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Plant Composition and Feed Value of First Cut Permanent Meadows

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Abstract: Permanent grasslands represent the main terrestrial ecosystem and serve as an important global reservoir of biodiversity, providing a wide range of benefits to humans and ecosystems. The effects of environment on permanent meadows (in our survey, they were centuries-old meadows that had not been plowed, mowed, or fertilized with manure) production have been adequately investigated in literature. However, plant species composition impact on potential feed value of first cut has still to be understood, in particular regarding different agronomic management. Our field trial was carried out in five farms, in a territory involved in the value chain of the Parmigiano Reggiano PDO (Val d'Enza, Northern Italy), over a two-year period (2017–2018). Differences in botanical composition, biomass, and *Pastoral Value* index (PV), which synthesizes grassland yield and nutritional parameters, were investigated in depth. The herbage dry matter (DM) yield was affected by year, farm, and their interaction factors. Its highest value across the two years was recorded in farm 5 (11.7 tons of DM ha^{-1}), which applied the highest rate of nitrogen fertilization. The botanical composition of the first cut has favored the presence of both *Poaceae* and 'other species' (each one around 40 plants per transect) compared to *Fabaceae* (seven plants per transect). However, higher numbers of *Fabaceae* plants (13 and 10) plausibly determined increases in PV in farms 3 and 5 (56.4 and 58.7, respectively). Although differences were observed among the most important nutritional parameters of grassland (crude protein, digestible and undigested neutral detergent fiber contents), suitable net energy for lactation (NE$_L$) values for feeding lactating cows were always recorded during the two years of survey. The present study provides a contribution of knowledge on how the botanical composition of permanent meadows may affect their potential nutritive value as fresh herbage for feeding dairy cows. Considering these results, the agronomic management should seek a level of plant biodiversity that at the same time might guarantee satisfactory yield and feed value, also in a context of climate change.

Keywords: yield; botanical composition; forage quality; agronomic performance

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1. Introduction

Grasslands serve as an important global reservoir of biodiversity, including many iconic and endemic species, but also provide a wide range of material and non-material benefits to humans. These benefits include food production and several ecosystem services, such as water supply and regulation, carbon storage and climate mitigation, pollination, and a host of cultural services [1–3]. Globally, permanent grassland is the main terrestrial ecosystem, covering 26% of the world land area [4] and 28% of the total utilized agricultural area of European Union in 2013 [5].

Naturalists have used phytosociology since the early 20th century to classify vegetation communities and to assess habitat conservation. The development of phytosociological classification required botanical relevés of all vascular species and a coefficient of abundance–dominance for each [6]. Phytosociology is the mainstream method for classifying vegetation communities as it can be applied to all ecosystems worldwide [7–9]. Vegetation classes are arranged into a hierarchical system, and their names follow scientific rules [10]. Once developed, the use of phytosociological classifications requires only a complete botanical relevé and less than an hour in each area. Phytosociology has also been used to predict agronomic characteristics, such as forage quality, and environmental characteristics by using indicator species [11]; however, coefficients of abundance–dominance correlate only weakly with each species' percentage of total grassland biomass (i.e., relative biomass), which can skew the assessment of agronomic characteristics [12,13].

Parmigiano Reggiano is an Italian hard cooked, Protected Designation of Origin (PDO) cheese made from raw bovine milk following a strict manufacturing protocol. Feeding with hay is a crucial element in the production area of Parmigiano Reggiano PDO cheese where regulations require at least 50% of the dry matter (DM) of the ration to come from fodder with at least 75% from local crops. The importance of hay in the production system of Parmigiano Reggiano is therefore significant; forages are extremely variable in chemical composition, digestibility, and intake potential [14,15] although the Italian hays are often of low quality due to the climatic conditions [16]. Feed nutritive value has been shown to be associated with nutrient contents and the amount of forage consumed [17], and these effects are correlated to the stage of maturity and the botanical composition [18,19]. In addition, biological parameters (such as in situ rumen incubations and neutral detergent fiber degradation characteristics) have been proposed to properly evaluate forages used in dairy cow diets [20]. It has been proved that biological measurements can be useful in improving the prediction of in vivo nutrient digestibility and the energy values of forages [21].

On Parmigiano Reggiano dairy farms, alfalfa occupies about half the utilized agricultural areas (47.6%). The second-most common crop, with a share of 26.4%, is winter wheat (*Triticum aestivum* L.), which is mostly produced for grain and has limited use as a forage [22], although the latter is arousing interest in recent years [18].

Permanent meadows, which are still found on the less-intensive farms, are generally cultivated without irrigation in the hills and with surface irrigation in the plains. When irrigated, permanent meadows provide an average of 13 tons (t) of dry matter (DM) ha^{-1} y^{-1}, well distributed over 5–6 cuts, while 2–3 cuts are common in non-irrigated meadows with an average production of 5–6 tons DM ha^{-1} y^{-1}, concentrated in spring [23]. Dairy slurry and manure are relatively inexpensive sources of nitrogen (N) and are thus widely used as organic fertilizers on permanent grassland [24,25]. In Northern Italy, around 60% of the farms applying liquid slurry use surface broadcasting during autumn or early winter [26].

