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Can tree leaves be an alternative source of feed for dairy ruminants?

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Abstract The importance of agroforestry systems is increasing as they promote sustainable agricultural practices to address climate change and food security. The study aimed to assess the potential of tree species as feed ingredients for dairy ruminants. Leaves from five tree species—*Fraxinus excelsior* L. (common ash), *Morus nigra* L. (black mulberry), *Robinia pseudoacacia* L. (locust tree), *Salix babylonica* L. (weeping willow) and *U. minor* Mill. (field elm)—were sampled in three different times in spring–summer season 2023 on a farm in Northern Italy. Proximate composition, analyses along with in vitro analyses, were conducted to determine net energy of lactation (NE_L) by gas production and fibre digestibility (NDFD). Results found wide variability among species for chemical and nutritive value. Fibre content (% DM) was highest in *U. minor* (57.2) and lowest in *M. nigra* (34.5), whereas crude protein (% DM) was highest in *R. pseudoacacia* (22.4), followed by *S. babylonica* and *M. nigra* (20.3, on average), *U. minor* (15.1) and *F. excelsior* (14.0). *Morus nigra* had the highest NDFD (75.3%) and NE_L (5.66 MJ/kg DM). Intermediate NE_L values were recorded for *F. excelsior* and *S. babylonica* (4.50 MJ/kg DM, on average), the lowest values in *U. minor* and *R. pseudoacacia* (3.90 MJ/kg DM, on average). The study indicated that most of the examined tree species can be used as dietary supplements due to their nutritional properties as they maintain their quality throughout the growing season. *Morus nigra* emerges as the most promising species due to its superior nutritive value.

Keywords Tree leaves · Dairy cattle · Circular economy · Feeding value

Introduction

The integration of crops and livestock systems with woody species on the same land unit can promote ecological intensification and the synergic use of resources and inputs (Paris et al. 2019a). Moreover, fodder trees and shrubs on dairy cattle farm can provide additional feed rich in macro and micronutrients (Luske et al. 2017a). To address global climate change and food security while maintaining or improving the environment, international researchers and policy makers are increasingly promoting agroforestry (Paris et al. 2019b). This interest is aimed

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in harnessing the multiple benefits of agroforestry. Literature highlights the positive effects of these systems in reducing pollutants in runoff water, decreasing nitrogen leaching and increasing carbon sequestration (Burgess and Rosati 2018; Paris et al. 2019b; Kendall et al. 2021). Thanks to their life-form deep root system, trees can maintain the high nutrient quality of foliage even during dry periods, when herbage quality decreases. Trees modify microclimatic conditions, including solar radiation, temperature, humidity, and wind speed, which can have beneficial effects on ecosystem functions and contribute to forage growth and animal welfare. Additionally, trees can provide alternative sources of both macro- and micronutrients for livestock (Emile et al. 2016; Kendall et al. 2021; Novak et al. 2017; Vandermeulen et al. 2018), and alternative feed during periods of low forage availability (Kendall et al. 2021; Vandermeulen et al. 2018). Woody species with low to moderate tannin concentrations could be beneficial for animal health and performance, limiting protein rumen degradability, reducing methane production, defending against bloat and mitigating gastrointestinal parasites (Ravetto Enri et al. 2020; Hassanat and Benchaar 2013; Mahieu et al. 2021; Vandermeulen et al. 2018).

Nevertheless, besides all these beneficial effects, the incorporation of tree fodder in animal diets might be limited by low digestibility, low palatability or potential toxicity (Vandermeulen et al. 2018). To fully integrate and utilize the forage potential of tree leaves, their nutritional composition must be comparable to grazed swards (Kendall et al. 2021). However, while a considerable number of studies have investigated the nutritional value of leaves and twigs from shrub and tree species in tropical and subtropical climates, particularly in low-income countries (Gebeyew 2015; Simbaya et al. 2020), research on tree leaves from temperate climates has generally been limited to chemical composition analysis and the determination of dry matter or organic matter digestibility (Emile et al. 2016; Luske and Van Eekeren 2014; Mahieu et al. 2021; Parissi et al. 2018; Smith et al. 2018).

This study aims to bridge this gap by providing a more comprehensive assessment. Specifically, it investigates the leaves of five common temperate tree species at three distinct stages of the growing season, integrating comprehensive chemical composition analysis with gas and methane production, fibre digestibility evaluations and net energy for lactation. By offering a detailed insight into seasonal variations in leaf composition, this approach provides essential data to assess the feasibility of incorporating tree leaves into dairy animal diets. Aligned with the objectives of the DairyMix Project (https://www.dairymix.eu/), this study explores the potential of tree species as a complementary feed source for dairy cattle, to promote land-use diversification and sustainable farming in the intensive dairy sector.

Materials and methods

Study farm

The study was conducted at the educational-experimental farm "Angelo Menozzi" (45°19′11"N 9°15′56"E, 90 m a.s.l.) of the Milan University Farms Functional Centre (CFAA), located in Landriano (Pavia) in the southwestern Lombardy, Northern Italy, one of the six case study farms of the Dairymix project in Italy. The climate is classified as *Cfa* according to Köppen's classification, which denotes a humid temperate climate with hot summers and no dry season.

Samples and tree tested

Initially, all the trees on the farm were identified and mapped. These trees were not planted specifically for silvopastoral purposes but are considered Trees Outside the Forest (TOF), found either as scattered individuals or in rows along field boundaries, canals, and roads. Based on a literature review (Luske et al. 2017b), the five most promising species, in terms of nutritive value, were selected. The study focused on the following species: *Fraxinus excelsior* L. (common ash), *Morus nigra* L. (black mulberry), *Robinia pseudoacacia* L. (black locust), *Salix babylonica* L. (weeping willow), and *Ulmus minor* Mill. (field elm). Leaves (*M. nigra, S. babylonica* and *U. minor*) or leaflets (*F. excelsior* and *R. pseudoacacia*), including petioles, were manually harvested at three different times during 2023: May 31, July 13, and September 5. corresponding to 110, 140 and 210 Cumulative Growing Degree Days (GDD) (Prislan et al. 2013; ARPA 2024) The plants selected for sampling were mature (25–55 years old based on farm information), fully developed according to the typical architecture of the species, in good health, and exhibiting good vegetative vigor. Leaf samples were collected in duplicate from 3 randomly selected plants per species, covering all orientation of the canopy.

Proximate, mineral and tannins composition of tree leaves

Approximately 1.5 kg of fresh leaves from each species and each harvest were collected, transported in a refrigerated container to the University laboratory, oven-dried at 55 °C until a constant weight was achieved, and ground to 1 mm using a Wiley mill (Pulverisette 19, Fritsch, Idar-Oberstein, Germany). The following analyses were performed:

- Dry Matter (DM) (AOAC Official Method 934.01, 2023)
- Ash (AOAC Official Method 942.05, 2023)
- Neutral Detergent Fibre (NDF): corrected for insoluble ash and with the addition of α-amylase (aNDFom; Mertens 2002)
- Acid Detergent Fibre (ADF): Van Soest et al. (1991), using the Ankom 200 fibre analyser (Ankom Technology Corp., Fairport, NY)
- Acid Detergent Lignin (ADL): Van Soest (1963)
- Crude Protein (CP): Dumas AOAC Official Method 990.03 (2023) using Rapid MAX N Exceed (Elementar Analysensystem GmbH, Langenselbold, Germany)
- Starch: AOAC Official Method 996.11 (2023)
- Soluble Sugars: Hall Method (Hall 2014)
- Ether Extract: AOAC Official Method 920.39 (2023)

For mineral determination, dried samples were weighted and digested using a microwave digestion system (Anton Paar MULTIWAVE-ECO) in Teflon tubes with 10 mL of 65% HNO_3 applying a one-step temperature ramp: the temperature increased to 175 °C in 20 min and maintained for 10 min. After a cooling time of 20 min, the mineralized samples were transferred to polypropylene test tubes, diluted 1:100 with a 1.3 M HNO_3 solution in MilliQ water and analysed by ICP-MS (AGILENT 7850 ICP-MS). A 200 µg L-1 internal standard solution (45Sc, 72Ge, 89Y, 103Rh, 159 Tb, 165Ho) was introduced before the nebulizer in the sample flow. Interferences were removed by a Collision Cell with He at 5 ml min-1.

