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Ensilibility and protein degradation characteristics of forage from mountain grasslands containing tanniferous species

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Abstract

Ensiling forage from species-rich mountain grasslands is challenging. Low concentrations of fermentable carbohydrates and the coarse morphological structure of the forage promote the activity of butyric acid forming bacteria. This is associated with the formation of ammonia from protein degradation, resulting in an insufficient pH decline. On the other hand, forage from species-rich swards may contain tanniferous plant species which contain varying contents of condensed tannins (CT). Therefore, the silage quality of forage prepared from species-rich mountain grasslands and the role CT may play in silage fermentation was studied. A set-up of two long-term mineral fertilization field experiments, located in the Jura mountains and the Alps in Switzerland were used to obtain forage with contrasting species and chemical composition. Collection was done during both the generative and vegetative growth stage from three differently fertilized swards: unfertilised ("0"), fertilized with phosphorus and potassium ("PK") or fertilized with PK and nitrogen ("NPK"). The forage was wilted to approximately 37% dry matter (DM), chopped to 2 cm lengths and ensiled for at least 65 days as laboratory-scale silages. The wilted forage was characterized by concentrations of crude protein between 117 and 130 g/kg DM and water-soluble carbohydrates varying from 84 to 148 g/kg DM. Concentrations of CT ranged from 6 g to 14 g/kg DM and those of soluble CT from 1.8 to 7.6 g/kg DM. All silages contained butyric acid, irrespective of the type of fertilization or harvest stage (range: 1.5 g to 16 g/kg DM). Concentrations of acetic and lactic acid ranged from 2.1 g to 15.0 g/kg DM and from 21.0 to 44.0 g/kg DM, respectively. Concentrations of unfermented sugar remained high and pH levels were above those expected. Formation of non-protein-N (NPN) increased in the range of 130 to 264 g/kg N from wilted to ensiled forage. The negative correlations of CT or soluble CT contents with ammonia-N or NPN in silage were found in both forage from the generative and vegetative harvests indicating a possible relationship with protein degradation during ensiling.

KEYWORDS
condensed tannin, forbs, long-term mineral fertilization, protein fraction, silage quality

1 | INTRODUCTION

In mountainous areas, climatic conditions can change rapidly and the required amount of dry weather for haymaking is often limited. Thus, over the last few decades haymaking has been gradually replaced by silage preparation due less time required for wilting and the higher forage quality (Wyss et al., 2016).

Mountain grasslands are often characterized by species-rich plant communities with potentially large proportions of forbs (Bruinenberg et al., 2006; Jeangros et al., 1999). However, only a few studies report the silage quality of forbs (Isselstein & Daniel, 1996; Weißbach, 1998) or swards with high proportions of forbs (Seng et al., 2008; Wyss et al., 2016). While some forb species may limit the preparation of high-quality silage when present in large proportions or harvested at an advanced stage of maturity (e.g., *Heracleum sphondylium* L.), other forbs such as *Anthriscus sylvestris* (L.) Hoffm., *Ranunculus acris* L., *Ranunculus repens* L. or *Taraxacum officinale aggr*., are characterized by a comparable ensilibility as ryegrasses (Wyss & Vogel, 1999). However, the silage quality of forage from species-rich grasslands is often inferior to silage prepared from intensively managed swards based on grass- or legume species (Ertekin et al., 2022; Ineichen et al., 2019; Lee et al., 2008).

Well conserved silages require sufficient acidification during fermentation through the microbial generation of fermentation acids, that is, primarily lactic acid and to a lesser extent, acetic acid (Kung Jr. et al., 2018). This process, however, requires substantial amounts of fermentable carbohydrates. These are present, for instance, in *Lolium* spp. species with particularly high concentrations of water-soluble carbohydrates (WSC) (Haigh, 1990; Humphreys, 1989). Mountain grassland is dominated by less intensive grass species (Ineichen et al., 2020) containing lower WSC contents and therefore increases the risk of insufficient silage acidification (Wyss, 2006).

Forbs may increase the buffering capacity of the forage due to higher concentrations of proteins, minerals and organic acids compared to grasses (Isselstein & Daniel, 1996). Increased concentrations of protein may lead to significant protein degradation during wilting and subsequent desmolysis of amino acids during ensiling (Petit & Tremblay, 1992). The latter particularly increases the formation of readily soluble nitrogen (N), such as ammonia-N (Givens & Rulquin, 2004) or toxic biogenic amines (Fusi et al., 2004), which increase the buffering capacity of the silage and counteract the rapid decline in silage pH required for silage conservation (Kung Jr. et al., 2018). Protein degradation may be partly due to proteolytic plant enzymes present in forage, acid hydrolysis driven by acid formation during fermentation and by proteolytic clostridia (Kung Jr. et al., 2018).

Morphologically forbs are a heterogenous functional group including leafier and more stem-like species. For instance, the bulky structure of mature wood cranesbill (*Geranium sylvaticum* L.), with woody stems, complicates compaction during ensiling and increases the risk of butyric acid formation and the propagation of yeasts or secondary fermentation. Whereas dandelion (*T. officinale*), with its leafy morphology and high WSC content, is a highly fermentable forb (Wyss et al., 2016).

Chemically, forbs may contain substantial amounts of plant phenolic compounds (Fraisse et al., 2007). Several studies have shown that tannins, which represent a chemically distinct group of phenolic compounds, may reduce protein degradation (Albrecht & Muck, 1991; Herremans et al., 2019; Martens et al., 2019) or butyric acid formation (Jayanegara et al., 2019). Tannins are chemically differentiated into hydrolysable and condensed tannins (CT, syn. proanthocyanidins). For CT particularly, the potential to interact with forage protein during ensiling is largely dependent on the proportion of soluble, protein or fibre bound CT (Girard et al., 2018; Kagiya et al., 2019). In species rich mountain swards, forbs and some legumes may contain substantial amounts of tannins (Jayanegara et al., 2011; Jeangros et al., 1994) and therefore affect silage fermentation quality.

The present study was conducted to assess the silage quality of species-rich swards with high forb proportions from two field experiments located in the Swiss mountains (Baumberger et al., 1996; Thomet & Koch, 1993). Each field experiment consisted of unfertilised swards ("0") and swards fertilized with either phosphorus and potassium ("PK") or PK and N ("NPK"). Long-term continuous treatments, at least 4 decades at each site, resulted in swards with distinct botanical compositions specifically in relation to the proportion of forbs (Ineichen et al., 2020). Forage was ensiled during both the generative and vegetative growth cycle over one growing season. In addition to silage quality, protein degradation was determined from the ensiled forage and compared to the tannin content in the corresponding fresh silage.

2 | MATERIALS AND METHODS

2.1 | Origin and characteristics of species-rich mountain swards

Forage was ensiled over the growing season of 2017 from two long-term mineral fertilization field experiments located in the Jura mountains (site "Jura"; characterized in Thomet & Koch, 1993) and the "Alps" (site "Alps" characterized in Baumberger et al., 1996) in Switzerland. The field experiment "Jura" is located in the canton of Solothurn (47°33′ N, 7°67′ E) at an altitude of 930 m a.s.l. and was established in 1972 (Thomet & Koch, 1993). Long-term fertilization included unfertilised swards ("0") or fertilized with P and K ("PK") or additional N ("NPK"). Each fertilizer treatment was repeated in three blocks with a plot size of 6 m × 2.65 m. To avoid confounding, measurements and forage were collected from an area of 6 m × 1.30 m, omitting 6 m × 0.68 m at each side. Fertilization included 80 kg P_2O_5 and 240 kg K_2O for PK and NPK, respectively, while NPK contained an additional 75 kg N per ha and year. Chemical forms of individual fertilizers and application procedures are described in detail in Ineichen et al. (2020). Forage was harvested three times, once in the generative stage (01 June 2017) and twice in the vegetative stage (03 August 2017 and 04 October 2017).

