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1 **Relationship between the chemical composition, nutritive value and the maturity stage**  
2 **of six temperate perennial grasses during their first growth cycle along an altitude**  
3 **gradient**

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21

22 **Abstract**

23

24 This work addresses the dynamic of phenological development on mean stage weight (MSW),  
25 chemical composition, (ash, nitrogen (N), neutral detergent fibre (NDF) and acid detergent  
26 fibre (ADF) contents, and pepsin-cellulase dry matter digestibility (PCDMD) for cultivars of  
27 six perennial grass species (timothy, ryegrass, cocksfoot, sheep's fescue, red fescue and  
28 meadow foxtail), during their first growth cycle. The study was performed over two years  
29 along 3 sites distributed along an altitudinal gradient. The dynamics of MSW, N, NDF, ADF  
30 and PCDMD for the species differed according to the environment. For a given sum of  
31 temperatures, at Clermont-Ferrand (CL; 350 m), MSW was delayed (note of 2.37) compared  
32 with Saint-Genès-Champanelle (SG; 850 m; note of 2.97) and Laqueuille (LA; 1100 m; note  
33 of 3.02). Consequently, the NDF and ADF contents were lower and the PCDMD value was  
34 higher in the cultivars established at CL (575 g/kg dry matter (DM), 287 g/kg DM and 0.64  
35 respectively) than in those at SG (653 g/kg DM, 349 g/kg DM and 0.51 respectively) and LA  
36 (653 g/kg DM, 343 g/kg DM and 0.52 respectively). In all sites, and for the two years, the  
37 PCDMD content of timothy (0.60), ryegrass (0.63) and cocksfoot (0.59) cultivars were higher  
38 than that of sheep's fescue (0.47), red fescue (0.53), and meadow foxtail (0.51). The only  
39 exception was identified for CL site in the first year of the study, when the dynamic of  
40 PCDMD was similar for all cultivars. Sum of temperatures and MSW were closely related to  
41 nutritive value of forage, but neither indicator fully described the nutritive value of perennial  
42 grasses.

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44 **Keywords:** sum of temperatures, phenological stage, perennial temperate grass, forage,  
45 quality

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55 **Abbreviations:** ADF: acid detergent fibre; CL: Clermont-Ferrand; DM: dry matter; GDD:  
56 Growing degree-days; LA: Laqueuille; MSW: mean stage weight; N: nitrogen; NDF: neutral  
57 detergent fibre; NIRS: near-infrared spectroscopy; PCDMD: pepsin-cellulase dry matter  
58 digestibility; SG: Saint-Genès-Champanelle;

## 59 **1. Introduction**

60

61 Permanent grasslands, traditionally used as forage for ruminants, are an important type of  
62 land use in Europe, covering more than a third of the European agricultural area (Huyghe et  
63 al., 2014). They are of great interest for the sustainable production of many agricultural  
64 systems. Permanent grasslands cover a diversity of environments and corresponding species  
65 compositions, but in general, their botanical composition is dominated by perennial grasses  
66 (Andueza et al., 2016a). Permanent grassland can be considered as a unique mixture of  
67 species with different maturity stages (Bruinenberg et al., 2002), and this complexity makes it  
68 difficult to characterize and understand their feed value. Maturity is considered as the most  
69 important factor influencing the nutritive value of permanent grasslands (Buxton, 1996;  
70 Bruinenberg et al., 2002). It varies with species (Jeangros and Amaudruz, 2005; Ansquer et  
71 al., 2009) and can be influenced by the environment (Andueza et al., 2010). The nutritive  
72 value of several species found in permanent grasslands when they are grown alone in artificial  
73 swards is well known (Andrieu et al., 1989). Furthermore, fewer literature references are  
74 found for native populations of these species or of other species such as Yorkshire fog  
75 (*Holcus lanatus* L.), red fescue (*Festuca rubra* L.) or golden oatgrass (*Trisetum flavescens*  
76 L.), found in the botanical composition of many permanent grasslands (Pontes et al., 2007a).  
77 Temperature is the primary environmental factor influencing plant development (Buxton,  
78 1996, Perotti, 2021), owing to its influence on enzyme reactions. Close relationships between  
79 nutritive value of forages and growth temperatures have been reported by Bertrand et al.,  
80 (2008); Ford et al., (1979) and by Contreras and Albretch (2006). Clear relationships between

