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

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

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Abstract

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

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

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

1. Introduction

Permanent grasslands, traditionally used as forage for ruminants, are an important type of land use in Europe, covering more than a third of the European agricultural area (Huyghe et al., 2014). They are of great interest for the sustainable production of many agricultural systems. Permanent grasslands cover a diversity of environments and corresponding species compositions, but in general, their botanical composition is dominated by perennial grasses (Andueza et al., 2016a). Permanent grassland can be considered as a unique mixture of species with different maturity stages (Bruinenberg et al., 2002), and this complexity makes it difficult to characterize and understand their feed value. Maturity is considered as the most important factor influencing the nutritive value of permanent grasslands (Buxton, 1996; Bruinenberg et al., 2002). It varies with species (Jeangros and Amaudruz, 2005; Ansquer et al., 2009) and can be influenced by the environment (Andueza et al., 2010). The nutritive value of several species found in permanent grasslands when they are grown alone in artificial swards is well known (Andrieu et al., 1989). Furthermore, fewer literature references are found for native populations of these species or of other species such as Yorkshire fog (*Holcus lanatus* L.), red fescue (*Festuca rubra* L.) or golden oatgrass (*Trisetum flavescens* L.), found in the botanical composition of many permanent grasslands (Pontes et al., 2007a). Temperature is the primary environmental factor influencing plant development (Buxton, 1996, Perotti, 2021), owing to its influence on enzyme reactions. Close relationships between nutritive value of forages and growth temperatures have been reported by Bertrand et al., (2008); Ford et al., (1979) and by Contreras and Albretch (2006). Clear relationships between maturity and nutritive value of different species have also been reported (Andrieu et al., 1989). Finally, Iannucci et al., (2008) and Rossignol et al., (2015) showed positive relationships between temperatures and phenological stage for some grasses, although these relationships can vary with latitude and altitude. Temperature influences also the phenology of grasses throughout morphological relative abundance of the different plant organs and functional modifications (leaf traits for example, Pontes et al., 2007b) and throughout changes in the chemical composition of these organs. The swift changes depend on the altitude because the daily temperature is different. Consequently, it is difficult to compare forage samples obtained in different environments. From a practical point of view, the maturity stage is the most frequent indicator used for predicting the nutritive value (Andrieu et al., 1989), but sometimes the relationships between botanical composition, environmental factors and maturity stage are not clear. According to the results obtained by Andueza et al. (2010), maturity stage do not fully explain how the nutritive value of forages in permanent grasslands changes during the first growth cycle. The first growth cycle of grasses in general, and for permanent grassland in particular, across the year is the most important source of forage of swards. According to Louault et al., (2002), in upland areas, the first grown cycle represents 2/3 of the biomass production. Climate change induce strong modifications in classic agronomic references for the farmers, due to the high variability of intra- and interannual climate in upland and lowland areas. Therefore, the quality and quantity of forage available along the growing season is variable, making the use of the herbaceous resource more difficult for farmers. Understanding the relationships between these factors is necessary to manage permanent grasslands efficiently.

The objective of this study was to compare the time course of phenological development, chemical composition and nutritive value of six temperate perennial grass species covering a large range of plant material found in permanent grasslands during their first growth cycle along an altitude gradient made up of three sites over two years. Our hypothesis is that accumulated temperatures (Ansquer et al., 2009) could be a better indicator than maturity stage for predicting the chemical composition and nutritive value of forage samples. The choice of sown pastures was made because the time course of chemical composition and nutritive value of native populations follows a similar pattern than sown pastures (Andrieu et al., 1989) and these ones becomes more readily available.

2. Materials and Methods

2.1 Plant material and environmental conditions

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

At each site, six cultivars of different grass species were sown in spring 2008 in plots of 6 × 1.5 m^2 in a randomized complete block design with three replications. On the CL site, the trial was reseeded in autumn 2008 because of poor emergence of seeds in spring. Cultivars of different species collected comprised timothy (*Phleum pratense* L., *cv*. Rasant), ryegrass (*Lolium perenne* L., *cv*. Milca), cocksfoot (*Dactylis glomerata* L., *cv*. Starly), sheep's fescue (*Festuca ovina* L., *cv*. Spartan), red fescue (*Festuca rubra* L., *cv*. Swing) and meadow foxtail (*Alopecurus pratensis* L., *cv*. Levocska). Fertilizer was applied to obtain a non-limiting nutrient status and avoid any confounding effects of site and fertility. All plots were fertilized with 100 and 150 kg/ha of P_2O_5 and K_2O respectively in May 2008 and March 2009 and 2010. They also received 120 kg/ha of N (ammonium nitrate) in March 2009 and 2010 and 40 kg/ha of N after each cut in July and September.

