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Temporal variations in leaf traits, chemical composition and *in vitro* true digestibility of four temperate fodder tree species

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Abstract

Context. Many tree and shrub species are underestimated fodder resources due to insufficient knowledge about their potential feeding value, especially for goats.

Aims. The present work aimed at assessing productive and nutritional attributes of the foliage of the following four temperate tree species widespread in Europe: *Acer pseudoplatanus*, *Fraxinus excelsior*, *Salix caprea* and *Sorbus aucuparia*.

Methods. Leaf length and biomass, proximate composition, fatty acid profile, phenolic composition and *in vitro* true dry matter digestibility were determined along the vegetative season.

Key results. The leaf length of the four species was significantly related to leaf biomass and can be considered as a proficient proxy for estimating leaf biomass. The differences found among the species were remarkable, although weakly related to temporal changes, especially when considering fatty acid and phenolic compositions. *Fraxinus excelsior* sprouts were the most productive, with a mean biomass of 13.2 g dry matter (DM) per sprout at the end of the growing season. Its foliage showed also the lowest phenolic concentrations (average total extractable phenols of 11.25 g/kg DM), resulting in the highest digestibility values (average *in vitro* true dry matter digestibility of 56.5 g/kg DM). Digestibility of *S. aucuparia* was similar, but its lower polyunsaturated fatty acid concentration (average value of 62.13 g/kg DM) could reduce the interest for this species as a feeding resource for goat dairy products with healthy properties. The lower digestibility found for *A. pseudoplatanus* and *S. caprea* (average values of 43.3 and 46.2 g/kg DM, respectively) may be related to their higher phenolic concentrations (average total extractable phenols of 45.9 and 47.3 g/kg DM, respectively).

Conclusions. The four species could represent an appealing feedstuff for goat nutrition, due to the valuable and complementary nutritional characteristics of their foliage.

Implications. The use of the studied species as fodder resource may be particularly relevant during drought periods and in the late summer when herbage quality decreases, especially in terms of crude protein and fatty acid profile.

Additional keywords: fatty acids, feed quality, goats, *in vitro* digestibility, tannins.

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Introduction

Tree and shrub foliage is an important component of small ruminant diet in many parts of the world. It plays an essential role for browsing animals, especially where livestock systems are based on rangeland and grazable forestland exploitation (Leng 1997). Due to their deep root system, trees can maintain the high nutrient quality of their foliage also in dry periods, when herbage quality decreases (Papachristou and Papanastasis 1994). For this reason, the interest for fodder tree species is rising also in European temperate regions where drought periods are increasing in length and frequency over large areas due to global warming (Vandermeulen *et al*. 2018*a*). In this context, the so called 'silvopastoral systems', integrating fodder trees and grasslands, are spreading as a possible solution to face climate changes and to support a sustainable and resilient agriculture, especially in marginal areas (Novak 2014; Seidavi *et al*. 2018).

The knowledge of the importance of tree foliage for ruminant nutrition has led to several studies aimed at evaluating leaf forage productivity, chemical composition and digestibility (e.g. Boufennara *et al*. 2012). Proximate and fatty acid (FA) composition, phenolic concentration and digestibility have been largely assessed for various fodder tree species in several environments such as savanna (Tefera *et al*. 2008), tropics (Arigbede *et al*. 2012), subtropics (Prakash *et al*. 2009), American temperate region (Ricklefs and Matthew 1982),

Mediterranean region (Kökten *et al*. 2012) and non-European alpine areas (Khanal and Subba 2001).

