	7.15	7.08	0.071	ns	ns	ns	ns
Lactic acid bacteria	6.15	6.91	6.64	6.93	6.99	6.78	6.65	6.68	0.160	ns	ns	ns	ns
Heterofermentative facultative <i>Lactobacillus</i>	7.31	6.95	7.50	7.41	7.20	6.95	7.07	6.95	0.113	ns	ns	ns	ns
Dextran-producing <i>Leuconostoc</i>	4.30	2.99	nd	nd	1.67	1.67	nd	nd	0.336	ns	ns	*	ns
Gram-positive catalase-positive bacteria	5.52	5.26	5.84	5.78	4.68	4.69	5.19	5.02	0.156	ns	*	ns	ns
<i>Enterococcus</i>	5.95 <sup>a</sup>	5.76 <sup>a</sup>	5.45 <sup>a</sup>	4.94 <sup>a</sup>	nd	nd	2.76 <sup>b</sup>	2.08 <sup>bc</sup>	0.433	ns	***	ns	**
<i>Enterobacteriaceae</i>	2.67	2.30	1.63	1.43	nd	nd	1.72	nd	0.178	ns	*	ns	•
Yeast	3.54	2.67	4.06	3.83	nd	nd	3.55	3.35	0.377	ns	•	*	ns
Mould	1.08	2.19	nd	nd	1.41	nd	nd	1.49	0.125	ns	ns	ns	ns

<sup>a</sup> Values reported are the mean from triplicate cheese samples ( $n = 3$ ); values with different superscript letters differ at  $P < 0.05$  by statistical Benjamini–Hochberg test ( $\bullet P < 0.10$ ;  $*P < 0.05$ ;  $**P < 0.01$ ;  $***P < 0.001$ ; ns, not significant). The non-significant interactions (S\*P and S\*T) are not reported in the table. Abbreviations are: EXT, extensive grazing system; SEMI, semi-intensive grazing system; S, grazing system; T, treatment of milk; P, period; nd, not determined (below detection threshold: 10 or 100 according to media); SEM, standard error of the means.

proteolysis was most advanced in R cheeses made in July, while no difference was observed among PA cheeses (Table 2). In September, the levels of heterofermentative facultative *Lactobacillus* on the cheese rind and of *Enterococcus* on the cheese core were higher in PA cheeses, while no difference was observed among R cheeses (Table 4).

For every milk treatment and period of cheese-making studied, the fat in DM content was significantly higher in EXT than in SEMI cheeses (+4.23%,  $P < 0.001$ ; Table 2), and the opposite was observed for cheese calcium and phosphorus contents. The maximal deformation strength was higher for SEMI than for EXT cheeses (+53.3 N cm<sup>-2</sup>,  $P < 0.001$ ). The latter were also brighter and less red than SEMI cheeses ( $P < 0.01$ ). The FA profiles of EXT and SEMI cheeses were very different, as they were for their respective milks (Table 3). The EXT cheese cores were higher in C18:0 (+4.1 g 100 g<sup>-1</sup>,  $P < 0.001$ ), cis9-C18:1 (+5.4 g 100 g<sup>-1</sup>,  $P < 0.001$ ), and cis-MUFA (+5.1 g 100 g<sup>-1</sup>,  $P < 0.001$ ), and lower in short- and medium-chain FA (-1.4 and -3.4 g 100 g<sup>-1</sup>, respectively,  $P < 0.001$ ), saturated FA (SFA; -3.4 g 100 g<sup>-1</sup>,  $P < 0.001$ ), trans11-C18:1 (-1.1 g 100 g<sup>-1</sup>,  $P < 0.001$ ), trans-MUFA (-1.3 g 100 g<sup>-1</sup>,  $P < 0.001$ ), and PUFA (-0.4 g 100 g<sup>-1</sup>,  $P < 0.01$ ) than SEMI cheese cores. The cis9-C18:1 to C16:0 ratio was significantly higher for EXT than for SEMI cheese cores ( $P < 0.001$ ). The fatty acid composition of cheese cores is detailed in Supplementary Table S4. Similar results were obtained for cheese rinds (Supplementary Table S5). The levels of microbial groups in cheeses were weakly impacted by the grazing system used, except for yeasts (Table 4). Their levels were higher in EXT than in SEMI cheese rinds (+0.71 log cfu g<sup>-1</sup>).