Total phenols (TP) and total tannins (TT) contents were determined according to the Folin-Ciocalteu method as reported by Makkar (2003a).

In vitro gas production, fermentative profile and nutritive value

Rumen fluid was collected from three dry cannulated cows housed in the experimental farm “Cascina Baciocca”, of the Milan University Farms Functional Centre (Cfaa) located in Cornaredo (Milan, Italy). The donor animals were handled as outlined by the Directive 2010/63/EU on animal welfare for experimental animals and all animal procedures were conducted under the approval of the University of Milan Ethics Committee for animal use and care and with the authorization of the Italian Ministry of Health (authorization no. 79/2022-PR). The cows were fed a diet of 75% grass hay and 25% commercial concentrate. Rumen liquor was collected 2 h after the morning feeding, strained through 4 layers of cheesecloth, poured into a flask, pre-warmed at 39 °C, and purged with CO_2. The buffer solution was prepared according to Menke and Steingass (1988). An amount of 300 mg of each sample was weighed in duplicate into serum bottles (120 mL), and each bottle inoculated with 40 mL of rumen inoculum and insufflated with CO_2 for 24 h. Standard samples with known gas production (GP) were also added for the computation of net energy of lactation (NE_L). Incubation was performed according to Battelli (2023). The CH_4 concentration was determined according to Battelli et al. (2023) using a Varian 3800 gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA, USA). The concentrations of short chain fatty acids (SCFA) were determined using a Varian CP-3800 gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA, USA), as reported in Battelli et al. (2024).

For the determination of ruminal digestibility and NE_L, the method of Menke and Steingass (1988) was used. The digestibility of NDF (NDFD) was evaluated after 48 h of in vitro incubation of the leaf samples using the DaisyII incubator jars (Ankom Technology, Macedon, NY, USA) following Spanghero et al. (2010). All the in vitro incubations were conducted in three runs.

NE_L and OMD (organic matter digestibility) values were calculated according to Menke and Steingass (1988):

$$NEL\left(\frac{MJ}{kgDM}\right) = 0.54 + 0.0959 * GP + 0.0038 * (CP\% * 10) + 0.0001733 * (EE\% * 10)^2$$

$$OMD = 15.38 + 0.8453 * GP + 0.0595 * (CP\% * 10) + 0.0675 * (Ash\% * 10)$$

Statistical analyses

The whole dataset was analysed using SAS software (Version 9.4, 2012; SAS Institute Inc.). The MIXED procedure was used to evaluate the effect of tree species, harvest times, and their interaction (Model 1) with the following model 1.

Model 1:

$$Y_{ijk} = \mu + S_i + H_j + FR_l + +SxH_{ij} + e_{ijk}$$

where: Y_{ijk} = dependent response variables, μ = general mean, S_i = tree species effect (i = *F. excelsior*, *M. nigra*, *R. pseudoacacia*, *S. babylonica*, *U. minor*), H_j = harvest time effect (j = 1—3), FR_l = field sample replication (l = 1—2), $(S \times H)_{ij}$ = interaction between tree species and harvest time, e_{ijk} = residual error.

For the in vitro analyses, the same model was used but the random effect of Run (n = 1–3) was added to the model.

Relationships among variables, related to chemical analysis and different tree species, were investigated through Pearson correlation and Multiple Correspondence Analysis (MCA). Variables were categorized into two levels, low and high, using the mean as the threshold.

Results and discussion

Proximate and mineral composition of tree leaves

Table 1 presents the average values of the proximate composition of the five tree species, across the three different harvesting times. The results of DM content highlight significant differences among the tree species studied ($p < 0.001$). The highest average value (across the three harvests) was found in *U. minor* (40.5%), whereas the lowest was observed in *M. nigra* (28.3%). There was a significant species*harvest interaction ($p < 0.001$); on average DM tended to increase with the sampling date except for *U. minor* and *R. pseudoacacia* which had an opposite trend. Regarding CP, a significant species effect was observed ($p < 0.001$), with some species showing average values (% DM) across the three harvests equal or higher than 20%, such as *R. pseudoacacia* (22.4), *S. babylonica* (20.0) and *M. nigra* (20.5), making them potential alternative to other common forage crop such as lucerne in ruminant diets. There was a significant species*harvest interaction for CP ($p < 0.001$); the CP content tended to decrease with the harvest time in *F. excelsior* and *M. nigra*. This agrees with Parissi et al. (2018) who analysed, among the others, *R. pseudoacacia* and *M. alba* leaves and stems in spring and autumn. The CP content of the second harvest of *R. pseudoacacia* showed the highest value compared to the other harvests. This difference in CP dynamics among species emphasizes the importance of considering species-specific harvest strategies to optimize the nutritional quality of tree foliage for animal consumption, especially for the species characterized by the higher CP content. The fibrous fractions (aNDFom, ADF and ADL) demonstrated a significant species-related effect ($p < 0.001$), with no significant influence of harvest or species*harvest interaction. The aNDFom content varied from 57.2% in *U. minor* to 34.5% in *M. nigra.* The difference in fibre content has important implications for feed formulation and animal nutrition. For instance, *M. nigra*, with its lower aNDFom, might be a more suitable feed source for animals requiring higher energy intake, while *U. minor*, with its higher aNDFom, could be more appropriate for animals with lower energy requirements such as dry cows or heifers. The ADF and ADL contents showed similar trends (only species effect), with the highest values in *S. babylonica* and *R. pseudoacacia*, and the lowest value in *M. nigra* which showed the lowest fibrous fraction contents. The high ADL content observed in

Table 1 Proximate composition (% on DM) of the five tree species across the three harvest times, expressed as LSMEANS (% of DM unless differently stated)

