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Use of willow (*Salix sp*) biomass as tree fodder: nutritional value and conservation methods

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Abstract This study evaluated the nutritional value and different methods of conservation of two willow materials—the hybrid clone Terra Nova (HTN) from a short-rotation coppice (SRC) system and the native *Salix atrocinerea* (SAT) from riparian stand—representing contrasting willow resources available in the study region. The leaf fraction (LE)—although representing < 50% of total stem biomass—, showed the highest nutritional value versus stems, leaf crude protein (CP) reached 23.7% dry matter (DM) in HTN and 17.7% DM in SAT, with in vitro organic matter digestibility (IVDMO) 52.75% in HTN and 38.8% in SAT; by contrast, edible stems showed much lower CP (3.3–5.8% DM) and IVDMO (11.8–31.2%). Leaf Relative Forage Value (RFV) was "Good" in HTN (110–115) and "Poor–Fair" in SAT (82). Sun-drying proved most effective at preserving nutritional quality, followed by formic acid-assisted ensiling. Both materials showed poor suitability for ensiling because of low water-soluble carbohydrate levels, requiring additive application to reach target pH (4.5 vs. > 5.0 in untreated silages). Willow leaf silages achieved CP levels of 18–22% DM (up to 27.8% in HTN with additive), while fermentation intensity remained low; SAT leaves showed a higher fermentability coefficient (44 vs. 28), consistent with better acidification. The HTN material proved suitable as a protein supplement for moderate-energy diets, whereas SAT was limited to maintenance-level feeding. Willow forage shows notable potential as a supplemental fodder resource for ruminant systems, although effective preservation requires additive use to maintain nutritional quality during storage.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10457-026-01486-8.

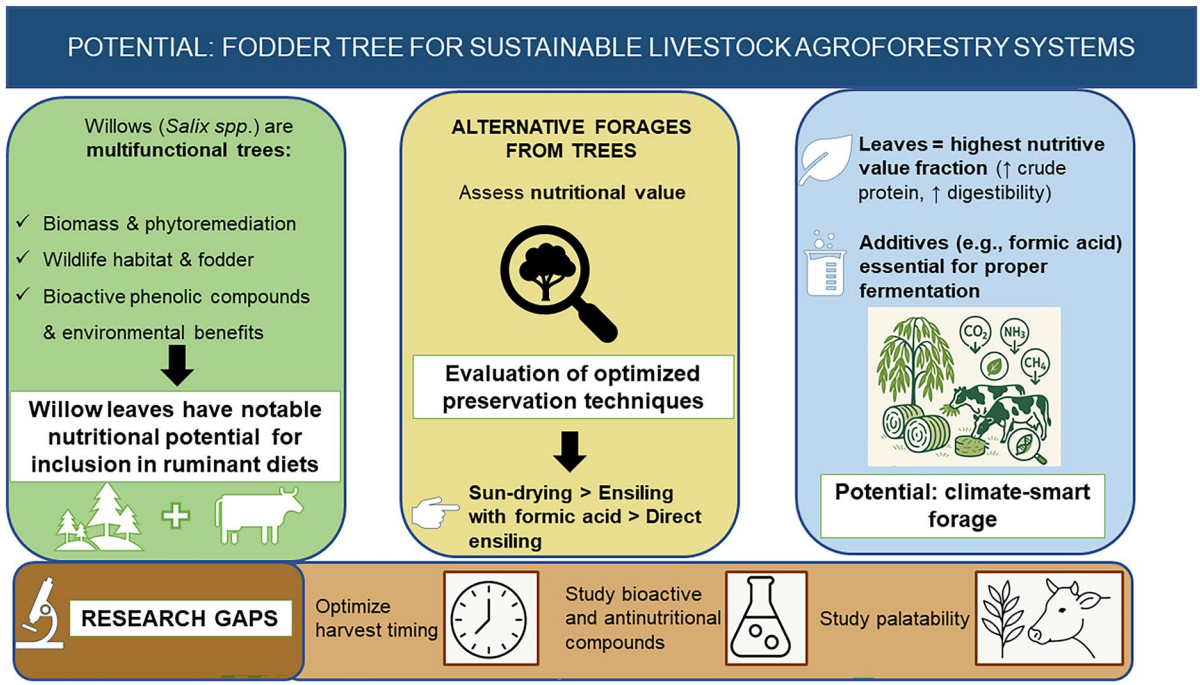
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Graphical abstract



Keywords Agroforestry · *Salix atrocinerea* · Ruminant nutrition · Silage quality · Short-rotation coppicing · Willow leaves

Abbreviations

ADF	Acid detergent fiber
ADL	Acid detergent lignin
AS	Additive silage
BC	Buffering capacity
CP	Crude protein
CT	Condensed tannins
DM	Dry matter
CS	Control silage
ES	Edible stems
FC	Fermentability coefficient
F	Fresh
FW	Fresh weight
HTN	*Salix* "Terra Nova" clone
IVDMO	In vitro Organic matter digestibility
LAB	Lactic acid bacteria
LE	Leaves
NDF	Neutral detergent fiber
NH_3-N	Ammoniacal nitrogen
NSC	Non-structural carbohydrates
OM	Organic matter
RFV	Relative forage value
SAT	Native *Salix atrocinerea*
SRC	Short-rotation coppicing
SUN	Sun-dried
TN	Total nitrogen
WO	Woody material
WSC	Water-soluble carbohydrates

Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), 'Agroforestry is a collective name for land-use systems and technologies where woody perennials (trees, shrubs, palms, bamboos, etc.) are deliberately used on the same land-management units as agricultural crops and/or animals, in some form of spatial arrangement or temporal sequence'. Agroforestry emerged as a formal concept in the 1970 s, although the practice itself represents a re-emergence of ancient farming

systems in which trees traditionally played key productive and ecological roles. Modern agroforestry has evolved into a multifunctional land-use strategy that enhances productivity, resilience, and environmental performance through systems such as forage banks and alley pastures. Notably, trees initially planted for soil conservation (e.g., *Salix* spp. and *Populus* spp.) are gaining attention as emergency fodder, a practice already adopted in different regions during critical situations for livestock feeding (Moore et al. 2003; Hejcman et al. 2016; Vandermeulen et al. 2018; Navale et al. 2022; Bonizzi et al. 2025).

The use of tree and shrub foliage as livestock feed during seasonal forage shortages is a globally widespread practice (Luske and van Eekeren 2018). Traditionally, tree fodder has been a critical component of ruminant nutrition and remains vital in certain European farming systems. Prominent examples include dried oak (*Quercus* spp.) leaves fed to sheep in Greece and stored carob (*Ceratonia siliqua*) pods utilized in Crete and Sicily (Eichhorn et al. 2006).