81 maturity and nutritive value of different species have also been reported (Andrieu et al.,  
82 1989). Finally, Iannucci et al., (2008) and Rossignol et al., (2015) showed positive  
83 relationships between temperatures and phenological stage for some grasses, although these  
84 relationships can vary with latitude and altitude. Temperature influences also the phenology  
85 of grasses throughout morphological relative abundance of the different plant organs and  
86 functional modifications (leaf traits for example, Pontes et al., 2007b) and throughout changes  
87 in the chemical composition of these organs. The swift changes depend on the altitude  
88 because the daily temperature is different. Consequently, it is difficult to compare forage  
89 samples obtained in different environments. From a practical point of view, the maturity stage  
90 is the most frequent indicator used for predicting the nutritive value (Andrieu et al., 1989), but  
91 sometimes the relationships between botanical composition, environmental factors and  
92 maturity stage are not clear. According to the results obtained by Andueza et al. (2010),  
93 maturity stage do not fully explain how the nutritive value of forages in permanent grasslands  
94 changes during the first growth cycle. The first growth cycle of grasses in general, and for  
95 permanent grassland in particular, across the year is the most important source of forage of  
96 swards. According to Louault et al., (2002), in upland areas, the first grown cycle represents  
97 2/3 of the biomass production. Climate change induce strong modifications in classic  
98 agronomic references for the farmers, due to the high variability of intra- and interannual  
99 climate in upland and lowland areas. Therefore, the quality and quantity of forage available  
100 along the growing season is variable, making the use of the herbaceous resource more  
101 difficult for farmers. Understanding the relationships between these factors is necessary to  
102 manage permanent grasslands efficiently.

103 The objective of this study was to compare the time course of phenological development,  
104 chemical composition and nutritive value of six temperate perennial grass species covering a  
105 large range of plant material found in permanent grasslands during their first growth cycle

106 along an altitude gradient made up of three sites over two years. Our hypothesis is that  
107 accumulated temperatures (Ansquer et al., 2009) could be a better indicator than maturity  
108 stage for predicting the chemical composition and nutritive value of forage samples. The  
109 choice of sown pastures was made because the time course of chemical composition and  
110 nutritive value of native populations follows a similar pattern than sown pastures (Andrieu et  
111 al., 1989) and these ones becomes more readily available.

112

## 113 **2. Materials and Methods**

114

### 115 *2.1 Plant material and environmental conditions*

116

117 The trials were conducted for two consecutive years (2009–2010) at three sites in close  
118 proximity forming an altitude gradient: Clermont-Ferrand (CL), 3°07E, 45°47N; 350 m a.s.l.,  
119 Saint-Genès Champanelle (SG), 3°01E, 45°43N; 850 m a.s.l. and Laqueuille (LA), 2°45E,  
120 45°39N; 1100 m a.s.l. in the French Massif central. Trials were established on browned  
121 fluvisol, combisol and andosol type soils at CL, SG and LA respectively.

122 At each site, six cultivars of different grass species were sown in spring 2008 in plots of 6 ×  
123 1.5 m<sup>2</sup> in a randomized complete block design with three replications. On the CL site, the trial  
124 was reseeded in autumn 2008 because of poor emergence of seeds in spring. Cultivars of  
125 different species collected comprised timothy (*Phleum pratense* L., cv. Rasant), ryegrass  
126 (*Lolium perenne* L., cv. Milca), cocksfoot (*Dactylis glomerata* L., cv. Starly), sheep's fescue  
127 (*Festuca ovina* L., cv. Spartan), red fescue (*Festuca rubra* L., cv. Swing) and meadow foxtail  
128 (*Alopecurus pratensis* L., cv. Levocska). Fertilizer was applied to obtain a non-limiting

129 nutrient status and avoid any confounding effects of site and fertility. All plots were fertilized  
130 with 100 and 150 kg/ha of P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O respectively in May 2008 and March 2009 and  
131 2010. They also received 120 kg/ha of N (ammonium nitrate) in March 2009 and 2010 and 40  
132 kg/ha of N after each cut in July and September.

133 The temperature and rainfall were collected from weather stations located about 50 m from  
134 the experiment sites. Herbage samples were collected with a grass shear (Gardena Ulm  
135 Germany) by harvesting the aboveground mass at 5 cm stubble height in a 20 cm × 112 cm  
136 area within each experimental plot. In spring and summer of 2009 and 2010, herbage samples  
137 from each experimental plot were collected 11 times using a thermal calendar (heat  
138 accumulation) at around 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300 and 1400  
139 growing degree-days (GDD, °C) (Table S1) in order to try to homogenise the harvestings in  
140 all environments. Heat accumulation was calculated as the sum of mean daily temperatures  
141 (°C) since 1 February, with temperatures bounded to a minimum of 0°C and a maximum of  
142 18°C (Ansquer et al., 2009). At each site, plant biomass was sampled when the GDD  
143 thresholds were reached. At LA, plots were also cut at September 8 and October 30 in 2008  
144 and 2009 respectively. At SG, plots were cut on October 29 and 20 in both years. At CL plots  
145 were cut on October 20 in 2009. Forage obtained was weighed and divided into two  
146 subsamples. The first subsample (125 g fresh material) was stored at -20 °C and used to  
147 characterize phenological stage. The second subsample was dried at 60 °C for 72 h to  
148 determine dry matter (DM) content, and then ground in a hammer mill through a 1 mm  
149 screen.