Natural and anthropogenic factors have accelerated climate change, with global surface temperatures predicted to increase by 1.8–5.7 °C by 2100 and more frequent heatwaves [27]. Global warming can have significant impacts on plant carbon (C) assimilation (photosynthesis) and C loss (respiration) since these processes are highly temperature sensitive. Photosynthesis and respiration regulate net primary productivity of ecosystems and the balance between these two processes determines whether an ecosystem is a net C sink or source [28]. High temperatures already impose major limitations on the productivity of temperate agricultural systems including pastures [29]; the response of these systems to predicted increases in global temperature will vary in both magnitude and direction across biomes, depending on species composition and prevailing climatic conditions [30–34].

In the Parmigiano Reggiano area, an average increase of yearly temperatures of about 1.5 °C was recorded in the 1991–2016 period. Cumulative observed precipitation increased only during autumn, while it slightly decreased in the other seasons, especially in the summer periods, during which an increase in the number of consecutive days without rain events was observed [35].

A better understanding of the consequences of the predicted climatic changes on forage production and vegetation characteristics of the main forage resources for livestock systems is urgent, to design the necessary strategies and adaptation pathways [36]. The study, carried out in Val d'Enza (Italy) territory, which represents an important resource for the value chain of Parmigiano Reggiano PDO, aims to provide a contribution to the body of knowledge on permanent meadows as a food source for lactating cows. Thus, botanical composition, yield, and nutritional quality of first-cut herbage in permanent grasslands were evaluated in this research.

2. Materials and Methods

2.1. Field Experiments and Soil Samples

The research was performed in the Central Po plain (Val d'Enza) between the rural areas of Parma and Reggio Emilia, in five different farms in the Emilia-Romagna region (Italy)—each containing three experimental sites—across two consecutive growing seasons (2017–2018). The altitude of these sites ranged between 32 and 40 m above sea level. Temperature and rainfall data were obtained from the regional meteorological agencies [37], recording an annual mean temperature of 14.9 °C in 2017 and 14.4 °C in 2018, and annual total rainfall of ~560 mm and ~920 mm (Figure 1). The total area of the permanent meadows of the five farms was: 1.58 ha, 2.60 ha, 1.01 ha, 3.21 ha, and 2.80 ha, respectively. Soils (Table S1) have been fertilized using farmyard manure applied on the sward in October in both survey years (100 kg N ha^{-1} in 2017, and 60 kg N ha^{-1} in 2018 in farm 1; 120 kg N ha^{-1} in 2017, and 84 kg N ha^{-1} in 2018 in farm 2; 120 kg N ha^{-1} in 2017, and 100 kg N ha^{-1} in 2018 in farm 3; 108 kg N ha^{-1} in 2017, and 98 kg N ha^{-1} in 2018 in farm 4; 205 kg N ha^{-1} in 2017, and 300 kg N ha^{-1} in 2018 in farm 5). The N content applied in farmyard manure was determined on individual farms (data shown in Supplementary Materials, Table S2).

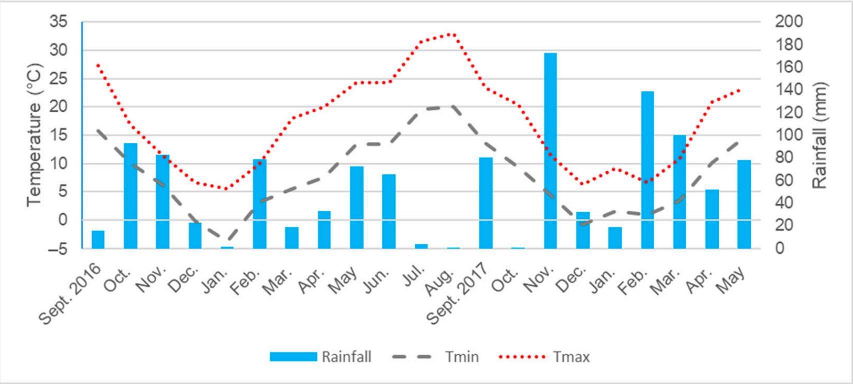

Figure 1. Monthly mean minimum (Tmin) and maximum (Tmax) air temperatures and total rainfall recorded during the two growing seasons.

The field trials were carried out on a silty clay loam (nine sites), clay loam (three sites), and silty loam (three sites) soils. Samples of the soils were taken in March 2017 in layers of 0–15 cm, 15–30 cm, and 30–60 cm, respectively; their basic physical and chemical properties are listed in Supplementary Materials, Table S1. The soil samples were sent to two laboratories (Tentamus Agriparadigma S.r.l., Ravenna, Italy; Alfa Solutions S.p.A., Reggio Emilia, Italy) for standard chemical and physical soil analysis, which included clay, sand, and silt content, as well as pH (in water), soil organic matter [38], exchangeable potassium (K), assimilable phosphorus (P), and total nitrogen (N).