Similarly, the *dehesa* silvopastoral system, distinctive to the Iberian Peninsula's southwest, was shaped by centuries of extensive grazing management (Olea et al. 2005). While traditional systems like the *dehesa* rely on slow-growing trees, modern bioenergy approaches exploit fast-growing species. Willow (*Salix* spp.) is suitable for cultivation in short-rotation coppice (SRC) systems for renewable energy production, thanks to its rapid regrowth capacity and high leaf biomass yield (Dimitriou and Rutz 2015; Daugaviete et al. 2022; Baker et al. 2022), a practice well-established in the UK, Western Europe, Canada and north-eastern United States (Smart and Cameron 2008). Beyond its use as a bioenergy feedstock, willow (*Salix* spp.) serves as valuable summer forage for livestock (Kemp et al. 2003) and shows potential as a strategic, drought-resilient fodder source for cattle (Moore et al. 2003). However, in temperate regions where climatic conditions typically support year-round grass growth, tree and shrub biomass has played a marginal role as forage resources. Progressive tree clearance for pasture expansion and the introduction of novel forage crops has led to the abandonment of these traditional systems in many humid regions of Europe (Robinson 1985). Despite the decline of these practices, it has long been recognized that ruminants in humid climates can utilize low-lignin biomass from certain tree and shrub species (e.g., *Populus, Salix, Alnus*) to meet part of their nutritional requirement (Hejcman et al. 2016; Vandermeulen et al. 2018). Willow has been traditionally utilized as livestock forage in temperate regions as supplemental feed for beef cattle and replacement stock during critical years (drought, extremely cold, volcanic eruptions) (Oppong et al. 2001; Hejcman et al. 2016). Several studies have characterized the nutritional composition and seasonal dynamics of willow foliage as livestock forage; for example, Kendall et al. (2021) sampled in June and discussed both composition and seasonality in the context of ruminant feeding, underscoring the need to align harvest timing with targeted nutritive attributes. Recent comparative analyses further show that willow can equal or exceed the nutritive value of other commonly used fodder tree species as for example ash and elm (Bonizzi et al. 2025). The renewed interest in willow as fodder is therefore relatively recent and is closely linked to its establishment for other primary purposes—such as erosion control, ecological engineering, or bioenergy production under short-rotation coppice (SRC) systems. In countries such as New Zealand, willows planted for soil conservation have been increasingly harvested and fed fresh to grazing cattle during critical dry periods (Moore et al. 2003), illustrating their practical value in modern feeding strategies. In parallel, SRC plantations and associated breeding programs have generated fast-growing hybrids with high leaf biomass yield and structural traits compatible with mechanized harvesting. These features, together with willow's resilience and multipurpose functionality, have positioned *Salix spp*. as promising candidates for integration into contemporary livestock–tree systems, not only as fresh forage but also as preserved fodder.

Recent agricultural policy reforms, particularly in the European Union, have both elevated the importance of forage trees and shrubs for ecosystem services and increased recognition of their potential as supplementary pastoral feed resources (Kuzovkina and Volk 2009; Vandermeulen et al. 2018). Agroforestry has gained recognition as a multifunctional land-use strategy that simultaneously optimizes economic outputs (food, feed, fuel, and fiber production) and delivers non-market benefits such as environmental conservation, carbon sequestration, and cultural landscape preservation. Trouillard et al. (2024) and Muyabe et al. (2025) indicated that agroforestry as a

sustainable solution to tackle challenges in livestock farming, integrating trees with pasture or crop systems, offering ecological and economic advantages such as improved biodiversity, soil fertility, and carbon sequestration. These authors also emphasize its role in climate resilience, providing shade and windbreaks that mitigate heat stress in livestock. Nevertheless, these data are conspicuously absent for native species, particularly regarding their nutritional profile after ensiling. Consequently, while multipurpose tree species are acknowledged as potential livestock forage resources, their full utilization is constrained by critical knowledge gaps concerning nutritive value and preservation methods.

Willows are pioneer species in many humid habitats of the northern temperate zone, exhibiting a near-global distribution with native representatives on all continents except Antarctica. Modern taxonomic studies estimate 450–520 extant *Salix* species globally, approximately 135 of which are native to the European continent and adjacent Arctic regions (Baker et al. 2022). The Atlantic bioclimatic region of northern Iberia represents a unique agricultural paradigm within Europe's humid zone, where the primary sector's strategic role in territorial cohesion has fostered production systems distinct from Mediterranean models. This is evidenced by: (1) specialized humidity-adapted cropping systems with Europe's most intensive dairy-forage operations, and (2) land organization patterns, marked by population dispersion, rural depopulation, and smallholder land fragmentation.

Agricultural production is increasingly focused on multifunctional cropping systems as valuable tools to mitigate climate change impacts, biodiversity loss, and food insecurity while enhancing climate-resilient production systems. In Galicia, native and naturalized tree species such as willow (*Salix* spp.), alder (*Alnus* spp.), hazel (*Corylus* spp.), and poplar (*Populus* spp.), have recently gained attention for their potential as multi-purpose species to boost ecosystem services (Swagemakers et al. 2017). Integrating these species into livestock systems aligns with both EU agro-environmental frameworks and Payment for Ecosystem Services schemes, including the "greening" measures of the Common Agricultural Policy (CAP), which incentivise sustainable land management practices.

Willows are widely distributed across the humid bioclimatic zones of Atlantic Europe and thrive under the mild, rainy conditions characteristic of north-western Iberia. The two species assessed in the present study—*Salix atrocinerea* (a native riparian species) and the fast-growing hybrid Salix "Terra Nova"—represent ecologically and functionally contrasting materials of particular interest in this regional context. *S. atrocinerea* is abundant and naturally available in riparian buffers, whereas the Terra Nova clone is cultivated under SRC management for its vigorous regrowth and high leaf-to-stem ratio. Evaluating both materials therefore provides relevant insight into the potential of locally available and commercially bred willow resources as tree fodder in Atlantic livestock systems.

While existing studies demonstrate the potential of willow foliage as a ruminant dietary supplement (Muklada et al. 2021; Wangui et al. 2024; Steffen et al. 2024), data remain limited for native or locally distributed species such as *S. atrocinerea*. Moreover, establishing nutritional value must be complemented by developing optimized preservation techniques to enable: (1) effective on-farm biomass storage, and (2) implementation of strategic seasonal feeding programs. Effective conservation management is crucial for preserves nutritional quality over time, benefiting animal health and productivity. Silage—a fermented, preserved feed produced from plant material—represents a particularly promising approach given its widespread use in ruminant production systems (Borreani et al. 2018).

The aims of this study were: (1) to characterize the nutritional value of three biomass fractions—leaves (LE), edible stems (ES $\leq$ 5 mm diameter) and woody material (WO $>$ 5 mm)—from two willow materials, and (2) to evaluate different conservation methods for the leaf fraction.

Materials and methods

Plant material, planting systems and study sites

This study evaluated two contrasting willow materials originating from different locations and management systems, which reflects the real availability of willow biomass resources in the study region. The native *S. atrocinerea* (SAT) was collected from an unmanaged riparian buffer, while the hybrid Salix "Terra Nova" (HTN) was obtained from a short-rotation coppice