150

## 151 *2.2 Determination of maturity stage*

152

153 Maturity stage was determined on 50 random tillers, adapting a method proposed by Moore et  
154 al., (1991). Each tiller was characterized by a maturity stage, and a numerical index was  
155 assigned to it (Andueza et al., 2019). The average stage of the population of tillers for each  
156 plot was calculated as follows:

157

$$158 \quad MSW = \frac{\sum (D_i W_i)}{W},$$

159

160 where *MSW* is the mean stage by weight, *D<sub>i</sub>*, is the code of stage *i* as defined in Andueza et  
161 al., (2019), *W<sub>i</sub>* is the number of plants in stage *I*, and *W* is the total number of plants.

162

### 163 *2.3 Determination of chemical composition and nutritive value*

164

165 Ground samples were analyzed for ash, nitrogen (N, according to AOAC, 1990), neutral  
166 detergent fibre (NDF, Van Soest et al., 1991) and acid detergent fibre (ADF) according to  
167 Van Soest and Robertson (1980) and pepsin cellulase digestibility (PCDMD, Aufrère and  
168 Michalet-Doreau (1983). Neutral detergent fibre and ADF analyses were performed on an  
169 Ankom system (Ankom® Tech. Co., Fairport, NY, USA). Analyses were conducted using  
170 near-infrared spectroscopy (NIRS), with spectra obtained on a monochromator (FOSS-  
171 NIRSystems 6500, Silver Spring, MD, USA) scanning in the spectral range 400–2500 nm.  
172 For ash and N, global calibration models obtained by Andueza et al., (2011) were used. For  
173 NDF, ADF and PCDMD, the models obtained by Andueza et al., (2016b) were used. Ninety-  
174 nine spectrally selected samples were chemically and biologically assayed and added to these  
175 models.

176



177 2.4 Statistical analysis

178

179 Data for MSW, NIRS estimates of chemical composition and PCDMD underwent to  
180 repeated-measures ANOVA according to the following model:

181

$$182 \quad Y_{ijklm} = \mu + L_i + B(L)_{ij} + Y_k + C_l + T_m + (L \times Y)_{ik} + (L \times C)_{il} + (L \times T)_{im} + (Y \times C)_{kl} + (Y \times T)_{km} + \\ 183 \quad (C \times T)_{lm} + (L \times Y \times C)_{ikl} + (L \times Y \times T)_{ikm} + (L \times C \times T)_{ilm} + (Y \times C \times T)_{klm} + (L \times Y \times C \times T)_{ijklm} + \varepsilon_{ijklm},$$

184

185 where  $Y$  is the dependent variable,  $\mu$  is the overall mean,  $L$  is the location or site (2 degrees of  
186 freedom df),  $B$  is the block nested to location (6 df),  $Y$  is the year (1 df),  $C$  is the cultivars (5  
187 df),  $T$  is the temperature (10 df),  $L \times Y$  is the interaction between location and year (2 df),  $L \times C$   
188 is the interaction between location and cultivar (10 df),  $L \times T$  is the interaction between  
189 location and temperature (20 df),  $Y \times C$  is the interaction between year and cultivar (5 df),  $Y \times T$   
190 is the interaction between year and temperature (10 df),  $C \times T$  is the interaction between  
191 cultivar and temperature (50 df),  $L \times Y \times C$  is the interaction between location, year and cultivar  
192 (10 df),  $L \times Y \times T$  is the interaction between location, year and temperature (20 df),  $L \times C \times T$  is  
193 the interaction between location, cultivar and temperature (100 df),  $Y \times C \times T$  is the interaction  
194 between year, cultivar and temperature (50 df),  $L \times Y \times C \times T$  is the interaction between location,  
195 year, cultivar and temperature (100 df) and  $\varepsilon$  is the experimental error. Block was considered  
196 as a random effect. Temperature was considered as a repeated measure.

197 Statistical analyses were performed using the Mixed procedure of the SAS statistical package  
198 (SAS, 1998). Differences between treatments were compared using its PDIFF option.

199

### 200 3. Results

201

202 The ombrothermic diagrams for each site and year are shown in Figure S1. Precipitation data  
203 bars drop below the temperature data curve during the period study indicating water  
204 deficiency at CL in March 2009 (Figure S1a) and in April in 2010 (Figure S1b). No other  
205 water deficiency periods were identified. Outputs of the ANOVA for MSW, N, NDF, and  
206 PCDMD are shown in Table 1 (outputs of the ANOVA for ash and ADF are shown in Table  
207 S2). The statistical analysis revealed significant ( $p < 0.05$ ) environmental, genotypic and  
208 genotypic  $\times$  environment interactions for all determinations. The effect of temperature  
209 accounted for the greatest proportion of variance for all the determinations, was largest for  
210 PCDMD and ADF (0.64 and 0.63 respectively), and accounted 0.57, 0.53 and 0.46 for N,  
211 NDF and MSW respectively. Ash content had the lowest variance explained by temperature  
212 and the greatest genotypic variance (0.21). For MSW, NDF and PCDMD, proportion of  
213 variance explained by cultivars ranged from 0.07 to 0.09, whereas for ADF and N it  
214 accounted for less than 0.04. The site effect explained 0.15 and 0.13 of the total variance for  
215 MSW and N respectively, whereas for ash, NDF, ADF and PCDMD, this proportion ranged  
216 from 0.05 to 0.08. The year effect explained only 0.01 or less for all determinations.