Values of sand, silt, and clay content among the 15 sites ranged from 6% to 24% (on average, 11%), from 49% to 66% (on average, 56%), and from 13% to 39% (on average, 32%), respectively, in the surface layer (0–15 cm). Clay content varied to a lesser extent in the middle layer (15–30 cm) among the 15 sites (31–42%, average 37%), unlike the other soil texture parameters (sand, 4–24%, on average 10%; silt, 39–62%, on average 52%). Soils in farms 1, 2 and 3 were the most stable ones in the 0–30 cm layer and were classified as silty clay loam. Conversely, farm 4 varied among the two upper layers, from silty clay loam (0–15 cm) to silty clay (0–30 cm), and farm 5 from silty loam (0–15 cm) to silty clay loam (15–30 cm). The texture of the lower layer (30–60 cm) was rather constant among the different farms and classified as silty clay loam. Generally, all analyzed soils were sub-calcareous, except for farms 3 (pH 7.07 in 0–15 cm, 7.32 in 15–30 cm) and 5 (pH 7.12 in 0–15 cm, 7.19 in 15–30 cm) which had a neutral reaction. Farms 3 and 5 also provided the highest levels of organic matter in the surface layer (0–15 cm), together with farm 4. All sites were rich in N content (3.6 g kg^{-1}, on average), and particularly farm 4 (4.7 g kg^{-1}), while farm 3 stood out for its high levels of both P (444 mg kg^{-1}) and K (652 mg kg^{-1}) in the first 15 cm layer (Supplementary Materials, Table S1).

2.2. Herbage Sampling Method, Measurements of Traits and Data Collection

In the period of trials, 30 surveys were carried out to characterize the vegetation composition of grasslands, which are mainly mowed to produce hay or to collect fresh grass to be fed directly to dairy cows. Each survey was conducted along a 25-m linear transect in which botanical composition was determined using the vertical point-quadrat method [12]. At every 50-cm interval along the transect, plant species touching a steel needle were identified and recorded. Since occasional species are often missed by this method, a complete list of all other plant species included within a 1-m buffer area around the transect line (vegetation plot) was also recorded [13] and plant nomenclature was done according to Pignatti [39]. The data on the botanical composition were analyzed by computing *Pastoral Value* index (PV; range 0–100), which synthesizes grassland yield and nutritional parameters as follows [40,41]:

$$Pastoral\ Value = 0.2 \sum_{i=1}^{i=n} CSi * ISi$$

where CSi is the single species per cent contribution to vegetation composition (species relative abundance) of each transect, and ISi the index of specific forage value (0–5). The indices of each species were assigned in accordance with Cavallero et al. [42], while an average value (compared to the botanical family to which it belongs) was assigned for the species which were not present in the list.

For each of the 30 transects, a sample of herbage of about 1 kg was taken and divided into grasses, legumes, and other species. In order to estimate the DM yield, each herbage portion was collected with the self-loading wagons and then weighed. The batches were then analyzed in the laboratory (using Near-Infrared Reflectance Spectroscopy and floristic analysis) and DM was attributed.

2.3. Near-Infrared Reflectance Spectroscopy Analysis

Before each cut (13–14 April 2017, and 22–23 April 2018), a sample strip (approximately 1 × 1 m) of fresh pasture was harvested from each site with a manual lawn mower. Total pasture production per hectare per year was calculated by weighing the individual forage wagons after each mowing. In order to estimate the nutritional value, with a specific NIRS curve developed for permanent meadows (both dry and fresh), herbage samples were ground to 2 mm and the following parameters were recorded: dry matter (%), ash (% of DM), crude protein content (CP, % of DM), neutral detergent-insoluble protein (NDIP, % of DM), acid detergent-insoluble protein (ADIP, % of DM), and soluble protein (SolP, % of DM) [43], neutral detergent fiber with amylase and sodium sulphite method [44] (aNDFom, % of DM), acid detergent fiber (ADF, % of DM), acid detergent lignin (ADL, % of DM), undigested NDF after 240 h (uNDF, % of aNDFom), as reported by Brogna et al. [45], digestible NDF evaluated after 24 h of in situ rumen incubations (dNDF, % of aNDFom), as reported by Palmonari et al. [46], fat (% of DM), starch (% of DM), sugar (% of DM), and net energy for lactation (NE_L, kcal kg DM^{-1}). These values were determined using the instrument Foss NIR-System 5000 monochromator (NIR-System, Silver Spring, MD, USA), by double scanning each sample in the 1098–2500 nm spectral region. Predictions were performed using the equation developed and validated by Brogna et al. [45]. Spectral data were processed using WinISI II V1.5 software (Infrasoft International, Port Matilda, PA, USA).

2.4. Data Analysis

The experimental data were analyzed using GenStat 17th software (VSN International, Hemel Hempstead, UK) for analysis of variance with fixed factors: farm, year, and their interaction. Means were compared using Duncan's test at $p \leq 0.05$. However, to give an idea of the yearly variability, all data recorded in the two growing seasons were also processed for a Principal Component Analysis. A biplot was then utilized in order to evaluate the existing relationships between farms, parameters, and years.

3. Results

3.1. Yield and Botanical Characterization of Permanent Meadows

Results about dry matter production, PV, and botanical diversity are represented in Table 1. Year, farm, and their interaction had an effect. All sites yielded 7.17 and 9.19 tons of DM ha^{-1} y^{-1} on average in 2017 and 2018, respectively, and all farms were distinguished in terms of production. Considering the interaction between year and farm (Supplementary Materials, Table S3), the highest yield was obtained in farm 5 in 2018 (12.58 tons of DM ha^{-1}), while the lowest one was obtained in farm 3 in 2017 (3.73 tons of DM ha^{-1}).

Table 1. Yield, Pastoral Value, and Dry Matter content of different species.