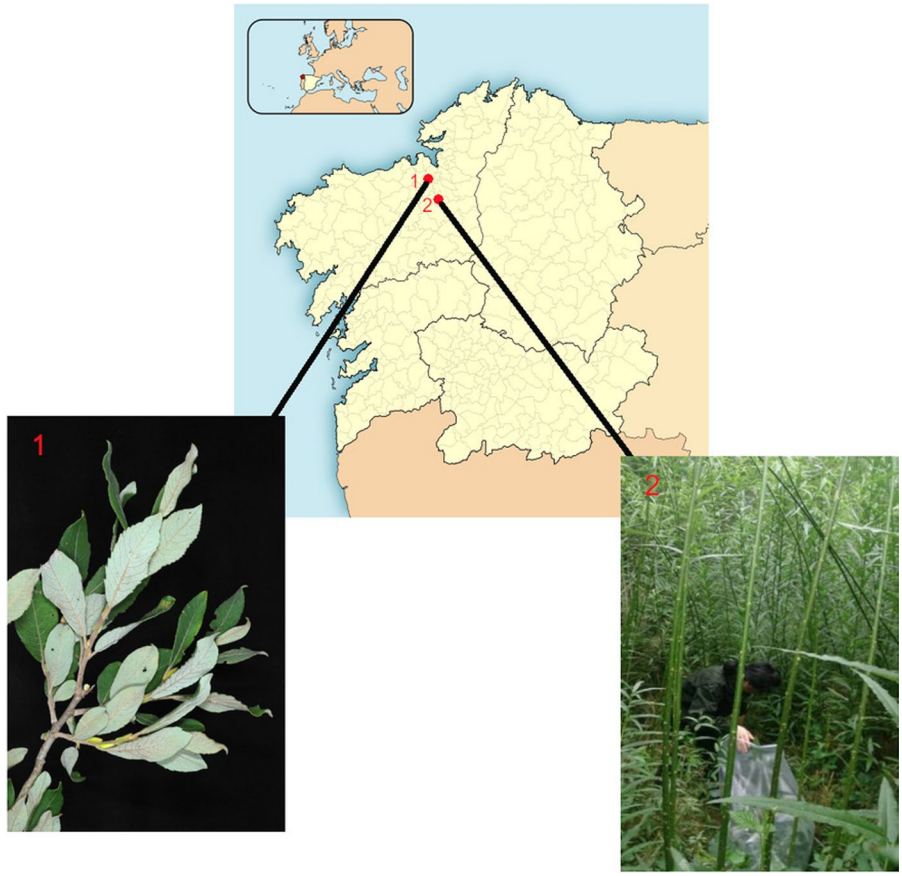


Fig. 1 Geographic locations of the study sites within the Atlantic biogeographic region: **1** *Salix atrocinerea* (SAT)riparian buffer planting and **2** *Salix* hybrid clone "Terra Nova" (HTN) short-rotation coppice (SRC) plantation

(SRC) plantation. These materials were not grown in the same place, nor under comparable management conditions.

Native riparian willow (S. atrocinerea, SAT)

S. atrocinerea (SAT), a widely distributed species across southwestern/central/northern Europe and northern Africa. In the study region (Galicia, NW Spain), SAT occurs naturally as riparian vegetation, predominantly along watercourses. Plant material was collected at the Agricultural Research Center "Centro de Investigaciones Agrarias de Mabegondo" (AGACAL-CIAM) (Fig. 1). The riparian planting had been established > 4 years prior to sampling and received no management intervention (no pruning, thinning or pollarding). Thus, the SAT biomass used in this study represents unmanaged, naturally growing willow typical of riparian buffers in the Atlantic bioclimatic region.

Hybrid willow Salix "Terra Nova" (HTN)

The hybrid clone *Salix* "Terra Nova" (HTN), a commercial clone (Kuzovkina and Volk 2009) developed by the European Willow Breeding Partnership for bioenergy production under marginal growing conditions. HTN clone was grown under SRC management at an experimental plantation in Mesía (Fig. 1), located < 30 km from the SAT site. Trees were planted at a density of 10,000 plants ha^{-1}. Biomass samples were collected from mother plants following coppicing at two distinct growth stages: two-year (HTN2) and four-year (HTN4) regrowth.

In contrast with SAT, the HTN material reflects intensively managed willow, specifically bred and cultivated for rapid regrowth and high leaf productivity.



Fig. 2 Plant material used in the study, different morphological fractions. **A**–**D** *Salix atrocinerea*: **A** whole branches, **B** leaf fraction **C** edible stem fraction, **D** woody material; **E**–**H** "Terra Nova" clone: **E** whole branches, **F** leaf fraction, **G** edible stem fraction, **H** woody material

Sampling and pre-processing protocols

Fresh plant material collection and processing (experiment A1)

Fresh biomass was harvested in early June from five randomly selected SAT trees. From each tree, six representative stems were collected (total n = 30 stems per species/age group). Stems were sampled evenly around the crown, collecting stems from all orientations (north, south, east and west). These stems were manually separated into three fractions (Fig. 2): leaves with petiole (LE), edible stems (ES; defined as ≤ 5 mm diameter, (Oppong et al. 2001) and woody material (WO > 5 mm diameter) for determining the proportional distribution of biomass fractions.

Following the same methodology, five HTN4 individuals were sampled in July, with six stems per plant processed into LE, ES, and WO fractions. Concurrently, five HTN2 individuals were harvested by cutting six stems at the coppicing point, separating only LE and ES fractions. For HTN2, no WO fraction was present because juvenile shoots did not surpass the 5-mm threshold used to define woody material; thus, only LE and ES were separated and analyzed.

After recording fresh weights for each fraction, representative aliquots were collected (n = 5 composite samples, one per tree). The ES and WO fractions were manually chopped with pruning shears and subsequently shredded using a forage chopper machine, followed by preliminary size reduction in a knife mill (Pulverisette 11, Fritsch GmbH, Idar-Oberstein, Germany) and final grinding to 1-mm particle size in a laboratory ball mill (Retsch PM 400, Retsch GmbH, Haan, Germany). Dry matter (DM) content was determined in a forced-air oven (Uni-therm, Russell-Lindsey Engineering, Birmingham, England) at 80 °C for 16 h following standard forage analysis procedures, constant weight was verified in representative subsamples to ensure complete drying.

Collection and processing of material for the conservation evaluation (A2 experiment).

Experiment A2 was carried out using only fresh leaves from SAT and two-year-old coppiced Terra Nova clone (HTN2). In both willow materials, the



Fig. 3 Silage preparation for control ensiling (CS) and formic acid-treated silage (AS): **A** fresh leaf material after mechanical homogenization using a guillotine chopper, scale bar 5 cm; **B** vacuum-sealed laboratory silos (500 g capacity in polyethylene bags)

leaf fraction consisted exclusively of leaves including their petioles; edible stems (ES) were not used in Experiment A2. In mid-July, approximately 10 kg of fresh leaves per species were collected, homogenized, and divided into four 2.5-kg aliquots randomly assigned to conservation treatments: fresh (F), sun-dried (SUN) and ensiling (control silage (CS) and additive silage (AS)).

The fresh leaves treatment (F) consisted of oven drying at 80 °C for 16 h and subsequent grinding to a particle size of 1 mm following standard forage analysis procedures, constant weight was verified in representative subsamples to ensure complete drying. The sun-dried leaves treatment (SUN) consisted of natural drying outside on a wire mesh for 72 h to a dry matter level of about 90% followed by grinding to 1 mm particle size.

For treatments involving silage (CS and AS), fresh leaves were manually chopped with a guillotine to homogenize the starting material, a theoretical chop length of 1.5–2.0 cm was used (Fig. 3A), which is recommended for forages harvested at approximately 30–40% dry matter to ensure adequate packing density and fermentation. The chopped leaves for direct ensiling (CS) received an equal volume of distilled water as a control treatment, while silage (AS) was sprayed with a commercial formic acid-based additive (ENSILE 3F®) at the manufacturer's recommended dose (3 g/kg fresh matter). Laboratory-scale silos were prepared with 500 g of material in vacuum-sealed polyethylene bags following (Johnson et al. 2005), with quintuplicate replicates per treatment (Fig. 3). A total of 20 laboratory silos were prepared and kept at room temperature for 30 days. After

opening, each silo was split into two aliquots: one oven-dried and ground (80 °C, 16 h, 1-mm) following Experiment A1 protocols, and the other was kept frozen (−20 °C) until fermentative quality analysis.

Chemical composition, digestibility and fermentation quality

Experiment A1 analyses

For chemical composition, in vitro digestibility and fermentation analyses, the six stems collected from each tree were composited into a single sample, resulting in n = 5 biological replicates for all analytical determinations. Dried and ground samples were analyzed in duplicate using wet chemistry analysis (Pereira-Crespo et al. 2022) for: organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), non-structural carbohydrates (NSC), water-soluble carbohydrates (WSC), in vitro organic matter digestibility (IVDMO; Tilley-Terry method), also, Relative Forage Value (RFV) was calculated as: RFV = (IVDMO × 120/NDF)/1.29 (adapted from Rohweder et al. (1978)). To aid interpretation, Supplementary Table 1 summarizes the commonly used RFV quality categories based on Rohweder et al. (1978) and Linn & Martin (1991).