217

Inset Table 1 near here

218

219 A strong interaction for MSW, N, NDF, ADF and PCDMD was Year  $\times$  Site (Tables 2, 3 and  
220 S3). It explained 0.03 for PCDMD and 0.02 for the other determinations, with only 0.005 of  
221 the degrees of freedom. For all the variables, this interaction was mainly due to the different  
222 behavior of the cultivars in the CL trial in the first year of the study compared to the behavior  
223 of the species at the SG and LA sites and CL in the second year. To a lesser extent, the

224 behavior of species at the LA site in the second year of the study also contributed to the  
225 significance of the interaction Year  $\times$  Site. Illustrating this, for MSW, the SG site showed  
226 mean values of 3.02 and 2.91 in the first and second year of the study respectively, against  
227 3.14 and 2.89 for the LA site, but the CL site showed MSW values of 2.22 and 2.53. Other  
228 strong interactions were Site  $\times$  Cultivar for MSW and ash. Meadow foxtail cultivar had a  
229 higher MSW value with respect to the other species in the CL trials than in the other trials  
230 whereas the difference between MSW values for sheep's fescue cultivar and others were  
231 lower in CL trials than in SG and LA trials (Table 2). Similarly, the ash content of this  
232 meadow foxtail cultivar with respect to the others was lower in the CL trials than in the SG  
233 and CL trials (Table S3).

234 Inset Table 2 near here

235 Inset Table 3 near here

236

237 The Site  $\times$  Temperature interaction can be extrapolated from Tables 2, 3 and S3. It was  
238 meaningful for ash (Table S3) and N (Table 3). At 400 and 500 GDD, cocksfoot and sheep's  
239 fescue cultivars showed the highest (4.06 and 3.57 g/kg DM respectively) and the lowest  
240 (3.13 and 2.85 g/kg DM respectively) N values, but at 1400 GDD the two cultivars showed  
241 similar N values of 1.58 and 1.63 g/kg DM respectively (Table 3). The Temperature  $\times$   
242 Cultivar interaction was important for MSW, ash, N, NDF, ADF contents and PCDMD  
243 (Figure 1). The Year  $\times$  Site  $\times$  Cultivar interaction was relevant for MSW and ash (Table 2,  
244 Table S3, Figure 2). At 400 GDD, sheep's fescue cultivar was the earliest, while meadow  
245 foxtail and red fescue cultivars showed an intermediate development. Cocksfoot, ryegrass and  
246 timothy started their development latest. Furthermore, at 1400 GDD, variability among  
247 cultivars was lower than that at the beginning of the cycle. At this accumulated temperature,

248 cocksfoot and meadow foxtail cultivars were among the least well-developed (3.3 and 3.1  
249 respectively), whereas ryegrass cultivar showed the highest MSW value (3.8).

250 Inset Figure 1 near here

251 Inset Figure 2 near here

252

253

254 The time course of maturity stages of different species across the growth cycle was not fully  
255 congruent with digestibility, cell wall content and N (Figure 1). In this way, the dynamic of  
256 MSW for ryegrass, cocksfoot and timothy was similar whereas the PCDMD of ryegrass was  
257 higher than that of cocksfoot and timothy. Otherwise, from 900 GDD the MSW of meadow  
258 foxtail cultivar was stable and it was lower than that of timothy, and ryegrass cultivars.  
259 During this period, the PCDMD of meadow foxtail cultivar was also lower than that of  
260 ryegrass and timothy. Furthermore, the PCDMD of meadow foxtail cultivar at the end of the  
261 cycle was the lowest (0.36). Although the relationship between the time course of heat  
262 accumulation and that of MSW, NDF, ADF and PCDMD was similar for the species within a  
263 site (Tables 2, 3, S3), there were differences among sites. Thus, trials performed at CL  
264 showed less developed plants characterized by higher digestibility and lower contents of NDF  
265 and ADF than that at the SG and LA sites (Tables 2, 3, S3). The differences among sites were  
266 particularly marked between the plants grown in CL in 2009 and the other environments. The  
267 greatest differences across the first growth cycle between the time course of MSW of different  
268 species in CL in 2009 and the other environments were for sheep's fescue and cocksfoot  
269 cultivars, however, ryegrass cultivar did not show a different time course compared to SG,  
270 LA and at CL in 2010 (Figure 2). Concerning the NDF, ADF contents and PCDMD of  
271 species grown at CL in 2009, the differences between them were lower than the between-  
272 species differences observed in other environments.