The interaction between the effects of the grazing system and milk pasteurisation (S\*T) was significant for some cheese characteristics: the DM content of SEMI cheeses was higher in PA than in R cheeses, contrary to the DM content of EXT cheeses (Table 2). Fat content in EXT cheeses was higher in R than in PA cheeses, while no difference was observed in SEMI cheeses. The indicator of primary proteolysis (WSN/N) in R cheeses was higher in EXT cheeses than in SEMI cheeses, while it was equivalent in all PA cheeses regardless of the grazing system used. The colour of R cheeses was less yellow than that of PA cheeses, in particular for EXT cheeses.

Concerning the period of cheese-making, the gross composition of ripened cheeses made in July and September was similar regardless of the grazing system used and any milk treatment (Table 2). Cheeses made in July were less white than those made in September ( $P < 0.001$ ). The concentrations of C18:0 (+1.1 g 100 g<sup>-1</sup>), cis9-C18:1 (+1.9 g 100 g<sup>-1</sup>), and cis-MUFA (+1.6 g 100 g<sup>-1</sup>) were slightly higher in July than in September cheese cores ( $P < 0.05$ ; Table 3). The level of dextran-producing *Leuconostoc* was higher in the cores of cheeses made in July, while the opposite effect was observed for yeasts ( $P < 0.05$ ; Table 4). In September, the concentrations of C16:0 and CLA were higher in SEMI than in EXT cheese cores (Table 3). During this period, SEMI cheese cores had lower concentrations of long-chain saturated FA,  $n-3$  FA, and  $n-6$  FA. Moreover, in the volatile fraction, the concentrations of butyric and caproic acids were higher for EXT than for SEMI cheeses, especially in September.

### 3.3. Cheese sensory properties

Cantal-type cheese sensory characteristics are presented in Table 5. For every grazing system and period of cheese-making studied, most sensory attributes were modified by the milk treatment used. In R cheese rinds, the spot salience (-3.0) and quantity (-3.3) were scored lower than for PA cheeses. The R cheese cores were less yellow (-0.4,  $P < 0.001$ ) and less veined (-0.6,  $P < 0.01$ ) than PA cheese cores. The R cheeses were considered by the panellists to be less firm to the touch (-0.9,  $P < 0.001$ ) and more granular in the mouth (+0.5,  $P < 0.001$ ) than PA cheeses. The R cheeses were characterised by a more intense taste (+0.9,  $P < 0.001$ ) with a more pronounced pungent taste (+0.7,  $P < 0.001$ ) than PA cheeses. The R cheeses also developed more intense odours (+0.9,  $P < 0.01$ ) characterised by the attributes “fermented cream” and “yeast”, while the PA cheeses were characterised by the attributes “fresh cream” and “butter”. The R cheeses also developed more persistent aromas (+0.8,  $P < 0.001$ ) described as more “rancid” and “stable” and less “butter”. The colour of R cheeses spots was slightly more ochre in September, contrary to those of PA cheeses, which were more ochre in July. Cheeses made in July from

**Table 5**  
Effects of grazing system, period of cheese-making and treatment of milk on the Cantal-type cheese sensory characteristics.<sup>a</sup>