Experiment A2 analyses

In order to determine ensilability, the fresh leaves (pre-ensilage samples) were analyzed for: dry matter (DM), Water-soluble carbohydrates content (WSC), Buffering capacity (BC). Then, the fermentation coefficient (FC) index of Weissbach et al. (1974) for pre-ensiled samples were calculated.

The silage samples dried and ground were analyzed for OM, CP, NDF, ADF, ADL, WSC, NSC, and IVDMO. Silage fermentation quality included the following parameters: pH, ammoniacal nitrogen (NH_3-N), soluble nitrogen (soluble N), fermentation acids (lactic, acetic, propionic and butyric) and alcohols (ethanol and propanol). All parameters related to the silage fermentation quality was determined by the methodologies described in Pereira-Crespo et al. (2021). All values are expressed as concentrations on a dry matter basis, except for NH_3-N and soluble N, which are expressed as a percentage of total N. Finally, the buffering capacity was performed on fresh sample according to the methodology described by Playne and McDonald (1966).

Statistical analysis

The data analysis was generated using SAS software, version 9.4 of the SAS System (SAS Institute Inc 2012). Data for nutritional quality parameters, silage quality, and fermentation characteristics were analyzed using one-way ANOVA (PROC GLM) with species and treatment as fixed effect. Post-hoc comparisons employed Duncan's Multiple Range Test at α = 0.05 significance level.

Results

Experiment A1: nutritional value of fresh willow biomass fractions

Table 1 displays the proportional distribution of biomass fractions across willow materials, native riparian SAT unmanaged and hybrid willow HTN under SRC management (HTN2 and HTN4, two- and four-year-old regrowth respectively). HTN2 produced no WO fraction, as two-year regrowth did not exceed the 5-mm cut-off used to define WO. The leaf fraction (LE) constituted the majority (44.7% FW) of *SAT* biomass, while the hybrid clone HTN showed lower leaf proportions (HTN4: 35.3% FW; HTN2: 21.1% FW). For HTN2, all non-leaf biomass was classified as edible stems (ES, 78.9% FW). In contrast, HTN4

Table 1 Proportional fresh weight (FW) distribution of biomass fractions (leaf [LE], edible stem [ES], and woody material [WO]) across *Salix* species (mean ± SD)

Species[a]	n	LE (% FW)	ES (% FW)	WO (% FW)
SAT	30	44.74 ± 2.35^{a}	22.92 ± 2.95^{b}	32.34 ± 5.28^{b}
HTN2	30	21.10 ± 1.72^{c}	78.90 ± 1.72^{a}	–
HTN4	30	35.34 ± 2.44^{b}	8.02 ± 1.01^{c}	56.63 ± 3.30^{a}
p-value	–	< 0.0001	< 0.0001	< 0.0001

[a]SAT: *Salix atrocinerea*; HTN2: Salix "Terra Nova" (2 year-old coppice); HTN4: salix "Terra Nova" (4-year-old coppice)

[a,b,c]Statistical analysis: one-way ANOVA (PROC GLM, SAS 9.4); post-hoc test: Duncan (α = 0.05) within a column, means without a common superscript differ at the significance level indicated

Table 2 Nutritional composition of willow biomass fractions by genotype (mean ± SD)

Fraction	Species	n	Nutritional composition[a]									
			DM (%)	OM (%)	CP (%)	ADF (%)	NDF (%)	ADL (%)	WSC (%)	NSC (%)	IVDMO (%)	RFV
Leaves	SAT	5	$36.42^{b} \pm 0.88$	$94.60^{a} \pm 0.19$	$17.69^{c} \pm 0.81$	$35.54^{a} \pm 1.90$	$44.40^{a} \pm 2.36$	$18.58^{a} \pm 1.48$	$10.59^{a} \pm 0.52$	$11.18^{a} \pm 0.69$	$38.81^{b} \pm 3.07$	$81.57^{b} \pm 8.83$
	HTN2	5	$29.16^{c} \pm 0.57$	$91.74^{c} \pm 0.21$	$23.73^{a} \pm 0.58$	$33.02^{b} \pm 1.50$	$44.60^{a} \pm 1.19$	$12.72^{c} \pm 0.97$	$5.78^{c} \pm 0.20$	$6.57^{c} \pm 0.49$	$52.75^{a} \pm 0.69$	$110.10^{a} \pm 3.89$
	HTN4	5	$42.40^{a} \pm 4.20$	$93.44^{b} \pm 0.50$	$20.83^{b} \pm 0.71$	$33.12^{c} \pm 0.84$	$41.16^{b} \pm 0.90$	$14.50^{b} \pm 0.37$	$9.04^{b} \pm 0.16$	$10.20^{b} \pm 0.26$	$50.72^{a} \pm 2.41$	$114.65^{a} \pm 5.49$
p-value			< 0.0001	< 0.0001	< 0.0001	0.032	0.0089	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Edible stems (< 5 mm diameter)	SAT	5	$37.26^{b} \pm 0.72$	96.10 ± 2.25	$5.80^{a} \pm 0.48$	$63.36^{b} \pm 2.19$	$72.83^{b} \pm 2.22$	$21.40^{a} \pm 1.02$	$7.31^{a} \pm 0.90$	$7.46^{a} \pm 0.62$	$24.24^{b} \pm 0.93$	$30.98^{b} \pm 4.45$
	HTN2	5	$43.95^{a} \pm 2.31$	97.48 ± 0.16	$3.28^{c} \pm 0.22$	$74.16^{a} \pm 0.62$	$86.70^{a} \pm 1.07$	$16.94^{c} \pm 0.58$	$2.15^{c} \pm 0.24$	$3.70^{b} \pm 0.62$	$11.00^{c} \pm 4.35$	$11.81^{c} \pm 4.71$
	HTN4	5	$43.60^{a} \pm 3.96$	96.28 ± 0.41	$4.86^{b} \pm 0.06$	$57.85^{c} \pm 1.24$	$68.10^{c} \pm 2.4$	$19.80^{b} \pm 0.35$	$5.82^{b} \pm 0.69$	$8.35^{a} \pm 0.82$	$31.16^{a} \pm 1.50$	$42.56^{a} \pm 1.78$
p-value			0.0029	0.2405 ns	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Woody material	SAT	5	44.44 ± 1.21	97.94 ± 0.22	2.94 ± 0.30	70.44 ± 0.70	81.15 ± 0.73	19.5 ± 0.57	5.12 ± 0.58	5.68 ± 1.18	13.18 ± 1.95	15.10 ± 2.87
	HTN2[b]	0	–	–	–	–	–	–	–	–	–	–
	HTN4	5	52.42 ± 2.46	98.36 ± 0.09	2.67 ± 0.05	67.72 ± 2.35	77.68 ± 2.40	18.6 ± 0.47	4.1 ± 0.84	7.51 ± 1.14	21.45 ± 1.18	25.74 ± 2.15
p-value			0.0002	0.0041	0.0831 ns	0.0379	0.0142	0.026	0.055 ns	0.0377	< 0.0001	< 0.0001

[a]Abbreviations: DM (dry matter), OM (organic matter), CP (crude protein), ADF (acid detergent fiber), NDF (neutral detergent fiber), ADL (acid detergent lignin), WSC (water-soluble carbohydrates), NSC (non-structural carbohydrates), IVDMO (in vitro organic matter digestibility), RFV (relative forage value)

[b] In HTN2, no woody material (WO) was obtained because all stems were ≤ 5 mm in diameter (operational threshold for WO in this study); only LE and ES were analyzed

[a,b,c] Different superscript letters within the same biomass fraction and parameter indicate statistically significant differences among willow species according to one-way ANOVA (PROC GLM, SAS 9.4) and Duncan's Multiple Range Test ($\alpha = 0.05$). ns = not significant ($p > 0.05$)

exhibited minimal ES content, with woody material (WO) representing the dominant biomass fraction.