273

274 **4. Discussion**

275

276 This study was performed using six cultivars belong to different species representative of the  
277 main grasses encountered in permanent grasslands in six different growing conditions (3  
278 altitudes and two years). Sum of temperatures was the main factor affecting the maturity  
279 stage, chemical composition and nutritive value of the six species, because plant processes are  
280 temperature-dependent (Johnson and Thornley, 1985; Bonhomme, 2000). Site and cultivar  
281 factors were also important in explaining the differences in maturity, chemical composition  
282 and nutritive value of forage samples in the trials. The variance explained by the year effect,  
283 even although it was significant, was lower than these explained by the site and temperature  
284 effects on maturity development, chemical composition and nutritive value despite the  
285 diversity of weather conditions among years (Figure S1). The variability of the maturity  
286 stages among years have been pointed out by Vuffray et al. (2016), who found differences in  
287 the flowering time of ten grassland species of 13 and 22 days (difference between earliest and  
288 latest dates) according to different areas over a period of 21 years. Andueza et al., (2010)  
289 reported significant differences between two permanent grasslands of 34, 25 and 10 % for CP,  
290 NDF and *in vivo* organic matter digestibility in one year, whereas in the preceding year,  
291 differences between grasslands were 7, 0 and 2 % for the same determinations. In the present  
292 study, the fact that herbage samples were aligned by the sum of temperatures could partially  
293 explain the results obtained, however, the interactions of species with environmental factors  
294 for all the studied determinations were highly complex. They indicate that the maturity stage,  
295 chemical composition and nutritive value were unstable across the wide range of growing  
296 conditions in the study.

297 One major result of this study is the different dynamic concerning the phenological  
298 development of the different species considered, mainly for sheep's fescue cultivar, in relation  
299 to other results published previously. The phenological development of the cultivar of this  
300 species in the present study occurred much earlier than as reported in Ansquer et al. (2009)  
301 and in Jeangros and Amaudruz (2005). Ansquer et al. (2009) reported that the differences in  
302 flowering date of five out of the six species used in this study ranged between 133 Julian days  
303 for cocksfoot and red fescue and 174 Julian days for timothy, the flowering date of ryegrass  
304 and sheep's fescue lying between these two extremes (140 and 149 Julian days respectively).  
305 Jeangros and Amaudruz, (2005) reported that meadow foxtail was the earliest species to attain  
306 the maturity stage of inflorescence emergence, whereas timothy was the latest species to  
307 attain this stage. These different results can be partially explained by the methodology used  
308 for determining the maturity stage. Ansquer et al., (2009) considered the flowering stage  
309 when 50 % of all reproductive tillers reached this stage, whereas Jeangros and Amaudruz,  
310 (2005) considered the stage of inflorescence emergence as attained when 50 % of all plants  
311 reached this stage. In the present study, an adaptation of the method of Moore et al., (1991)  
312 was used to determine maturity stage. This methodology based on a random selection of 50  
313 tillers representatives of all plants of the grassland is more precise, and evaluates the mean  
314 maturity stage of the plot. It would be better-suited to research applications (Kalu and Fick,  
315 1980) and more specifically to comparing different plots or establishing relationships with  
316 their nutritive value.

317 An unexpected result of the present study was the different evolution of chemical composition  
318 and nutritive value of the species in the trial performed at CL, particularly in the first year of  
319 the study and to a lesser extent in the second one compared to the time course of species in  
320 the other trials. Thus, in the CL trial in 2009 the maturity development of the studied species  
321 was delayed compared with the other trials. In parallel, CP, NDF and ADF contents of species

322 in this trial were lower, and PCDMD content was higher than that obtained in the other trials.  
323 In the LA trial in 2010 the maturity stage of the different species was similar to that seen in  
324 the other trials up to 900 GDD, but then it did not progress compared to the results of the  
325 other trials. The differences in the management of these trials can explain these results. Trials  
326 were established in spring 2008 and were cut twice before the start of the experiment except  
327 for the CL trial, which was established in autumn 2008 because of sprouting problems in  
328 spring, and this trial was therefore not cut before the start of the experiment. The tables of  
329 forage nutritive value used in France (Andrieu et al., 1989) reported a different maturity  
330 development of forages during the seeding year than during the full harvest year, but they do  
331 not distinguish between the maturity stage during the full harvest year of forages seeded in  
332 spring or in autumn. Rossignol et al., (2015) reported an effect of plant resource strategy of  
333 species on their phenology that could be related by the different dynamic of chemical  
334 composition and nutritive value of species when they were established in CL in the first year  
335 of the present study. The delayed maturity stage and the higher forage quality of the CL trial,  
336 particularly that of 2009 in relation to those of SG and LA, could not be explained by the  
337 average temperatures of the growing cycle of this trial compared to those of the SG and LA  
338 trials as stated by Buxton, (1996). Photoperiod could partially explain these results, because  
339 the first growth cycle of the CL trials was developed under the influence of shorter  
340 photoperiods than those of SG, and specially LA, although in the literature, the effect of  
341 photoperiod on the quality of forages is not clear. Juan et al., (1993) found that lucerne  
342 (*Medicago sativa* L.) grown under a 13 h photoperiod showed a higher leaf/stem ratio than  
343 lucerne grown under a 16 h photoperiod, but according to Wilson, (1982), long photoperiods  
344 may be associated with high forage quality. Maturity stage of grass species is influenced by  
345 photoperiod. Thus, flowering initiation of grasses depends on day length, which could explain

346 the results obtained here on MSW, but also chemical composition and nutritive value between  
347 sites.