Sample	Harvest	DM %	ASH	aNDFom	ADF	ADLom	CP	Soluble sugars	EE	NFC
Fraxinus excelsior L Common ash	1st harvest	35.3^{c}	9.73^{ef}	40.4^{de}	32.5^{bcdef}	16.2^{bc}	15.9^{ef}	4.63^{cde}	2.00^{efg}	32.0^{ab}
	2nd harvest	41.4^{a}	7.55^{hi}	39.6^{de}	29.2^{def}	10.2^{def}	12.5^{h}	4.60^{cde}	3.20^{bcd}	37.2^{a}
	3rd harvest	41.2^{a}	8.10^{h}	39.1^{de}	28.6^{ef}	10.1^{ef}	13.6^{gh}	3.65^{def}	4.67^{a}	34.5^{a}
Morus nigra L Black mulberry	1st harvest	24.3^{g}	14.6^{c}	34.5^{ef}	26.6^{f}	7.53^{f}	23.1^{a}	7.25^{a}	1.44^{g}	26.3^{bc}
	2nd harvest	30.6^{ef}	16.6^{b}	36.7^{ef}	29.4^{def}	6.71^{f}	19.0^{d}	5.97^{abc}	2.25^{efg}	25.5^{bcd}
	3rd harvest	29.9^{ef}	20.0^{a}	32.3^{f}	28.5^{ef}	5.97^{f}	19.5^{cd}	5.10^{bcd}	2.18^{efg}	26.0^{bcd}
Robinia pseudoacacia L Black locust	1st harvest	35.9^{c}	5.97^{j}	44.3^{cd}	34.5^{abcde}	21.6^{a}	21.4^{b}	3.36^{def}	2.81^{bcde}	25.5^{bcd}
	2nd harvest	33.2^{d}	7.54^{hi}	46.8^{bc}	36.9^{abc}	23.5^{a}	24.2^{a}	1.80^{f}	2.71^{bcde}	18.8^{ef}
	3rd harvest	35.0^{c}	7.22^{i}	44.9^{cd}	32.3^{bcdef}	19.7^{ab}	21.5^{b}	3.00^{ef}	3.53^{b}	22.8^{cde}
Salix babylonica L Weeping willow	1st harvest	29.5^{f}	9.5^{fg}	52.1^{a}	38.9^{ab}	20.7^{ab}	20.6^{bc}	7.33^{a}	2.47^{def}	15.4^{fg}
	2nd harvest	30.7^{e}	9.2^{fg}	48.5^{bc}	39.8^{a}	20.2^{ab}	20.3^{bcd}	6.70^{ab}	2.51^{cdef}	19.5^{def}
	3rd harvest	32.5^{d}	8.9^{g}	47.7^{bc}	37.9^{ab}	18.6^{abc}	19.1^{cd}	5.20^{bcd}	2.40^{def}	21.9^{cdef}
Ulmus minor Mill Field elm	1st harvest	41.4^{a}	10.2^{e}	52.6^{b}	31.1^{cdef}	14.2^{cde}	14.0^{g}	7.19^{a}	1.75^{fg}	21.4^{cdef}
	2nd harvest	41.2^{a}	12.9^{d}	59.3^{a}	33.3^{abcde}	14.4^{cde}	16.5^{e}	5.06^{bcd}	2.12^{efg}	9.20^{gh}
	3rd harvest	39.0^{b}	14.3^{c}	59.7^{a}	35.3^{abcd}	15.5^{bcd}	14.7^{fg}	4.04^{cde}	3.33^{bc}	7.97^{h}
Standard error of LSMEANS		0.39	0.21	1.96	2.20	1.77	0.48	0.66	0.27	2.17
p-value species		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
p-value harvest		<0.001	<0.001	0.4509	0.6519	0.2080	0.0024	0.0026	<0.001	0.3244
*p-value species*harvest*		<0.001	<0.001	0.2008	0.6331	0.4621	<0.001	0.3976	0.0077	0.0083

^{abcdefghij}Different letters on the same column within the species correspond to different Least Squares Means ($p<0.05$)

DM = Dry Matter

aNDFom = Neutral Detergent Fibre corrected for insoluble ash and using α-amylase; ADF = Acid Detergent Fibre; ADLom = Acid Detergent Lignin corrected for insoluble ash

CP = Crude Protein; EE = Ether Extract

NFC = Non-Fibrous Carbohydrates

S. babylonica and *R. pseudoacacia* raises concerns about protein availability; therefore, despite potentially adequate crude protein levels in these species, a significant portion of that protein may be bound to lignin and thus unavailable for digestion. This finding highlights a critical consideration for feed formulation: while *M. nigra* appears promising due to its low fibre content, *S. babylonica* and *R. pseudoacacia*, despite other beneficial characteristics, may require specific processing or supplementation strategies to improve protein utilization and overall nutritional value. Regarding starch content (data not reported in the table) there was a significant species effect ($p < 0.001$), although the values were overall low. The highest average value (% DM) across the harvests was observed in *F. excelsior* (6.79) followed by *U. minor* (4.36), *M. nigra* (4.23), *S. babylonica* (3.69), and *R. pseudoacacia* with the lowest value (2.82). For soluble sugars, *S. babylonica* and *M. nigra* had the highest sugar content and were statistically similar, while *R. pseudoacacia* showed the lowest value. Although the species*harvest interaction was not significant, the harvest effect was significant ($p = 0.003$) with a slight decrease in soluble sugars from the first to the third harvest.

There were also differences among species for the ash content with the highest average value across the harvests ($p < 0.001$, SE = 0.12) in *M. nigra*, 17.1% on DM, and the lowest in *R. pseudoacacia* (6.91% on DM). A significant species*harvest interaction was observed for the ash content: the ash increased over time in *M. nigra* and in *U. minor*, whereas the opposite trend was seen in *F. excelsior*. For the other species, no significant differences in ash content were observed among harvest dates. Minerals play crucial roles in various physiological processes, including bone development, enzyme activity, and immune function. However, excessively high ash content can also negatively impact digestibility by diluting the energy and nutrient density of the feed.

Overall, the results of the chemical composition underlined significant differences among species for the main chemical constituents. The composition of *M. nigra* falls within the ranges reported in the review by Hassan et al. (2020). For *F. excelsior*, a comparison with the values reported by Ravetto Enri et al. (2020) for leaves collected in Piedmont (Italy) shows notable similarities, except for the ADF and ADL fibrous fractions, which are higher in the present study. The different altitude (88 m a.s.l. in the present study vs. 1300 m in the Ravetto Enri's study) and the different climate according to the Koppen's classification—Cfa (temperate, no dry season, hot summer) in the present study vs. Cfb (temperate, no dry season, warm summer) in the cited study—likely influenced these differences. Warmer temperatures at lower altitudes can accelerate phenological stages and promote leaf lignification, leading to increased fibre content (Vitasse et al. 2009; Lee et al. 2018). In the leaves of tropical tree species, Rothman et al. (2015) found that rising temperatures and altered rainfall patternsmole *M. nigra* collected in Lusignan (France), comparable values were found, although fibrous fractions were consistently higher in our study. Overall, in terms of CP value, *M. nigra, R. pseudoacacia* and *S. babylonica* provide a satisfactory CP content and comparable to that of legume forages such as lucerne. Except for *F. excelsior* and *U. minor*, protein levels are higher than the average CP value (16.8%) found in herbaceous species studied by Mahieu et al. (2021). In general, chemical characteristics, such as CP, tend to worsen slightly during the growing season. Due to their relatively long vegetative growth period, woody species exhibit a more gradual foliage maturation process compared to herbaceous plants. Consequently, the decrease in crude protein concentration during summer is more gradual (Mahieu et al. 2021). This relative stability is a significant advantage for using tree leaves as forage, as delaying the harvest does not lead to a drastic drop in their quality.