Nutritional value of biomass fractions across willow species

Leaf fraction (LE)

Differences between the two willow materials evaluated significantly affected all nutritional parameters defining leaf quality (Table 2). The leaf fraction showed notably high crude protein content coupled with moderate-low digestibility, resulting in intermediate energy potential as inferred from IVDMO. Leaves from the hybrid clone HTN exhibited significantly higher crude protein (CP) and in vitro organic matter digestibility (IVDMO) than those from SAT. Although fiber content (ADF, NDF) differed significantly among the two willow materials evaluated, the absolute variations were minor. However, HTN2 leaves displayed substantially lower acid detergent lignin (ADL) compared to other species and other age classes. The hybrid clone HTN2 also showed significantly reduced water-soluble carbohydrates (WSC) and non-structural carbohydrates (NSC) relative to both HTN4 and SAT.

Relative Forage Value (RFV) assessments revealed superior quality in HTN leaves, with mean values of 114.6 (HTN4), 110.1 (HTN2), and 81.5 (SAT). According to standard RFV categories (Prime > 150, Premium 125–150, Good 103–124, Fair 86–102, Poor < 86; see Supplementary Table 1), both HTN4 and HTN2 fall within the "Good" forage class, while SAT leaves lie within the "Poor–Fair" range. These RFV values indicate higher predicted intake potential and digestibility for HTN leaves, making them more suitable as supplementary forage, whereas SAT leaves are better suited for maintenance-level feeding.

Table 3 Ensiling characteristics of fresh willow leaves from *Salix atrocinerea* (SAT) and hybrid clone Terra Nova 2-year-old coppice (HTN2). Means ± SD

Parameter[a]	HTN2	SAT
n	5	5
DM (%)	26.44 ± 0.50	38.91 ± 0.10
WSC (%DM)	5.95 ± 0.55	10.38 ± 0.49
BC (meq/kg DM)	248 ± 4.95	157 ± 2.33
FC	28	44

[a]DM: dry matter, WSC: water-soluble carbohydrate, BC: buffering capacity, FC: fermentability coefficient (Weissbach et al. 1974)

Table 4 Nutritional composition and in vitro organic matter digestibility of willow leaf (*Salix atrocinerea* [SAT] vs. *Salix* × Terra Nova [HTN2]) under different conservation treatment (fresh leaves, sun-dried, control-ensiled and additive silage with formic acid addition). Means ± SD

Parameters[a]	Conservation method							
	Fresh leaves		Sun-dried		Control silage		Additive silage	
	HTN2	SAT	HTN2	SAT	HTN2	SAT	HTN2	SAT
n	5	5	5	5	5	5	5	5
DM (%)	26.44 ± 0.50	38.91 ± 0.10	89.77 ± 0.00	91.02 ± 0.00	25.86 ± 0.34	38.4 ± 0.85	27.02 ± 0.68	39.89 ± 0.29
OM (% DM)	91.96 ± 0.05	94.54 ± 0.05	91.78 ± 0.08	94.78 ± 0.13	91.60 ± 0.10	94.46 ± 0.05	92.23 ± 0.24	94.76 ± 0.05
CP (% DM)	24.50 ± 0.70	16.28 ± 0.14	24.25 ± 0.58	17.38 ± 0.38	24.82 ± 0.22	17.62 ± 0.69	27.78 ± 0.58	18.72 ± 0.13
ADF (% DM)	30.62 ± 0.79	41.42 ± 0.33	29.52 ± 0.58	40.64 ± 0.69	32.60 ± 1.90	44.32 ± 0.98	28.75 ± 0.37	39.78 ± 0.81
NDF (% DM)	42.26 ± 2.39	46.78 ± 0.77	40.26 ± 0.34	44.66 ± 1.56	44.74 ± 1.73	50.92 ± 0.80	44.80 ± 0.61	47.60 ± 0.81
ADL (% DM)	10.60 ± 0.48	24.06 ± 0.21	9.76 ± 0.22	22.96 ± 0.36	12.28 ± 1.10	26.26 ± 0.65	10.13 ± 0.15	22.56 ± 0.77
WSC (% DM)	5.95 ± 0.55	10.38 ± 0.49	6.91 ± 0.46	10.49 ± 0.61	2.67 ± 1.01	7.09 ± 0.56	5.70 ± 0.42	9.11 ± 0.65
NSC (% DM)	7.84 ± 0.45	12.45 ± 0.84	7.97 ± 0.70	12.84 ± 0.58	3.75 ± 0.47	7.25 ± 0.74	5.68 ± 0.43	9.28 ± 0.88
IVDMO (%)	56.06 ± 1.48	37.33 ± 1.08	53.00 ± 1.74	33.48 ± 1.43	49.70 ± 2.35	30.00 ± 2.08	53.34 ± 1.06	36.88 ± 0.94
RFV	123.80 ± 9.44	74.22 ± 1.07	122.48 ± 4.78	69.78 ± 3.48	103.43 ± 5.91	54.84 ± 4.26	109.80 ± 1.65	72.09 ± 2.26

[a]DM (dry matter), OM (organic matter), CP (crude protein), ADF (acid detergent fiber), NDF (neutral detergent fiber), ADL (acid detergent lignin), WSC (water-soluble carbohydrates), NSC (non-structural carbohydrates), IVDMO (in vitro organic matter digestibility), RFV (relative forage value)

Table 5 Analysis of variance for the effects of species and conservation method on willow leaf nutritional traits. *P*-values obtained from one-way ANOVA (PROC GLM, SAS 9.4)

Parameters[2]	Source of variation[1]		
	Species	Conservation method	Species × conservation method
	P-value	*P*-value	*P*-value
DM	***	***	***
OM	***	***	***
CP	***	***	***
ADF	***	***	ns
NDF	***	***	ns
ADL	***	***	ns
WSC	***	***	ns
NSC	***	***	ns
IVDMO	***	***	ns
RFV	***	***	**

[1] ns: not significant; *, significant at the 0.05 probability level; **, significant at the 0.01 probability level; ***, significant at the 0.001 probability level

[2]DM (dry matter), OM (organic matter), CP (crude protein), ADF (acid detergent fiber), NDF (neutral detergent fiber), ADL (acid detergent lignin), WSC (water-soluble carbohydrates), NSC (non-structural carbohydrates), IVDMO (in vitro organic matter digestibility), RFV (relative forage value)

Although CP and IVDMO are not components of the RFV formula, their higher values in HTN further support its superior nutritive profile and their potential use as a moderate-energy protein supplement.

Edible stem fraction (ES)

Significant interspecific differences emerged for all chemical composition parameters (except organic matter), particularly for CP and in vitro organic matter digestibility. HTN4 stems demonstrated superior overall nutritional quality compared to SAT, while HTN2 showed exceptionally low IVDMO.

Non-edible fraction (woody material, WO))

The WO fraction demonstrated limited nutritional value with minimal energy potential (IVDMO: 13.28 and 21.45%) and protein value (CP: ~ 3%DM), with only marginal (albeit statistically significant) differences between SAT and the hybrid clone HTN4. Although RFV was originally developed for herbaceous forages and is not strictly applicable to woody biomass, the index was used here for comparative purposes. Both SAT and HTN4 exhibited RFV values far below the "Poor" category (< 86), indicating extremely low predicted intake potential, although HTN4 showed slightly higher values than SAT (25.74 vs. 15.10).