348 The relationships between maturity stage and temperature accumulation were linear for most  
349 environments with the exception of LA in 2010 in which the MSW index did not increase  
350 after 900 GDD, and thus PCDMD remained stable from this accumulated temperature up to  
351 1400 GDD. Rossignol et al., (2015) stated that the accumulated temperature for reaching a  
352 maturity stage varied with latitude and altitude. In the present study, these results are difficult  
353 to explain, but although the same stages of maturity were reached in all environments, the  
354 number of tillers in most advanced maturity stages was lower in LA in 2010, the number of  
355 less mature tillers being higher than that obtained in the other environments. Murphy and  
356 Briske, (1992) and Zobel et al (2000) state that perennial grasses can persist for several years  
357 in the sward by vegetative multiplication. Matthew et al., (2000), have reported a seasonal  
358 pattern of tiller distribution (vegetative vs. reproductive). It is well known that grass  
359 phenology is influenced by abiotic factors other than temperature, such as photoperiod,  
360 precipitation or nutrient availability (Hovenden et al., 2008; Martinelli and Galasso, 2011),  
361 but these factors do not explain the results found in this study. Other factors such as  
362 management (particularly date of cut or grazing performed in the previous autumn), may help  
363 explain the different dynamic of maturity stage observed at LA in 2010. In autumn 2009 this  
364 trial was cut 10 days later than the trials in the other locations, this location being at a higher  
365 altitude than SG and CL.

366 With advancing maturity stage, the proportion of cell walls in grass and permanent grassland  
367 increases, while that of crude protein decreases (Andueza et al., 2010; Andueza et al., 2013).  
368 Consequently, forage digestibility decreases with increased maturity stage. Rate of decline in  
369 digestibility depends on species (Bruinenberg et al., 2002) and temperature (Deinum et al.,  
370 1981). In our experiment, the effect of temperature is clear when the changes in quality



371 parameters are compared between years. Thus Hill et al., (1995) found that quality parameters  
372 of tall fescue (*Festuca arundinacea* Schreb.) were better predicted from climatological data  
373 than from morphological staging. In the present experiment, when the effect of temperature  
374 and maturity stage on the chemical composition and PCDMD were analyzed, we found that  
375 digestibility and chemical composition were closely related with the maturity stage (MSW)  
376 than with temperature, particularly in CL in 2009 and LA 2010 from 900 GDD, suggesting  
377 that factors other than temperature could be important in explaining the forage quality.

378 Although the relationships between the dynamic of NDF, ADF and PCDMD contents within  
379 each species and the time course of the maturity stage were clear, decreasing the content of  
380 PCDMD with the progress of the growth cycle and the accumulation of temperature, the  
381 relationships were less clear when the six species cultivars were considered together. Thus,  
382 perennial ryegrass cultivar showed the highest PCDMD values and the lowest NDF and ADF  
383 content between 400 and 1200 GDD. This species was among those that showed the latest  
384 maturity stage between 400 and 700 GDD, but it was classified among the species that  
385 reached the stage “inflorescence emerged” at 1200 GDD. Similarly, the dynamic of maturity  
386 stage of cocksfoot and timothy cultivars was similar, but the PCDMD content of this last  
387 species was higher than that of cocksfoot across almost all the studied period (up to 1100  
388 GDD).

389

## 390 **5. Conclusions**

391

392 This experiment set out to improve our knowledge of the relationships between maturity  
393 stage, sum of temperatures and nutritive value of temperate perennial grasses. The results of  
394 the current study can serve as a basis to compare forages obtained at different locations or

395 years in relation to their nutritive value. The environment and more specifically the altitude  
396 influence the development of growth and the quality of perennial grasses. Thus, at the same  
397 sum of temperature, grasses grown at CL characterized by low altitude present less mature  
398 stages and higher quality than those grown at SG and LA, which were characterized by  
399 medium and high altitudes respectively. Our findings show that factors related to plot  
400 management could influence grass development response and thereby forage quality,  
401 however, they will be different according to the species. These findings are important for  
402 farmers, but also for researchers interested in comparing the nutritive value of forages  
403 obtained in different pedo-climatic conditions, and could be useful to refine advice to farmers  
404 considering local conditions. More research should be done in order to identify the  
405 management practices that could influence the nutritive value of forages. This work  
406 conducted in pure stands could also provide baseline data for further research on the  
407 complementarity of species according to the environment for the use together in permanent  
408 grassland or multi-specific grasslands. Furthermore, these results could serve for choosing the  
409 more appropriate vegetal material for a possible restauration of degraded grasslands according  
410 to their adaptation to the environment.

411

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413

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546



547 **Table 1.** Proportion of variance explained (V) and statistical significance of *F* ratios (Signif) from the analysis of variance for phenological stage  
 548 note (MSW), nitrogen (N), neutral detergent fibre (NDF), and pepsin-cellulase dry matter digestibility (PCDMD).