According to these and other studies, tree and shrub foliage is increasingly recognised as a high-quality feedstuff for small ruminants, either offered as sole feed or as dietary supplement (Leng 1997). Tree foliage has a superior mineral composition compared with that of some grasses (e.g. dry grasses), making foliage a significant source of minerals for the animals and rumen microbiota (Leng 1997). Some foliage has high protein contents and is, therefore, used as protein source to supplement other feeds, particularly when ruminants are offered roughages of a low nutritive value (Simbaya 2002; Kongmanila 2012). Tree and shrub foliage contains endogenous secondary compounds able to bind with proteins and protect them from microbial attack in the rumen afterwards. For this reason, foliage can represent a source of by-pass protein for the ruminant that, escaping rumen degradation, can be digested in the intestine, enhancing the animal protein status (Leng 1997). Plant secondary compounds are also able to alter the balance of microorganisms in the rumen (Vasta and Bessa 2012). Therefore, besides variations in FA, differences in concentrations of plant secondary compounds may also confer specific intrinsic sensory and chemical attributes to ruminant-derived dairy and meat products (Iussig *et al.* 2015*a*). Some plant secondary compounds contained in a large number of fodder tree species, such as phenolic compounds, are also characterised by potential anti-nutritional effects (Waghorn 2008). Among phenolic compounds, tannins can cause either adverse or beneficial effects on nutrient utilisation, health and animal production, in relation to their molecular characteristics and concentrations (Min *et al.* 2003). Particularly, tannins (depending on the type, chemical characteristics and amount ingested) can (1) increase intestinal escape protein availability, (2) reduce methane production, (3) defend against bloat and (4) wane gastrointestinal parasites (Mueller-Harvey 2006; Vandermeulen *et al*. 2018*a*). Francisco *et al*. (2015) reported also that inclusion of low concentrations (up to 16.3 g/kg DM) of condensed tannins (CT) in lamb diet could wax antioxidant properties in meat, improving its stability after storage. Similarly, milk from goats fed with low concentrations of hydrolysable tannins (HT) showed an increasing trend of conjugated linoleic acid isomers and oleic acid, as a result of an enhancement of Δ9-desaturase activity (Abo-Donia *et al*. 2017).

Temporal variations have also been analysed, since tree foliage productivity, chemical composition and digestibility can significantly change due to phenological advancement and climatic variations (Papachristou and Papanastasis 1994; Ndondo *et al*. 2004; Kökten *et al*. 2012).

In the last decades, extensive goat rearing has spread in European alpine areas, particularly in marginal areas (Battaglini *et al*. 2014; Álvarez-Martínez *et al*. 2016). Here, due to their increasing presence and resistance to browsing and drought, extensive shrublands and small trees can provide abundant leaf fodder, which represents the basic component of goat diet. Particularly, a general increase of tree leaf consumption has been observed in late summer, when the availability and nutritive value of herbage decrease (Castro and Fernández Núñez 2016). Moreover, goats are well adapted to exploit low-quality forages due to their ruminal bacterial population, suitable for degradation of highly lignified material and resistance to tannin toxicity, resulting in an increased tree foliage digestibility (Manousidis *et al*. 2016).

Various research has been conducted on European temperate tree species (see, among others, Luske and van Eekeren 2015; Emile *et al*. 2016; Vandermeulen *et al*. 2018*b*). These plants have been a widespread feeding resource since Neolithic (Gómez García and Fillat 1984; Kühn *et al*. 2013; Hejcman *et al*. 2014), especially for goat nutrition (Leng 1997; Castro and Fernández Núñez 2016). However, the leaf biomass production of fodder tree species in these environments is poorly documented. Moreover, only a limited number of authors experienced the assessment of foliage production using allometric equations based on leaf traits (Forrester *et al*. 2017). In addition, there is a lack of knowledge on the FA profile of such tree foliage, even if this feature is of increasing interest for the possible effects on the nutraceutical properties of the derived dairy and meat products (Glasser *et al*. 2013).

The present study aimed to fill the gap of knowledge about the above-mentioned topics. For this purpose, we selected, in the western Italian Alps, the following four temperate tree species that are widespread on European uplands (San-Miguel-Ayanz *et al*. 2016): *Acer pseudoplatanus* L. (sycamore maple), *Fraxinus excelsior* L. (ash), *Salix caprea* L. (goat willow) and *Sorbus aucuparia* L. (rowan). These trees are among the browses most preferred and used by goats, either directly by browsing or by being fed after cutting (Guinier 1950; Thiebault 2005; Iussig *et al*. 2015*a*, 2015*b*). The objective of the work was to characterise their leaves across the whole vegetative season in terms of leaf traits (i.e. leaf length and biomass), proximate composition (i.e. dry matter (DM), ash, crude protein (CP), ether extract (EE), and fibre fractions), FA profile, phenolic compounds (i.e. total extractable phenols (TEP), non-tannin phenols (NTP), total tannins (TT; CT and HT) and *in vitro* true DM digestibility (IVTD).