Concerning minerals (Table 2), significant differences emerged between tree species ($p < 0.001$). Some minerals, such as Mg, Ca, and K, show less variation between harvests, while others, like Na, Zn, Fe, and Cu, vary more, suggesting that seasonal and ecological factors influence their concentrations. On average, *F. excelsior*'s mineral content aligns with Luske and van Eekeren (2018), except for lower P and Zn but higher Ca levels. Mineral values of *S. babylonica* resemble results from Kendall et al. (2021), and *U. minor* shows similar content to the study of Smith et al. (2018), although with higher P and Mg in our findings. In total mineral content, *M. nigra* and *R. pseudoacacia* had the highest values, correlating with ash content. *Morus nigra* generally showed the highest levels of major minerals (Mg, P, K, Ca), while *S. babylonica* had the highest Na, Zn, Mn, Cu, and Co, *U. minor* the highest Fe, and *R. pseudoacacia* the

Table 2 Mineral composition of tree leaves, average value across the harvests expressed as LSMEANS on DM

	Major minerals (g kg-1)					Trace elements (mg kg-1)					
	Ca	P	K	Mg	Na	Fe	Mn	Zn	Cu	Co	Se
Fraxinus excelsior L. Common ash	17.9^{c}	1.68^{c}	15.7^{c}	3.25^{b}	0.02^{d}	107^{b}	46.1^{b}	15.5^{d}	5.72^{c}	0.15^{c}	0.03^{c}
Morus nigra L. Black mulberry	35.3^{a}	3.08^{a}	26.8^{a}	3.67^{a}	0.04^{c}	125^{a}	25.3^{d}	29.4^{c}	6.31^{c}	0.12^{c}	0.09^{a}
Robinia pseudoacacia L. Black locust	14.4^{d}	2.10^{b}	12.9^{d}	1.93^{d}	0.02^{d}	87.4^{c}	34.9^{c}	33.1^{bc}	6.25^{c}	0.13^{c}	0.09^{a}
Salix babylonica L. Weeping willow	16.5^{cd}	2.26^{b}	22.9^{b}	2.59^{c}	0.12^{a}	99.0^{bc}	56.0^{a}	59.6^{a}	10.1^{a}	0.9^{a}	0.07^{ab}
Ulmus minor Mill Field elm	21.1^{b}	1.76^{c}	14.9^{cd}	3.55^{ab}	0.11^{b}	140^{a}	15.8^{e}	37.4^{bc}	7.65^{b}	0.35^{b}	0.04^{bc}
Standard error of LSMEANS	1.85	0.17	1.61	0.24	6.95	5.66	3.17	3.04	0.51	0.08	0.02
p-value species	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0136
p-value harvest	0.0034	< 0.001	0.5939	0.0802	< 0.001	< 0.001	< 0.001	0.0023	< 0.001	0.1109	0.0029
*p-value species*harvest*	< 0.001	0.0149	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.0174	0.2359	0.0826

highest Se value. All the five species considered were lower in Cu than the values reported by Luske and Van Eekeren (2015) for *L. perenne* (8.9 mg kg^{-1} on DM), except for *S. babylonica* which, across the three harvests, showed a value of 10.1 mg kg^{-1} (SE 0.2).

Therefore, while *M. nigra* may offer a good source of minerals, its high ash content should be considered in diet formulations to ensure adequate energy intake and further research is needed to investigate the specific mineral bioavailability to different animal species. The critical aspect regarding the mineral content of the leaves, as previously highlighted, is its correlation with ash content, which may impact digestibility by diluting the energy and nutrient density of the feed.

Tree leaves generally contain higher concentrations of Ca, S, Zn, Cu and Se compared to grasses (Luske and van Eekeren 2018). Studies with lucerne show some tree leaves have higher levels of Ca, Mg, and Mn (Kazemi and Mokhtarpour 2021), highlighting their richness in both macro and micro elements. Species, environmental conditions, such as elevation, season, soil composition, moisture and pH, significantly impact mineral concentration in leaves (Sardans et al. 2016; Luske and van Eekeren 2018). Luske and van Eekeren (2018) suggest that the high Zn and Se concentrations in tree leaves likely originate from deeper soil layers underscoring the role of trees in supplying microelements to agricultural systems.

Trace minerals are crucial for immune function, fertility, and growth of dairy cows. During the dry period they can improve cow health and reduce risks of infections and mastitis (Machado et al. 2013). The contribution of tree leaves to the mineral supply for animals depends on their mineral concentration but also on intake rate, dietary proportion and bioavailability (Luske and van Eekeren 2018). Utilization of minerals by the animals is further influenced by antinutritional factors (e.g. tannins) which can interfere with absorption (Luske and van Eekeren 2018; Naumann et al. 2017). The absorption and utilization by the animals of Ca and P are influenced by their proportion in the diet. The ideal Ca:P ratio in dairy cattle diets ranges between 1.5:1 and 2:1 (NASEM 2021) with wider ratios impairing the utilization of both, increasing the risk of hypocalcemia. In this study, the Ca:P ratio in the tree leaves ranged from approximately 7:1 to 12:1. Therefore, when tree leaves make up a significant part of the diet, P supplementation may be required to optimize the use of both minerals.

Gas production, methane and fermentative profile

Total GP after 24 h of incubation (Table 3) was significantly different among species ($p < 0.001$), with average values (ml/200 mg DM) across harvests ranging from a maximum of 39.5 in *F. excelsior*, statistically similar to *M. nigra* (33.6) and *S. babylonica* (36.7), to a minimum of 26.9 in *U. minor*, comparable to *R. pseudoacacia* (29.0). The fermentative activity and subsequent GP, as a measure of digestibility and fermentation efficiency, varied significantly among the studied plant species. Such differences are likely attributable to variations in chemical composition, fibre content, and secondary metabolites present in the plant material, underscoring the importance of species selection when considering the incorporation of tree fodder into ruminant diets, as even minor differences in chemical composition or secondary metabolites among species can significantly influence fermentation patterns and gas production.

The effect of harvest time was not statistically significant, although a significant species*harvest interaction was observed ($p = 0.0062$). Specifically, the harvest date did not affect GP for *M. nigra* and *U. minor,* whereas GP increased with harvest date in *R. pseudocacia* and *S. babylonica.* The increase in GP over time for *S. babylonica* can be attributed to a progressive increase in NFC (Table 2), which provide readily fermentable substrates for rumen microbes. For *R. pseudoacacia*, the observed slight increase in GP is possibly due to a reduction in fibre lignification, as indicated by the declining ADL/NDF ratio over time (from 0.487 to 0.439 respectively from first to third harvest). This reduced lignification would improve the accessibility of structural carbohydrates to microbial fermentation, enhancing GP. For *F. excelsior,* GP was lowest in the second harvest compared to the first and third. This could be due to seasonal variations in the leaves' chemical profile, particularly in the concentration of secondary metabolites. Such compounds, which plants produce in response to environmental or physiological factors, can inhibit microbial activity or reduce the availability of fermentable substrates. Environmental conditions or plant stress during the second harvest may

Table 3 Gas production (GP), methane (CH_4), Neutral Detergent Fibre Digestibility (NDFD), and Net Energy for lactation (NE_L) in the five tree species and across the three different harvests expressed as Lsmeans