The field experiment "Alps" is located in the canton of Bern (47°33′ N, 7°67′ E) at an altitude of 1340 m a.s.l. and was

established in 1956. Long-term fertilization included the same treatments as at site Jura. Each fertilizer treatment was repeated in four blocks with a plot size of 10 m × 5 m, respectively. One block was not sampled to avoid confounding caused by the shading effect of trees growing next to the experimental field. Fertilization included 60 kg P_2O_5 and 180 kg K_2O for treatments PK and NPK, respectively, while treatment NPK included an additional 80 kg of N per ha and year. Chemical forms of individual fertilizers and application procedures are described in detail in Ineichen et al. (2020). Forage was harvested twice per year, once during the generative (16 June 2017) and once during the vegetative growth periods (23 August 2017).

TABLE 1 Yield and plant species number and composition of the mountain grasslands in the Jura and the Alps in relation to long-term mineral fertilization.

Sward	Jura[b]			Alps[c]		
Fertilization[a]	0	PK	NPK	0	PK	NPK
Yield (t/ha and year)	2.35	3.40	5.35	1.14	3.51	6.32
Generative (t/ha)	0.88	1.48	2.55	0.82	2.43	4.05
Vegetative (t/ha)	1.47	1.92	2.80	0.33	1.08	2.27
Species number (*n*)	39	29	24	44	39	37
Grasses % (*n*)	83 (10)	75 (9)	65 (7)	48 (16)	45 (11)	50 (10)
Agrostis capillaris	–	–	–	6	6	–
Arrhenatherum elatius	–	33	17	–	–	–
Avenula pubescens	–	–	–	–	–	6
Bromus erectus	40	2	7	5	–	–
Carex montana	–	–	–	5	–	–
Cynosurus cristatus	–	–	–	7	–	–
Dactylus glomerata	–	8	18	–	5	10
Festuca pratensis	–	–	–	–	6	6
Festuca rubra	36	20	12	13	16	19
Holcus lanatus	–	3	3	–	–	–
Trisetum flavescens	–	8	8	–	–	4
Legumes % (*n*)	3 (4)	9 (4)	1 (4)	3 (2)	12 (3)	5 (4)
Lathyrus pratensis	–	2	–	–	–	–
Trifolium pratense	2	3	–	–	11	–
Trifolium repens	–	3	–	–	–	4
Forbs % (*n*)	14 (25)	16 (16)	34 (13)	49 (26)	43 (25)	45 (20)
Alchemilla vulgaris	–	–	–	–	–	4
Crepis aurea	–	–	–	12	–	–
Crepis biennis	–	2	2	–	–	–
Geranium sylvaticum	3	8	26	–	–	11
Hieracium auricula	–	–	–	4	–	–
Hypochaedris radicata	–	–	–	–	5	–
Leontodon hispidus	2	–	–	–	–	–
Plantago lanceolata	–	3	4	–	9	14
Ranunculus nemorosus	–	–	–	4	–	–
Rumex acetosa	–	–	2	–	–	6
Sanguisorba minor	5	–	–	6	–	–
Taraxacum officinale	–	2	–	–	7	–

[a]Fertilization including no application of fertilizers ("0"), fertilization of phosphorus and potassium ("PK") and fertilization of PK and nitrogen ("NPK").
[b]Plant species composition determined according to Klapp (1930). Arithmetic means displayed for species with a contribution of the above-ground dry matter biomass ≥2%.
[c]Plant species composition determined according to Daget and Poissonet (1969). Arithmetic means displayed for species with an abundance of ≥4%. Abundance of plant species was defined as the frequency of a species in relation to the total number of plant species present along a longitudinal transversal.

The plant species composition of each plot was determined during the generative growth stage (Table 1). At the field experiment Jura, plant species composition was assessed based on the procedure by Klapp (1930). Percent values represent arithmetic means displayed for species with an estimated contribution of the above-ground dry matter (DM) biomass ≥2%. At the field experiment Alps, plant species composition was determined according to the procedure by Daget and Poissonet (1969). Percent values represent arithmetic means, displayed for species with an abundance of ≥4%. The abundance of each plant species was defined as the frequency of a species in relation to the total number of species present along a longitudinal transversal.

2.2 | Sampling procedure and ensiling of experimental swards

At each field experiment, swards were mowed at 4 cm above ground level. The yield was determined at each plot using a spring scale. A sample was collected to determine the DM content of the fresh forage to calculate DM yield, respectively. Forage was wilted for up to 12 h to a DM content of 35%. The wilted forage (~4 kg per treatment) was transported to the Agroscope research station (Posieux, FR) and mechanically chopped to a particle size of approximately 2 cm. Approximately 600 g from the chopped plant material was then ensiled in laboratory scale silos (1.5 L) for each plot without the addition of ensiling additives. To account for fermentation gas losses, silos were weighed twice during the first week after ensiling and then once per week for the duration of the experiment. Fermentation lasted at least 95 days for forage from all harvests, except for forage from the second vegetative harvest at site Jura, which was ensiled for 65 days. From the remaining chopped plant material, two subsamples were taken each. One subsample of ~400 g was dried at 60°C for 24 h for the analysis of gross chemical composition. The other subsample of ~300 g was frozen at −20°C until lyophilized (Delta 1–24 LSC; Christ, Osterode, Germany). Subsequently, all samples, were milled through a 1.0-mm screen (Brabender rotary mill; Brabender GmbH & Co. KG, Duisburg, Germany) for analysis of protein and tannin fractions.

2.3 | Near infrared and chemical analysis

Gross chemical composition of both wilted and ensiled forages was determined with near-infrared spectroscopy (NIRS) (NIR-Flex N-500, Büchi, Flawil, Switzerland) based on previous calibrations for forage from species-rich swards (Ampuero-Kragten & Wyss, 2014). Net energy concentration for lactation (NEL) in wilted and ensiled forages was determined using a regression (Agroscope, 2018) for swards with unknown botanical composition accounting for crude fibre (CF), ash, crude protein (CP) and DM concentration based on NIRS data. Fermentation acids and silage ethanol concentration were analysed by liquid chromatography (Ultimate, Thermo Fisher Scientific, Reinach, Switzerland). Mineral composition was determined by the forage testing lab Dairy One (Ithaca, New York, USA). Silage pH was determined using a pH electrode and ammonia (NH_3) concentration was measured using an ammonia selective electrode (model: No 6.020202.110, Metrohm Schweiz AG, Zofingen, Switzerland). In both wilted and ensiled forages, N samples were analysed using the Dumas method (ISO, 2008; method 16,634–1) on a C/N analyser (Trumac CNS; Leco Instruments, St. Joseph, MI, USA); the results were multiplied by 6.25 to obtain the CP concentration. Based on this CP concentration, protein fractions including fractions A, B_1, B_2, B_3 and C were determined according to Licitra et al. (1996) at the Landwirtschaftliche Kommunikations- und Servicegesellschaft mbH, Lichtenwalde, Germany. Concentrations of CT were determined based on a modified protocol from Terrill et al. (1992) described in detail in Kagiya et al. (2019) allowing the differentiation of CT into soluble (sCT), protein-bound (pCT) and fibre-bound proanthocyanidins (fCT) at the Christian-Albrechts-University laboratory in Kiel (Germany). Determination of CT concentration and fractionation was based on a proanthocyanidin standard by purification with Sephadex LH-20 gel chromatography (GE Healthcare, Solingen, Germany) using 10 g DM from 50 randomly selected sainfoin samples from a previous experiment (Malisch et al., 2015). The purity of the standard was determined using LC–MS/MS analysis.

