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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 m² 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 P₂O₅ and K₂O 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_i*, is the code of stage *i* as defined in Andueza et
161 al., (2019), *W_i* 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, μ 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 ε 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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418

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422

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545

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 [†]	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 [‡]	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

[†]DF = degree of freedom; [‡]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 [†]	CL	SG	LA	sem [†]	CL	SG	LA	sem [†]	CL	SG	LA	sem [†]
<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

[†]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 [†]	CL	SG	LA	sem [†]	CL	SG	LA	sem [†]	CL	SG	LA	sem [†]
<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

[†]sem = standard error of mean

576

577

578

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).

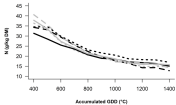
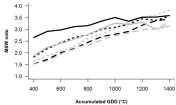
586

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).

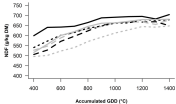
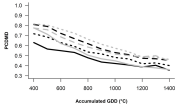
592

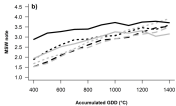
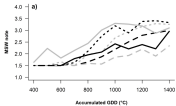
593

594



— Po - - Lp ··· Pr - · - Dg — Ap





— Pp - - Lp — Fe — Og - - Fr — Ag

