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Abstract

This study examined the relationships between botanical composition and forage parameters (yield and forage quality variables) in 153 permanent grasslands located in the Massif Central of France. Grasslands were sampled at two vegetation stages in the first growth cycle. Botanical composition, yield, ash, crude protein, neutral detergent fibre, acid detergent fibre, acid detergent lignin, organic matter digestibility (OMD) and voluntary intake (VI) were estimated for each sample. Temporal variability in species–forage parameter relationships were accounted for using innovative multivariate analyses applied mainly in ecological science. Crude protein and OMD were weakly correlated when each harvest time was analysed separately. Species–forage parameter relationships remained stable during the first growth cycle. The stability of these relationships indicates that permanent grasslands dominated by competitive species were associated with high yield and forage quality values whereas permanent grasslands composed of conservative species and/or high proportions of senescent material were associated with high structural carbohydrate values and low yield, OMD and VI values. Based on these relationships, we propose a typology of permanent grasslands along with a set of indicator species enabling non-specialist botanists to easily classify grasslands for grassland management purposes.

Keywords: floristic composition, chemical composition, organic matter digestibility, voluntary intake, permanent grasslands

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Introduction

The EC definition of permanent grassland is a plot 'that has not been included in the crop rotation of the holding for five years or longer', yet European permanent grassland is usually kept for longer than five years and is not sown, although, in some cases, it may be oversown or resown depending on management intensity. Permanent pastures, as part of permanent grasslands are used to feed livestock and are typically composed of a mixture of native self-seeded grasses and forbs. These botanical communities are maintained by agricultural practices (livestock grazing and/or mowing) but are not substantially modified by intensive inorganic fertilization, drainage or herbicide use (Reheul *et al.*, 2007). They are also called unimproved permanent grasslands, but if one or more of these practices is used on them, they are reclassed as semi-improved or improved permanent grasslands according to degree of use.

Permanent grasslands are a major feature of land use in Europe, covering more than a third of the European agricultural area (FAO, 2008). Forage from permanent grasslands is a key component of ruminant diets and also plays a key role in differentiating products derived from livestock fed with this type of forage (Martin *et al.*, 2005).

The feed value of the main forage grass species found in temporary grasslands is well established (Baumont *et al.*, 2007), but there is a lack of adequate estimates of the feed value of forage obtained from permanent grasslands (Bruinenberg *et al.*, 2002; Pelve *et al.*, 2012). The large diversity of indigenous grasses and dicotyledons in permanent grasslands and their broad variability in phenology and competitiveness (Rossignol *et al.*, 2014) may explain the difficulty in obtaining good estimates of nutritive value. Seasonal changes in plant abundances further complicate efforts to predict forage quality. Indeed, Todorova and Kirilov

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(2002) reported huge variation (19–69% of total yield) in the proportion of *Agrostis capillaris* in different periods of the first growth cycle of an *Ag. capillaris–Festuca fallax* grassland. Likewise, Andueza *et al.* (2013) reported important variations in the floristic composition of two botanically different extensively managed permanent grasslands studied at three dates during the first growth cycle in the Massif Central of France. This complexity is the reason why the variability in the feed value of permanent grasslands, and its evolution over the first growth cycle, are still poorly understood.

This study set out to (i) describe the relationship between the botanical composition of permanent grasslands (seminatural unimproved and semi-improved pastures and also grasslands improved in the past but unploughed for at least 5 years) and forage parameters:, i.e., yield, chemical composition and feed value, (ii) determine the stability of this relationship over the first growth cycle and (iii) characterize permanent grasslands on the basis of these variables. These objectives were achieved using innovative multivariate analyses that take into account how the relationship changes over time.

Materials and methods

Grasslands and sampling

A total of 153 permanent grasslands over 5 years old with no history of ploughing were randomly selected from locations in the Auvergne region of central France. The sampled grasslands were representative of different types of soil [granitic ($n = 78$) and volcanic ($n = 75$)], altitude [ranging from 300 to 1350 m a.s.l. ($n = 83$ for altitude > 900 m a.s.l., $n = 70$ for an altitude < 900 m a.s.l.)] and annual rainfall [ranging from 480 to 1440 mm ($n = 23$ for an annual rainfall > 1000 mm, $n = 130$ for an annual rainfall < 1000 mm)]. Plots included grasslands dominated by *Lolium perenne* or *Dactylis glomerata* but also *Holcus lanatus*, *Festuca rubra* or *Ag. capillaris*. The mean ± s.d. soil chemical and physical properties of the studied area according to Hulin *et al.* (2011) were as follows: 45 ± 14% sand, 34 ± 10% loam, 21 ± 8% clay, 7·2 ± 5·6% organic matter, 5·9 ± 0·34 pH, 280 ± 73 mg per kg Olsen–P status, 350 ± 200 mg per kg K status and 22·7 ± 10·39 CEC. Each grassland was sampled twice in 2005 during the first cycle of growth: once when farmers made silage (Silage stage, SS), that is, around the heading stage of dominant grass species, and once when they made hay (Hay stage, HS), that is, around the flowering stage of dominant grass species. In each grassland, we used different plots for SS and HS samples. Sampling was performed on surfaces left uncut by farmers when they decided to harvest the grasslands between the two sampling periods. On average, HS sampling came three weeks after the SS sampling. For each sampling, five randomized 0·25 m² subplots (0·5 × 0·5 m) were cut within each grassland at 5 cm above ground level using a small lawnmower. Forage obtained in the five subplots was pooled to give a single representative sample per grassland, then weighed and divided into two subsamples. The first subsample (125 g fresh material) was stored at −20°C and used to characterize botanical composition. The second subsample was dried at 60°C for 72 h to assess dry-matter (DM) content, then ground in a hammer mill through a 0·8-mm screen.