Item	**Harvest**	**Farms**					**Mean**	**Year**	**Farm**	**Year × Farm**
		1	**2**	**3**	**4**	**5**		***p*-Value**	***p*-Value**	***p*-Value**
Yield (t of DM ha^{-1})	2017	5.9 ^{d}	3.7 ^{e}	7.3 ^{c}	8.1 ^{b}	10.9 ^{a}	7.2 ^{b}	<0.001	<0.001	<0.001
	2018	6.4 ^{d}	6.7 ^{e}	10.0 ^{c}	10.3 ^{b}	12.6 ^{a}	9.2 ^{a}			
Pastoral Value	2017	43.8 ^{b}	39.2 ^{b}	52.6 ^{a}	44.6 ^{b}	48.7 ^{a}	45.8	n.s.	<0.001	n.s.
	2018	41.4 ^{b}	32.9 ^{b}	60.3 ^{a}	44.9 ^{b}	68.7 ^{a}	49.7			
Plants per transect (number)	2017	102 ^{a}	79 ^{b}	73 ^{c}	112 ^{a}	71 ^{c}	87	n.s.	<0.001	0.020
	2018	131 ^{a}	93 ^{b}	59 ^{c}	93 ^{a}	62 ^{c}	88			
Poaceae (plants per transect)	2017	23	47	40	53	39	40	n.s.	n.s.	n.s.
	2018	38	45	34	35	45	40			
Fabaceae (plants per transect)	2017	10 ^{ab}	0 ^{b}	13 ^{a}	5 ^{ab}	11 ^{a}	8	n.s.	0.025	n.s.
	2018	3 ^{ab}	1 ^{b}	13 ^{a}	9 ^{ab}	10 ^{a}	7			
Other species (plants per transect)	2017	69 ^{a}	32 ^{b}	20 ^{c}	54 ^{b}	21 ^{c}	39	n.s.	<0.001	n.s.
	2018	89 ^{a}	46 ^{b}	12 ^{c}	48 ^{b}	8 ^{c}	41			

Each survey was conducted along a 25-m linear transect in which botanical composition was determined using the vertical point–quadrat method [12]. Different letters within each row indicate significant differences (within a variable) between treatments and/or year according to Duncan's range test ($p \leq 0.05$). n.s. = not significant.

The PV did not vary between 2017 and 2018 (45.8 and 49.7 on average of all sites, respectively), but differences were recorded among the five farms. The highest value was recorded in farms 3 and 5, while the second group included farms 1, 2, and 4.

In farms 1 and 4, a higher total number of plants was observed along the transects, while farm 3 was included in the second cluster; in farms 2 and 5, lower values were recorded. As for the yield, an effect of year ×farm interaction was recorded (Supplementary Materials, Table S3), and maximum (131) and minimum (59) values were recorded in farm 1 and farm 3 in 2018, respectively. Table 1 shows the division of plants that was observed (including *Poaceae*, *Fabaceae*, and other species). The first group was not affected by the two experimental factors, nor by their interaction. The highest number of *Fabaceae* was recorded in farms 3 and 5, the second group included farms 1 and 4, while in farm 3 the numbers of *Fabaceae* observed were lower. The highest variability among the five farms was recorded for the 'other species' category ($p < 0.001$). The average number of 'other species' fluctuated between 12 plants per transect on farm 3 (in 2018) and 89 plants per transect on farm 1 (also in 2018). As for *Fabaceae*, the farms have been clustered into three different groups in relation to the number of plants belonging to other species: farm 1 had the highest number of observations, farms 2 and 4 formed the second cluster, and finally farms 3 and 5. During the two years of the survey, across 40 plants that were observed per transect, the mean number of *Poaceae* and 'other species' was always found without variations.

The botanical composition of the permanent meadows was as follows: in the year 2017, 47.8% of *Poaceae*, mainly Italian ryegrass (*Lolium multiflorum* Lam.), rough bluegrass (*Poa sylvicola* Guss.), and foxtail grass (*Alopecurus utriculatus* (L.) Pers.); 10.1% of *Fabaceae*, mainly white clover (*Trifolium repens* L.); and 42.1% of other botanical families, mainly common dandelion (*Taraxacum officinale* (L.) Weber), and *Ranunculus* spp.

By contrast, in the year 2018, the composition was: 50.1% of *Poaceae*, mainly Italian ryegrass (*Lolium multiflorum* Lam.), rough bluegrass (*Poa sylvicola* Guss.), and field meadow foxtail (*Alopecurus pratensis*); 10.3% of *Fabaceae*, mainly red clover (*Trifolium pratense* L.); 39.6% of other botanical families, mainly *Ranunculus* spp., common dandelion (*Taraxacum officinale* (L.) Weber) and cut-leaf Crane's-bill (*Geranium dissectum* L.). Figure 2. shows botanical composition of the sample taken for analysis (numerical contribution of different species and DM%). A summary of the botanical composition of individual plant species in monitored permanent grassland has been reported in Table S4 (Supplementary Materials).