Item	Raw				Pasteurised				SEM	Effects and significance				
	July		September		July		September			S	T	S*T	P	P*T
	EXT	SEMI	EXT	SEMI	EXT	SEMI	EXT	SEMI						
<b>Appearance of cheese rind</b>														
White-grey colour	4.5 <sup>bc</sup>	4.6 <sup>bc</sup>	4.2 <sup>c</sup>	4.1 <sup>c</sup>	6.1 <sup>a</sup>	5.6 <sup>a</sup>	6.2 <sup>a</sup>	5.3 <sup>ab</sup>	0.115	**	***	*	ns	ns
Spot colour (white-ochre)	5.7 <sup>c</sup>	6.0 <sup>c</sup>	6.0 <sup>c</sup>	6.4 <sup>bc</sup>	7.6 <sup>a</sup>	7.5 <sup>ab</sup>	7.2 <sup>ab</sup>	7.4 <sup>ab</sup>	0.070	ns	***	ns	ns	*
Spot salience	4.4	4.4	4.0	4.4	7.1	7.3	7.5	7.0	0.063	ns	***	ns	ns	ns
Spot quantity	4.1	4.3	3.0	4.2	7.0	7.7	6.9	7.3	0.074	*	***	ns	ns	ns
<b>Appearance of cheese core</b>														
White-yellow colour	5.6	6.3	5.4	5.8	6.4	6.7	5.6	6.0	0.061	*	***	ns	***	•
Core veined	3.6	3.8	3.8	4.3	4.4	4.8	4.2	4.6	0.117	ns	**	ns	ns	ns
Core marbled	4.6	5.2	3.5	4.0	4.6	4.5	3.4	3.7	0.110	ns	ns	ns	***	ns
<b>Odour</b>														
Intense	6.3	5.7	5.5	5.6	5.2	5.0	4.9	4.6	0.093	ns	**	ns	•	ns
Fresh cream	0.2	0.4	0.3	0.0	0.8	0.7	0.9	0.7	0.073	ns	**	ns	ns	ns
Fermented cream	1.8	1.5	0.9	1.1	0.9	0.9	0.6	0.4	0.043	ns	**	ns	•	ns
Butter	0.2	0.2	0.1	0.2	0.6	0.5	0.5	0.3	0.034	ns	***	ns	ns	ns
Egg	0.2	0.1	0.3	0.3	0.1	0.1	0.1	0.1	0.027	ns	ns	ns	ns	ns
Swiss type cheese	0.4 <sup>ab</sup>	0.3 <sup>b</sup>	0.6 <sup>ab</sup>	0.4 <sup>ab</sup>	0.7 <sup>ab</sup>	0.8 <sup>a</sup>	0.4 <sup>ab</sup>	0.5 <sup>ab</sup>	0.067	ns	•	ns	*	*
Yeast	1.3	0.8	0.6	0.6	0.5	0.7	0.5	0.4	0.042	ns	**	ns	ns	ns
Vanilla	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.025	ns	ns	ns	ns	ns
Plastic	0.2	0.1	0.2	0.2	0.1	0.1	0.1	0.0	0.008	ns	ns	ns	ns	ns
<b>Texture</b>														
Firm	4.6	4.9	4.9	5.0	5.8	5.7	5.5	5.9	0.066	ns	***	ns	ns	ns
Elastic	3.8 <sup>d</sup>	4.5 <sup>c</sup>	4.9 <sup>abc</sup>	5.5 <sup>a</sup>	4.8 <sup>bc</sup>	5.3 <sup>ab</sup>	5.3 <sup>ab</sup>	5.0 <sup>abc</sup>	0.103	ns	***	*	ns	***
Creamy	5.2	5.0	5.5	5.1	4.8	4.3	5.0	4.6	0.068	*	•	ns	ns	ns
Adhesive	2.1	1.9	2.6	1.8	2.6	1.6	2.7	2.3	0.097	**	ns	ns	ns	ns
Granular	1.7	1.4	1.1	1.0	1.0	1.0	0.5	0.9	0.054	ns	***	ns	•	ns
<b>Taste</b>														
Intense	6.7	6.4	6.4	6.1	5.6	5.7	5.4	5.4	0.080	ns	***	ns	*	ns
Salty	5.4	5.5	5.5	5.4	5.4	5.3	5.4	5.2	0.031	*	ns	ns	ns	ns
Sour	1.7	1.8	1.8	1.7	1.6	1.2	2.0	1.4	0.088	*	ns	•	ns	ns
Bitter	2.2	2.2	2.5	2.0	2.0	1.8	1.9	1.7	0.090	ns	ns	ns	ns	ns
Pungent	2.3	2.2	2.2	1.5	1.5	1.2	1.4	1.2	0.078	ns	***	ns	ns	ns
<b>Aroma</b>														
Fresh cream	0.1	0.0	0.1	0.1	0.2	0.1	0.3	0.1	0.024	ns	ns	ns	ns	ns
Fermented cream	1.1 <sup>a</sup>	0.6 <sup>b</sup>	0.5 <sup>b</sup>	0.4 <sup>b</sup>	0.4 <sup>b</sup>	0.6 <sup>b</sup>	0.2 <sup>b</sup>	0.4 <sup>b</sup>	0.041	ns	***	**	ns	ns
Butter	0.0	0.1	0.1	0.1	0.2	0.3	0.2	0.1	0.017	ns	**	ns	*	•
Swiss type cheese	0.2	0.2	0.2	0.1	0.3	0.4	0.2	0.3	0.048	ns	ns	ns	•	ns
Mushroom	0.2	0.0	0.1	0.1	0.2	0.1	0.1	0.0	0.012	ns	ns	ns	ns	ns
Stable	0.8	0.5	0.4	0.3	0.2	0.3	0.1	0.1	0.023	ns	***	ns	ns	ns
Rancid	0.6	0.6	0.5	0.4	0.2	0.2	0.2	0.3	0.035	ns	***	ns	ns	ns
Plastic	0.1 <sup>ab</sup>	0.2 <sup>ab</sup>	0.3 <sup>a</sup>	0.2 <sup>ab</sup>	0.2 <sup>ab</sup>	0.2 <sup>ab</sup>	0.1 <sup>ab</sup>	0.0 <sup>b</sup>	0.006	ns	ns	ns	•	*
Persistent	6.3	5.9	6.2	6.0	5.5	5.0	5.4	5.4	0.085	ns	***	ns	ns	ns