2.4 | Statistical analysis

Data were analysed with R Foundation for statistical computing (2018). The function lmer for linear mixed models, from the package lmerTest (Kuznetsova et al., 2017), was used. Gross nutrient and mineral composition, silage fermentation quality, tannin concentration and protein fractions were analysed using the following model (Y) including fertilization (f), harvest (h) and their interaction ($f \times h$). Plots were included as a random factor.

$$Y \sim f + h + f \times h + 1 \,|\, \text{plot}$$

The factor fertilization included three levels (0, PK and NPK) and the factor harvest included two levels (generative and vegetative), respectively. The first harvest was considered the generative stage, while the consecutive growth(s) were considered as the vegetative stage. In the case of site Jura, data from the first and second regrowth were averaged arithmetically per plot prior to statistical analysis.

Homogeneity of variances and normality distribution of residuals were checked graphically. If data were transformed before analysis of variance, p-values and superscripts are based on transformed values, but the non-transformed arithmetic means, and standard errors of the means are presented. Differences among means were considered significant at $p < .05$ using the Tukey–Kramer test (R package lsmeans Lenth, 2016).

3 | RESULTS

3.1 | Botanical, chemical and mineral characteristics of ensiled forage

The number of plant species increased from NPK to PK to unfertilised swards in both field experiments (Table 1). On average, the proportion of grasses, legumes and forbs accounted for 74 ± 9%, 5 ± 4% and 21 ± 11% for the swards at site Jura and for 48 ± 3%, 7 ± 5% and 46 ± 3% for the swards at the Alps. On average, DM concentrations of wilted forage ranged from 30% to 42% and 31% to 39% for the forage ensiled from Jura and the Alps, respectively (Table 2). Concentrations of CP varied between 110 and 144 g/kg DM across swards for both field experiments and those of sugar between 80 and 138 g/kg DM. Net energy concentrations for lactation (MJ NEL/kg DM) varied across forages from 4.6 in NPK fertilized swards from Jura (vegetative harvest) to 6.1 in PK fertilized swards from the Alps (generative harvest). Mineral concentrations of Ca, P and K were similar across forages, but P was numerically higher in forages harvested from the Alps compared to site Jura.

3.2 | Silage fermentation quality

3.2.1 | Jura

Across swards, silage DM concentrations ranged from 29% to 43% for forage ensiled from Jura (Table 3). Silages pH was highest in unfertilised swards of the vegetative harvest compared to all other treatments ($p < .05$). Silage pH was higher in unfertilised, or PK fertilized swards compared to swards fertilized with NPK of the generative harvest ($p < .05$), while in the vegetative harvest, silage pH was higher in

TABLE 2 Chemical and mineral composition (g/kg dry matter, DM) and net energy for lactation (MJ NEL/kg DM) of wilted forage prior to ensiling from the grasslands located in the Jura and the Alps in relation to long-term mineral fertilization.

Grassland	Harvest	Generative			Vegetative		
	Fertilization[a]	0	PK	NPK	0	PK	NPK
Jura	DM (%)	39	31	30	42	40	39
	CP	123	123	115	134	134	115
	Sugar	120	124	138	110	123	137
	NDF	478	453	486	460	421	450
	ADF	298	310	344	268	281	304
	ADL	37	44	37	39	45	42
	EE	30	29	29	41	38	36
	Ash	69	85	81	100	108	109
	NEL	6.0	5.9	5.8	6.0	6.1	5.9
	Ca	9.1	11	8.6	14	17	15
	Mg	1.9	1.8	1.8	2.4	2.2	2.2
	P	1.9	3.1	3.0	1.9	3.3	2.8
	K	14	18	17	13	16	14
Alps	DM (%)	39	35	35	35	35	31
	CP	125	130	117	110	129	144
	Sugar	116	101	80	86	91	91
	NDF	487	525	610	471	472	494
	ADF	311	328	388	307	307	327
	ADL	48	46	48	61	59	65
	EE	34	36	29	38	41	34
	Ash	74	91	89	81	106	116
	NEL	5.8	5.5	4.8	5.8	5.8	5.9
	Ca	8.5	7.7	6.2	14	13	11
	Mg	3.5	2.3	1.9	4.7	3.1	2.8
	P	2.1	3.7	3.3	1.8	4.3	4.3
	K	15	30	30	12	26	28

Note: Values show arithmetic means.
Abbreviations: ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre.
[a]Fertilization including no application of fertilizers ("0"), fertilization of phosphorus and potassium ("PK") and fertilization of PK and nitrogen ("NPK").

TABLE 3 Silage chemical composition (g/kg dry matter, DM) of forage from the grasslands located in the Jura and the Alps in relation to long-term mineral fertilization.

Grassland	Harvest Fertilization[†]	Generative			Vegetative			SEM	*p*-value[‡]		
		0	PK	NPK	0	PK	NPK		*f*	*h*	*f x h*
Jura	DM (%)	36	29	29	42	40	39	1.4	**	***	NS
	pH	5.05^{b}	4.86^{b}	4.53^{c}	5.29^{a}	4.90^{b}	4.63^{bc}	0.07	***	*	***
	Lactic acid	21^{c}	33^{b}	44^{a}	26^{bc}	30^{bc}	30^{bc}	2.0	***	*	**
	Acetic acid	2.6	2.6	4.3	2.2	2.9	2.8	0.28	NS	NS	NS
	Butyric acid	7.5^{bc}	16^{a}	14^{ab}	7.2^{bc}	10.9^{abc}	9.6^{bc}	1.17	NS	***	*
	Ethanol	7.6	9.0	7.3	4.9	3.2	4.5	0.59	NS	***	NS
	NH_3-N (g/kg total N)	42	47	41	45	33	32	0.3	NS	*	*NS*
	CP	129	127	118	134	138	118	3.4	NS	NS	NS
	NDF	485	460	461	440	393	394	9.7	*	***	NS
	ADF	326	344	344	274	287	284	7.8	NS	***	NS
	Sugar	87^{ab}	85^{b}	100^{ab}	81^{b}	103^{ab}	109^{a}	4.5	NS	*	*
	NEL (MJ/kg DM)	5.4	5.4	5.4	5.4	5.7	5.7	0.05	NS	***	NS
Alps	DM (%)	38	34	34	35	34	32	0.6	*	*	NS
	pH	4.99	4.82	5.00	5.32	5.24	4.92	0.05	NS	NS	NS
	Lactic acid	30^{b}	41^{a}	33^{ab}	33^{ab}	27^{b}	36^{b}	1.6	NS	NS	*
	Acetic acid	4.0^{b}	6.3^{b}	3.8^{b}	3.8^{b}	8.6^{b}	15^{a}	1.07	**	***	***
	Butyric acid	2.1^{bc}	2.6^{bc}	5.1^{ab}	6.2^{a}	1.5^{c}	2.8^{bc}	0.55	NS	NS	**
	Ethanol	5.1	4.0	4.6	6.5	6.1	6.2	0.50	NS	NS	NS
	NH_3-N (g/kg total N)	43	57	65	46	48	60	0.3	**	NS	NS
	CP	129^{ab}	129^{ab}	123^{b}	114^{b}	131^{ab}	147^{a}	2.8	*	NS	**
	NDF	484^{bc}	507^{b}	584^{a}	458^{bc}	430^{c}	465^{bc}	13.3	*	***	**
	ADF	334^{b}	353^{ab}	401^{a}	325^{b}	312^{b}	319^{b}	8.5	*	**	*
	Sugar	95^{a}	75^{abc}	58^{c}	78^{abc}	87^{ab}	60^{bc}	3.8	**	NS	**
	NEL (MJ/kg DM)	5.5^{ab}	5.2^{b}	4.7^{b}	5.4^{ab}	5.6^{a}	5.6^{a}	0.08	NS	***	***