	Units	GP 24 h ml/200 mg	CH_4 ml/200 mg	CH_4 % on total gas production	NDFD %	OMD %	NE_L MJoule / kg DM
Fraxinus excelsior L. Common ash	1st harvest	47.9^{a}	10.98^{a}	23.4^{bcd}	49.3^{d}	70.9^{c}	5.69^{b}
	2nd harvest	29.1^{bcdef}	4.82^{e}	17.5^{e}	38.6^{e}	50.9^{h}	3.81^{hi}
	3rd harvest	41.6^{abcd}	8.55^{abcde}	22.2^{bcde}	51.5^{d}	52.6^{fgh}	4.11^{fgh}
Morus nigra L. Black mulberry	1st harvest	38.2^{bc}	8.45^{bcd}	22.4^{cde}	82.2^{a}	81.5^{a}	6.27^{a}
	2nd harvest	32.2^{bcdef}	7.41^{bcde}	22.8^{cde}	74.6^{b}	75.3^{b}	5.60^{b}
	3rd harvest	30.3^{cdef}	7.12^{cde}	23.3^{bcd}	69.1^{c}	73.5^{bc}	5.11^{c}
Robinia pseudoacacia L. Black locust	1st harvest	25.0^{f}	6.63^{de}	26.4^{ab}	8.1^{g}	51.5^{gh}	3.68^{i}
	2nd harvest	27.6^{ef}	7.47^{bcde}	27.7^{a}	9.4^{g}	54.0^{fg}	3.78^{hi}
	3rd harvest	34.5^{bcde}	8.75^{bc}	25.6^{abc}	21.7^{f}	54.0^{fgh}	3.96^{ghi}
Salix babylonica L. Weeping willow	1st harvest	29.8^{def}	6.46^{de}	22.0^{de}	39.9^{e}	61.7^{d}	4.57^{de}
	2nd harvest	39.0^{abcd}	9.22^{abc}	22.9^{bcde}	42.2^{e}	61.6^{d}	4.60^{d}
	3rd harvest	41.4^{ab}	9.86^{ab}	23.6^{bcd}	41.1^{e}	58.0^{e}	4.21^{efg}
Ulmus minor Mill Field elm	1st harvest	26.7^{f}	6.32^{e}	24.5^{bcd}	51.3^{d}	54.9^{f}	3.89^{ghi}
	2nd harvest	26.1^{f}	5.91^{e}	22.1^{de}	53.3^{d}	61.4^{d}	4.37^{def}
	3rd harvest	27.8^{ef}	6.52^{de}	23.2^{cd}	49.1^{d}	55.2^{ef}	3.71^{i}
Standard error of LSMEANS	5.43	1.59	3.18	2.10	1.25	0.15	
p-value species		<0.001	0.0152	<0.001	<0.001	<0.001	<0.001
p-value harvest		0.1784	0.3504	0.4222	0.0041	<0.001	<0.001
*p-value species*harvest*		0.0062	0.0036	0.3024	<0.001	<0.001	<0.001

^{abcdefghi}Different letters on the same column within the species correspond to different least squares means ($p < 0.05$)

have influenced the production of these compounds, thereby reducing GP.

The average GP value of *M. nigra* across the harvests (33.6 ml/200 mg DM; SE = 2.69) was comparable to the value (37.2 ml/200 mg DM) reported by Olfaz et al. (2018) and slightly lower than the value (41.2 ml/200 mg DM) reported by Gemeda and Hassen (2015). However, both studies were conducted on sample of *Morus alba* L. The GP values are in line with those reported by Spanghero et al. (2019) for forages used in ruminant diets in Northern Italy (44.6 and 32.4 ml/200 mg DM for maize silage and meadow hay, respectively) and by Patra and Yu (2013) for lucerne hay (33.6 ml/200 mg DM). These comparisons highlight the potential of *M. nigra* as a viable forage source for ruminants, particularly when considering its comparable fermentability to widely utilized forage types; however further research is needed to determine the optimal inclusion level of *M. nigra* in ruminant diets. The GP values had a positive correlation with NDFD (R = 0.81).

As far as the methane is concerned, a significant effect was observed only for species ($p = <0.001$), with *R. pseudoacacia* showing the highest average value across the harvests (26.6% of total GP), while the other four tree species had similar values, with an average of 22.5%. No consistent trend was observed in the species × harvest interaction across the harvests. The high CH_4 percentage in *R. pseudoacacia* is likely due to the low fermentability of its leaves, which results in low GP value and reduced VFA production. In the rumen, methanogenesis affects the efficiency of VFA production; increased methane production often indicates greater hydrogen removal by methanogens, which can reduce the hydrogen available for VFA production. Consequently, higher methane proportions can be associated with lower total VFA production.

Gürsoy (2023) compared Italian ryegrass with leaves from willow, vine, and plane trees. The methane percentage of willow leaves was 12.4%, lower than the average value obtained in the present study. However, direct comparisons should be made

Table 4 Results of Volatile Fatty Acids (VFA) analysis in relation with species and harvest

	Units	pH 24 h	VFA total mmol/L	Acetic %VFA	Propionic %VFA	Butyric %VFA	Valeric %VFA	Iso-valeric %VFA	Acetic:Propionic
Fraxinus excelsior L. Common ash	1st harvest	6.70^{c}	69.6^{ab}	55.0^{f}	15.4^{cde}	8.83^{ab}	2.30^{bcd}	1.35^{cd}	3.59^{cd}
	2nd harvest	6.21^{d}	57.8^{def}	52.7^{h}	19.2^{a}	8.66^{bc}	1.29^{g}	1.14^{g}	2.80^{f}
	3rd harvest	6.69^{bc}	58.2^{def}	53.8^{g}	17.2^{b}	9.17^{a}	1.64^{f}	1.19^{efg}	3.20^{e}
Morus nigra L. Black mulberry	1st harvest	6.75^{abc}	74.2^{a}	55.6^{cdef}	14.5^{ef}	8.26^{cd}	3.02^{a}	1.55^{ab}	3.86^{ab}
	2nd harvest	6.74^{bc}	64.6^{bcd}	55.3^{ef}	15.5^{cd}	7.92^{d}	2.85^{a}	1.43^{bc}	3.61^{cd}
	3rd harvest	6.76^{abc}	68.7^{abc}	55.4^{def}	15.0^{cdef}	8.12^{d}	2.92^{a}	1.58^{a}	3.71^{bcd}
Robinia pseudoacacia L Black locust	1st harvest	6.82^{ab}	52.4^{f}	56.7^{b}	14.7^{def}	8.10^{d}	2.09^{cde}	1.27^{defg}	3.86^{ab}
	2nd harvest	6.81^{ab}	56.1^{ef}	56.6^{b}	14.5^{ef}	8.10^{d}	2.38^{bc}	1.34^{cd}	3.90^{ab}
	3rd harvest	6.79^{abc}	58.4^{def}	56.2^{bcd}	14.8^{cdef}	8.11^{d}	2.44^{b}	1.41^{c}	3.80^{bc}
Salix babylonica L. Weeping willow	1st harvest	6.79^{ab}	62.5^{cde}	56.5^{bc}	15.0^{cdef}	7.90^{d}	2.22^{bcd}	1.26^{defg}	3.77^{bc}
	2nd harvest	6.72^{bc}	63.5^{bcd}	56.4^{bc}	15.1^{cdef}	7.92^{d}	2.29^{bcd}	1.31^{cdef}	3.74^{bcd}
	3rd harvest	6.79^{abc}	62.1^{cde}	55.3^{ef}	15.7^{c}	8.17^{d}	2.47^{b}	1.34^{cd}	3.53^{d}
Ulmus minor Mill Field elm	1st harvest	6.77^{abc}	61.4^{de}	57.7^{a}	14.3^{f}	7.91^{d}	1.84^{ef}	1.18^{fg}	4.04^{a}
	2nd harvest	6.81^{ab}	65.1^{bcd}	56.6^{b}	15.1^{cdef}	7.90^{d}	2.05^{de}	1.25^{defg}	3.75^{bcd}
	3rd harvest	6.83^{a}	59.5^{de}	56.0^{bcde}	15.2^{cdef}	8.26^{cd}	2.22^{bcd}	1.32^{cde}	3.70^{bcd}
Standard error of LSMEANS	0.11	12.72	1.07	0.53	0.64	0.16	0.25	0.12	
p-value species		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
p-value harvest		<0.001	0.1896	<0.001	<0.001	0.0754	0.0759	0.0697	<0.001
*p-value species*harvest*		<0.001	0.0051	0.0022	<0.001	0.7511	<0.001	0.0144	<0.001

^{abcdefg}Different letter on the same column within the species correspond to different least squares means ($p<0.05$)

cautiously due to differences in species, growing conditions, and experimental setups. Indeed, there's a need for more research specifically focused on methane production from tree leaves in temperate climates. Comparison with forages used in temperate climates showed similar methane percentages: for example, Patra and Yu (2013) reported an average methane percentage for lucerne of 23.9 and 23.1 for ryegrass harvested at mid maturity (Macome et al. 2017). These results suggest that methane emissions from tree leaves, including those in this study, are generally comparable to those from common forage species. Although phenolic compounds present in tree leaves might be expected to lower methane production, the observed methane percentages align closely with those of conventional forages, indicating that tree leaves could be a viable alternative feed source with no substantial disadvantage in terms of methane output.