<sup>a</sup> Values reported are the mean from triplicate cheese samples ( $n = 3$ ); values with different superscript letters differ at  $P < 0.05$  by statistical Benjamini–Hochberg test ( $\bullet$   $P < 0.10$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; ns, not significant). The non-significant interaction ( $S^*P$ ) is not reported in the table. Abbreviations are: EXT, extensive grazing system; SEMI, semi-intensive grazing system; S, grazing system; T, treatment of milk; P, period; SEM, standard error of the means.

R milks were less elastic than those made in September, while no difference was observed in PA cheeses. In July, the R cheeses had a less “Swiss type cheese” odour than PA cheeses, while no difference was observed in cheeses made in September.

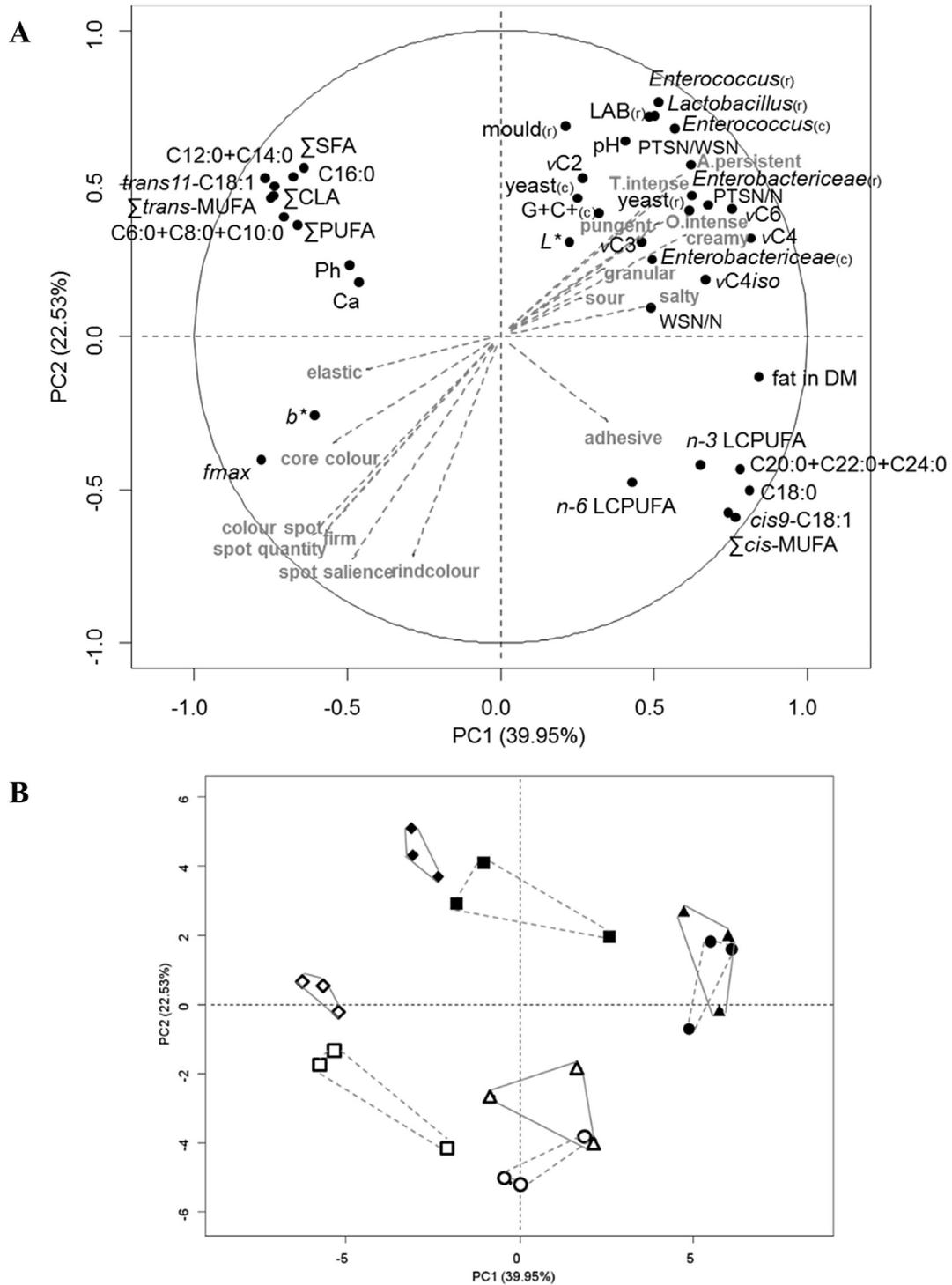
A significant effect of the grazing system used was observed only for six out of thirty-five sensory attributes, regardless of the milk treatment and the period of cheese-making. The quantity of spots on the rind was slightly lower, and the cheese cores were less yellow ( $P < 0.05$ ) for EXT than for SEMI cheese rinds. The latter were creamier ( $P < 0.05$ ) and more adhesive ( $P < 0.01$ ). Moreover, EXT cheeses were slightly saltier and more sour ( $P < 0.05$ ) than SEMI cheeses. No sensory attributes related to odour and aroma were affected by the grazing system used.