Experiment A2: effect of conservation method on willow leaf nutritional value

Ensilability of fresh leaves

Forage ensilability depends on dry matter (DM), water soluble carbohydrates (WSC) and buffer capacity (BC) values. Table 3 summarizes the ensilability

Table 6 Fermentation parameters of leaf fraction silage from two willow species (*Salix atrocinerea* and hybrid clone Terra Nova in a 2-year-old coppice system) with different ensiling treatments (mean ± SD)

Parameters	Direct-ensiled		Formic-ensiled	
	HTN	SAT	HTN	SAT
n	5	5	5	5
pH	5.56 ± 0.02	5.40 ± 0.08	4.55 ± 0.04	4.50 ± 0.04
Lactic acid (% DM)	0.26 ± 0.06	0.19 ± 0.02	0.16 ± 0.01	0.16 ± 0.01
Acetic acid (% DM)	0.90 ± 0.02	0.16 ± 0.02	0.13 ± 0.02	0.07 ± 0.01
Propionic acid (% DM)	0.01 ± 0.002	0.00 ± 0.00	0.65 ± 0.044	0.37 ± 0.025
Butyric acid (% DM)	0.09 ± 0.038	0.01 ± 0.004	0.01 ± 0.005	0.01 ± 0.004
Ethanol (% DM)	1.78 ± 0.11	1.60 ± 0.14	0.04 ± 0.01	0.06 ± 0.01
Propanol (% DM)	0.08 ± 0.007	0.05 ± 0.009	0.05 ± 0.031	0.04 ± 0.007
Total N (% DM)	3.97 ± 0.11	2.66 ± 0.09	4.57 ± 0.12	2.95 ± 0.05
N-NH3 (% N total)	0.04 ± 0.003	0.01 ± 0.001	0.68 ± 0.058	0.37 ± 0.035
Soluble N (%N total)	0.45 ± 0.01	0.13 ± 0.00	1.04 ± 0.05	0.45 ± 0.04

HTN: *Salix* hybrid clone Terra Nova; SAT: *Salix atrocinerea*

Table 7 Analysis of variance for treatment effects on willow leaf silage fermentation quality. *P*-values obtained from one-way ANOVA (PROC GLM, SAS 9.4)

Fermentation parameters	Source of variation		
	Species	Conservation method	Species x conservation method
	P-value	*P*-value	*P*-value
pH	***	***	*
Lactic acid (% DM)	*	***	*
Acetic acid (% DM)	***	***	***
Propionic acid (% DM)	***	***	***
Butyric acid (% DM)	***	***	***
Ethanol (% DM)	ns[a]	***	*
Propanol (% DM)	*	*	ns[a]
Total N (% DM)	***	***	**
N-NH3 (% N total)	***	***	***
Soluble N (%N total)	***	***	***

[a] ns: not significant; *, significant at the 0.05 probability level; **, significant at the 0.01 probability level; ***, significant at the 0.001 probability level

of leaves (pre-ensiled) from *Salix atrocinerea* and the hybrid clone 'Terra Nova'. HTN2 exhibited higher buffering capacity (248 meq/kg DM) compared to SAT (157 meq/kg DM). Conversely, SAT showed significantly greater fermentability coefficient (FC) values (SAT: 44 vs. HTN2: 28), indicating that the leaves of HTN2 will be more difficult to ensile.

Chemical composition and in vitro digestibility of willow leaves across conservation treatments

Table 4 present descriptive means and Table 5 shows significant interactions for the chemical composition and nutritional parameters. The species × conservation method interaction was significant for DM, OM, CP and RFV (Table 5), indicating that these parameters responded differently to conservation treatments in SAT and HTN2. In SAT, descriptive means (Table 4) show that additive-treated silage (AS) tended to preserve CP and RFV more effectively than sun-drying (SUN), whereas in HTN2 the pattern was reversed, with SUN maintaining higher CP and RFV than AS. These differences reflect the significant interaction detected for these variables, while for the remaining nutritional traits (ADF, NDF, ADL, WSC, NSC, IVDMO) no interaction was detected and the two willow materials responded similarly to conservation method. Formic acid ensiling increased crude protein (CP) content relative to fresh leaves: from 24.50 to 27.78% DM for HTN2, and from 16.28 to 18.72% DM for SAT. The in vitro digestibility decline associated with ensiling was markedly more pronounced in control ensiled (CS) versus AS treatments. For the hybrid clone HTN2, RFV values (Supplementary Table 1) ranged from 103 to 123 depending on treatment, which places all HTN2 conserved materials within the "Good" forage category (103–124). In contrast, SAT leaves consistently presented RFV values between 55 and 75 across treatments, corresponding to the "Poor–Fair" categories (< 86–102). Although crude protein and in vitro digestibility are not components of the RFV formula, their trends parallel the observed RFV differences and further support the superior nutritive potential of HTN compared with SAT.

Silage fermentation quality

The fermentation patterns observed (Table 6) align with the nutritional differences previously described for the two willow materials, particularly the higher crude protein content and lower in vitro organic matter digestibility of SAT compared with the clone HTN. However, despite these nutritional contrasts, clear species-dependent differences in fermentation behavior emerged (Table 7). SAT silages demonstrated superior preservation characteristics compared to the HTN2. SAT produced silages with significantly lower pH values (4.7 vs. 5.1), lower concentrations of fermentation end-products (e.g., lactic acid: 3.2% vs. 5.8% DM; acetic acid: 1.1% vs. 2.4% DM), and reduced ammoniacal N (8.4% vs. 12.1% of total N) and soluble N (32.6% vs. 38.9% of total N), indicating lower proteolytic activity during fermentation.

The addition of formic acid (AS treatment) consistently enhanced preservation quality in both willow materials, reducing silage pH from > 5.0 in direct-ensiled (CS) controls to approximately 4.5. However, all treatments exhibited limited fermentation activity, as reflected by the limited accumulation of lactic and acetic acids, suggesting inherent resistance of willow leaves to anaerobic degradation across conservation method. The differential response between SAT and HTN2 suggests that species-specific biochemical

properties, linked to their contrasting fermentability coefficient values, may influence fermentation dynamics more strongly than preservation methods alone. SAT, with lower buffering capacity and higher WSC, achieved more favorable fermentation profiles, while HTN2 demonstrated more restricted acidification, consistent with its poorer fermentability coefficient.

Discussion

The nutritional composition of the fresh willow biomass analyzed aligned with those reported in the literature for other *Salix* spp. species (Kemp et al. 2001, 2003; Oppong et al. 2001; Luske et al. 2017; Luske and van Eekeren 2018). Among the different fractions of fresh willow biomass, the leaf fraction (LE) exhibited the highest nutritional value, with elevated crude protein levels (CP: 23.73% DM for HTN2, 20.83% DM for HTN4, and 17.69% DM for SAT), and moderate digestibility for HTN (IVDMO: 52.75% for HTN2 and 50.72% for HTN4) and low digestibility (38.81%) for SAT. The contrasting digestibility patterns observed between HTN2 and HTN4 edible stems can be explained by differences in shoot maturity and structural development under SRC management. The HTN clone has been specifically selected in breeding programs for strong juvenile vigor, rapid regrowth capacity, and relatively lower lignification compared with unmanaged willow materials (Kuzovkina and Volk 2009). However, in its second-year post-coppice (HTN2), stems may undergo an early and accelerated phase of structural thickening, characterized by a rapid increase in cell wall deposition and the formation of primary walls rich in less accessible cellulose. This developmental stage can temporarily reduce stem digestibility despite moderate lignin concentrations. In contrast, the older HTN4 stems displayed a more favorable anatomical configuration, with a higher leaf-to-stem ratio and a more advanced differentiation of supporting tissues, resulting in improved IVDMO values compared with both HTN2 and SAT. Similar age-dependent effects on stem nutritive value have been reported for willow by Oppong et al. (2001) and undefined 2020). These ontogenetic patterns likely account for the unusually low stem digestibility observed in HTN2, while explaining why HTN4 maintained superior forage quality relative to the native species.