549

550		DF <sup>†</sup>	MSW		N		NDF		PCDMD	
551			V	Signif	V	Signif	V	Signif	V	Signif
552	Site	2	0.15	<0.001	0.13	<0.001	0.06	<0.001	0.05	<0.001
553	Year	1	<0.01	0.320	<0.01	0.007	0.01	<0.001	0.01	<0.001
554	Cult <sup>‡</sup>	5	0.09	<0.001	<0.01	0.001	0.07	<0.001	0.08	<0.001
555	Temp	10	0.46	<0.001	0.57	<0.001	0.53	<0.001	0.64	<0.001
556	Year*site	2	0.02	<0.001	0.02	<0.001	0.02	<0.001	0.03	<0.001
557	Site*cult	10	0.02	<0.001	<0.01	0.017	0.01	<0.001	<0.01	<0.001
558	Site*temp	20	0.01	<0.001	0.06	<0.001	0.02	<0.001	0.01	<0.001
559	Year*cult	5	<0.01	<0.001	<0.01	<0.001	0.01	<0.001	<0.01	<0.001
560	Year*temp	10	<0.01	<0.001	0.02	<0.001	0.02	<0.001	0.01	<0.001
561	Temp*cult	13	0.04	<0.001	0.02	<0.001	0.04	<0.001	0.02	<0.001
562	Year*site*cult	10	0.04	<0.001	<0.01	<0.001	0.01	<0.001	0.01	<0.001
563	Year*site*temp	20	<0.01	<0.001	0.02	<0.001	0.03	<0.001	0.01	<0.001
564	Site*temp*cult	100	0.03	<0.001	<0.01	<0.001	0.02	<0.001	0.01	<0.001
565	Year*temp*cult	50	0.01	0.006	<0.01	0.005	0.01	<0.001	0.01	<0.001
566	Year*site*temp*cult	100	0.01	0.060	<0.01	0.311	0.02	<0.001	0.01	<0.02

567

<sup>†</sup>DF = degree of freedom; <sup>‡</sup>Cult = cultivar; temp = temperature;

568 **Table 2.** Means for phenological stage note (MSW) and neutral detergent fibre (NDF, g/kg dry matter) for cultivars of six species and  
569 accumulated temperature (growing degree-days (GDD, °C)) obtained in the trials at Clermont-Ferrand (CL), Saint-Genès-Champanelle (SG) and  
570 Laqueuille (LA) in the two years of the study.

	MSW								NDF							
	Year1				Year 2				Year1				Year 2			
	CL	SG	LA	sem <sup>†</sup>	CL	SG	LA	sem <sup>†</sup>	CL	SG	LA	sem <sup>†</sup>	CL	SG	LA	sem <sup>†</sup>
<i>Species</i>																
timothy	2.1	2.7	2.8	0.06	2.3	2.8	2.8	0.06	529	640	647	5.1	577	654	655	4.9
ryegrass	2.3	2.9	3.2	0.06	2.3	3.0	2.9	0.06	538	596	615	5.0	547	593	601	4.9
sheep's fescue	2.0	3.8	3.7	0.06	3.3	3.4	3.1	0.06	572	704	698	5.3	641	708	683	5.1
cocksfoot	1.8	2.6	2.8	0.06	2.1	2.8	2.7	0.06	568	637	644	4.9	598	655	657	4.9
red fescue	2.5	3.2	3.3	0.06	2.6	2.9	2.8	0.06	555	665	651	5.1	616	672	644	4.9
meadow foxtail	2.6	2.9	3.0	0.06	2.6	2.7	3.0	0.06	568	656	663	5.0	590	654	670	4.9
<i>Temperature (accumulated GDD, °C)</i>																
400	1.5	1.8	2.2	0.07	1.7	1.9	1.9	0.06	426	582	596	5.8	519	523	539	5.2
500	1.6	2.3	2.4	0.06	1.8	2.1	2.4	0.06	522	587	612	5.6	500	560	565	5.2
600	1.6	2.6	2.7	0.06	2.0	2.4	2.6	0.06	504	611	637	5.3	534	621	619	5.2
700	1.7	2.8	2.9	0.06	2.3	2.7	2.7	0.06	524	641	629	5.3	560	634	648	5.3
800	2.0	2.9	3.2	0.06	2.4	2.8	2.8	0.06	566	641	640	5.3	584	682	661	5.2
900	2.2	3.1	3.2	0.06	2.7	2.9	3.3	0.06	590	650	679	5.4	583	689	692	5.3
1000	2.6	3.2	3.3	0.06	2.7	3.2	3.3	0.06	591	672	679	5.5	626	687	699	5.3
1100	2.6	3.3	3.6	0.06	2.9	3.2	3.2	0.06	606	679	688	5.3	631	694	691	5.3
1200	2.8	3.6	3.5	0.06	3.1	3.5	3.3	0.06	593	689	682	5.3	676	702	691	5.3
1300	2.8	3.7	3.7	0.06	3.0	3.6	3.1	0.06	579	693	667	5.3	666	708	690	5.3
1400	3.0	3.8	3.9	0.06	3.2	3.7	3.3	0.06	605	693	676	5.4	666	715	690	5.3

<sup>†</sup>sem = standard error of mean

571

572

573 **Table 3.** Means for nitrogen content (N, g/kg dry matter) and pepsin-cellulase dry matter digestibility (PCDMD) for cultivars of six temperate  
 574 grass species and accumulated temperature (growing degree-days (GDD, °C)) obtained in the trials at Clermont-Ferrand (CL), Saint-Genès-  
 575 Champanelle (SG) and Laqueuille (LA) in the two years of the study.