The  $S^*T$  interaction affected few cheese sensory properties. Among PA cheeses, the EXT cheeses had greyer rinds than the SEMI cheeses, while no difference was observed between R cheeses from the two grazing systems. The SEMI cheeses were more elastic than EXT cheeses, in particular for the R cheeses. The “fermented cream” aroma was higher for EXT cheeses than SEMI cheeses in R cheeses, while no difference was observed among PA cheeses.

Concerning the period of cheese-making, the differences between cheeses made in July and in September concerned mainly the appearance of the cheese core, regardless of the grazing system and the milk treatment used. Cheeses made in September were judged as having a whiter ( $+0.6$ ,  $P < 0.001$ ) and a more marbled core ( $+1.1$ ,  $P < 0.001$ ) than July cheeses. The latter had a more intense taste ( $P < 0.05$ ). The period had slight effects on the sensory attributes of odour, texture, and aroma.

#### 3.4. Relationships between sensory characteristics and the biochemical and microbial composition of Cantal-type cheeses

PCA was performed to summarise the main relationships between sensorial, biochemical and microbial data accounting for effects attributable to the grazing system, milk treatment, and period of cheese-making used (Fig. 1). The first and second principal components accounted for 62% of the total variance. Generally, the sensory variables corresponding to aroma, odour, and taste were positively correlated (Fig. 1A). These variables were also positively correlated with the levels of microbial groups,



**Fig. 1.** Principal component analysis (PCA) performed on biochemical and microbial data significantly different for at least one of 3 factors (grazing system, period of cheese-making, treatment of milk) from 24 cheese samples. Panel A: projection of 39 active variables (●) and the 16 highest discriminant sensorial attributes used as non-active variables (---). Abbreviations are: DM, dry matter; Ca, calcium; Ph, phosphorous; N, nitrogen; WSN, water-soluble nitrogen; PTSN, phosphotungstic acid soluble nitrogen; vC2, volatile acetic acid; vC3, volatile propionic acid; vC4iso, volatile isobutyric acid; vC4, volatile butyric acid; vC6, volatile caproic acid; fmax, strength to max deformation; FA, fatty acids; CLA, conjugated linoleic acid; SFA, saturated FA; PUFA, poly-unsaturated FA; LCPUFA, long-chain PUFA; MUFA, mono-unsaturated FA; (r), cheese rind; (c), cheese core; LAB, lactic acid bacteria; G+C+, Gram-positive catalase-positive bacteria; O., odour; T., taste; A., aroma. Panel B, plot of sample distribution is projected on principal components (PC) 1 and 2 (●, ○, EXT cheeses in July; ▲, △, EXT cheeses in September; ■, □, SEMI cheeses in July; ◆, ◇, SEMI cheeses in September). The closed symbols correspond to R cheeses and the open symbols to PA cheeses. The dotted line corresponds to cheeses made in July and the continuous line to cheeses made in September.