Materials and methods

Study area

The study was conducted at Oasi Zegna, located in the Piedmont Region, north-western Italy (45°40′N, 8°09′E), within the boundaries of Valle Sessera Site of Community Interest (SCI IT11300002). The study area is characterised by a suboceanic climate (Köppen's classification: Cfb), with annual mean temperature of 7.3°C and precipitation of 1700 mm (mean value for the years 2002–2015 of the pluviometric station of Bielmonte). Dominant soils have originated from siliceous parent rock. In the study area, tree stands are mixed broadleaved populations, dominated by *Fagus sylvatica* L. (European beech) and *Betula pendula* Roth (silver birch), currently managed by occasional selective cutting.

Experimental design

For each investigated species (i.e. *A. pseudoplatanus*, *F. excelsior*, *S. caprea* and *S. aucuparia*), a group of four trees was selected in December 2014, during the vegetative dormancy, in a 500 × 150 m^2 area with homogeneous

exposure (north–north-west) and elevation (1270–1320 m asl). Within each species group, the trees were located at a maximum distance of 125 m from each other and had a similar height, stem diameter, age, and no disease evidence. The selected trees were protected from animal browsing by using deer fences during the whole trial.

Leaf traits (i.e. leaf length and biomass) were recorded 12 times, while leaf sampling for laboratory analyses (i.e. chemical composition and IVTD) occurred four times during the trial, as detailed in Table 1.

Leaf length and biomass

Four sprouts at bud stage per tree were selected at a maximum height of 1.80 m (i.e. as far as goats can browse buds and leaves, assuming a bipedal stance; Iussig *et al*. 2015*b*). Each sprout was located in a different cardinal direction to avoid differences due to light exposure. The elongation of emerging leaves was monitored on each sprout, from the budburst of the earliest sprout until all trees reached the maximum vegetative phenological stage, according to the extended BBCH scale (Hack *et al*. 1992). At each survey date, following the survey schedule provided in Table 1, the phenological stage (vegetative and, when present, reproductive) of each individual was recorded. In addition, one sprout, comparable to the monitored one in terms of leaf number and development, was harvested and transported to the laboratory. The emitted leaves were then separated from the sprout and weighed. Leaf traits (i.e. leaf length and biomass) were computed by cumulating the values of all unfolded leaves per sprout in each survey date, accounting for a total of 16 replicates per species (four trees × four sprouts).

Growing degree-days

Four HOBO data loggers (Onset Corporation, Pocasset, MA, USA), placed near the centroid of each species group, recorded air temperature every 30 min for the whole trial. Heat units from 1 January 2015 to the end of the trial, expressed as cumulative growing degree-days (GDD), were calculated for each species group from daily air temperature, by cumulating all mean daily temperatures of >5°C (Prislan *et al*. 2013). For each sprout, the GDD corresponding to the 25%, 50%, 75% and 100% of the final leaf length and biomass were assessed by data interpolation.

Table 1. Survey schedule

All surveys were conducted in 2015. DOY, day of the year; GDD, growing degree-days, expressed as the mean ± s.e. of the four tree species

Date	DOY	GDD	Survey
15 Apr.	105	137.7 ± 2.76	Leaf traits (1st)
23 Apr.	113	205.0 ± 3.90	Leaf traits (2nd)
28 Apr.	118	238.6 ± 3.94	Leaf traits (3rd)
6 May	126	309.0 ± 4.23	Leaf traits (4th)
12 May	132	391.4 ± 4.86	Leaf traits (5th)
19 May	139	482.0 ± 5.27	Leaf traits (6th)
29 May	149	576.6 ± 5.82	Leaf traits (7th)
4 June	155	661.3 ± 5.98	Leaf traits (8th) and chemical analyses (1st)
18 June	169	869.5 ± 6.16	Leaf traits (9th)
2 July	183	1086.0 ± 5.99	Leaf traits (10th) and chemical analyses (2nd)
29 July	210	1623.5 ± 6.16	Leaf traits (11th) and chemical analyses (3rd)
25 Aug.	237	2054.3 ± 6.61	Leaf traits (12th) and chemical analyses (4th)

Leaf sampling

Since all the selected sprouts ended leaf emission (i.e. 4 June 2015), one leaf sample per tree (i.e. a total of four replicates per species) was collected for four dates from June to August, as detailed in Table 1. Such time interval approximately corresponds to the goat browsing season in these grazable forestlands. A quantity of ~400 g of fresh leaves (including petioles and rachises; Hejcmanová *et al*. 2014) was harvested all around the canopy, bottom-up to 1.80-m height, so as to simulate goat browsing (Iussig *et al*. 2015*b*). All leaves damaged by pathogens (insects, fungi) were avoided. The samples were placed in sealed polyethylene bags, immediately stored at 4°C in a portable refrigerator and transported to the laboratory. In the laboratory, each sample was divided into two homogeneous aliquots of ~200 g each. The samples were freeze-dried (Freeze Drying Equipments, Criofarma, Torino, Italy), grounded with a cutting mill to pass through a 1-mm-screen sieve (Pulverisette 15, Fritsch GmbH, Idar-Oberstein, Germany) and used for further laboratory analyses.