Determination of botanical composition

First, each sample was sorted into fresh forage and senescent material (Sm). Botanical composition of fresh forage was then determined by hand-sorting the samples into plant species. Species identification and nomenclature followed Flora Europea (Tutin *et al.*, 1964–1980). Individual species and Sm were then dried at 60°C for 72 h and weighed. Proportion of each species and Sm was expressed as species and Sm contribution to total yield.

Determination of chemical composition and feed value

Ash and crude protein (CP) contents, *in vivo* organic matter digestibility (OMD) and voluntary intake (VI) by sheep were estimated by near-infrared reflectance spectroscopy (NIRS) according to the global model proposed by Andueza *et al.* (2011). Specific NIRS models were also built for the estimations of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents. The reference methods used were proposed by Van Soest *et al.* (1991) for NDF and Van Soest and Robertson (1980) for ADF and ADL and performed on an Ankom analyzer (Ankom® Tech. Co., Fairport, NY, USA). For all determinations, before running the NIRS predictions, we first calculated Mahalanobis distance (H) between the spectra of each sample and the average calibration spectra to check the calibration models were compatible with the samples to be predicted. No samples were discarded (H > 3). Predicted values for chemical composition, OMD and VI of samples were within the range of values of the databases used to develop the calibration equations. Standard errors of cross-validation were 14·0, 12·1 and 6·0 g kg DM^{-1}, and coefficients of determination of cross-validation were 0·96, 0·94 and 0·90 for NDF, ADF and ADL respectively. Ash content, CP, VI, OMD, NDF, ADF and ADL were included in the analysis together with yield as variables representing forage quality.

Data analysis

Differences between sampling periods for forage parameter determinations were tested by ANOVA. The species proportions and forage parameters for each grassland and period of sampling generated two sequences of tables: one sequence of two tables for the species proportions at both SS and HS sampling periods and another sequence of two tables for the eight forage parameters, again at both the SS and HS sampling periods (Figure 1). The common relationship between botanical composition and forage parameters of permanent grassland and the stability of this relationship over both sampling periods were investigated using STATICO, a special kind of multivariate analysis (Simier *et al.*, 1999; Thioulouse *et al.*, 2004).

STATICO is a three-step method in which each table is first analysed separately by principal component analysis. The relationship between species and forage parameter is assessed for each period using a co-inertia analysis on each species–forage parameter pair of tables (Doledec and Chessel, 1994). Finally, a partial triadic analysis (Thioulouse and Chessel, 1987) is run on the sequence of cross-tables produced by the co-inertia analyses to assess the relationships that are stable along the sequence of cross-tables, that is over the two sampling periods here. The main outputs of this analysis are the interstructure, compromise and intrastructure. The compromise gives a picture of the common relationship between floristic composition and forage parameters over the two periods. The interstructure gives the importance of each period in the construction of the compromise, while the typological value gives an idea of how much the compromise expresses the information from each period. The intrastructure (trajectories) summarizes the variability of the species–forage parameters relationships around the common structure defined by the compromise between the two sampling periods (Thioulouse *et al.*, 2004; Thioulouse, 2011).

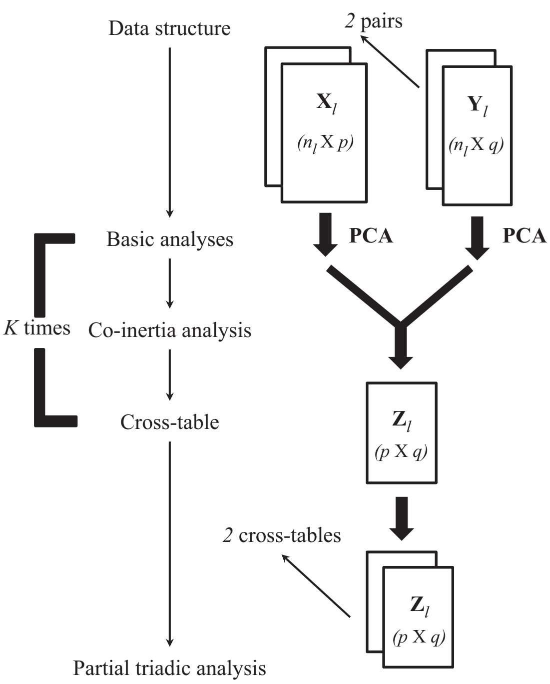

Figure 1 STATICO flow chart (adapted from Thioulouse *et al.*, 2004).

Before implementing the STATICO analysis, botanical composition data were Hellinger-transformed to factor out double absence of species being considered a resemblance between sites (Legendre and Gallagher, 2001), and forage parameter data were standardized (divided by the variance). For each sampling period, the significance of the relationship between botanical composition and forage parameter tables revealed by the co-inertia analyses was tested using a Monte Carlo test. Then, a grassland classification scheme was constructed using a hierarchical cluster analysis based on the row coordinates in the forage parameters space of a co-inertia analysis (Dray *et al.*, 2003) performed between botanical composition and forage parameters of the SS-period sampling. This method classifies swards by maximizing intragroup homogeneity and intergroup diversity. The number of groups was proposed by the scree test (Cattell, 1966). Multivariate analyses were carried out with GNU-project R software version R 2.13.2 (R Foundation for Statistical Computing, http://www.R-project.org) using the ADE4 package (Thioulouse *et al.*, 1997). Finally, the effect of these groups on the variables related to botanical composition and forage parameters was tested by ANOVA. The model included group and sampling period as fixed effects and grassland as random effect. Period of sampling was considered as a repeated measure. Arcsine square root transformation was performed on percentage species before statistical analysis to improve normality and homoscedasticity (Sokal and Rohlf, 1981). ANOVAs were performed using SAS statistical software (SAS, 2000).