Note: Values show arithmetic means and standard error of means (SEM). Within a row, means with different superscripts differ significantly at $p < .05$.
Abbreviations: ADF, acid detergent fibre; CP, crude protein; NDF, neutral detergent fibre; NEL, net energy for lactation.
[†]Fertilization including no application of fertilizers ("0"), fertilization of phosphorus and potassium ("PK") and fertilization of PK and nitrogen ("NPK").
[‡]*f*: fertilization; *h*: harvest; *f* × *h*: fertilization× harvest; NS, not significant.
*$p < .05$; **$p < .01$; ***$p < .001$.

unfertilised swards compared to PK or NPK fertilized swards ($p < .05$). Lactic acid concentrations increased from 21 g/kg DM in forage from unfertilised swards to 33 g/kg DM when fertilized with PK, to 44 g/kg DM when fertilized with NPK from the generative harvest ($p < .05$), while lactic acid concentrations did not differ in silages from the vegetative harvest ($p > .05$). Within the generative harvest, butyric acid concentrations where significantly higher in swards fertilized with PK compared to unfertilised swards but not compared to swards fertilized with NPK. In contrast, butyric acid concentrations of the vegetative harvest did not differ between treatments ($p > .05$). Silages contained higher ethanol concentrations during the generative (8.0 g/kg DM) than during the vegetative harvest (4.2 g/kg DM) ($p < .05$). Total NH_3-N, related to total N, was greater for silages harvested in the generative stage (43 g/kg total N) than those in the vegetative stage (39 g/kg total N) ($p < .05$).

Both NDF and ADF concentrations in silages were greater during the generative (469 and 338 g/kg DM) versus the vegetative (409 and 278 g/kg DM) stage. Silage from unfertilised swards had significantly higher NDF concentrations (465 g/kg DM) than silages from swards fertilized with PK (424 g/kg DM) or NPK (428 g/kg DM). Sugar concentrations did not differ between the fertilization treatments of the generative harvests, but silages fertilized with NPK had higher sugar concentrations in the vegetative harvest than those from unfertilised swards ($p < .05$). Concentrations of NEL were not affected by fertilization type, however, increased in silages from generative (5.4 MJ NEL/kg DM) to vegetative (5.6 MJ NEL/kg DM) ($p < .05$) harvests.

3.2.2 | Alps

Across swards, silage DM concentrations ranged from 32% to 38% for forage ensiled from the Alps (Table 3). Silages prepared from swards fertilized with PK had higher lactic acid concentrations compared to

silages from unfertilised but not from NPK fertilized swards from the generative harvest ($p < .05$). In contrast, forage harvested during vegetative growth did not differ in silage lactic acid concentrations. Silages prepared from swards fertilized with NPK from the vegetative harvest had significantly higher acetic acid concentrations compared to all other treatments. Formation of butyric acid was higher in silages prepared from unfertilised swards from the vegetative harvest compared to all other treatments ($p < 0.05$), except those fertilized with NPK from the generative harvest ($p > .05$). Swards fertilized with NPK had higher silage NH_3-N concentrations (63 g/kg total N) compared to those from unfertilised swards (44 g/kg total N) ($p < .05$), while those from PK fertilized swards (53 g/kg total N) did not differ by fertilization type.

The concentration of CP was highest in silages from swards fertilized with NPK from the vegetative harvest and significantly different to silages from NPK fertilized swards from the generative harvest and silages prepared from unfertilised swards from the vegetative harvest ($p < .05$). Concentration of NDF was highest in silages prepared from swards fertilized with NPK during generative growth compared to all other treatments ($p < .05$). Otherwise, within harvest, silages did not differ in NDF concentrations. Silages from swards fertilized with NPK from the generative harvest had significantly higher ADF concentrations compared to all other silages except for those fertilized with PK from the same harvest. Sugar concentration was significantly higher in silages prepared from unfertilised swards from the generative harvest than from swards fertilized with NPK from both harvests. Concentrations of NEL did not differ within harvest ($p > .05$). Silages prepared from PK or NPK fertilized swards from the vegetative harvest had higher NEL concentrations than silages from PK or NPK fertilized swards fertilized with PK and NPK from the generative harvest ($p < .05$).

3.3 | Composition of condensed tannins, protein fractions and protein degradation

3.3.1 | Jura

Wilted forage

Condensed tannin concentration was higher in forage harvested during the vegetative stage (13 g/kg DM) compared to generative growth (11 g/kg DM) (Table 4) ($p < .05$). The same pattern was observed for the concentration of sCT (generative: 4.8 g/kg DM and vegetative: 6.8 g/kg DM) and fCT (generative: 2.6 g/kg DM and vegetative: 2.0 g/kg DM) ($p < .05$). The concentration of pCT was significantly higher in fertilized forage (3.9 g/kg DM, average

TABLE 4 Composition of condensed tannins (CT) (g/kg DM) and protein fractions (A, B_1, B_2, B_3 and C) (g/kg nitrogen (N)) of wilted forage from the grasslands located in the Jura and the Alps in relation to long-term mineral fertilization.

Grassland	Harvest / Fertilization[†]	Generative			Vegetative			SEM	*p*-value[‡]		
		0	PK	NPK	0	PK	NPK		*f*	*h*	*f* x *h*
Jura	CT	10.3	11.0	10.9	10.3	13.8	13.4	0.5	NS	*	NS
	sCT	5.4	4.5	4.4	5.8	7.6	7.0	0.37	NS	***	NS
	pCT	2.2	3.9	3.8	2.9	3.9	4.1	0.20	***	*NS*	NS
	fCT	2.7	2.6	2.7	1.6	2.3	2.3	0.16	NS	*	NS
	A	166	179	171	116	136	157	6.5	NS	***	NS
	B_1	74	55	53	117	94	88	7.0	NS	***	NS
	B_2	441	528	587	375	445	506	17.8	***	***	NS
	B_3	253	165	137	314	216	157	16.2	***	**	NS
	C	66	73	52	78	109	92	5.4	NS	***	NS
Alps	CT	9.9^{bc}	7.7^{bc}	7.0^{c}	5.9^{c}	7.1^{c}	10.3^{a}	0.44	NS	NS	***
	sCT	4.0^{ab}	2.3^{b}	2.7^{ab}	1.8^{b}	2.9^{ab}	4.2^{a}	0.26	NS	NS	***
	pCT	3.3^{a}	3.3^{a}	2.7^{ab}	2.6^{ab}	2.4^{b}	3.2^{a}	0.11	NS	*	**
	fCT	2.6^{ab}	2.1^{abc}	1.6^{bc}	1.5^{c}	1.8^{abc}	2.9^{a}	0.16	NS	NS	***
	A	136	190	215	124	155	178	8.4	***	**	NS
	B_1	77	57	42	122	80	83	6.7	***	***	NS
	B_2	399	446	447	399	391	421	9.5	NS	NS	NS
	B_3	309	232	218	258	264	221	10.7	*	NS	NS
	C	79	75	78	97	110	97	5.4	NS	**	NS

Note: Values show arithmetic means and standard error of means (SEM). Within a row, means with different superscripts differ significantly at $p < .05$.
Abbreviations: CT, condensed tannin (proanthocyanidins); fCT, fibre-bound CT; pCT, protein-bound CT; sCT, soluble CT.
[†]Fertilization including no application of fertilizers ("0"), fertilization of phosphorus and potassium ("PK") and fertilization of PK and nitrogen ("NPK").
[‡]*f*: fertilization; *h*: harvest; *f* × *h*: fertilization× harvest; NS, not significant.
*$p < .05$; **$p < .01$; ***$p < .001$.

of PK and NPK) compared to forage from unfertilised swards (2.6 g/kg DM).