The Relative Feed Value (RFV) is a standardized index that integrates key nutritional factors (intake potential and digestibility) into a single value. This provides a rapid and reliable assessment tool for evaluating forage quality (Rohweder et al. 1978; Linn and Martin 1991). Based on the RFV ranges proposed by these authors (Supplementary Table 1), the leaf fraction qualifies as "Good" forage (HTN) or "Poor–Fair" forage (SAT), while edible stems and especially woody material fall consistently within the "Poor" category (< 86), confirming their limited feeding value. These results align with those reported by other authors (Oppong et al. 2001). The relatively low cell wall content (NDF: 40–45%) yielded an RFV comparable to medium-quality alfalfa (RFV 110–120), while crude protein levels substantially exceeded typical alfalfa values (16–20% DM). Although RFV does not incorporate crude protein, the CP concentrations observed in SAT (17.7% DM) fall within ranges typical for moderate-quality forages, whereas HTN2 and HTN4 (> 20% DM) show protein levels comparable to high-quality hays. These protein values, combined with RFV classifications ("Good" for HTN and "Poor–Fair" for SAT), further illustrate the superior nutritive potential of HTN foliage. The leaf lignin values (ADL 12–18% DM) in willow were significantly higher than those typically observed in conventional forage crops, while non-structural carbohydrate content (NSC 6–11% DM; WSC 5–10% DM) was substantially lower, reflecting fundamental differences in cell wall composition and energy reserves.

The woody material (WO) and edible stem (ES) fractions exhibited significantly higher values for DM, ADF, NDF, and ADL, coupled with lower CP, WSC, NSC, IVDMO, and RFV compared with the leaf fraction (LE). These findings align with studies on other *Salix* species, where leaves consistently showed lower fiber content and higher in vitro digestibility than stems (Muklada et al. 2021). Willow forage quality followed an anatomical gradient (leaves > thin stems > thick stems) and this suggests willow forage quality is maximized during early growth stages, when stems remain slender and leaf proportion is maximized (Larsen et al. 2020). However, despite this gradient, both edible stems (ES) and woody material (WO) fell within the lowest forage category based on RFV, contributing limited nutritional value

compared with leaves. This highlights an important practical trade-off: harvesting leaves alone would maximize forage quality but is labor-intensive and technically demanding, particularly under field conditions, whereas harvesting whole shoots substantially increases harvest efficiency and biomass yield but inevitably reduces overall forage quality due to the inclusion of high-fiber stem fractions. These considerations illustrate the need to balance nutritional objectives with operational feasibility when integrating willow into fodder systems.

Multiple studies (Oppong et al. 2001; Luske and van Eekeren 2018; Larsen et al. 2020; 2025); Bonizzi et al. 2025) have demonstrate that both harvest season and stem maturity significantly influence willow forage quality. Seasonal progression during the growing period typically results in a decline in crude protein (CP) concentration and a concomitant increase in dry matter (DM), patterns that align with the characteristics of our experimental material. In our study, material for Experiment A1 was harvested in early June, whereas Experiment A2 was collected in mid-July. It should be noted that Experiments A1 and A2 were conducted in early June and mid-July, respectively. Although the experimental design did not include replicated harvest dates and was not intended for formal temporal comparison, the cited phenological changes may partly explain the lower WSC levels and limited fermentation intensity observed in the July material used in A2.

Therefore, the seasonal context should be viewed as interpretative rather than comparative, yet it provides valuable insight into the mechanisms underlying the observed differences between experiments. As highlighted by Wangui et al. (2024), harvest timing is a key management lever for maximizing forage protein content, with early growing season harvests providing the highest nutritional value. Recent results from Larsen et al. (2025) further illustrate the inherent trade-off between biomass yield and quality in willow and poplar: early harvests produce protein-rich but lower-yielding biomass, whereas late-season harvests increase yield at the expense of feeding value. These temporal patterns in chemical composition and digestibility are driven by the phenological development and climatic adaptation of woody species. Consequently, selecting species-specific harvest windows adapted to local agroclimatic conditions becomes essential for quality forage production.

The observed species-specific differences in leaf forage quality align with prior studies demonstrating interspecific variability in willow nutritive value (Oppong et al. 2001; Mahieu et al. 2021). The hybrid willow clone HTN exhibited superior nutritional quality compared to SAT, showing higher crude protein content, greater in vitro organic matter digestibility (IVDMO). Management practices represent a critical determinant of willow biomass composition, independent of seasonal and genetic factors, Smith et al. (2014) established that trees managed under short rotation coppicing (SRC) systems produce greater amounts of young growth characterized by higher leaf-to-stem ratios and reduced lignin content, whereas mature tree canopies show reduced nutritional potential. In our study, while *Salix atrocinerea* branches exhibited higher leaf proportions, the coppiced hybrid 'Terra Nova' displayed superior forage quality indicators—including higher crude protein content (18.2% vs. 14.7%), lower lignin (ADL 11.4% vs. 15.8%), and greater relative feed value (RFV 125 vs. 98) compared to unmanaged specimens. These differences reflect distinct practical applications: HTN foliage serves as an effective protein supplement for moderate-energy, whereas SAT is limited to maintenance diets due to its lower digestibility. Therefore, this evidence indicates that SRC management can optimize the nutritional profile of select willow clones beyond their inherent leaf-stem allocation patterns.

Studies in the UK (Smith et al. 2014) have evaluated forage production from the edible fraction of *Salix* spp. biomass in integrated agroforestry systems designed for dual-purpose forage and bioenergy production. Their findings indicate potential applicability in beef cattle and sheep feeding regimes, though the naturally low water-soluble carbohydrate (WSC) content in willow may limit successful fermentation. In our case, regarding ensilability, the fermentation coefficient (FC) was substantially higher in SAT foliage compared to HTN2. This superiority in SAT can be attributed to (1) higher dry matter content, (2) higher water-soluble carbohydrate levels, and (3) lower buffering capacity in its leaves compared to the HTN. According to Weissbach et al. (1974), FC values >45 indicate easily ensilability. SAT leaves, with FC values approaching this critical threshold, therefore, they demonstrate inherently better ensiliability

characteristics than HTN2, thereby enabling optimal silage fermentation.

The silage analyses revealed crude protein (CP) contents ranging from 17.62 to 27.78% DM, neutral detergent fiber (NDF) from 44.74 to 50.92% DM, and in vitro organic matter digestibility (IVDMO) from 36.88 to 53.34% (Table 5). The relative energy potential of willow is limited by its low IVDMO values, which renders it nutritionally insufficient as a primary feed source for high-performance ruminants; however, it should be noted that our use of "energy potential" refers exclusively to digestibility-based indicators (i.e., IVDMO-derived assessments) rather than to calculated metabolizable energy (ME). Nevertheless, these findings align with Smith et al. (2014), who reported similar limitations for *S. viminalis* silage (42% Organic matter digestibility and 18% CP). In our study, formic-acid-treated HTN2 silage exceeded these benchmarks while SAT silage achieved comparable values.

Regarding conservation methods for fresh willow leaves, sun-drying—as anticipated—significantly increased dry matter (DM) content, thereby enhancing forage stability against microbial degradation. Our results indicate that, when considering both willow materials together, sun-drying most effectively preserved the original nutritional value of fresh willow leaves, followed by formic acid-assisted ensiling. However, the significant species × conservation method interaction (Table 5) showed that this general pattern did not apply equally to both species. In SAT, formic acid-treated silage resulted in slightly higher crude protein and RFV values than sun-dried leaves, whereas in the hybrid clone HTN2 the opposite trend was observed, with sun-dried material retaining superior nutritive value. These contrasting responses explain the apparent discrepancy and highlight the species-specific nature of preservation outcomes.