Results

Yield, forage quality and species diversity

Yield and forage quality characteristics for both harvesting periods of the sampled grasslands are reported in Table 1. Estimated values for ash content, CP content, OMD and VI showed higher average values at SS than at HS, whereas average values for yield, NDF, ADF and ADL were higher at HS than at SS.

Table 1 Mean, standard deviation (s.d.), minimum (min) and maximum (max) values obtained for yield and NIRS-estimated values for ash, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), organic matter digestibility (OMD) and voluntary intake (VI) of permanent grasslands sampled at two sampling periods (sp): silage stage (SS) and hay stage (HS).

	SS				HS					
	Mean	s.d.	Min	Max	Mean	s.d.	Min	Max	s.e.	sp
Yield (t DM ha^{-1})	4·22	2·45	0·37	9·39	5·42	2·81	0·49	10·69	0·21	***
Ash (g kg DM^{-1})	84	12·0	61	124	75	10·1	58	103	0·89	***
CP (g kg DM^{-1})	114	27·0	67	200	84	20·0	47	168	1·92	***
NDF (g kg DM^{-1})	560	44·0	428	717	607	31·6	477	685	3·09	***
ADF (g kg DM^{-1})	296	29·7	221	466	328	19·2	272	376	2·02	***
ADL (g kg DM^{-1})	41	14	12	89	46	8·2	28	79	0·91	***
OMD (g g^{-1})	0·71	0·06	0·52	0·79	0·62	0·04	0·50	0·74	0·00	***
VI (g per kg BW$^{0·75}$)	69·4	7·13	51·2	83·8	64·4	6·43	47·9	79·3	5·49	***

s.e., Standard error; ***$P < 0·001$.

In total, 125 different plant species (Table 2) were identified in the 306 samples (153 plots * 2 sampling periods), including thirty-six species of grasses (between 3 and 15 species per sample), seventy-eight species of forbs (0–23 species per sample) and eleven species of legumes (0–5 species per sample). Proportion of grasses ranged from 5 to 99% at SS and from 12 to 97% at HS. Proportion of forbs ranged from 0 to 66% at both SS and HS. Proportion of legumes ranged from 0 to 55% at SS and from 0 to 47% at HS. Proportion of Sm ranged from 0 to 81% at SS and from 0 to 47% at HS.

Relationship between botanical composition and forage parameters

Co-inertia analyses showed a significant correlation ($P < 0·001$) between botanical composition and forage parameter for each sampling period, allowing us to implement the STATICO analysis.

Interstructure and typological values

The interstructure factor map (Figure 2a) allows an examination of how the relationships between species and forage parameters change according to the two different periods. The eigenvalue of the first axis (81% of overall inertia) clearly prevailed over the eigenvalue of the second axis (19% of overall inertia). The arrows on the interstructure factor map (Figure 2a) pointed in the same direction on the first axis which showed that the costructure between forage parameters and botanical composition of permanent grasslands was similar throughout the SS and HS periods. The data from both sampling periods thus had the same influence on the compromise. However, the typological value showed that the compromises better expressed the information contained in tables derived from the SS sampling period ($\cos^2 = 0·95$) than from the HS sampling period ($\cos^2 = 0·82$).

Compromise

The compromise expressed the relationship between species and forage parameters over the two periods. The projection of variables (species and forage parameters) on the first two axes of the compromise enabled us to spot associations among species (Figure 2c) and among forage parameters (Figure 2b). Furthermore, comparing the coordinates of the species and forage parameters on the first two axes of the compromise enabled us to highlight which of the species were related to forage-quality variables and yield. In this sense, the first axis was dominant, accounting for 72% of overall inertia, whereas the second axis accounted for just 11% of inertia. Looking at the factor map of forage parameters (Figure 2b), the first axis displayed an opposition between estimated NDF, ADF and ADL values on one side and estimated OMD, VI and yield of permanent grassland on the other, thus expressing a gradient of forage quantity and quality. The second axis was characterized by an estimated CP value gradient. Ash content was closely correlated with CP content. In Figure 2c, species were ranked according to their proportion in permanent grasslands. Running a comparison of both compromise figures allowed us to characterize permanent grasslands according to the relationship between forage parameters and characteristic species. Thus, proportion of Sm was positively associated with permanent grasslands characterized by high NDF, ADF and ADL values, low OMD and yield values and a high proportion of spe-

Table 2 Mean, standard deviation (s.d.) and maximum (Max) values obtained for the most representative species (proportion of dry matter, as %) of permanent grasslands sampled at two sampling periods: ensilage stage (SS) and hay stage (HS).