The concentrations of protein fraction A (172 vs 136 g/kg N) and B_2 (519 vs 442 g/kg N) were significantly greater in wilted forage harvested in generative compared to vegetative stages, while this pattern was inversed in B_1 (60 vs 100 g/kg N), B_3 (185 vs 229 g/kg N) and C (64 vs 93 g/kg N). Fraction B_2 was lowest in forage from unfertilised swards (409 g/kg N), intermediate in forage from PK fertilized swards (487 g/kg N) and highest in forage from NPK fertilized swards (547 g/kg N) ($p < .05$). The inverse was found with fraction B_3, which decreased in wilted forage from unfertilised swards (284 g/kg N) to PK (191 g/kg N) ($p < .05$) and from PK to NPK (147 g/kg N) ($p < .05$).

Ensiled forage

Protein fraction A (362 vs 325 g/kg N) and B_2 (423 vs 371 g/kg N) were greater in silages harvested in generative than in vegetative stages (Table 5) ($p < .05$). Additionally, silages from swards fertilized with NPK had a greater concentration of the B_2 fraction (448 g/kg N) than silages prepared from unfertilised swards (353 g/kg N) ($p < .05$), while those from PK fertilized swards (390 g/kg N) did not differ from either fertilization type ($p > .05$). Protein fraction B_1 was greater in silages prepared from swards fertilized with NPK from the vegetative harvest compared to all other treatments. In contrast, fractions B_3 (115 vs 153 g/kg N) and C (73 vs 101 g/kg N) were lower in silages from the generative compared to the vegetative harvest ($p < .05$).

Protein degradation during ensiling

The difference in protein fraction B2 (ΔB2) was similar within the vegetative harvest, but in the generative harvest, unfertilised swards had a lower increase in fraction B2 than forage from PK or NPK fertilized swards ($p < .05$) (Table 5). The B_3 fraction had the highest degradation in forage from unfertilised swards (−128 g/kg N), was intermediate in forage from PK (−54 g/kg N) and lowest in forage fertilized with NPK (−37) ($p < .05$). Protein fraction C was unaffected during ensiling ($p > .05$).

3.3.2 | Alps

Wilted forage

Within the generative harvest, concentrations of CT, sCT, pCT and fCT were not affected by fertilization type ($p > .05$) (Table 4). In

TABLE 5 Composition of protein fractions (A, B_1, B_2, B_3 and C) of ensiled forage and differences (Δ) to wilted forages (g/kg nitrogen (N)) from the grasslands located in the Jura and the Alps in relation to long-term mineral fertilization.

Grassland	Harvest Fertilization[†]	Generative			Vegetative			SEM	*p*-value[‡]		
		0	PK	NPK	0	PK	NPK		*f*	*h*	*f* × *h*
Jura	A	396	356	334	379	308	290	20.6	NS	*	NS
	B1	33^{b}	25^{b}	24^{b}	37^{b}	42^{b}	69^{a}	4.1	NS	***	***
	B2	384	410	474	322	370	421	14.6	*	***	NS
	B3	127	116	102	185	157	118	9.1	NS	**	NS
	C	60	93	66	77	123	102	7.1	NS	***	NS
	ΔA	230	177	163	263	172	133	19.8	NS	NS	NS
	ΔB_1	−41	−30	−29	−80	−52	−19	7.6	NS	NS	NS
	ΔB_2	-57^{a}	-118^{b}	-113^{b}	-53^{a}	-75^{ab}	-85^{ab}	7.8	*	**	*
	ΔB_3	−126	−49	−35	−129	−59	−39	12.8	*	NS	NS
	ΔC	−6.0	20	14	−1.0	14	10	3.44	NS	NS	NS
Alps	A	378^{ab}	423^{a}	437^{a}	420^{a}	365^{ab}	307^{b}	13.8	NS	**	**
	B1	17	27	22	28	42	36	2.6	*	***	NS
	B2	317^{ab}	334^{ab}	311^{bc}	280^{c}	318^{ab}	351^{a}	7.9	NS	NS	**
	B3	202^{a}	134^{b}	151^{b}	171^{ab}	148^{b}	183^{ab}	6.8	***	NS	*
	C	86	82	79	101	127	123	7.7	NS	**	NS
	ΔA	242^{ab}	233^{ab}	222^{ab}	296^{a}	210^{ab}	129^{b}	15.9	**	NS	*
	ΔB1	−60	−30	−20	−94	−38	−47	7.0	***	**	NS
	ΔB2	-82^{a}	-112^{ab}	-136^{b}	-119^{ab}	-73^{a}	-70^{a}	9.6	NS	NS	*
	ΔB3	−107	−98	−67	−87	−116	−38	8.9	*	NS	NS
	ΔC	7.7	7.0	1.00	4.0	17	26	4.02	NS	NS	NS

Note: Values show arithmetic means and standard error of means (SEM). Within a row, means with different superscripts differ significantly at $P < 0.05$.
Abbreviations: CT, condensed tannin (proanthocyanidins); fCT, fibre-bound CT; pCT, protein-bound CT; sCT, soluble CT.
[†]Fertilization including no application of fertilizers ("0"), fertilization of phosphorus and potassium ("PK") and fertilization of PK and nitrogen ("NPK").
[‡]*f*: fertilization; *h*: harvest; *f* × *h*: fertilization× harvest; NS, not significant.
*$p < .05$; **$p < .01$; ***$p < .001$.

contrast, forage fertilized with NPK contained greater concentrations of CT, sCT and fCT than forage from unfertilised swards ($p < .05$) and also greater ones then swards fertilized with PK except in the case of fCT.

Protein fraction A (180 vs 152 g/kg N) and B_3 (253 vs 248 g/kg N) were greater in forage harvested in generative than in vegetative stages and lower for protein fractions B_1 (59 vs 95 g/kg N) and C (77 vs 101 g/kg N) ($p < .05$). Protein fraction A was lowest in forage from unfertilised swards (130 g/kg N), intermediate in swards fertilized with PK (173 g/kg N) and highest in swards fertilized with NPK (197 g/kg N) ($p < .05$). Forage from swards fertilized with either PK or NPK (average of both, 66 g/kg N) had lower concentrations of protein fraction B_1 than forage from unfertilised swards (100 g/kg N) ($p < .05$). Protein fraction B_3 was higher in unfertilised swards (284 g/kg) compared to NPK fertilized swards (220 g/kg N) ($p < .05$) but did not differ from those fertilized with PK (248 g/kg N) ($p > .05$).