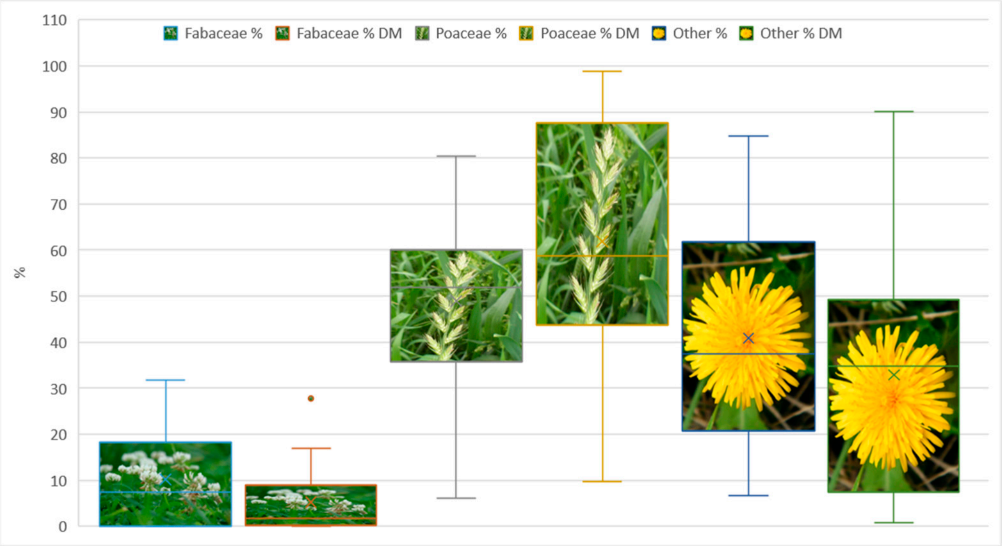

Figure 2. Botanical composition of the sample taken for analysis (numerical contribution of different species and DM%). *Fabaceae*% = % of total plants observed along the transect, *Fabaceae*% DM = % of Dry Matter, *Poaceae*% = % of total plants observed along the transect, *Poaceae*% DM = % of Dry Matter, Other% = % of total plants observed along the transect, Other% DM = % of Dry Matter.

3.2. Nutritional Composition of Permanent Meadows

The nutritional characteristics of fresh grass from stable meadows are shown in Table 2. The lowest ash content was found in the year 2017, with an average value of 8.3% of DM (vs. 10.29% of DM in 2018) in all sites. Both CP and neutral detergent-insoluble protein were higher in farm 2 than in the remaining ones and had a greater mean value in the second year of survey (14.2 vs. 12.1% of DM and 3.8 vs. 2.0% of DM, respectively). The increase in CP content and other correlated parameters (NDIP and SolP) in farm 2 could be affected by the increased presence of 'other species' in the second survey year, since no other changes of agricultural technique was adopted in this farm. Differences in soluble protein content given by year × farm interaction are shown in Table S3 (Supplementary Materials). The SolP content did not show a linear trend between the farms but varied across the two surveyed years, being averagely higher in 2018 (4.7 vs. 3.8% of DM). The same happened for both the neutral detergent and acid detergent fiber contents (49.4 vs.

44.7% of DM, and 33.2 in 2018 vs. 29.4% of DM in 2017, respectively). Sugar content had the opposite behavior, lowering its mean value in the second year (9.2 vs. 13.3% of DM in 2017). Meanwhile, uNDF and dNDF were evaluated after 240 h of in situ rumen incubations and varied oppositely over the two years, with higher average values of uNDF in 2017 (10.6 vs. 13.5% of aNDFom) and dNDF in 2018 (78.6 vs. 69.8% of aNDFom). Farms 1, 3, and 4 were grouped in the first cluster for uNDF, secondly farm 5 and then farm 2. Regarding dNDF, the greatest value was recorded in farm 2 and differed from farms 1 and 4. NE_L, fat, starch, ADL and ADIP were not affected by the two experimental factors, nor by their interaction.

Table 2. Statistical analysis of Near-Infrared Reflectance Spectroscopy data.