	SS			HS		
Species	**Mean**	**s.d.**	**Max**	**Mean**	**s.d.**	**Max**
Grasses						
Agrostis capillaris	8·27	9·21	39·82	12·43	12·99	60·81
Alopecurus pratensis	3·30	8·91	49·78	2·89	7·28	35·00
Anthoxanthum odoratum	1·23	3·00	21·24	0·68	1·81	13·35
Arrhenatherum elatius	3·88	8·46	47·14	4·98	11·52	60·82
Bromus mollis	3·76	8·45	47·32	1·48	4·00	31·83
Dactylis glomerata	10·49	14·41	79·52	11·84	17·63	87·75
Elytrigia repens	1·50	4·70	32·81	2·14	6·38	44·98
Festuca arundinacea	0·87	4·49	35·94	1·11	5·84	41·89
Festuca pratensis	1·61	6·21	48·75	2·22	8·11	56·95
Festuca rubra	7·31	12·50	61·02	6·15	10·85	59·90
Holcus lanatus	6·25	12·16	80·98	7·02	11·89	59·39
Lolium perenne	6·53	10·72	57·72	5·76	10·72	69·00
Phleum pratense	0·89	2·61	15·62	1·61	4·50	23·46
Poa pratensis	4·23	5·68	41·28	4·46	5·73	32·60
Poa trivialis	2·98	5·89	41·08	1·85	3·32	20·03
Trisetum flavecens	3·54	0·41	5·01	4·61	6·68	37·26
Forbs						
Cerastium spp.	1·60	2·34	10·83	1·20	1·82	8·48
Gallium mollugo	0·63	3·30	29·63	0·59	2·52	24·62
Heracleum sphondylium	1·00	3·82	23·77	1·05	6·02	52·49
Plantago lanceolata	1·22	3·14	22·24	0·56	1·44	8·00
Ranunculus acris	1·34	3·04	22·73	0·88	2·07	11·00
Rumex acetosa	0·54	2·50	22·16	0·33	1·37	10·00
Taraxacum officinale	4·42	7·39	61·08	1·54	2·81	16·36
Urtica dioica	0·46	4·32	52·80	0·81	5·59	49·35
Legumes						
Lathyrus pratensis	0·45	1·43	10·98	0·37	1·24	8·49
Trifolium pratense	0·68	4·68	55·80	0·61	4·04	47·00
Trifolium repens	1·27	2·45	15·77	1·04	2·71	21·00
Vicia sativa	0·42	1·80	17·06	0·61	2·29	17·00
Other						
Senescent material	6·12	10·80	80·91	6·11	4·97	26·11

Minimum values are 0·00 for all species, with the exception of senescent material at HS which presented a minimum value of 0·37%. Twenty grasses, sixty-eight forbs, seven legumes and two monocotyledons not grasses are not listed.

cies such as *Fe. rubra* and *Elytrigia repens*. In contrast, permanent grasslands characterized by high proportions of *L. perenne* were associated with high estimated OMD, VI and yield values and low ADL and structural carbohydrate contents. Abundance of *D. glomerata*, *Taraxacum officinale* and *Poa trivialis* was positively correlated with abundance of *L. perenne*. High proportions of *Alopecurus pratensis*, *Festuca pratensis*, *Ag. capillaris*, *Ho. lanatus*, *Fe. rubra* and *Bromus mollis* were associated with low CP content, whereas high proportions of *T. officinale*, *Urtica dioica*, *E. repens*, Sm and *Arrhenatherum elatius* were correlated to high CP and ash content values.

Intrastructure

The intrastructure indicated the reproducibility of the compromise for each of the two sampling periods, making it possible to analyse how the species–forage parameters relationship differed between the two sampling periods. The projection of the forage parameter variables and the projection of the species on the compromise axes are shown in the trajectories factor map (Figure 3). The two periods were plotted on separate graphs to show (3a) projection of forage parameters in SS, (3b) projection of species proportions in SS, (3c) projection of forage parameters in HS and

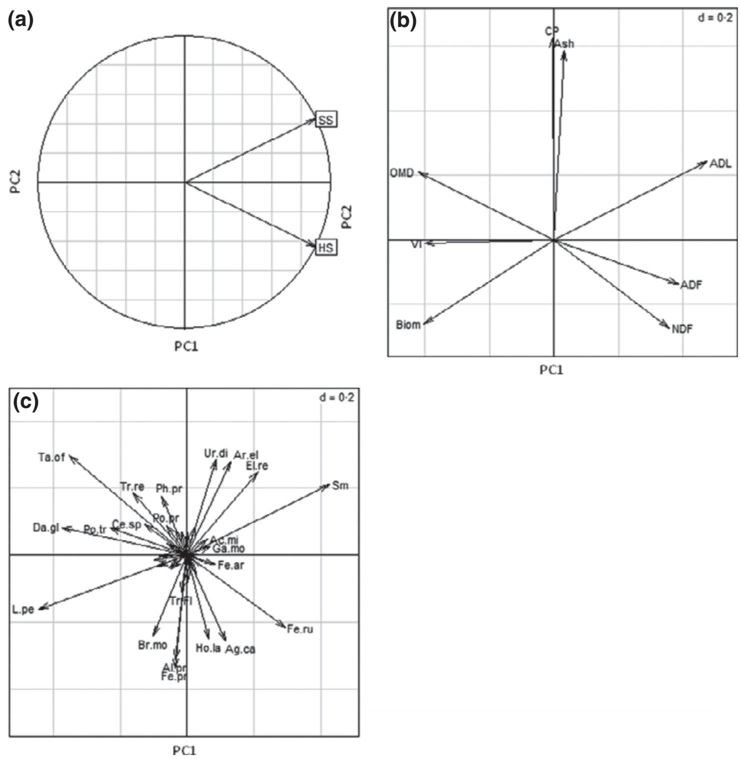

Figure 2 Interstructure (a) and compromise factor maps of the STATICO analysis of forage parameters (b) and botanical composition (c). The interstructure map shows the importance of each sampling period in the compromise. The compromise factor maps show the projection of forage parameter variables (b) and species (c) on the first and second axes, and the stable part of the species-forage parameter variables. d values give grid size. Ag.ca, *Agrostis capillaris*; Br.mo, *Bromus mollis*; Ce.sp, *Cerastium* spp; El.re, *Elytrigia repens*; Da.gl, *Dactylis glomerata*; Fe.pr, *Festuca pratensis*; Fe.ar, *Festuca arundinacea*; Fe.ru, *Festuca rubra*; Ph.pr, *Phleum pratense*; Po.pr, *Poa pratensis*; Ar.el, *Arrhenatherum elatius*; Ga.mo, *Gallium mollugo*; Tr.fl, *Trisetum flavescens*; Ac.mi, *Achillea millefolium*; Ho.la, *Holcus lanatus*; Sm, Senescent material; Ur.di, *Urtica dioica*; Po.tr, *Poa trivialis*; Ta.of, *Taraxacum officinale*; L.pe, *Lolium perenne*; Tr.re, *Trifolium repens*; Al.pr, *Alopecurus pratensis;* Biom, yield; ADL, acid detergent lignin; ADF, acid detergent fibre; NDF, neutral detergent fibre; CP, crude protein; OMD, organic matter digestibility; VI, voluntary intake; SS, silage stage; HS, hay stage; PC1, principal component 1; PC2, principal component 2; d value gives grid size.