Ensiled forage

Protein fraction A of ensiled forage did not differ within silages of the generative harvest ($p > .05$) (Table 5). Silages prepared from forage fertilized with NPK had a significantly lower A protein fraction compared to forage from unfertilised forage when harvested during vegetative growth (Table 5). Fraction B_1 was significantly greater in silages harvested in vegetative (35 g/kg N) compared to generative stages (22 g/kg N) ($p < .05$) and lower in silages prepared from unfertilised swards (23 g/kg N) compared to NPK fertilized swards (29 g/kg N) but not to PK fertilized swards (35 g/kg N). Within silages harvested in the generative stage, fraction B_2 did not differ depending on fertilization type. However, silages prepared from unfertilised swards harvested in the vegetative stage had a significantly lower concentration of fraction B_2 compared to those fertilized with PK or NPK and harvested at the same stage. Fraction B_3 was greater in silages from unfertilised swards compared to PK or NPK fertilized swards when harvested in a generative stage ($p < .05$), this difference was no longer present in silages harvested in vegetative growth ($p > .05$). Protein fraction C increased in silages harvested in a generative stage (82 g/kg N) compared to those harvested in a vegetative stage (117 g/kg N) ($p < .05$).

Protein degradation during ensiling

The increase in protein fraction A was significantly higher in forage from unfertilised swards ensiled in the vegetative stage compared to NPK fertilized swards ensiled at the same stage ($p < .05$), while there were no differences to all other treatments (Table 5). Protein fraction B_1 (ΔB_1) decreased significantly in silages prepared from forage in the vegetative stage (−60 g/kg N) compared to those prepared in the generative stage (−37 g/kg N). In silages from unfertilised swards, ΔB_1 decreased significantly (−77 g/kg N) compared to silages from PK (−34 g/kg N) or NPK (−34 g/kg N) fertilized swards. Protein fraction B_2 showed a lower decrease in silages prepared from forage from unfertilised swards compared to those from NPK fertilized swards during the generative harvest ($p < .05$), this effect was absent in vegetative harvest silages. The decrease in protein fraction B_3 (ΔB_3) was similar in silage prepared from forage from unfertilised (−97 g/kg N) or PK (−107 g/kg N) fertilized swards, while the ΔB_3 was significantly lower in silages prepared from forage from NPK fertilized swards (−53 g/kg N). Protein fraction C remained unaffected during ensiling ($p > .05$).

3.4 | Concentration of condensed tannins and protein degradation

Contents of NH_3-N (related to total N, %) and sCT or CT were negatively correlated in both the generative (sCT: $r = -0.68$, $R^2 = 0.466$, $p < .05$; CT: $r = -0.64$, $R^2 = 0.411$, $p < .05$) and vegetative harvests (sCT: $r = -0.52$, $R^2 = 0.267$, $p < .05$; CT: $r = -0.51$, $R^2 = 0.259$, $p < .05$). (Figure 1). The correlation between protein fraction A (related to total N, g/kg N) and sCT or CT was also negatively correlated in both the generative (sCT: $r = -0.56$, $R^2 = 0.313$, $p < .05$; CT: $r = -0.52$, $R^2 = 0.269$, $p < .05$) and vegetative (sCT: $r = -0.57$, $R^2 = 0.322$, $p < .05$; CT: $r = -0.66$, $R^2 = 0.435$, $p < .05$) harvests (Figure 2). In contrast, no relationship between ΔA (i.e., the difference between protein fraction A from ensiled to wilted forage) (Figure 3) was observed for both sCT and CT (sCT: $r = -0.32$, $R^2 = 0.101$, N.S.; CT: $r = -0.23$, $R^2 = 0.075$, N.S.) in the generative harvest, whereas there was a correlation for the vegetative harvest (sCT: $r = -0.51$ $R^2 = 0.257$, $p < .05$; CT: $r = -0.63$, $R^2 = 0.395$, $p < .05$).

4 | DISCUSSION

4.1 | Botanical characteristics of ensiled forage

Continuous long-term fertilization has led to established swards, characterized by high plant species numbers with a significant proportion of forb species (Ineichen et al., 2020). These swards were used to investigate the potential of ensiling forage from species-rich mountain grasslands. As expected, unfertilised swards showed increased plant species, while fertilization of PK increased the proportion of legume species (Silvertown et al., 2006). Irrespective of fertilization type, swards at site Jura were dominated by just a few grass species, except for swards fertilized with NPK as previously reported by Ineichen et al. (2020). These swards were characterized by large proportions of wood cranesbill, which possibly evolved due to an excess fertilization of N relative to the low harvest frequency applied in the long-term fertilization regime. On the contrary, swards at the Alps site were fairly evenly distributed with respect to plant species and proportion of plant functional groups. Thus, forage species such as wood cranesbill with large proportions of stems, present in all silages from the Jura site, are more difficult to compact during ensiling when compared to the leafier forages from the Alps (Ineichen et al., 2020).

4.2 | Quality of ensiled forage

Silages from the present study demonstrated acceptable energy concentrations, however, this may be a major limit when feeding forage

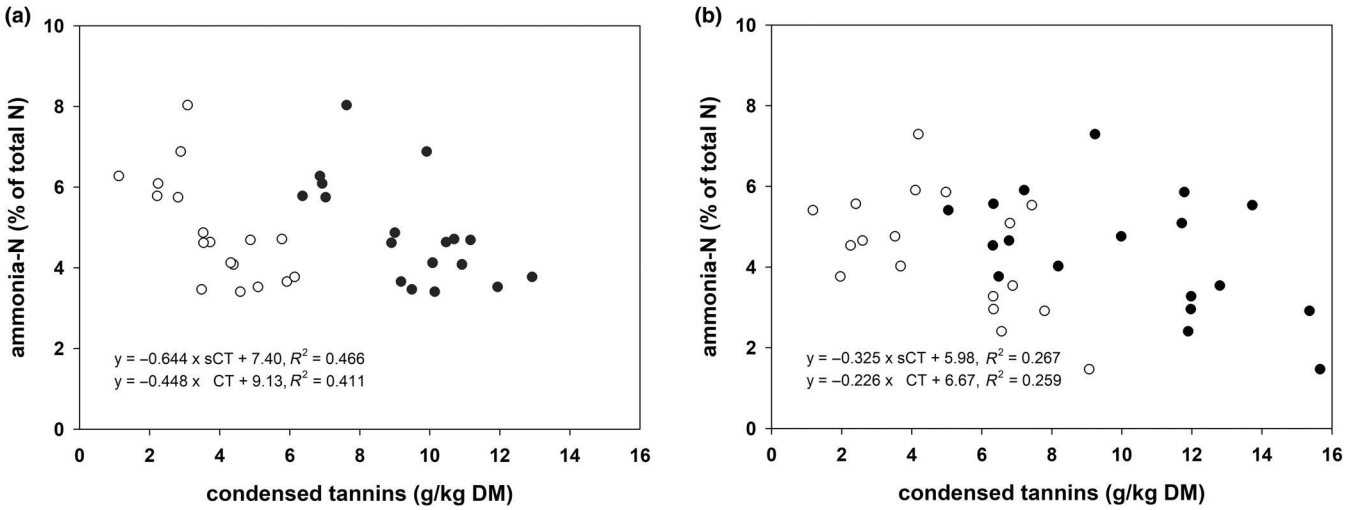

FIGURE 1 Correlation of soluble (sCT) and condensed tannins (CT) in wilted forage and concentration of ammonia-N (NH_3-N, % of total N) in silages prepared from grasslands of the Jura mountains and the Alps from a generative (a) and vegetative harvest (b). Data from both mountain grassland sites, and fertilization treatments were pooled.