Item	Harvest	Farms					Mean	Year	Farm	Year × Farm
		1	2	3	4	5		*p*-Value	*p*-Value	*p*-Value
DM	2017	17.8	18.0	22.6	19.8	26.2	20.3	n.s.	n.s.	n.s.
	2018	17.4	15.9	24.0	22.2	17.0	16.1			
Ash	2017	7.9	8.2	8.8	8.1	8.5	8.3^{b}	<0.001	n.s.	n.s.
	2018	10.2	10.7	9.4	9.8	10.9	10.2^{a}			
CP	2017	11.8^{b}	13.0^{a}	12.9^{b}	10.3^{b}	12.7^{b}	12.1^{b}	0.016	0.049	n.s.
	2018	13.3^{b}	18.5^{a}	12.9^{b}	13.1^{b}	13.4^{b}	14.2^{a}			
NDIP	2017	1.5^{b}	2.2^{a}	2.3^{ab}	1.4^{b}	2.6^{ab}	2.0^{b}	<0.001	0.010	n.s.
	2018	3.5^{b}	5.2^{a}	3.9^{ab}	3.0^{b}	3.4^{ab}	3.8^{a}			
ADIP	2017	1.4	1.4	1.4	1.6	1.2	1.4	n.s.	n.s.	n.s.
	2018	1.2	1.4	1.5	1.1	1.2	1.3			
SolP	2017	3.8	3.9	4.3	2.3	4.6	3.8^{b}	0.003	n.s.	0.041
	2018	4.8	5.5	4.1	4.6	4.5	4.7^{a}			
aNDFom	2017	46.5	41.5	45.5	42.7	47.5	44.7^{b}	0.018	n.s.	n.s.
	2018	49.3	48.5	48.6	46.9	53.8	49.4^{a}			
ADF	2017	31.0	28.3	28.3	30.3	28.9	29.4^{b}	0.003	n.s.	n.s.
	2018	33.3	32.3	35.5	31.0	34.0	33.2^{a}			
ADL	2017	5.6	4.3	4.7	5.5	4.2	4.9	n.s.	n.s.	n.s.
	2018	4.3	4.4	5.6	4.2	4.5	4.6			
uNDF	2017	16.3^{a}	10.9^{b}	12.9^{a}	15.6^{a}	11.5^{ab}	13.5^{a}	0.002	0.037	n.s.
	2018	10.4^{a}	7.8^{b}	12.0^{a}	10.9^{a}	11.8^{ab}	10.6^{b}			
dNDF	2017	65.0^{bc}	73.2^{a}	71.4^{abc}	63.3^{c}	75.8^{ab}	69.8^{b}	<0.001	0.021	n.s.
	2018	78.6^{bc}	84.2^{a}	75.0^{abc}	76.8^{c}	78.3^{ab}	78.6^{a}			
Lipid	2017	2.6	2.0	2.5	2.4	2.8	2.4	n.s.	n.s.	n.s.
	2018	2.4	2.7	2.4	2.5	2.8	2.6			
Starch	2017	2.9	1.8	2.7	1.7	2.1	2.2	n.s.	n.s.	n.s.
	2018	2.1	1.7	2.1	2.6	1.9	2.1			
Sugar	2017	12.7	14.9	12.7	13.4	12.7	13.3^{a}	<0.001	n.s.	n.s.
	2018	9.9	7.7	8.9	11.2	8.2	9.2^{b}			
NE_L	2017	5.5	5.8	5.6	5.5	5.7	5.6	n.s.	n.s.	n.s.
	2018	5.6	5.8	5.4	5.7	5.4	5.6			

DM = dry matter (%), Ash = ash content CP = crude protein content, NDIP = neutral detergent-insoluble protein, ADIP = acid detergent-insoluble protein, SolP = soluble protein, aNDFom, neutral detergent fiber with α-amylase, sodium sulfite, and correcting for ash contamination, ADF = acid detergent fiber, ADL = acid detergent lignin (% DM), uNDF = undigested NDF after 240 h of in situ rumen incubations, dNDF = digestible NDF evaluated after 24 h of in situ rumen incubations (% aNDFom), Lipid = lipid content, Starch = starch content, Sugar = sugar content (% DM), NE_L = net energy for lactation (MJ kg DM^{-1}). Different letters within each row indicate significant differences (within a variable) between treatments and/or year according to Duncan's range test ($p \leq 0.05$). n.s. = not significant.

3.3. Relationships between Years and Agronomic Parameters

The correlations between years and measured parameters were studied using PCA analysis and the result is shown as a biplot in Figure 3. The sum of the contributions of the two first principal components accounted for 62.47% of the total variability. PC1 revealed 37.75% of the total variation and differentiated all farms by the year, except for farm 5, which was represented in the right side of the plot in both years. The second year of the experiment was largely related to yield, PV, and most of the composition parameters, including ash, CP and total proteins, fats, NDIP, acid and neutral detergent fibers, and *Poaceae*-belonging species. Conversely, farms 1–4 in 2017 were on the left side of the graph together along with DM, sugars, ADL and ADIP, starch, *Fabaceae*, and other species. PC2 explained 24.72% of the total variance and differentiated farm 2 in both years, farm 1 in 2018 (1–2), and farm 4 in 2017 (4–1) (on the lower side) from all remaining year × locations. In fact, on the upper side, farms 5 and 3 in both years, farm 1 in 2017 (1–1), and farm 4 in 2018 (4–2) were grouped together with yield, PV, DM, fats, starch, acid and neutral detergent fibers, ADL, and *Fabaceae*. On average, over the course of two years, farm 2 was related to net energy for lactation, farm 3 with DM and starch, and farm 5 with fats, acid, and neutral detergent fibers. Some relations can also be highlighted between the evaluated parameters and grassland production. The yield was positively associated with parameters such as PV, *Fabaceae* number of plants and fats, acid, and neutral detergent fibers contents. The total yield of first-cut grassland was negatively correlated with sugars, ADIP, the number of plants belonging to 'other species' and, to some extent, *Poaceae* as well as net energy for lactation.