Table 4 shows the results for VFA and pH in the leaves of the five tree species after 24 h of incubation. All investigated effects were significant ($p<0.001$). On average across the harvests (SE = 0.09), *R. pseudoacacia* and *U. minor* showed the same pH value (6.80) which was the highest while *F. excelsior* had the lowest one (6.53). *Robinia pseudoacacia* exhibited a high pH along with high CP content but low GP values. Regarding VFAs, the species effect was always significant ($p<0.001$) as well as the species*harvest interaction, except for butyric acid as percentage of VFAs ($p=0.0751$). Across the harvests (SE = 12.5), *M. nigra* exhibited the highest total VFA, 69.2 mmol/L, suggesting a greater overall extent of fermentation and potentially higher energy provision to the animal in agreement with GP data, and *R. pseudoacacia*, on the other hand, showed the lowest average value, 55.6 mmol/L, further supporting the earlier observation of its lower fermentability. The lower total VFA production and altered VFA profile in *R. pseudoacacia*, combined with its high pH and low GP, further reinforce the conclusion that its nutritional value as a direct feed source may be limited without appropriate processing or supplementation strategies to enhance fermentability and

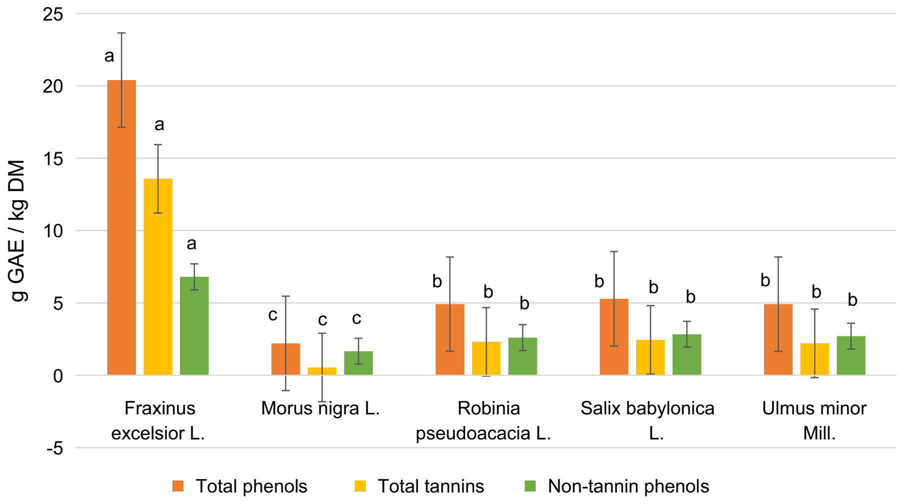

Fig. 1 Average values (LSMEANS values) across the harvests of different type of tannins and phenols in the five tree species. Different letters between bars of the same colour mean $P<0.05$

nutrient availability. Across the harvests (SE = 1.02), acetic acid, expressed as a percentage of total VFAs, showed the highest value in *U. minor* (56.8%) and the lowest in *F. excelsior* (53.8%) but the difference is numerically low. Propionic acid, key precursor for gluconeogenesis, exhibited the highest values across the harvests (SE = 0.41) in *F. excelsior* (17.3%), and the lowest in *R. pseudoacacia* (14.7%). As far as butyric acid is concerned, *F. excelsior* had the highest value (8.89% of total VFAs) which was statistically different from the other species, whose average across the harvests was 7.84% on total VFAs.

Overall, despite the observed differences in VFA profile were not so wide, the acetic acid to propionic acid ratio was significant different for all effects included in the model. On average across the harvests (SE = 0.09), *F. excelsior* showed the lowest acetic to propionic acid ratio (A:P ratio; 3.19) and the lowest A:P ratio was recorded in the second harvest for *F. excelsior* (2.80; SE = 0.12) and it represented the overall minimum observed. The A:P ratio is a key indicator of rumen fermentation pathways and their impact on both energy utilization and methane production. A lower A:P ratio generally favours propionate production over methane production, leading to a more efficient energy conversion for the animal. Acetate formation generates hydrogen, which increases the hydrogen available to methanogens, thereby negatively impacting methane mitigation. Conversely, propionate formation acts as a hydrogen sink, reducing the hydrogen available for methane production (Jo et al. 2022) and causing a shift in hydrogen away from the methane pathway (Nowak et al. 2022). Additionally, a decrease in ruminal propionate production can reduce blood glucose levels by decreasing gluconeogenesis, ultimately resulting in reduced milk lactose production (Jo et al. 2022).

On average, a general decrease in the ratio was noted from the first harvest (3.82) to the last (3.59) (SE = 0.09). In contrast, *R. pseudoacacia* and *U.minor* showed the highest ratio values among the species (3.85 and 3.82, respectively) confirming a greater negative potential for methane production for *R. pseudoacacia* and an expected lower efficient energy conversion for these two species.

Tannins and phenols

Figure 1 shows the results of TP, TT and non-tannins phenols, all expressed as g/kg dry matter, as gallic acid equivalents across the harvests. It is important to note that tannin results may be underestimated due to the analytical method employed, which exclusively quantifies tannins using catechin (Del Pino et al 2005). Harvest, species and the harvest*species interaction had a significant effect on TP and TT contents ($p < 0.001$ for all variables). This differs from the findings of Parissi et al. (2018), who reported that season had no significant effect on TP and TT in tree leaves. Among the species, *F. excelsior* showed the highest average TP content (20.4 g GAE / kg DM; SE = 0.30), statistically higher than that of the other species whereas *M. nigra* recorded the lowest TP content (2.21 g GAE/kg DM). *F. excelsior* not only had the highest TP and TT values but also the lowest acetic acid percentage on total VFAs. For *F. excelsior* the TT content was higher for the second harvest (data non shown) which was that with the lowest GP suggesting a direct inhibitory effect of tannins on rumen microbial fermentation. While *F. excelsior* exhibited high GP overall, the marked reduction in GP during the second harvest, when tannins peaked, indicates that the negative effects of tannins on microbial activity can outweigh any potential benefits at high concentrations. This is a critical consideration for animal feeding, as it suggests that the availability of energy from *F. excelsior* can vary significantly depending on the time of harvest. Conversely, *M. nigra* showed the lowest TP and TT contents while exhibiting, as previously noted, the highest GP and volatile fatty acid (VFA) production suggesting a more stable and readily fermentable feed resource. The low tannin content in *M. nigra* likely contributes to better nutrient availability and potentially higher feed intake.

The results of total TP and TT concentrations for *R. pseudoacacia*, *S. babylonica* and *U. minor* showed intermediate values among that of *F. excelsior* and *M. nigra*.

The values of TP and TT of *F. excelsior* are higher than the values reported by Ravetto Enri et al. (2020) on the same species; conversely all values for *S. babylonica* are lower than those reported by Ravetto Enri et al. (2020), who, however, analysed *Salix caprea.*

Our results showed significant differences among harvests, with higher values in the second and third compared to the first. According to Kumar et al. (2021), plants produce secondary metabolites, such as phenolic compounds and tannins, under environmental stresses, including drought. This may explain the increase in total tannins in the present study, observed as temperatures rose, between the first to the third harvest.