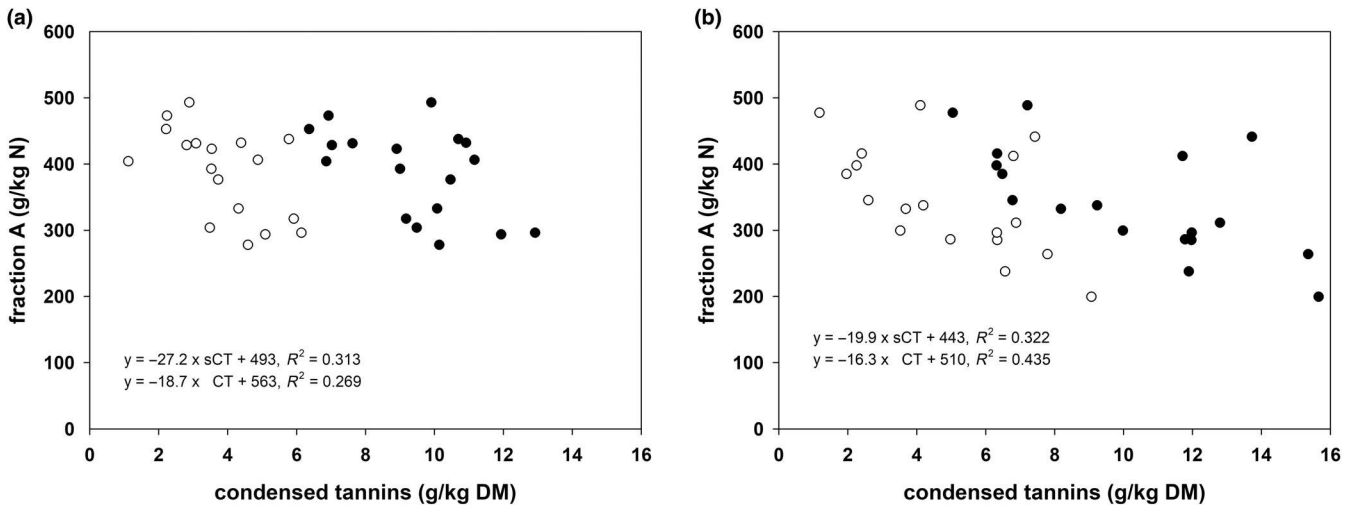

FIGURE 2 Correlation of soluble (sCT) and condensed tannins (CT) in wilted forage and protein fraction A (g/kg N) in silages prepared from grasslands of the Jura mountains and the Alps from a generative (a) and vegetative harvest (b). Data from both mountain grassland sites, and fertilization treatments were pooled.

from species-rich grasslands to ruminants (Hammond et al., 2014). Contrastingly, silage CP concentrations were moderate and similar to silages prepared from other mountain grasslands (Wyss et al., 2016). Similar CP concentrations were found in forage analysed by Gierus et al. (2016) from a long-term grassland fertilization experiment including no fertilization, PK or PK combined with three different levels of N fertilization. Forage CP concentrations in their experiment did not react to the fertilization type as was the case in silages from site Jura, while minor differences were found in silages from the Alps. Overall, silage quality did not follow a clear pattern with regards to fertilization type or harvest cycle related effects at either site. Differences between the generative and vegetative harvests were negligible. Similar observations were made by Wyss et al. (2016) who ensiled forage from several species-rich mountain grasslands.

High levels of N fertilization decrease the concentration of WSC (Nadeau et al., 2019; Tremblay et al., 2005). Forage WSC concentrations were similar across fertilization treatments at both grassland sites. Sugar concentrations of the wilted forage, prior to ensiling, were moderate when compared to forage from intensively managed ryegrass swards (Miller et al., 2001). The relatively high residual sugar concentrations of the silages resulted in a low production of fermentation acids and consequently in an insufficient pH decline. This indicates that the potential fermentative capacity of the silages was not fully exploited. The microbial activity was therefore not primarily hampered by a lack of sugar. However, that is, specific plant secondary

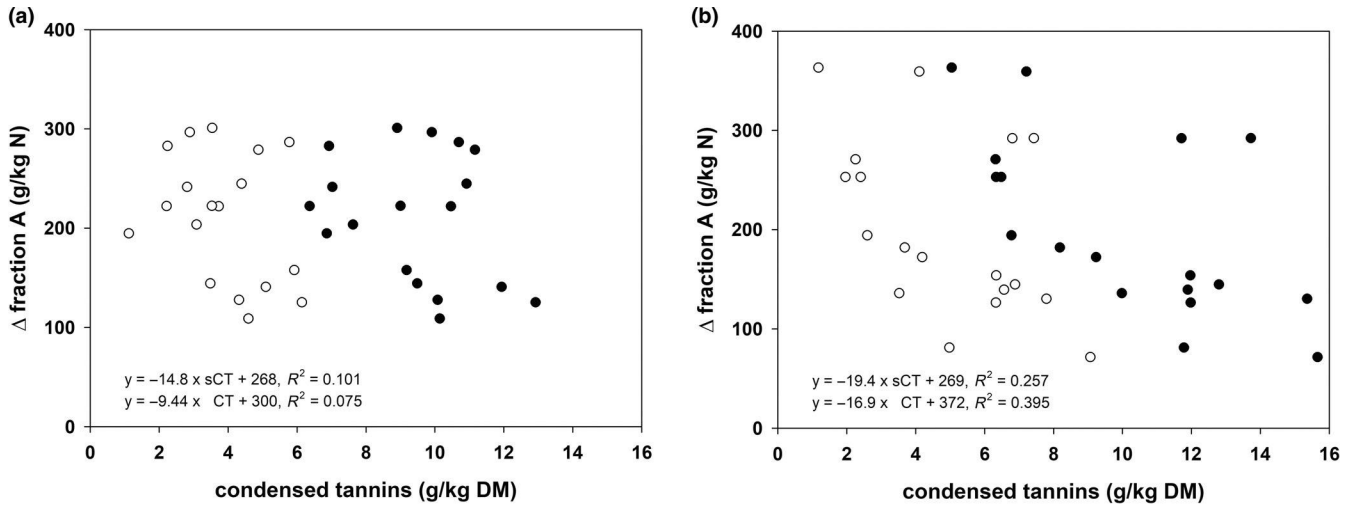

FIGURE 3 Correlation of soluble (sCT) and condensed tannins (CT) in wilted forage and the increase in protein fraction A (g/kg N) during ensiling in silages prepared from grasslands of the Jura mountains and the Alps from a generative (a) and vegetative harvest (b). Data from both mountain grassland sites, and fertilization treatments were pooled. N.S., not significant.

metabolites may have impaired the microbial activity and in particular the formation of lactic and acetic acid producing bacteria (Jeangros et al., 1994). Herremans et al. (2019) related higher residual sugar concentrations in silages to the inhibition of microbial activity due to the presence of tannins at doses of 8 g kg^{-1} DM of chestnut tannin and 10 g kg^{-1} DM of powdered oak tannin extract. In the present study, CT concentrations were higher than total tannin concentrations determined in the extracts by Herremans et al. (2019). On the other hand, other authors have reported higher formations of lactic acid with the addition of chestnut tannins (Tabacco et al., 2006). A meta-analysis by Jayanegara et al. (2019), showed a linear decrease in the formation of butyric acid with greater tannin concentration. However, this effect was pronounced at CT concentrations above 30 g CT per kg DM, three times higher than the CT concentrations of the forage used in this study.