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

2.2 Determination of maturity stage

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

$$MSW = \Sigma (D_i W_i)/W,$$

where *MSW* is the mean stage by weight, D_i, is the code of stage *i* as defined in Andueza et al., (2019), W_i is the number of plants in stage *I*, and *W* is the total number of plants.

2.3 Determination of chemical composition and nutritive value

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

2.4 Statistical analysis

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

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

where Y is the dependent variable, μ is the overall mean, L is the location or site (2 degrees of freedom df), B is the block nested to location (6 df), Y is the year (1 df), C is the cultivars (5 df), T is the temperature (10 df), $L\times Y$ is the interaction between location and year (2 df), $L\times C$ is the interaction between location and cultivar (10 df), $L\times T$ is the interaction between location and temperature (20 df), $Y\times C$ is the interaction between year and cultivar (5 df), $Y\times T$ is the interaction between year and temperature (10 df), $C\times T$ is the interaction between cultivar and temperature (50 df), $L\times Y\times C$ is the interaction between location, year and cultivar (10 df), $L\times Y\times T$ is the interaction between location, year and temperature (20 df), $L\times C\times T$ is the interaction between location, cultivar and temperature (100 df), $Y\times C\times T$ is the interaction between year, cultivar and temperature (50 df), $L\times Y\times C\times T$ is the interaction between location, year, cultivar and temperature (100 df) and ε is the experimental error. Block was considered as a random effect. Temperature was considered as a repeated measure.

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

3. Results

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

Inset Table 1 near here

A strong interaction for MSW, N, NDF, ADF and PCDMD was Year × Site (Tables 2, 3 and S3). It explained 0.03 for PCDMD and 0.02 for the other determinations, with only 0.005 of the degrees of freedom. For all the variables, this interaction was mainly due to the different behavior of the cultivars in the CL trial in the first year of the study compared to the behavior of the species at the SG and LA sites and CL in the second year. To a lesser extent, the behavior of species at the LA site in the second year of the study also contributed to the significance of the interaction Year × Site. Illustrating this, for MSW, the SG site showed mean values of 3.02 and 2.91 in the first and second year of the study respectively, against 3.14 and 2.89 for the LA site, but the CL site showed MSW values of 2.22 and 2.53. Other strong interactions were Site × Cultivar for MSW and ash. Meadow foxtail cultivar had a higher MSW value with respect to the other species in the CL trials than in the other trials whereas the difference between MSW values for sheep's fescue cultivar and others were lower in CL trials than in SG and LA trials (Table 2). Similarly, the ash content of this meadow foxtail cultivar with respect to the others was lower in the CL trials than in the SG and CL trials (Table S3).

Inset Table 2 near here

Inset Table 3 near here

The Site × Temperature interaction can be extrapolated from Tables 2, 3 and S3. It was meaningful for ash (Table S3) and N (Table 3). At 400 and 500 GDD, cocksfoot and sheep's fescue cultivars showed the highest (4.06 and 3.57 g/kg DM respectively) and the lowest (3.13 and 2.85 g/kg DM respectively) N values, but at 1400 GDD the two cultivars showed similar N values of 1.58 and 1.63 g/kg DM respectively (Table 3). The Temperature × Cultivar interaction was important for MSW, ash, N, NDF, ADF contents and PCDMD (Figure 1). The Year × Site × Cultivar interaction was relevant for MSW and ash (Table 2, Table S3, Figure 2). At 400 GDD, sheep's fescue cultivar was the earliest, while meadow foxtail and red fescue cultivars showed an intermediate development. Cocksfoot, ryegrass and timothy started their development latest. Furthermore, at 1400 GDD, variability among cultivars was lower than that at the beginning of the cycle. At this accumulated temperature, cocksfoot and meadow foxtail cultivars were among the least well-developed (3.3 and 3.1 respectively), whereas ryegrass cultivar showed the highest MSW value (3.8).