It has been reported that condensed tannins at 20–50 g/kg of dry matter may enhance ruminal protein bypass with minimal effects on feed intake (Ravetto Enri et al. 2020; Fonseca et al. 2023) whereas higher level can decrease dry matter intake and digestibility. The TT content of samples of the present study was always lower than 15 g/kg DM hence intake limitations or negative effects on digestibility are unlikely, even with high leaf inclusion. Moreover, it has to be remarked that tannins from tree species can reduce methane production without significantly affecting rumen fermentation efficiency (Hassanat and Benchaar 2013; Fonseca et al 2023). This aligns with the findings of Jayanegara et al. (2011) who reported a negative correlation between methane emissions and TT content. In the present study, while a negative correlation ($R = -0.35$) was observed between TT and total GP, a significant reduction in methane production was not detected in most samples. This is likely attributable to the generally low TT levels observed, with the exception of *F. excelsior* at the second harvest.

In vitro analyses: neutral detergent fibre digestibility (NDFD)

The NDFD was significantly influenced by species ($p < 0.001$), harvest ($p = 0.004$) and species*harvest ($p < 0.001$) (Table 3). Among the species, *M. nigra* exhibited the highest NDFD value across the harvests (75.3%; SE = 1.67), making it the species with the most digestible fibre in our study, although a decrease in digestibility was observed over the different harvests: from 82.2% in the first harvest to 69.1% in the third. In contrast, the lowest NDFD value was found in *R. pseudoacacia* followed by *S. Babylonica.* The NDFD of *R. pseudoacacia* showed an opposite trend to *M. nigra*, with an increase in NDFD from the first harvest (8.1%) to the last (21.7%), consistent with the GP data and in agreement with the value (17.4%) reported by Dong et al. (2019). Despite its very low digestibility, which limits its potential as a feed ingredient compared to herbaceous species, *R. pseudoacacia* possesses some advantages. As observed by Snyder et al. (2007), this species is highly adaptable to various soil types, tolerant to drought, and characterized by rapid growth, making it a valuable candidate for inclusion in silvopastoral systems especially for the supply of protein even if its fibre contribution to the diet is limited.

Fraxinus excelsior and *U. minor* also showed interesting patterns in NDFD across harvests. In agreement with the GP and tannin content data (as previously discussed), *F. excelsior* exhibited a reduced NDFD in the second harvest (38.6%) compared to the first and third harvests (mean value of 50.4%). The coincident peak in tannins during the second harvest likely contributed to this reduced fibre digestibility, further highlighting the inhibitory effect of tannins on rumen microbial activity. This effect is supported by

Battelli et al. (2024) who observed in in vivo tests that increasing levels of inclusion of tannins in the diet of dairy goats caused a linear decrease in diet digestibility. *Ulmus minor*, on the other hand, showed the lowest NDFD in the first harvest (41.1%) compared to the second and third harvests (52.3%), indicating a different pattern of fibre development or a response to environmental conditions over the growing season. These variations in NDFD among species and across harvests underscore the importance of considering both species selection and harvest management when utilizing tree leaves as feed resources. While *M. nigra* demonstrates good potential as a source of digestible fibre, its seasonal variation in digestibility needs to be considered. *Robinia pseudoacacia*, despite its low NDFD, can still play a role in silvopastoral systems due to its other desirable characteristics. Finally, the fluctuating NDFD of *F. excelsior*, likely influenced by tannin content, highlights the need for further research to optimize its utilization as a feed resource.

The NDFD values from this study can be compared to analyses conducted on forages collected in the same area (Po plain valley) and analysed in the same laboratory by Chiaravalli et al. (2019) who obtained a value of 57.8% for permanent meadow, lower than that observed for *M. nigra* of the present study. The NDFD of *M. nigra* is also comparable to the 75.1% obtained by Pirondini et al. (2015) for Italian ryegrass silage, which is considered the forage with the highest fibre quality in Northern Italy. Moreover, *M. nigra* L., *U. minor* and *F. excelsior* have a higher NDFD than the 40.4% reported by Pirondini et al. (2015) for lucerne hay.

To the authors' knowledge, this study is among the first to report detailed in vitro fibre digestibility values for tree leaves. Few studies are available on this topic: Dong et al. (2020) investigated the in vitro digestibility of *M. alba* silage leaves obtaining values lower than that of the present study. Gürsoy (2023) reported a decrease in NDFD when willow, vine, and plane leaves were added to Italian ryegrass. In our study, *M. nigra* exhibited NDFD values across the three harvest times higher than those reported by Gürsoy (2023), confirming the excellent level of NDFD in *M. nigra*.

The average OMD results across the harvests are in line with those obtained for NDFD, with *M. nigra* and *R. pseudoacacia* showing the best (76.8%; SE = 0.76) and the worst value (53.2%; SE = 0.77), respectively. A statistically significant decline is observed ($p < 0.001$) across species, from the first harvest (64.1%; SE = 0.68) to the last (58.7%; SE = 0.67) highlighting the importance of harvest timing for maximizing the nutritional value of the tree leaves studied.

Similarly, on *M. alba* leaves Eshetu et al. (2018) showed a decrease in OMD according to the harvest date (from 86.9 to 75.9%) whereas McWilliam et al. (2005) indicate OMD values in willow of 63% in summer to values of 58% in late autumn. Gürsoy (2023) reported an OMD value for Italian ryegrass of 73.1%, which in our study was surpassed only by *M. nigra*. When compared to other feed in the INRAE database (2018), *M. nigra* also exhibited higher OMD values than several other common feedstuffs, including among others lucerne dehydrated (60.6%), grass dehydrated (71.6%), oats (71.2%), wheat bran (69.1%) and middlings (73.6%). Emile et al. (2016) reported values of in vitro DM digestibility (highly correlated to OMD) for *U. minor x resista* of 64%, for *M. alba* of 84% and for *R. pseudoacacia* of 48% which agree with the ranking of our study.

Net energy for lactation (NEL)

The NE_L values for each species and harvest times were significantly affected by species, harvest and their interaction ($p < 0.001$) (Table 3). In agreement with previous data (total VFA and NDFD) across the harvests, *M. nigra* had the highest NE_L, slightly exceeding values found by Olfaz et al. (2018) while *R. pseudoacacia* and *U. minor* had the lowest NE_L with no significant differences between them. *Salix babylonica* showed slightly had a NE_L content lower than *M. nigra* but higher than *R. pseudoacacia* and *U. minor* and higher than NE_L of *S. alba* studied by Gürsoy (2023) in Turkey. For F. excelsior, for the reasons already described, the NE_L was lower for the second harvest than for first and third. Except for *U. minor* and *R. pseudoacacia*, the nutritional values exceeded the INRAE (2018) average of 4.91 MJ/kg for lucerne, indicating their potential as viable alternatives or complements to traditional forages. Under similar conditions, Amodeo (2007) observed average NE_L values of 5.33 MJ/kg DM for permanent meadow hay and 5.05 MJ/kg DM for lucerne hay, while Gürsoy (2023) reported an average of 5.87 MJ/kg DM for Italian ryegrass. Notably, the NEL values recorded for *M. nigra* in this study exceeded these benchmarks,