Ensiling did not substantially alter leaves´ chemical composition, corroborating findings by Muklada et al. (2021) and Steffen et al. (2024), who reported successful willow forage ensiling without drastic quality loss. However, both ensiling methods reduced leaf digestibility, particularly in untreated silage, consistent with results from Larsen et al. (2024) where in vitro organic matter digestibility (IVDMO) declined by 19–31% compared to fresh biomass. Previous studies on willow silage (Smith et al. 2014; Larsen et al. 2020; 2024); Muklada et al. 2021; Steffen et al. 2024) highlight this genus's inherently poor ensilability, evidenced by persistently elevated pH in untreated silage. Our findings corroborate these observations, suggesting that the low water-soluble carbohydrate (WSC) content in willow leaves likely explains its suboptimal fermentation process. Both our findings and prior works (Muklada et al. 2021; Larsen et al. 2024) highlight the critical role of additives in achieving adequate acidification (target pH ≤ 4.0) for effective willow silage preservation.

The interaction between willow species and preservation method revealed that formic acid-treated silage of SAT leaves showed slightly superior forage value compared to sun-dried leaves from the same species—a pattern not observed in the HTN material. This differential response may be attributed to SAT's higher water-soluble carbohydrates (WSC) content compared to HTN.

For optimal silage preservation, a dry matter (DM) content ≥ 30% is required to inhibit clostridial activity and reduce energy and nitrogen losses compared to fresh forage (Borreani et al. 2018). In this study, the mean leaf DM content of hybrid HTN (26.4%) fell below this optimal threshold, whereas SAT (39.9%) met the target DM level for optimal preservation by ensiling. Consequently, the fresh leaves of SAT demonstrated superior fermentation quality, exhibiting significantly lower pH and ammonia-N value than HTN. Despite these differences, all silage treatments displayed consistently low fermentation intensity. For HTN, the low WSC content (5.9% DM) in fresh leaves likely restricted fermentation. Although SAT contained substantially higher WSC levels (10.3% DM), both species showed remarkably similar fermentation profiles. This unexpected convergence indicates that willow silage fermentation is constrained by factors other than WSC availability. The uniformly low fermentation activity across treatments could alternatively stem from the 30-day anaerobic storage period, which—although sufficient for conventional forages—proves inadequate for willow biomass, presumably due to its distinct biochemical properties.

Notably, published studies on willow ensiling report required storage durations ranging from 42 days (Steffen et al. 2024) to several months (Smith et al. 2014; Muklada et al. 2021; Larsen et al. 2024). The limited fermentation observed in our study resulted in significantly lower concentrations of

fermentation-derived acids compared to conventional ruminant forage silages. Conversely, ammonia-N and soluble N levels—key indicators of proteolytic activity during ensiling—remained well below typical values. According to INRA (2023), normal ranges are 5–8% of total N for N–NH_3 and around 50% of total N for soluble N. These lower values suggest well-preserved protein quality in willow leaf silage, likely due to restricted fermentation processes.

Contrary to established patterns in other forage silages—where formic acid addition typically reduces protein degradation (Borreani et al. 2018; Muck et al. 2018), formic acid-treated willow leaf silage exhibited increased proportions of total N as ammonia-N and soluble N compared to untreated controls. This paradoxical response underscores the fundamentally distinct biochemical behavior of willow during ensiling and likely reflects protein–tannin interactions that limit proteolysis. This interpretation is consistent not only with previous findings in willow silages, where condensed tannins form stable complexes that reduce ammonia-N formation (Mueller-Harvey 2006); Larsen et al. 2020; Steffen et al. 2024), but also with broader research showing that both condensed and hydrolysable tannins suppress proteolytic activity across diverse forage species (Jayanegara et al. 2019; Ke et al. 2022).

The low concentrations of acetic and propionic acids also observed in willow leaf silages—both critical for suppressing aerobic spoilage microorganisms- suggest inherent aerobic instability, particularly in untreated silage. This metabolic profile indicates that supplementation with homo- or heterofermentative lactic acid bacteria (LAB) inoculants could potentially enhance both fermentation efficiency and aerobic stability, thereby improving post-opening silage preservation after opening (Muck et al. 2018). Furthermore, to fully evaluate willow's potential as forage, future research should investigate qualitative parameter evolution during ensiling, including production of volatile compounds that may influence silage palatability.

This study compared willow materials that were harvested from two contrasting environments and management systems (a short-rotation coppice plantation for HTN and a naturally occurring riparian stand for SAT). As a consequence, the differences observed between HTN and SAT cannot be attributed solely to species effects, but rather to the combined influence of genotype, site conditions and management history. These constraints are inherent to the real availability of willow biomass resources in the region, but they should nonetheless be considered when interpreting the results. Future studies should therefore evaluate both materials under controlled and identical agronomic conditions to allow a fully standardized species-level comparison.

The livestock sector's growing requirements demand comprehensive research to identify optimal dietary nutrients that simultaneously (1) safeguard animal health, (2) maximize product quality, and (3) minimize environmental impact. Within this framework, securing sustainable local protein sources has emerged as a critical priority for modern animal production systems. Our findings show that willow foliage represents a viable supplemental protein source, with silages reaching crude protein concentrations of 18–22% DM, and that additive-assisted ensiling is an effective strategy to preserve its nutritional value. Further research should evaluate long-term intake responses and palatability across ruminant species to determine its practical feeding relevance.

Willow is a tannin-containing tree fodder (Oppong et al. 2001; Luske and van Eekeren 2018), and although this study did not characterize these compounds, their potential roles in protein protection, nitrogen utilization and rumen fermentation warrant further attention. Future research should therefore quantify the composition and stability of condensed tannins and other phenolic metabolites across conservation methods, and assess their functional effects on ruminal metabolism. Such work will be essential to better define the nutraceutical and climate-smart potential of willow foliage within sustainable livestock feeding strategies.

Conclusions

Although the leaf fraction (LE) represented < 50% of total stem biomass in the studied willow materials, they emerged as the most nutritionally valuable component, combining high crude protein with moderate-to-low energy potential as inferred from IVDMO. By contrast, edible stems (ES) and woody material (WO) showed substantially lower nutritional quality. Significant interspecific variation was observed, the

hybrid clone HTN (SRC) exhibited higher leaf CP and higher IVDMO than the native SAT (riparian origin), indicating superior leaf nutritive quality in the SRC material.

Regarding preservation of the leaf fraction, sun-drying best retained the original nutritional value, followed by formic-acid–assisted ensiling, whereas direct ensiling was the least effective. These findings demonstrate that additive use is essential for willow silage production, as limited fermentation intensity may compromise aerobic stability.

Looking ahead, future research should focus on management strategies to increase leaf-to-stem ratios (e.g., harvest timing) and on the role and preservation of bioactive compounds (such as condensed tannins, phenolics and salicin) in relation to their practical use in ruminant nutrition.

In summary, willow should be considered a multipurpose tree resource for integration into silvopastoral systems, where its leaf fraction can complement ruminant diets while contributing ecosystem services (e.g., riparian buffering, shade, biodiversity support, and carbon sequestration). Willow's value lies in its climate-smart functionality as part of diversified agroforestry designs, providing locally available protein from foliage without compromising the species' other functions.

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Data availability The data that support the findings of this study are available from the authors upon reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval No animals were directly used in the experiments presented in this study. However, the in vitro digestibility assays (Tilley & Terry, 1963; Alexander & McGowan, 1966) required rumen inoculum obtained from two rumen-cannulated donor cows maintained under institutional care at the Centro de Investigaciones Agrarias de Mabegondo (CIAM), Spain. The collection and use of rumen fluid from these donor animals were covered by institutional ethical approval (Protocol Nº 2016/01) and cow care and handling were performed by trained personnel according to the instructions for experimental animal protection included in Spanish legislation.

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