Proximate composition

AOAC (2000) procedures were used to determine DM (Method no. 930.15), ash (Method no. 942.05), CP (Method no. 984.13), acid detergent fibre (ADF) and acid detergent lignin (ADL; Method no. 973.18). The EE was determined following Method no. 920.39 of AOAC (2003). Neutral detergent fibre (NDF) was analysed according to Van Soest *et al*. (1991); α-amylase (Sigma Aldrich, Saint Louis, MO, USA), but no sodium sulfite was added and results were corrected for residual ash content. The proximate composition was expressed as g/kg DM.

Fatty acid composition

The FA composition was assessed using a combined direct transesterification and solid-phase extraction method, as described by Alves *et al*. (2008). The FA methyl esters were separated, identified and quantified as detailed by Renna *et al*. (2014). The FA composition was expressed as g/kg DM.

Phenolic composition

The TEP and phenol fractions (NTP and CT) were determined using standard protocols, as detailed in Iussig *et al*. (2015*a*). The absorbance was recorded at 725 nm (TEP and NTP, expressed as gallic acid equivalents) and 550 nm (CT, expressed as leucocyanidin equivalents) using a UV–VIS spectrophotometer (Shimadzu UVmini-1240, Shimadzu Corporation, Kyoto, Japan). The TT were computed as the difference between TEP and NTP. The HT were estimated as the difference between TT and CT. The phenolic composition was expressed as g/kg DM.

In vitro *true dry matter digestibility*

Four slaughtered adult male Alpine goats were used as rumen fluid donors. The male goats belonged to an extensive dairy goat farm located in Rueglio (Torino, Italy). They were fed on alpine grazable forestlands rich in fodder tree and shrub species, including those under investigation in the present trial. Slaughtering procedures followed Council Regulation (EC) No. 1099/2009 of the European Union. They occurred four times (once per each male goat), every three weeks between October and December 2015. A representative sample of the rumen contents was collected from each animal into a thermos container, which was kept at 39°C using a warm water-containing cooler, and immediately transported to the laboratory, where the sample was filtered through four layers of cheesecloth under continuous flush of CO_2 at 39°C. The IVTD was determined according to the ANKOM Daisy procedure (ANKOM 2017). Following the slaughtering time schedule, four runs were performed. For each run, all 64 leaf samples were digested using the rumen fluid of one goat, accounting for a total of eight replicates per plant species and sampling date (two trees × four rumen fluids).

Leaf samples (0.25 g) were weighed into filter bags (ANKOM® #F57; pore size 25 µm, ANKOM Technology Corporation, Fairport, NY, USA), which were then heat-sealed. Rumen fluid was diluted into the buffer medium in a proportion of 1 : 4 (v/v). Thereafter, a volume of 2 L of buffered rumen fluid was transferred into four 5-L jars at 39°C under anaerobic conditions. Each jar, containing leaf samples and one blank, was placed in a revolving incubator (ANKOM Daisy^{II} digestion system, ANKOM Technology Corporation) at 39°C for 48 h, under continuous rotation. After incubation, the samples were rinsed with cold water and subjected to an extraction with NDF solution at 100°C for 1 h, so as to remove microbial debris and any remaining endogenous products.

Statistical analyses

To evaluate species precocity in leaf development at different phenological stages, one-way ANOVA was used to analyse for differences among the GDD corresponding to budburst and to 25%, 50%, 75% and 100% of the final leaf trait values. Moreover, to test for differences of final leaf length and biomass values among species at the end of the growing season, a one-way ANOVA was performed. For both analyses, the sprout was considered as the statistical unit and tree species as a fixed factor.

The relationship between leaf length and biomass was then explored with linear regressions, using a separate dataset for each species. The regression equations were based on the best-fitting model and the sprout was considered as the statistical unit.