proteolysis, and VFA concentration. All these variables were negatively correlated with the sensory variables corresponding to firmness and rind appearance. No FA concentration was significantly correlated with the variables cited above. The sums of SFA, PUFA, and CLA were negatively correlated with those of LCPUFA and *cis*-MUFA. On the first axis, R cheeses were characterised by higher levels of microbial groups, higher VFA concentrations, and higher levels of proteolysis in relation to stronger odours and tastes and more persistent aromas than PA cheeses (Fig. 1B). PA cheeses had higher firmness and appearance estimators than R cheeses. On the second axis, EXT and SEMI cheeses were mainly differentiated by their FA profiles.

## 4. Discussion

### 4.1. Grazing systems

The originality of this study is that the same milking machine and the same facilities and conditions of cheese-making and ripening were used for both grazing systems, in contrast with most field studies, and thus grazing system is the major factor explaining potential differences in milk and cheese microbial and chemical compositions.

The main influence of grazing systems concerned cheese texture, which is known to vary according to cheese fat in DM, calcium and phosphorus contents, FA profile, and proteolysis (Fox, Law, McSweeney, & Wallace, 1999). The higher fat in DM of EXT than SEMI cheeses is concordant with their creamier and more adhesive textures and is directly linked to the higher fat to protein ratio of the corresponding milks. The grazing diet in the EXT system is characterised by no concentrate distribution. The lower energetic density and the higher fibre content of the diet in EXT systems (Supplementary Table S1) reduced the protein content of the milk produced and increased the fat content of the milk, respectively. These observations were particularly more pronounced in September, when the EXT cows grazed on mature grass. Moreover, the energy expended by cows walking was higher in the EXT system, where the grazing surface was the largest. The higher body reserve mobilisation of EXT cows may contribute to the higher *cis*9-C18:1 content in their milk because *cis*9-C18:1 is the primary FA released from body reserves during their mobilisation (Kay et al., 2005). In contrast, the greater milk yield of SEMI cows could reflect the improved nutritional status of these cows, as previously reported by Pires et al. (2015).

Cheese FA profiles may also contribute to the differences found in cheese texture according to the grazing system. The influence of FA composition on cheese texture is mainly linked to C16:0 and *cis*9-C18:1 concentrations, which are the major milk saturated and monounsaturated FAs and have high and low melting points, respectively. In our study, the EXT cheeses (versus SEMI cheeses) were creamier, which is in agreement with their higher *cis*9-C18:1 to C16:0 ratios (Martin et al., 2005). As previously reported by many authors (Bodas et al., 2010; Lucas et al., 2006), the FA profiles of cheeses reflect those of milks. The latter can be modulated by diet characteristics such as the amount of concentrate or the botanical composition of grassland pastures (Chilliard et al., 2007; Ferlay, Martin, Pradel, Coulon, & Chilliard, 2006). Our results confirm that the concentrations in milk of short- and medium-chain FA and *trans*-C18:1 isomers are higher in SEMI than EXT milks, and the concentrations of *cis*9-C18:1 and *n*-3 LCPUFA are lower due to the combined effects of concentrate distributed to SEMI cows (Chilliard et al., 2007) and the lower botanical diversity of grasslands (Farruggia et al., 2014). These effects could be due partly to the leafier herbage grazed in the SEMI system. When herbage matures, the concentrations of *trans*11-C18:1 and *cis*9*trans*11-CLA decrease

while that of C16:0 increases (Coppa et al., 2015). We can notice that in EXT milks and cheeses, the lower concentration of the major atherogenic FA (C12:0, C14:0, C16:0) combined to the higher concentration of *cis*9-C18:1 and C18: 3*n*-3 can be considered as an advantage from a nutritional point of view (Bodas et al., 2010; Gómez-Cortés et al., 2009). On the contrary, the higher content of *trans*11-C18:1 and *cis*9*trans*11-CLA in SEMI milks and cheeses may be considered as an advantage regarding their putative role in the prevention of cancer (Shingfield, Bernard, Leroux, & Chilliard, 2010). However, the differences in milk FA profiles between the two grazing systems were limited because both diets were based essentially on grazed grass, as was previously shown by Coppa et al. (2011b).