(3d) projection of species proportions in HS. The stable part of the species–forage parameter dynamics revealed by the compromise analysis was better expressed for SS. Both species and forage parameters showed lower variability in HS than in SS, although there was strong similarity in the structure of the forage parameters and botanical composition. Consequently, for both sampling periods, yield and estimated VI and OMD values were mainly opposed to ADL but also to ADF and NDF in the first axis, whereas the second axis was explained by a gradient of CP values. There were no differences in botanical composition between the two sampling periods. However, Sm was less correlated with forage parameters in HS than in SS. *Dactylis glomerata* was more correlated with OMD in SS but it was more correlated with yield in HS.

A grassland typology according to botanical composition and forage parameters

The relationship between floristic composition and forage parameters as described prompted us to base the grassland typology on co-inertia analysis at the SS sampling period. The co-inertia and hierarchical cluster analyses led to a typology of permanent grasslands grouped into four classes according to the aggregate of proportion of different species and feed-quality values. Significant differences ($P < 0{\cdot}01$) between classes were found for all forage parameters (Table 3) and for the more representative species of each class (Table 4), except for proportion of *F. pratensis*. Significant differences ($P < 0{\cdot}01$) between periods were found for all forage parameters and for proportions of *Ag. capillaris,*

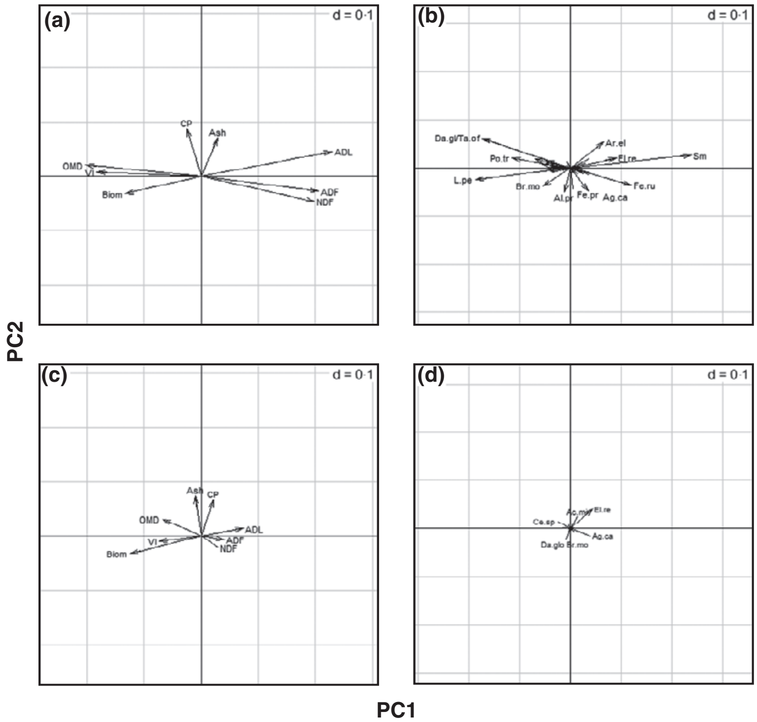

Figure 3 Trajectories factor map projection of the coordinates of the eight forage parameters and 125 species proportions on the principal component factor map of the compromise. PC1 = first principal component. PC2 = second principal component. The two periods [Silage stage (SS) and Hay stage (HS)] have been plotted on separate graphs to show (a) projection of forage parameters in SS, (b) projection of species proportions in SS, (c) projection of forage parameters in HS, (d) projection of species proportions in HS. d values give grid size. Ag.ca, *Agrostis capillaris*; Br.mo, *Bromus mollis*; Ce.sp, *Cerastium* spp; El.re, *Elytrigia repens*; Da.gl, *Dactylis glomerata*; Fe.pr, *Festuca pratensis*; Fe.ru, *Festuca rubra*; Ar.el, *Arrhenatherum elatius*; Ac.mi, *Achilea millefolium*; Sm, Senescent material; Po.tr, *Poa trivialis*; Ta.of, *Taraxacum officinale*; L.pe, *Lolium perenne*; Al.pr, *Alopecurus pratensis;* Biom, yield; ADL, acid detergent lignin; ADF, acid detergent fibre; NDF, neutral detergent fibre; CP, crude protein; OMD, organic matter digestibility; VI, voluntary intake.

B. mollis, T. officinale and *Ranunculus acris*. Class × period interaction was significant ($P < 0{\cdot}01$) for all forage parameters with the exception of yield and CP content, and for proportion of *T. officinale* and Sm. On the factor map of the co-inertia analysis (Figure 4), classes 1 and 4 were opposed to classes 2 and 3 according to the first axis (i.e., gradient of forage quantity and quality), while classes 1 and 3 were opposed to classes 2 and 4 on the second axis (i.e., gradient of CP).

Class 1 ($n = 28$) was characterized by low yield and low feed value. Average values were 1·7 and 2·6 t DM per ha yield, 117 and 88 g DM CP, 0·66 and 0·61 OMD and 65·9 and 61·3 g per kg $BW^{0.75}$ VI for SS and HS respectively (Table 3). This class was chiefly characterized by abundant senescent material in SS (Sm 20%) decreasing to 7% in HS and high proportions of *Ar. elatius, Ho. lanatus, E. repens, Ag. capillaris, Al. pratensis, U. dioica* and *Gallium mollugo* in both sampling periods (Table 4).