General linear models accounting for repeated measures were performed on proximate composition, FA profile (namely, total FA (TFA), groups of FA, and main represented individual FA), phenolic composition, and IVTD of each tree species. Tree was considered as the statistical unit, species as a fixed factor, and sampling date as a repeated measure.

To test for differences among species during the vegetative season, a one-way ANOVA was performed on the same variables (i.e. proximate composition, FA profile, phenolic composition and IVTD) for each of the four sampling dates, using tree as the statistical unit and tree species as the fixed factor. Additionally, the temporal variations in terms of proximate composition, FA, phenolic composition and IVTD within the same species were tested performing a one-way ANOVA, with tree as the statistical unit and sampling date as a fixed factor.

Assumptions of normality and homogeneity of variance were checked with Shapiro–Wilk and Levene's tests respectively. Variables that were not normally distributed were log-transformed before further statistical analysis; results are presented as non-transformed data. When normal distribution or homogeneity of variances were not met, even after log-transformation, the non-parametric Kruskal–Wallis test or Welch one-way ANOVA were used, respectively. When significant differences were found, Tukey's, Steel's and Tamhane's *post hoc* tests were performed for ANOVA, Kruskal–Wallis and Welch one-way ANOVA, respectively.

All statistical analyses were performed using SPSS (Version 24.0, IBM SPSS Statistics, Armonk, NY, USA). Significance was set at $P < 0.05$.

Results

Leaf traits

A total of 479 leaves was monitored during the trial. Mean number of emitted leaves per sprout was eight for *A. pseudoplatanus* and *S. caprea*, nine for *F. excelsior* and five for *S. aucuparia*.

The vegetative season started at 135 GDD (105th day of the year), with *S. caprea* unfolding of the first leaves (Table S1, available as Supplementary material to this paper). Budburst occurred first (at a lower GDD) for *S. caprea* and *S. aucuparia*, followed by *F. excelsior* and then by *A. pseudoplatanus* (Table 2). *Salix caprea* and *S. aucuparia* developed earlier in terms of leaf length and biomass for most of the considered stages, followed by *F. excelsior* and, then, by *A. pseudoplatanus*.

Differences among tree species in terms of final leaf traits were similar between leaf length and leaf biomass, with *F. excelsior* showing the greatest values, followed by *A. pseudoplatanus*, *S. aucuparia* and *S. caprea* (Fig. 1).

For each of the four species, a relationship between leaf length and leaf biomass was demonstrated. A remarkable amount of variance was explained by the regressions leaf length versus leaf biomass (regression parameters and equations are shown in Fig. 2).

Proximate composition

The proximate composition was significantly different among the considered fodder tree species (Fig. 3). The average DM contents of *S. caprea* and *S. aucuparia* leaves were higher than those of *A. pseudoplatanus* and *F. excelsior* leaves. The ash content in *S. aucuparia* leaves was always lower than in the leaves of the other species. In *F. excelsior*, ash increased across time, displaying the highest values at the end of the season (Table 3). The highest EE values were observed in *A. pseudoplatanus*, followed by *S. caprea*, *F. excelsior* and *S. aucuparia*. *Salix caprea* was found to be the most fibrous species, with the highest concentrations of NDF, ADF and ADL. *Fraxinus excelsior* showed the lowest concentrations of ADF and ADL, while intermediate amounts of these fractions were found in *A. pseudoplatanus* and *S. aucuparia* leaves. Concerning NDF, *F. excelsior* showed values similar to those

Table 2. Growing degree-days (GDD, mean ± s.e.) for each development stage

Data are provided for budburst and cumulative leaf length and biomass per sprout at 25%, 50%, 75% and 100% of the final recorded value of each species. Different letters within a row indicate significant differences ($P < 0.001$) among tree species