	N								PCDMD							
	Year1				Year 2				Year1				Year 2			
	CL	SG	LA	sem <sup>†</sup>	CL	SG	LA	sem <sup>†</sup>	CL	SG	LA	sem <sup>†</sup>	CL	SG	LA	sem <sup>†</sup>
<i>Species</i>																
timothy	25	18	18	1.0	25	18	27	1.0	0.72	0.57	0.55	0.934	0.67	0.55	0.56	0.911
ryegrass	23	17	20	1.0	22	18	29	1.0	0.71	0.61	0.58	0.914	0.67	0.61	0.61	0.911
sheep's fescue	26	18	21	1.0	21	16	25	1.0	0.66	0.40	0.41	0.976	0.50	0.41	0.44	0.941
cocksfoot	25	18	23	1.0	25	19	28	1.0	0.70	0.57	0.55	0.911	0.63	0.54	0.53	0.911
red fescue	31	18	22	1.0	26	17	29	1.0	0.68	0.48	0.51	0.940	0.56	0.48	0.50	0.911
meadow foxtail	24	18	22	1.0	24	19	27	1.0	0.63	0.45	0.47	0.914	0.56	0.49	0.47	0.911
<i>Temperature (accumulated GDD, °C)</i>																
400	25	37	31	0.9	45	36	41	0.9	0.84	0.71	0.68	1.039	0.77	0.77	0.75	0.930
500	44	26	27	0.9	38	29	35	0.9	0.78	0.69	0.63	0.993	0.77	0.72	0.73	0.930
600	40	22	24	0.9	30	22	30	0.9	0.79	0.62	0.59	0.941	0.72	0.62	0.63	0.930
700	35	19	22	0.9	27	18	29	0.9	0.75	0.56	0.59	0.941	0.67	0.58	0.56	0.941
800	25	16	20	0.9	24	16	27	0.9	0.69	0.54	0.54	0.941	0.62	0.49	0.51	0.930
900	24	15	20	0.9	21	15	25	0.9	0.65	0.50	0.47	0.952	0.63	0.46	0.45	0.941
1000	23	13	19	0.9	17	14	24	0.9	0.65	0.45	0.47	0.981	0.54	0.45	0.42	0.941
1100	19	13	18	0.9	16	13	24	0.9	0.61	0.43	0.43	0.941	0.51	0.43	0.43	0.941
1200	17	12	18	0.9	15	13	23	0.9	0.60	0.40	0.41	0.941	0.44	0.39	0.41	0.941
1300	16	12	18	0.9	15	12	23	0.9	0.61	0.38	0.44	0.941	0.44	0.38	0.41	0.941
1400	15	11	17	0.9	14	12	22	0.9	0.56	0.36	0.37	0.963	0.42	0.38	0.41	0.941

<sup>†</sup>sem = standard error of mean

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579 Figure captions

580

581 Figure 1. Dynamic of maturity stage (MSW), nitrogen (N), pepsin-cellulase dry matter  
582 digestibility (PCDMD) and neutral detergent fibre (NDF) along the first growing cycle  
583 (between 400 and 1400 growing degrees-days (GDD)) for 6 temperate cultivars of different  
584 grass species (timothy; Pp, ryegrass; Lp, cocksfoot; Dg, sheep's fescue; FO, red fescue; Fr,  
585 and meadow foxtail; Ap),(mean values of 3 sites and 2 years).

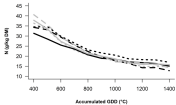
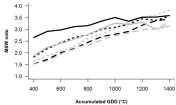
586

587 Figure 2. Dynamic of maturity stage (MSW) and pepsin cellulase dry matter digestibility  
588 (PCDMD) (between 400 and 1400 growing degrees-days (GDD)) of the six cultivars of  
589 different grass species (timothy; Pp, ryegrass; Lp, cocksfoot; Dg, sheep's fescue; FO, red  
590 fescue; Fr, and meadow foxtail; Ap) grown in Clermont-Ferrand in 2009 (a and c  
591 respectively) and in the other environments (average values; b and d respectively).

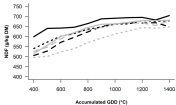
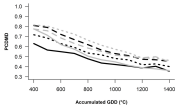
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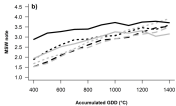
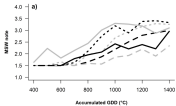
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— Po - - Lp - · - Pp — Dg · · · Pr — Ap





— Pp - - Lp — Fe — Og - - Fr — Ag