Class 2 ($n = 49$) was characterized by high yield and high feed-value parameters (except CP). Average values were 4·51 and 5·98 t DM per ha yield, 108 and 81 g kg DM^{-1} CP, 0·72 and 0·62 OMD and 71·4 and 65·7 g per kg $BW^{0.75}$ VI for SS and HS respectively (Table 3). The characteristic species of Class 2 were *Ag. capillaris, L. perenne, D. glomerata, Fe. rubra, Ho. lanatus, Al. pratensis, T. officinale* and *Cerastium* spp. (Table 4).

Class 3 ($n = 48$) was characterized by high yield and high feed-value parameters. Average values were 5·83 and 6·98 t DM per ha yield, 121 and 86 g kg DM^{-1} CP, 0·75 and 0·64 OMD, 73·3 and 66·3 g per kg $BW^{0.75}$ VI, and 535 and 600 g per kg NDF for SS and HS respectively (Table 3). The characteristic species of Class 3 were *D. glomerata, T. officinale* and *L. perenne*.

Table 3 Mean values and statistical effects for yield and NIRS-estimated values for ash, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), organic matter digestibility (OMD) and voluntary intake (VI) in four classes of permanent grassland sampled at two periods during the first growth cycle: silage stage (SS) and hay stage (HS).

	SS				HS							
	Class 1	Class 2	Class 3	Class 4	Class 1	Class 2	Class 3	Class 4	s.e.	Class	Period	Class × Period
Yield (t DM ha^{-1})	1·67	4·51	5·83	3·43	2·61	5·98	6·98	4·52	0·36	***	***	ns
Ash (g kg DM^{-1})	94·3	80·3	84·8	76·8	77·2	73·2	78·3	69·8	1·68	***	***	**
CP (g kg DM^{-1})	117	108	121	107	88	81	86	79	3·89	**	***	ns
NDF (g kg DM^{-1})	597	550	535	583	619	604	600	608	5·67	***	***	***
ADF (g kg DM^{-1})	319	288	283	314	340	324	325	330	3·75	***	***	***
ADL (g kg DM^{-1})	53	36	35	49	48	44	44	52	1·63	***	**	***
OMD (g g^{-1})	0·66	0·72	0·75	0·68	0·61	0·62	0·64	0·60	0·01	***	***	**
VI g per kg $BW^{0·75}$)	65·9	71·4	73·3	61·3	61·3	65·7	66·3	60·3	0·99	***	***	*

s.e., Standard error; ns, $P > 0·05$; $*P < 0·05$; $**P < 0·01$; $***P < 0·001$.

Taraxacum officinale decreased significantly between SS and HS (Table 4).

Class 4 ($n = 28$) was differentiated from other classes by low feed-value parameters, including low CP. Average values were 3·43 and 4·52 t DM per ha yield, 107 and 79 g per kg DM CP, 0·68 and 0·60 OMD, 61·3 and 60·3 g per kg $BW^{0·75}$ VI, and 583 and 608 g per kg NDF for SS and HS respectively (Table 3). The characteristic species of Class 4 were *Fe. rubra*, *Ag. capillaris*, *Ho. lanatus*, *G. mollugo* and Sm (Table 4).

Discussion

The correlation between forage parameters and botanical composition

We found very broad variability in the chemical composition and feed value of the grassland-derived forages used in this study. The range of values in our dataset was similar to the range reported by Baumont *et al.* (2007) for green forages from the first growth cycle in French permanent grasslands. This large range in terms of both species identity and relative abundance reflects the diversity of plant communities sampled in this study.

An interesting finding of this study was the lack of correlation between CP and other feed-value parameters. Several studies have reported that CP and OMD decrease in a similar way with time (Andrieu *et al.*, 1981; Andres *et al.*, 2005). Our data did not confirm this pattern, even though both variables were well correlated ($r = 0·86$) when the two sampling periods were combined, but this correlation almost disappeared when the CP–OMD linkage was investigated separately for each sampling period. Accordingly, for a given point in time, grassland forage can be characterized as either low OMD and high CP or low OMD and low-CP.

A surprising finding of this study was the positive correlation between Sm and CP content. The possible presence of fungal diseases of grasses on Sm could explain this result. According to Queiroz *et al.* (2012), plants characterized by moderate rust diseases showed lower CP content than disease-free plants whereas plants characterized by high rust infection showed higher CP content than disease-free plants. Potter (1987) also reported that the presence of crown rust increased the CP content of perennial and Italian ryegrasses. According to Dimmock and Gooding (2002), rust causes physical damage to plants thereby causing leaves to senesce prematurely through desiccation. This reduces translocation but greatly increases retention of amino acids which could increase the CP content of forage.

Table 4 Mean values and statistical effects for proportion of different species in four classes of permanent grasslands sampled at two periods during the first growth cycle: ensilage stage (SS) and hay stage (HS).