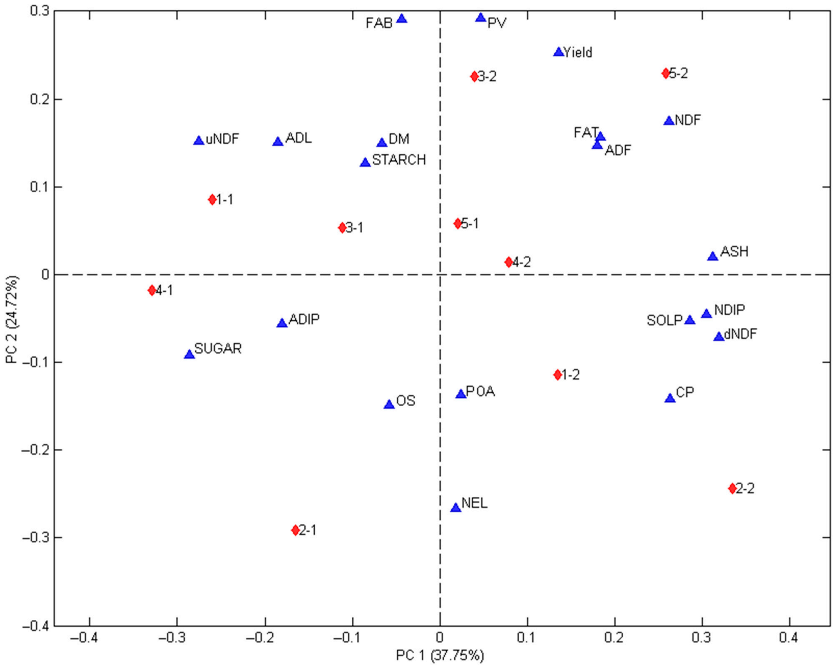

Figure 3. Biplot of Principal Component Analysis of the harvests carried out in 2017 and 2018. Farm × year combinations were named as follows (Farm–Year): 1-1 = Farm 1 in 2017, 1-2 = Farm 1 in 2018, 2-1 = Farm 2 in 2017, 2-2 = Farm 2 in 2018, 3-1 = Farm 3 in 2017, 3-2 = Farm 3 in 2018, 4-1 = Farm 4 in 2017, 4-2 = Farm 4 in 2018, 5-1 = Farm 5 in 2017, 5-2 = Farm 5 in 2018. The studied parameters were as follows: ADF = acid detergent fiber, ADIP = acid detergent-insoluble protein, ADL = acid detergent lignin, ASH = ash content; CP = crude protein content, DM = dry matter, dNDF = digestible NDF evaluated after 24 h of in situ rumen incubations, FAB = number of *Fabaceae* plants per transect, FAT = fats content, POA = number of *Poaceae* plants per transect, NDIP = neutral detergent-insoluble protein, NDF = neutral detergent fiber with α-amylase, sodium sulfite, and correcting for ash contamination, NEL = net energy for lactation, OS = number of plants belonging to 'other species' per transect, PV = pastoral value, SOLP = soluble protein, STARCH = starch content, SUGAR = sugars content, uNDF = undigested NDF after 240 h of in situ rumen incubations, Yield = grassland yield.

4. Discussion

Although the plant species composition and potential feed value of permanent meadows is well documented, an important research effort has still yet to be devoted to understanding their potential for the feeding of dairy cows and, additionally, the effects of climate change on the characteristics of grassland. Climate change is having a big impact on the yield and on the quality of forage and cereal crops [47,48]. Permanent grassland represents around 70% of all agricultural land And, in comparison with other agroecosystems, grasslands are more sensitive to climate change. However, digital and precision agriculture as well as crop simulation models can help farmers to adopt adaptation strategies suitable to reduce the negative impacts related to climate change [49,50].

In the Mediterranean area under drought conditions, the maximum decrease in yield in terms of dry matter was recorded, while an opposite trend has been observed in Northern Europe with an increase of about 25% of yield in term of dry matter, due to an increase of photosynthetic rate and water use efficiency by 40.8% and 62.1%, respectively. *Poaceae* reported a more sensitive response to drought stress than *Fabaceae*, particularly in terms of photosynthetic parameters and yield [51]. In the present studied area, the increasing yearly mean temperature and decrease in summer precipitation are of particular concern, as their effect on botanical diversity can influence not only the overall sward production, but also the nutritional quality of grasslands and, thus, their feed value. According to a recent review [36], in the Apennines grassland systems, several knowledge gaps emerged on the impacts of climate change on plant functional traits, future habitats suitability, and grasslands loss. The current study analyzed different aspects related to permanent meadows (yields, floristic composition, and forage value) over two consecutive years. The farm's manure management did not change in the survey; the timing and mode of application were the same.

4.1. Yield and Botanical Composition

The relationship between species richness and aboveground net primary productivity is still a central and much-debated issue in community ecology [52].

Variability in yield among the five farms was detected across the two years and unsurprisingly an increase of N rate led to both greater forage yield and nutritive value [53], as was also observed in the PCA analysis. Grassland management intensification can greatly influence N dynamics between aboveground and belowground compartments mainly due to the large amount of available N forms, which are repeatedly added to soils [54]. Plausibly, the higher rainfall amount enhanced dry matter production in 2018 across all sites, and the highest production was recorded in farm 5, where the greatest amount of N was applied. Additionally, a very high organic matter content in the soil surface layer (0–15 cm) probably ensured a better soil fertility [55]. Although a negative relation between forage nutritional value and yield has long been recognized by several authors [56–58], we observed a different trend. In fact, in the second year of survey, where high yields were recorded, the PV value was higher in three out of five farms. Across the two years, farm 4 was the second most productive site, where the maximum number of plants per transect was detected. The numerical contribution of the various species can be very different from their yield contribution. Causes of the increase in production are often related to the presence of a substantial percentage of *Poaceae* plants, which have a high rate of development and consequently make a significant contribution of DM. However, *Poaceae* contribution did not vary in our study, so the differences in production between the farms may also be related to the higher number of plants belonging to *Fabaceae* (mainly white and red clovers) present in farm 3 and 5 (around 10–13) across the two years of experiment. *Fabaceae* are generally short-lived perennial herbaceous plants, typically growing up to 30 cm tall, and may represent the main cause of the high PV indexes found in the above mentioned sites; the PV index ranges from 0 to 100 and synthetizes production and nutritional parameters of the pasture composition. Conversely, the lowest yield and PV were detected in farm 2, where *Fabaceae* composition was approximately nil, thus revealing a stage of degradation of the grassland to be eventually repaired by additional seeding, as reported by Nerušil [59].