Inset Figure 1 near here

Inset Figure 2 near here

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

4. Discussion

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

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

An unexpected result of the present study was the different evolution of chemical composition and nutritive value of the species in the trial performed at CL, particularly in the first year of the study and to a lesser extent in the second one compared to the time course of species in the other trials. Thus, in the CL trial in 2009 the maturity development of the studied species was delayed compared with the other trials. In parallel, CP, NDF and ADF contents of species in this trial were lower, and PCDMD content was higher than that obtained in the other trials. In the LA trial in 2010 the maturity stage of the different species was similar to that seen in the other trials up to 900 GDD, but then it did not progress compared to the results of the other trials. The differences in the management of these trials can explain these results. Trials were established in spring 2008 and were cut twice before the start of the experiment except for the CL trial, which was established in autumn 2008 because of sprouting problems in spring, and this trial was therefore not cut before the start of the experiment. The tables of forage nutritive value used in France (Andrieu et al., 1989) reported a different maturity development of forages during the seeding year than during the full harvest year, but they do not distinguish between the maturity stage during the full harvest year of forages seeded in spring or in autumn. Rossignol et al., (2015) reported an effect of plant resource strategy of species on their phenology that could be related by the different dynamic of chemical composition and nutritive value of species when they were established in CL in the first year of the present study. The delayed maturity stage and the higher forage quality of the CL trial, particularly that of 2009 in relation to those of SG and LA, could not be explained by the average temperatures of the growing cycle of this trial compared to those of the SG and LA trials as stated by Buxton, (1996). Photoperiod could partially explain these results, because the first growth cycle of the CL trials was developed under the influence of shorter photoperiods than those of SG, and specially LA, although in the literature, the effect of photoperiod on the quality of forages is not clear. Juan et al., (1993) found that lucerne (Medicago sativa L.) grown under a 13 h photoperiod showed a higher leaf/stem ratio than lucerne grown under a 16 h photoperiod, but according to Wilson, (1982), long photoperiods may be associated with high forage quality. Maturity stage of grass species is influenced by photoperiod. Thus, flowering initiation of grasses depends on day length, which could explain the results obtained here on MSW, but also chemical composition and nutritive value between sites.

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

With advancing maturity stage, the proportion of cell walls in grass and permanent grassland increases, while that of crude protein decreases (Andueza et al., 2010; Andueza et al., 2013). Consequently, forage digestibility decreases with increased maturity stage. Rate of decline in digestibility depends on species (Bruinenberg et al., 2002) and temperature (Deinum et al., 1981). In our experiment, the effect of temperature is clear when the changes in quality parameters are compared between years. Thus Hill et al., (1995) found that quality parameters of tall fescue (*Festuca arundinacea* Schreb.) were better predicted from climatological data than from morphological staging. In the present experiment, when the effect of temperature and maturity stage on the chemical composition and PCDMD were analyzed, we found that digestibility and chemical composition were closely related with the maturity stage (MSW) than with temperature, particularly in CL in 2009 and LA 2010 from 900 GDD, suggesting that factors other than temperature could be important in explaining the forage quality.

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

5. Conclusions

This experiment set out to improve our knowledge of the relationships between maturity stage, sum of temperatures and nutritive value of temperate perennial grasses. The results of the current study can serve as a basis to compare forages obtained at different locations or years in relation to their nutritive value. The environment and more specifically the altitude influence the development of growth and the quality of perennial grasses. Thus, at the same sum of temperature, grasses grown at CL characterized by low altitude present less mature stages and higher quality than those grown at SG and LA, which were characterized by medium and high altitudes respectively. Our findings show that factors related to plot management could influence grass development response and thereby forage quality, however, they will be different according to the species. These findings are important for farmers, but also for researchers interested in comparing the nutritive value of forages obtained in different pedo-climatic conditions, and could be useful to refine advice to farmers considering local conditions. More research should be done in order to identify the management practices that could influence the nutritive value of forages. This work conducted in pure stands could also provide baseline data for further research on the complementarity of species according to the environment for the use together in permanent grassland or multi-specific grasslands. Furthermore, these results could serve for choosing the more appropriate vegetal material for a possible restauration of degraded grasslands according to their adaptation to the environment.