	SS				HS						
	Class 1	Class 2	Class 3	Class 4	Class 1	Class 2	Class 3	Class 4	Class	Period	Class × period
Agrostis capillaris	5·47	12·78	2·99	12·23	7·54	16·71	8·43	16·66	***	**	ns
Bromus mollis	1·07	5·14	5·90	0·39	0·74	1·39	2·73	0·21	***	**	ns
Cerastium spp.	0·71	2·03	2·14	0·79	1·19	1·54	1·34	0·35	***	ns	ns
Elytrigia repens	7·18	0·05	0·54	0·02	9·82	0·04	1·04	0·01	***	ns	ns
Dactylis glomerata	4·61	6·31	23·55	1·30	5·93	8·32	22·56	5·50	***	ns	ns
Festuca pratensis	0·21	1·76	2·20	1·76	2·05	2·20	2·41	2·09	ns	ns	ns
Festuca arundinacea	3·02	0·22	0·14	1·08	4·35	0·08	0·00	1·56	***	ns	ns
Festuca rubra	1·61	6·58	0·16	26·56	0·90	6·89	0·61	19·61	***	ns	ns
Phleum pratense	0·56	0·70	1·46	0·57	0·55	1·30	2·63	1·47	**	ns	ns
Anthoxanthum odoratum	0·00	2·04	1·40	0·77	0·04	1·10	0·66	0·59	**	ns	ns
Arrhenatherum elatius	13·55	0·93	3·15	0·60	15·94	2·27	3·89	0·65	**	ns	ns
Gallium mollugo	1·58	0·19	0·03	1·51	0·17	0·37	0·00	2·38	***	ns	ns
Lathyrus pratensis	0·00	0·58	0·21	1·06	0·05	0·062	0·02	0·86	***	ns	ns
Heracleum sphondylium	0·00	0·95	2·21	0·00	0·00	1·24	2·07	0·00	**	ns	ns
Holcus lanatus	13·85	5·75	3·45	4·34	14·04	4·44	7·19	4·19	***	ns	ns
Senescent material	19·94	2·12	2·00	6·36	7·39	4·92	5·49	7·99	***	ns	***
Urtica dioica	2·13	0·08	0·15	0·00	4·31	0·06	0·00	0·00	***	ns	ns
Poa trivialis	0·92	3·04	5·75	0·17	0·82	2·04	3·17	0·31	***	ns	ns
Taraxacum officinale	1·19	3·41	9·84	0·13	1·00	1·45	2·76	0·18	***	***	***
Plantago lanceolata	0·32	2·47	0·54	1·09	0·04	0·98	0·17	1·00	***	ns	ns
Lolium perenne	0·69	11·63	8·36	0·32	1·82	9·45	7·33	0·50	***	ns	ns
Ranuculus acris	0·10	1·64	1·91	1·09	0·29	1·53	0·96	0·21	***	**	ns
Rumex acetosa	0·00	0·84	0·82	0·06	0·00	0·51	0·52	0·04	**	ns	ns
Trifolium repens	0·00	1·83	1·91	0·48	0·07	1·37	1·71	0·29	***	ns	ns
Alopecurus pratensis	5·75	4·44	2·52	0·19	4·01	5·23	1·33	0·055	***	ns	ns

ns, $P > 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$.

Our results further confirmed the well-known negative correlation between structural carbohydrate–lignin contents and OMD, VI and yield. Several papers have reported similar relationships between ADF and ADL content and OMD (Demarquilly *et al.*, 1995) and between cell-wall content and VI (Mertens, 1994) in forages.

Looking at the relationship between botanical composition and forage parameters, the first factor map of the compromise revealed a species gradient based on ability to compete using natural resources (nutrients, light etc.; Grime, 1977) associated with a feed-value gradient. Permanent grasslands rich in competitive grasses such as *L. perenne* and *D. glomerata* associated with high forage parameter values (Schubiger *et al.*, 2001; Bruinenberg *et al.*, 2002) were opposed to more conservative grasses such as *Fe. rubra* and *Ag. capillaris* associated with low forage parameter values (Frame, 1991; Pavlu *et al.*, 2006; Pontes *et al.*, 2007). The presence of these functional groups of species (Cruz *et al.*, 2002) is generally associated with a gradient of several environmental variables (precipitation, relative irradiation, soil type, air temperature and nitrogen) (Ejrnaes and Bruun, 2000; Jeangros *et al.*, 2000) and/or with different management history [fertilization, drainage (Hopkins, 1986) and intensity of management (Pavlu *et al.*, 2006)].

Other major species playing an important role in this study were forbs. The feed value of some of these species is not yet sufficiently described in the literature. Forbs may contain secondary compounds which could modify – positively or negatively – the feed value of permanent grasslands. The few papers citing feed values for this functional group do not always

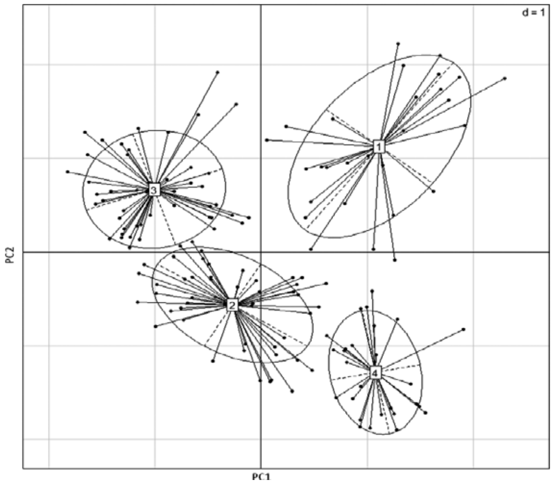

Figure 4 The four classes of permanent grassland charted in the two-dimensional space of the co-inertia analysis defined by principal component axes 1 and 2. values give grid size. PC1 = principal component 1; PC2 = principal 2; d value gives grid size.

give consistent results. Schubiger *et al.* (2001), using the *in vitro* method of Tilley and Terry (1963), reported OMD values for *T. officinale* that were similar to ryegrass, whereas Wilman and Riley (1993) and Derrick *et al.* (1993), using *in vivo* digestibility, reported lower values than for ryegrass. Derrick *et al.* (1993) however, reported similar intake values between *T. officinale* and leafy ryegrass. In addition, Seither *et al.* (2012) found that the nutritive value of herbage from diverse grasslands was in some cases higher than grass-dominated swards, but Hofmann and Isselstein (2005) concluded that the introduction of forbs in a permanent grassland decreased forage digestibility. In our study, the presence of *T. officinale* was positively correlated with abundance of *L. perenne* and was associated with high OMD and VI values, as suggested by Tilley and Terry (1963). Other species associated with high-nutritive forage were *Cerastium* spp, *Ra. acris* and *Heracleum sphondylium*. However, the forbs that negatively correlated to high-nutritive forage included *G. mollugo* and *U. dioica* as reported by Hofmann and Isselstein (2005).