4.2. Nutritional Composition

To increase cattle feed efficiency, the best strategy should be adopted; to do this, a rapid assessment of the nutritional composition of the forage should be available for farmers [18]. The amounts of CP (and fractions), ADF, aNDFom, and their specific fractions in DM influence the quality of the grassland and, thus, its effects on animal nutrition. The lowest ash content was recorded in 2017 and may be attributed to the earlier sampling during the first survey year. Other hypotheses are the influence of the floristic composition or a contamination of the samples with soil due to heavy rain recorded before the last sampling, which may have affected the overall ash content. Cornell Net Carbohydrate and Protein System has become widely used to manage livestock nutrition and its parameters were affected by the years of survey. Higher levels of CP, NDIP, and SolP were found in the second year. A close empirical negative correlation exists between forages uNDF and dNDF, which indicates that uNDF is a useful parameter for the prediction of the nutritive value of fresh grass. CP, NDIP, and SolP are attributable to the different floristic composition and indirectly attributable to the recorded PV. On the other hand, the results observed for the different fiber fractions (dNDF, and uNDF) are compatible with a different phenological stage of the plants at the time of sampling, and we can therefore assume an influence of the autumn–winter season. Moreover, the value of uNDF can also be used to estimate NDF digestibility, impacting on the performance of dairy cows. In fact, a one-unit increase in NDF digestibility was related to a 0.17 kg increase in dry matter intake and a 0.25 kg increase in 4% fat-corrected milk [14]. The value of uNDF (undigested NDF after 240 h of in situ rumen incubations) was extremely low; uNDF is also negatively correlated with sugar, which is significantly present in early growth stage hay. Conversely, we recorded very high values of dNDF (digestible NDF evaluated after 24 h of in situ rumen incubations), confirming an excellent nutritive value of fodder [60]. Data demonstrates that the early cutting (13–14 April 2017, and 22–23 April 2018) was an important factor determining the nutrient content and fiber digestibility of herbage plants in the permanent meadows studied. The mean of the Near-Infrared Reflectance Spectroscopy composition data was generally comparable to those summarized by the Dairy NRC [60]. The recorded nutritional composition is talso ypical of the *Poaceae* species which represents a very important percentage of DM in the surveyed grasslands, as shown in Figure 2.

In the light of climate change scenario, agronomic management should be revised and improved to guarantee high yield and quality of permanent meadows. Durant and Doublet [61] found that, in general, over-sowing tended to provide benefits in terms of the total annual forage yield and quality, while fertilization provided no real benefit in terms of forage quality. However, there was no persistence of introduced species in the sward. Hence, over-sowing and fertilizer applications are limited and short-lived agronomic approaches.

Finally, interest in grassland ecosystems has received increased attention from the scientific community in recent years and its development has accelerated in the recent years. Thus, future research on meadow ecosystems should consider a multidisciplinary approach that incorporates different dimensions and multi-trophic levels, including economic aspects and ecosystem services, functions and integrity, as well as cultural value [62].

5. Conclusions

Our study gave some valuable insights into the permanent grassland system. Results showed that all five farms analyzed had a satisfactory first-cut yield, confirming the positive effect of increasing nitrogen fertilization rates. Although differences were observed among the main nutritional parameters of grassland (CP, dNDF, and uNDF), suitable NE_L values for feeding lactating cows were consistently recorded during the two years of survey, restating the importance of permanent meadows for feeding lactating cows. Interestingly, a positive relation between forage nutritive value and yield was recorded in most sites. Regarding the botanical composition of the first cut, we noticed a preference for *Poaceae* and ‘other species’ over the temperature-sensitive *Fabaceae*, both in terms of seed germination and growth. However, no differences in the mean number of plants per transect were observed within the two years of survey, or the three groups analyzed. These findings reiterate the necessity of conducting future research to evaluate the effect of farm and manure management on species richness, especially since its relationship with aboveground net primary productivity remains a central and debated issue in community ecology. Additionally, the implications of climate change on the characteristics of the first cut should be further investigated, with particular attention paid to water scarcity and the prolongation of the vegetative phase in autumn and winter. Particular attention should then be focused on agronomic techniques that are able to increase the soil's water retention in order to avoid yield reduction in a year with scarce rainfall.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13030681/s1. Table S1, Soil characteristics of the grassland survey sites; Table S2, Chemical characteristics of the raw livestock manures; Table S3, Statistical analysis of Near-Infrared Reflectance Spectroscopy data with details on the interaction Farm × Year; Table S4, Botanical composition of individual plant species in monitored permanent grassland.

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