The yellower cores of SEMI cheeses compared with EXT cheeses may be linked to a higher fat concentration of carotenoids in SEMI cheeses because these pigments are known to have a considerable effect on milk and cheese yellow colour (Nozière et al., 2006). Little or no change in the concentration of carotenoids between milks and cheeses was observed by these authors. The putative higher carotenoids concentration of SEMI milk fat is in accordance with the fact that, due to the higher stocking rate and rapid rotation among paddocks, the SEMI cows were allowed to graze herbage at leafy stage rich in carotenoids (Nozière et al., 2006), especially in September.

The cheeses from the two studied grazing systems were not clearly differentiated according to their flavours or their odours, even if EXT cheeses were slightly saltier and sourer than SEMI cheeses, in agreement with Coppa et al. (2011b). This result contrasts with studies reporting that the most diversified grasslands give intense and diversified aromas to cheeses (Coulon et al., 2004; Martin et al., 2005). Surprisingly, the cheese flavour seems unaffected by the more advanced secondary proteolysis and by the higher concentration of volatile butyric acid in EXT cheeses, which is usually associated to strong cheese flavour (Cornu et al., 2009). We can suppose that the quantitative differences of proteolysis or VFA concentrations observed between the grazing systems were too weak to be perceived by the panellists. Moreover, microbial activities play a key role in the development of cheese flavours (Montel et al., 2014). Although the levels of several microbial groups were higher in EXT than in SEMI milks, microbial levels in cheeses were weakly modified by the grazing system, except for yeast levels, which were higher in EXT cheese rinds. The same starter cultures were added to all cheeses for acidification and ripening, which may have impeded the development of the raw milk microbiota, thereby lessening the differences between grazing systems.

The high spot quantities observed in our study on SEMI cheese rinds were previously reported in Cantal-type cheeses manufactured with milks with high PUFA contents (Coppa et al., 2011b). Lerch et al. (2015) also observed differences in rind appearance in Saint-Nectaire type cheeses associated with *Mucor* (mould) development related to the FA composition of milk. Although the antimicrobial properties of FA and their derivatives have been known for many years (Kabara, Swieczkowski, Conley, & Truant, 1972), their effects on cheese surface microbiota have not been evaluated (Monnet, Landaud, Bonnarme, & Swennen, 2014). We can hypothesise that the FA profile of SEMI cheese rinds may confer an advantage to ripening starters that produce typical Cantal cheese spots, such as the mould *S. casei* (Coppa et al., 2011a), which could explain the observed differences in cheese rind appearance.

### 4.2. Milk treatment

The sensory properties of cheese were drastically modified by milk pasteurisation in our study. The more intense and diversified flavour of raw milk than pasteurised milk cheeses was previously

reported by many authors using similar (Buchin et al., 1998; Cornu et al., 2009) or different cheese models (Bachmann et al., 2011; Montel et al., 2014; Van Hekken, 2012). The drop in the VFA concentrations in pasteurised milk cheeses observed in our study, and more generally the differences in the balance of volatile compounds as previously reported by Buchin et al. (1998) and Demarigny, Beuvier, Buchin, Pochet, and Grappin (1997) were closely related to the low levels and diversity of microorganisms in pasteurised milks and to the associated catabolic reactions in pasteurised milk cheeses. This may contribute to their more intense and diversified flavours as found in our study. An optimum flavour is achieved faster in raw milk cheeses due to the enhanced microbial activities in these cheeses (Bachmann et al., 2011; Grappin & Beuvier, 1997). Cornu et al. (2009) suggested that softer odours such as “cream” and “fresh cream” in pasteurised milk Cantal-type cheeses would be a result of the decreased quantity of short-chain fatty acids released, which are odour-active compounds.

The more advanced proteolysis of raw milk cheeses made in July compared with pasteurised milk cheeses may explain their less elastic texture and their lower firmness as reported by Gaya, Medina, Rodriguez-Marin, and Nunez (1990). Proteolysis levels and the elasticity of pasteurised milk cheeses manufactured in July and September were similar, which confirms the negative association observed in PCA between proteolysis and cheese elasticity. Nevertheless, some authors found that proteolysis was lower in raw milk cheeses because of their lower moisture content (Grappin & Beuvier, 1997), which we did not observe in this study.