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

	DF[†]	MSW		N		NDF		PCDMD	
		V	Signif	V	Signif	V	Signif	V	Signif
Site	2	0.15	<0.001	0.13	<0.001	0.06	<0.001	0.05	<0.001
Year	1	<0.01	0.320	<0.01	0.007	0.01	<0.001	0.01	<0.001
Cult[‡]	5	0.09	<0.001	<0.01	0.001	0.07	<0.001	0.08	<0.001
Temp	10	0.46	<0.001	0.57	<0.001	0.53	<0.001	0.64	<0.001
Year*site	2	0.02	<0.001	0.02	<0.001	0.02	<0.001	0.03	<0.001
Site*cult	10	0.02	<0.001	<0.01	0.017	0.01	<0.001	<0.01	<0.001
Site*temp	20	0.01	<0.001	0.06	<0.001	0.02	<0.001	0.01	<0.001
Year*cult	5	<0.01	<0.001	<0.01	<0.001	0.01	<0.001	<0.01	<0.001
Year*temp	10	<0.01	<0.001	0.02	<0.001	0.02	<0.001	0.01	<0.001
Temp*cult	13	0.04	<0.001	0.02	<0.001	0.04	<0.001	0.02	<0.001
Year*site*cult	10	0.04	<0.001	<0.01	<0.001	0.01	<0.001	0.01	<0.001
Year*site*temp	20	<0.01	<0.001	0.02	<0.001	0.03	<0.001	0.01	<0.001
Site*temp*cult	100	0.03	<0.001	<0.01	<0.001	0.02	<0.001	0.01	<0.001
Year*temp*cult	50	0.01	0.006	<0.01	0.005	0.01	<0.001	0.01	<0.001
Year*site*temp*cult	100	0.01	0.060	<0.01	0.311	0.02	<0.001	0.01	<0.02

[†]DF = degree of freedom; [‡]Cult = cultivar; temp = temperature;

Table 2. Means for phenological stage note (MSW) and neutral detergent fibre (NDF, g/kg dry matter) for cultivars of six species and accumulated temperature (growing degree-days (GDD, °C)) obtained in the trials at Clermont-Ferrand (CL), Saint-Genès-Champanelle (SG) and 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†
Species																
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
Temperature (accumulated GDD, °C)																
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

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

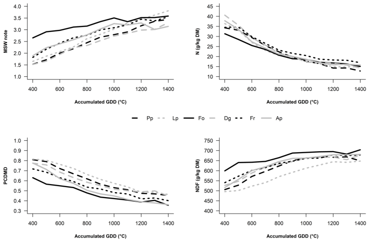
	N								PCDMD							
	Year1				Year 2				Year1				Year 2			
	CL	SG	LA	sem[†]	CL	SG	LA	sem[†]	CL	SG	LA	sem[†]	CL	SG	LA	sem[†]
Species																
timothy	25	18	18	1.0	25	18	27	1.0	0.72	0.57	0.55	0.934	0.67	0.55	0.56	0.911
ryegrass	23	17	20	1.0	22	18	29	1.0	0.71	0.61	0.58	0.914	0.67	0.61	0.61	0.911
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
Temperature (accumulated GDD, °C)																
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

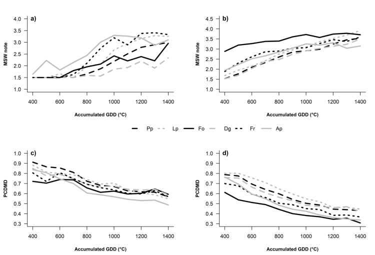
Figure captions

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

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



MSW note
N (g/kg DM)
PCDMD
NDF (g/kg DM)
Accumulated GDD (°C)
Pp
Lp
Fo
Dg
Fr
Ap


a)
b)
c)
d)
MSW note
PCDMD
Accumulated GDD (°C)
Pp
Lp
Fo
Dg
Fr
Ap