Another interesting finding was that the relationships between botanical composition and forage parameters remained stable during the period of the first growth cycle despite the fact that differences in nutritive value and yield of permanent grasslands were smaller in HS than in SS. This means that forage parameters for permanent grassland composed mainly of competitive species were higher than forage parameters for permanent grassland composed of conservative species during both SS and HS, despite the contrasting patterns of change in feed value of these groups. Duru *et al.* (2008) and Andueza *et al.* (2010) have already shown that permanent grasslands composed of species that employ resource–capture strategies (competitive species), such as *L. perenne* and *D. glomerata*, had higher digestibility values at the beginning of the cycle but also showed a stronger decline than permanent grasslands composed of species that employ resource–conservation strategies, such as *Fe. rubra* and *Ag. capillaris*. Similarly, Andueza *et al.* (2013) found the same pattern for VI on two different permanent grasslands. In this study, we showed similar patterns, but the novelties are as follows: first, that the grasslands dominated by competitive species preserve their higher forage quality (OMD and VI) compared to grasslands dominated by conservative species despite the sharp decline observed between SS and HS for OMD; secondly, that the trends identified using our database can be generalized to a large range of permanent grasslands.

Characterization of permanent grasslands

The permanent grasslands selected and analysed in this study can be categorized into four plant community classes that provide different forage qualities. The co-analysis of botanical composition and forage parameters underlined the association between permanent grasslands with high proportions of competitive species such as *D. glomerata, L. perenne, T. officinale and Cerastium* spp. with high yield and feed values (classes 2 and 3), especially when they are cut or grazed early (as shown by the comparison at SS vs. HS). On the other hand, permanent grasslands composed mainly of *Fe. rubra, Ag. capillaris, Ar. elatius* and *Ho. lanatus* with high proportions of Sm were associated with high fibre contents and low feed values (classes 1 and 4), particularly in the early stages of the growth cycle (SS). Our analysis also showed that grassland types can be further discriminated according to their forage CP and ash contents, allowing us to separate Class 1 from 4 and Class 2 from Class 3. This study thus demonstrates that each class can be characterized by a given species assemblage. However, several species can be found in different classes and in significant proportions (e.g. *Ag. capillaris* in Class 2 and Class 4 or *L. perenne* in Class 2 and Class 3). This result underlines that grassland class cannot be determined by a single characteristic species but rather by the co-occurrence of a set of characteristic species. Permanent grasslands dominated by *Ag. capillaris* and *Fe. rubra* (Class 4) are thus characterized by low feed value, whereas permanent grasslands dominated *by L. perenne* and *Ag. capillaris* (Class 2) showed high feed value. Forbs can also be indicative species despite their lower proportions in the classes. *T. araxacum officinale*, *Cerastium* spp. and *He. sphondylium* were found in high feed-value classes whereas *G. mollugo* and *U. dioica* belonged to low feed-value classes. Consequently, an important result of this study was that we were able to identify several indicator species or species associations characterizing the yield and feed value of permanent grasslands: *D. glomerata* and *T. officinale* for grasslands with high yield potential and high nutritive value, *Ag. capillaris* and *L. perenne* for grasslands with similar characteristics but low CP content, *Fe. rubra* and *Ag. capillaris* for permanent grasslands with low yield and low feed-value parameters, and the association of high proportions of Sm with species such as *E. repens*, *Ho. lanatus* or *Ar. elatius* indicated permanent grasslands with low yield, OMD and intake values but high CP content. This typology differs from that proposed by Daccord *et al.* (2006), who also linked nutritive value to botanical composition of permanent grasslands. However, the typology proposed by Daccord *et al.* (2006) was mainly based on the proportions of different families in the grasslands, although they did take into account the proportion of certain individual species like ryegrass.

Senescent material was found to be associated with permanent grasslands of Class 1. This can be explained by the fact that Class-1 pastures were associated with more extensive management (data not shown) than the other more intensively managed grassland classes, which would equate to a higher quantity of rejections at the end of autumn especially in the SS. The difference of Sm between SS and HS in this class is a result that can be explained by the increase in green forage between SS and HS periods in relation to Sm.

An outcome of this study is the proposed permanent grasslands typology scheme using forage quality and yield based on a set of common grassland species including grasses and forbs. This typology, based on assemblages of species easily identified in permanent grasslands, can be used without deep knowledge of botany. We anticipate these results to be complementary to other tools available, such as the functional classification of permanent grasslands according to grass growth strategies (Cruz *et al.*, 2002). Practical use of this permanent grasslands classification by farmers and land managers (earlier use of grasslands composed of competitive species and then later use of permanent grasslands composed of conservative species) would allow more sustainable and efficient farm-scale management of permanent grasslands.

Conclusions

Applying STATICO – an original statistical method mainly used in ecological science – enabled us to identify the relationships between botanical composition and forage parameters (yield and forage quality) of permanent grasslands at two important maturity stages of the first growth cycle. We established relationships between the dominant species and forage quality parameters of permanent grasslands in a large range of samples and proved that these correlations remain stable over the first growth cycle. This study highlights links between the abundance of certain species in permanent grasslands and their forage quality. We identified a set of indicator species or species associations that characterized the yield and forage quality of permanent grasslands. These findings offer a useful tool for more sustainable and efficient management of permanent grasslands that can also be used in addition to other functional typology tools that farmers already use in practice.

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