Sensory analysis highlighted higher spot quantity and salience on the rinds of pasteurised milk cheeses compared with raw milk cheeses. The same ripening starters that contribute to the development of spots on Cantal cheeses rind were inoculated on the cloths of both the raw and the pasteurised milk cheeses. However, among the microbial groups potentially involved in ripening and comprising species added as ripening starters, the levels of Gram-positive catalase-positive bacteria tended to be higher on the rinds of pasteurised milk cheeses, while the levels of yeasts and moulds were lower on the rinds of those cheeses. Although we do not know the precise levels of the ripening starter strains reached in each cheese, we can hypothesise that they grow more easily on the rinds of pasteurised cheeses because the levels of raw milk microbiota in these cheeses are reduced by pasteurisation.

In our trial, the cores of the pasteurised cheeses were considered the yellowest for every grazing system and cheese-making period studied. This effect of pasteurisation on cheese colour is infrequently mentioned in the literature, although it is a decisive factor for consumers when deciding to purchase cheeses. Despite the low temperature and the short duration of milk heating (72 °C, 20 s), we propose the hypothesis that this colouration could be linked to a non-enzymatic Maillard reaction (Van Boekel, 1998) caused by the interaction between reducing sugars and amino acids.

#### 4.3. The combined effect of grazing system and milk treatment

Regarding cheese flavour, some authors have previously reported interactions between milk production conditions and milk heat treatment (Cornu et al., 2009). In particular, in cheeses derived from pasture or winter diets, Verdier-Metz et al. (2002) observed a higher effect of diet on cheese flavour when using raw milk than when using pasteurised milk. This suggests that this effect could be ascribed to milk components such as native enzymes from milk (e.g., LPL or plasmin) or milk microbiota that are modified by milk pasteurisation. In our study, the fermented cream aroma was more pronounced in EXT than in SEMI cheeses made from raw milk, whereas this effect was not observed with pasteurised milk cheeses. Similarly, differences in proteolysis and elasticity in raw

milk cheeses between grazing systems were observed, while all pasteurised cheeses had similar characteristics. This difference in raw milk cheeses cannot be explained by milk LPL, because LPL did not vary in milk according to the grazing systems or the period of cheese-making. Levels of lactic bacteria (lactic acid bacteria and heterofermentative facultative *Lactobacillus*) differed in milk according to the grazing systems used, which could have affected the extent and nature of amino acids released by the degradation of peptides (action of microbial proteinases and peptidases) through proteolysis, and thus their transformation to catabolic products involved in cheese flavour (Kieronczyk, Skeie, Olsen, & Langsrud, 2001; Marilley & Casey, 2004). With regard to the level of microbial groups in ripened cheeses, only few interactions with milk treatment were observed. Where there were interactions, differences between periods of cheese-making were only observed in pasteurised milk cheeses. Therefore, the interactions between milk production conditions and milk heat treatment reported previously are only partly supported by our results. One explanation could be that the grazing systems used were too similar to exert a significant effect on the sensory properties of cheese.

#### 4.4. Period of cheese-making

The effect of the cheese-making period on the sensory properties of cheese is weak in our study because the animal diet and cheese-making facilities were similar at both periods. Our result is contradictory to other studies (Agabriel et al., 2004; Esposito et al., 2014; Giaccone et al., 2016) where this effect was noteworthy because of the combination of different elements (season, feeding, management system).

### 5. Conclusion

In this experiment, we aimed to test whether the effects of farming systems on the sensory properties of cheese are partly eliminated when milk is pasteurised prior to cheese-making. This hypothesis was confirmed only for proteolysis, elasticity, and fermented cream aroma of cheeses. These differences could be associated to the different levels of lactic bacteria in raw milk of EXT and SEMI systems. Nevertheless, the two grazing systems were too similar to exert a significant effect on the sensory properties of cheeses. The effect of milk pasteurisation was the greatest when compared with other factors. The grazing systems tested resulted in important differences in milk FA profile, and hence in cheese texture. We noticed differences in the levels of several microbial groups in milks that were no longer present in the associated cheeses. The addition of the same starter cultures in all cheeses may explain that the cheese flavour was faintly affected. Although the effects of grazing systems on cheese rind appearance were not explained by the levels of microbial groups in cheese, we hypothesise that the grazing systems used may induce a modification in the balance of microbial species present and their associated microbial activities. Further research integrating a metabarcoding approach (sequencing of phylogenetic biomarker amplicons) will be needed to better understand the effects of the factors tested.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.idairyj.2016.09.007>.

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