All silages produced from the Jura mountains had elevated butyric acid concentrations, which indicate an inferior silage quality as described by McPherson and Violante (1966). Due to the lack of N fertilization in unfertilised and PK fertilized swards, the forage is likely characterized by generally lower nitrate concentrations. Therefore, an impairing effect of nitrate on butyric acid forming bacteria may be missing. Contrastingly, the formation of ethanol was below critical thresholds, indicating decreased activity of yeast cells (Kung Jr. et al., 2018). In a field-study by Wyss et al. (2016), the quality of silages from mountain grasslands were determined by average silage characteristics including a pH of 4.8, 45 g lactic acid, 9 g acetic and butyric acid, and 6 g ethanol per kg DM. The source of increased butyric acid formation was unlikely due to excessive soil contamination since ash concentrations were below critical levels. However, the use of silage additives specifically targeting the activity of butyric acid forming bacteria would represent a useful means to increase the silage quality of the swards investigated. Additionally, the formation of ammonia is unlikely to indicate an increased proteolytic activity of clostridia with levels ranging from 32.4 to 65.2 g/kg N (Kung Jr. et al., 2018). Furthermore, DM contents of the silages were in a range that is not typically associated with wet silages and increased butyric acid forming bacterial activity. The formation of butyric acid is most likely attributed to a sugar-based limitation of lactic acid formation bacteria with a conversion of lactic acid to butyric acid (Kung Jr. et al., 2018).

4.3 | Protein degradation of silages

Protein degradation may occur in silage with elevated protein concentrations such as intensively managed leys of *Lolium* spp. or mixtures containing clover (Givens & Rulquin, 2004). Based on the consideration of ammonia-N relative to total N concentrations, proteolysis was below critical thresholds in all silages and comparable to silages from a previous field study (Wyss et al., 2016). An ammonia-N suppressing effect of tannin extracts (Salawu et al., 1999; Cavallarin et al., 2002; Tabaco et al., 2006) or from tanniferous legumes (Albrecht & Muck, 1991) has been reported by several authors. However, CT concentrations in the present study were far below those determined effective to supress the formation of readily soluble N by Jayanegara et al. (2019). Concentrations of CT may vary depending on the botanical composition of the sward (Jayanegara et al., 2011). Concentrations of tannins in species-rich swards are therefore highly dynamic and the effects on silage quality are not evident at every harvest. Wood cranesbill has been found to occur in large proportions in NPK fertilized swards and is particularly rich in tannins (Ineichen et al., 2020). In previous assessments of the forage quality at both grassland sites, Jura and Alps, the Alps' autumn harvest did not contain detectable levels of CT (Ineichen et al., 2020), this is possibly due to the low proportions of wood cranesbill.

Both the plant species and the time of harvest affect the concentration and composition of sCT and pCT as reported by Girard et al. (2018). From a variety of herbal species studied, *Sanguisorba minor* Scop., *Lotus corniculatus* L. and *Onobrychis viciifolia* Scop. showed increased protein precipitation capacities when related to their CT concentrations (Hamacher et al., 2013). Therefore, in a mixed sward, the distribution of sCT and pCT and their affinity for protein precipitation is majorly dependent on the specific plant species present.

4.4 | Protein fractions of silages

When the wilting of fresh forage occurs rapidly, proteolytic activity by plant proteases is reduced, and subsequently less soluble N fractions are formed (Nadeau et al., 2019). According to Kirchhof et al. (2010) and Wyss et al. (2017), the distribution of the protein fractions A, B and C is plant species specific and dependent on the growth stage at harvest. Therefore, the individual proportions of plant species in a sward become relevant. From the wilted to the ensiled forage of the mountain grasslands, protein fraction A increased on average by 121% (from 160 g/kg CP to 355 g/kg CP) across all treatments. Givens and Rulquin (2004) reported a reduction of true protein from fresh to ensiled ryegrass-dominated forage of 64.3%, which is approximately 10% higher than in the forage from the mountain grassland swards.

In the case of fresh forage, high N fertilization rates have been reported to increase protein fraction A (Gierus et al., 2016; Tremblay et al., 2005). Hence, it would be expected that the highest proportion of protein fraction A be observed in NPK fertilized swards. However, with one exception (vegetative harvest of ensiled forage from site Alps), protein fraction A did not respond to the type of fertilization. This is likely related to moderate N fertilization of the field experiments although Nadeau et al. (2019) observed similar results as found in the present study with higher N fertilization levels. In the present experiment, forage ensiled during the vegetative growth stage had lower proportions of protein fraction A, although total forage protein concentrations were similar between harvests.

Wyss et al. (2017) suggested the inclusion of forage species containing bioactive compounds such as CT or polyphenol oxidase in multi-species swards. They demonstrated that the increase in protein fraction A was more than doubled in *Medicago sativa*, a species containing limited bioactive compounds compared to red clover or sainfoin. All mountain grassland swards contained CT but at moderate concentrations in the current investigation. When considering the reducing effect of polyphenol oxidase on protein degradation (Lee et al., 2008), an effect would be expected in swards fertilized with PK at the Alps site where it had a relevant proportion. However, such a trend was not found in the present study when considering the proportion of protein fraction A or its increase during ensiling. In contrast, there was a linear correlation in swards with higher CT concentrations and the increase in protein fraction A, demonstrating significantly reduced proteolytic activity. Swards with elevated concentrations of CT or sCT, may therefore present a means to reduce the increase of protein fraction A and reduce the buffering effect caused by the formation of NPN compounds. This is crucial as, in the present study, fermentable carbohydrates were limited in the forage to generate sufficient acidification to obtain well conserved silages. Furthermore, CT have been associated with reduced ruminal protein degradation (Waghorn, 2008) and improved N use efficiency (Wang et al., 2018). Feeding silages from species-rich swards as those investigated in the present study may therefore improve animal performance as well.

Nadeau et al. (2019) observed an increase in the proportion of the protein fractions B1 and B2 with increasing N fertilization rates from 0, 100 and 200 kg N per ha and year for the generative harvest of wilted forage, while protein fraction B_3 was not affected. Decreasing the degradation of protein fraction B_3 is beneficial, since it has a low ruminal degradability and increases the proportion of bypass protein to the small intestine (Licitra et al., 1996). In forage harvested from the mountain grasslands, the reduction in protein fraction B_3 was affected by fertilization and was lower when fertilizer included N. Kirchhof et al. (2010) indicated, that an advanced maturity stage increased protein fraction B_3 (and also C), which maybe an explanation for the observation made in this study. Fertilization with N may promote the phenological development and result in a more mature forage when fertilized with N. However, as indicated by the growth stage at harvest should be considered and swards fertilized with N may be at an advanced stage of maturity. In previous studies, protein fraction C was found to be unaffected by fertilization type (Gierus et al., 2016; Wyss et al., 2017), this too was the case for both wilted and ensiled forage in the present study. However, protein fraction C was higher in the vegetative compared to the generative harvest.

5 | CONCLUSIONS

The silage quality from species-rich mountain grasslands did not follow a clear pattern of fertilization type or harvest cycle related effects. This may be due to the large variation in sward botanical and chemical composition of the forage investigated. In general, acidification was moderate to low with comparably high residual sugar contents. Energy concentrations of the silages were acceptable, however, butyric acid concentrations where elevated. Silages would benefit from the use of a silage additive to counteract the insufficient acidification and activity of butyric acid formation bacteria. Protein fractions were influenced by type of fertilization and harvest cycle. The negative correlations of CT or soluble CT contents with ammonia-N or NPN in silage were found in both forage from the generative and vegetative harvests indicating a possible relationship with protein degradation during ensiling. Whether this effect would translate also in animal performance was not investigated in the current study. Future research should also investigate the influence of the epiphytic flora on the microbial fermentation. Additionally, determination of the fermentation coefficient, the buffering capacity and the use of silage additives would further increase the understanding of successful ensiling of forage from species-rich swards.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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