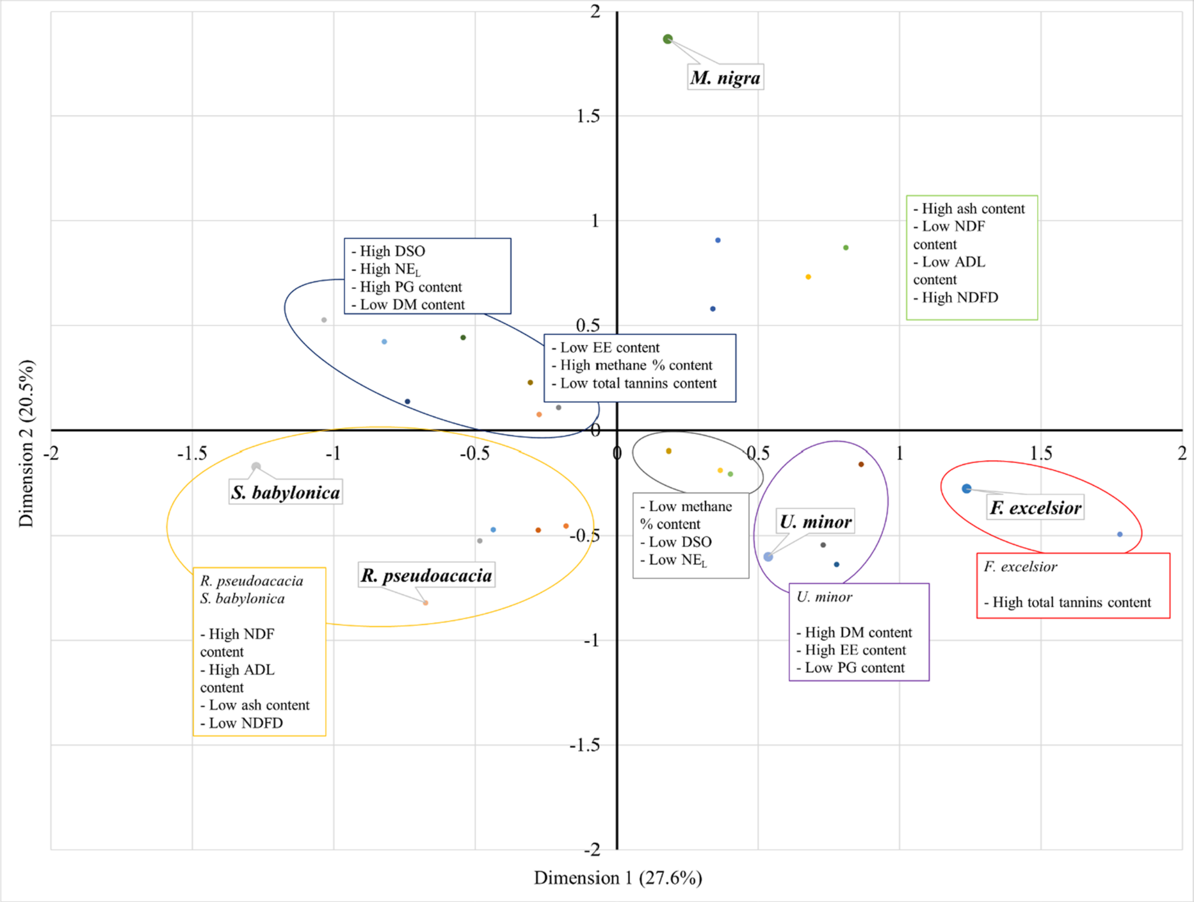

Fig. 2 Multiple Correspondence Analysis highlighting the relationships among tree species and chemical analysis

highlighting its suitability as a high-energy forage that could replace or complement permanent meadow or lucerne hays in livestock diets. This substitution could be particularly valuable in systems where traditional forages are less available or expensive. Across harvests, NE$_L$ decreased significantly, from the first harvest (4.82 MJ/kg DM; SE = 0.08) and the third one (4.22 MJ/kg DM) in the third one.

Multiple correspondence analysis

A multiple correspondence analysis (MCA) (Fig. 2) was performed to explore the multivariate relationships among the analyzed variables, providing a comprehensive overview of the similarities and differences among the tree species in terms of their nutritional profiles and in vitro fermentation characteristics. These summarized findings can important implications for selecting appropriate tree species for inclusion in ruminant diets and for developing effective feed management strategies to optimize nutrient utilization and minimize environmental impact.

The MCA revealed distinct groupings of the species based on their characteristics. *M. nigra* does not exhibit a strong relationship with the other species analysed; it appears in the same quadrant of low fibre fractions, high ash content and high NDFD level. This positioning reinforces the previous findings and suggests that *M. nigra* has a unique nutritional profile, being a readily digestible source of nutrients with a lower fibre content and a higher mineral content compared to the other species. From an animal feeding perspective, this profile confirms that *M. nigra* could be a valuable source of readily available energy and minerals, potentially suitable for inclusion in diets requiring high nutrient density, such as those for lactating animals or growing animals. In contrast, *R. pseudoacacia* and *S. babilonica* are grouped together in the opposite quadrant, where high levels of fibrous fractions, and low levels of ash and NDFD are present. This grouping indicates that these two species share similar characteristics related to fibre content and digestibility. Their high fibre content suggests a slower rate of digestion and potentially lower energy availability compared to *M. nigra*. This should be considered when formulating diets containing these species, as they might require supplementation with more readily digestible feedstuffs to meet animal energy requirements.

Fraxinus excelsior is positioned near high total tannin content, highlighting the dominant role of tannins in shaping the nutritional profile of *F. excelsior* differently than the other species investigated. *Ulmus minor* appears in the same quadrant of *F. excelsior*, closely associated with high levels of DM and EE, and low CP content.

Finally, the MCA confirms a key finding regarding methane production: methane content (as a percentage of total gases) is inversely related to tannin content. This observation reinforces the association between high tannin levels and reduced methane production, likely due to the inhibitory effects of tannins on methanogenic archaea or shifts in rumen fermentation pathways.

Conclusions

In conclusion, the study revealed that the leaves of all the examined tree species have good potential as forage for ruminant animals based on their nutritional quality, with the possible exception of *R. pseudoacacia. M. nigra* is the most promising species among those studied, thanks to its high NE_L and protein content, and high digestibility, despite its relatively high ash content. The results showed a slight decline in nutritional characteristics as the season progressed, although this deterioration was not noticeable in all species and was far less pronounced than commonly observed in herbaceous forage species. This is a positive aspect for the tree species studied as it would not limit the harvesting period to a narrow timeframe.

Since the tree species are present and often neglected on farms, and given their notable nutritional potential, comparable to that of the best herbaceous species, it would be worthwhile to further investigate their use as supplements in ruminant diets through palatability and digestibility trials. In the case of inclusion in lactating animal diets, it would also be important to study potential impacts on milk quality and quantity, as well as on the quality of cheese.

Including tree fodder in ruminant diets offers advantages over conventional forages: diversification of agricultural land, better use of water and soil nutrients, enhanced carbon sequestration, and longer growing seasons. Moreover, replacing purchased forages with tree fodder can lower costs and increase farm circularity. However, estimating sustainable yields is crucial to fully assess the suitability and competitiveness of tree species as forage. However, challenges remain concerning the methods of leaf harvesting, preservation, and distribution. Drying or ensiling the leaves could offer practical approaches to preserve this seasonal product, developing dedicated harvesting and preservation systems with specialized mechanization.

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Data availability Data are available on request.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval Animal procedures were conducted under the approval of the University of Milan Ethics Committee for animal use and care, with the authorization of the Italian Ministry of Health (authorization no. 79/2022-PR) in compliance with the standards laid down by the Animal Welfare Board (OPBA).

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