Development stage	*Acer pseudoplatanus* (GDD)	*Fraxinus excelsior* (GDD)	*Salix caprea* (GDD)	*Sorbus aucuparia* (GDD)
		Budburst		
	466.8 ± 4.55a	266.5 ± 2.96b	166.0 ± 2.14c	167.7 ± 2.81bc
		Leaf length		
25%	481.0 ± 4.74a	333.9 ± 2.50b	218.6 ± 2.09c	203.2 ± 2.64c
50%	514.3 ± 4.96a	411.5 ± 2.34b	301.2 ± 2.78c	281.2 ± 4.17c
75%	589.5 ± 5.42a	493.8 ± 3.13b	424.6 ± 4.09c	401.6 ± 2.62c
100%	1136.9 ± 18.95	1257.9 ± 17.70	1109.5 ± 14.50	1107.7 ± 14.90
		Leaf biomass		
25%	571.7 ± 6.81a	444.1 ± 2.77b	385.2 ± 4.48bc	334.2 ± 3.94c
50%	700.2 ± 7.31a	648.8 ± 16.86ab	569.1 ± 7.11bc	492.9 ± 5.27c
75%	1003.6 ± 16.46	968.1 ± 23.08	820.1 ± 18.68	912.0 ± 24.56
100%	1901.4 ± 13.49a	1720.1 ± 23.52b	1725.6 ± 16.63b	1759.9 ± 17.23ab

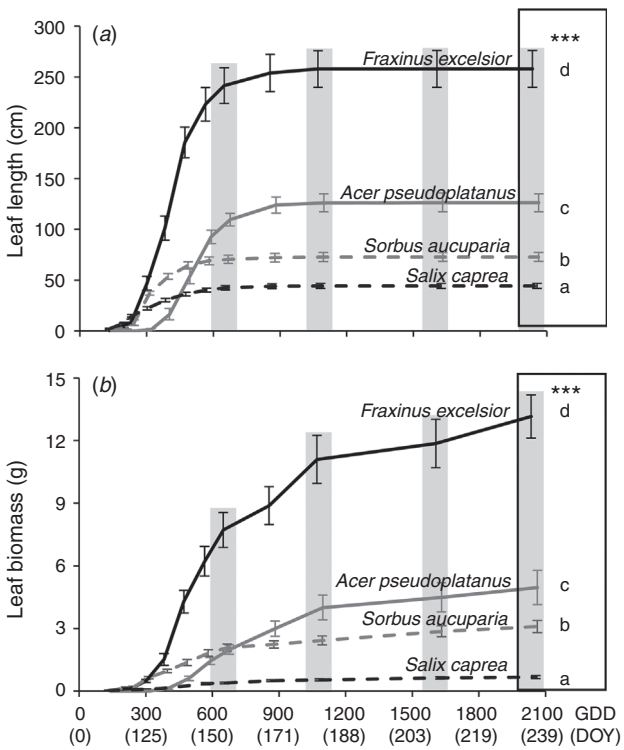

Fig. 1. Cumulative leaf development. Increase of cumulative leaf length (*a*) and leaf biomass (*b*) per sprout in relation to growing degree-days (GDD) and day of the year (DOY, expressed as a mean for the four tree species). Grey highlights indicate leaf sampling dates for laboratory analyses. Error bars represent the s.e. of the means, while different letters indicate significant differences among the tree species. ***, $P < 0.001$.

observed in *S. caprea*, whereas the lowest concentrations were found in *A. pseudoplatanus* and *S. aucuparia*.

The statistical analyses performed on the temporal variations in the proximate composition highlighted a general variability related to phenology advancement, with an overall decrease in CP and an increase in fibre content (Table 3).

Fatty acid profile

Regarding the FA profile of the leaves, the following 15 FA were detected in all samples: C12:0, C14:0, C15:0, C16:0, C16:1 *c*9, C18:0, C18:1 *c*9 (n9), C18:1 *c*11, C18:2 *c*9*c*12 (n6), C18:3 *c*6*c*9*c*12 (n6), C18:3 *c*9*c*12*c*15 (n3), C20:0, C20:1 *c*11, C22:0 and C20:4 *c*5*c*8*c*11*c*14 (n6). Among them, six FA, namely, palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 *c*9), linoleic acid (C18:2 n6), α-linolenic acid (C18:3 n3) and γ-linolenic acid (C18:3 n6), comprised 92–97% of TFA and were then considered for further statistical analyses. The remaining FA were cumulated in the 'Other FA' group. In all tree species, C18:3 n3, C18:2 n6, and C16:0 were the main detected FA (Fig. 4). In *S. aucuparia*, the lowest total monounsaturated FA (MUFA, together with *S. caprea*), polyunsaturated FA (PUFA) and TFA concentrations were detected (Fig. 4). Concerning individual FA, *S. aucuparia* showed the highest C18:0 and the lowest C18:3 n6 and C18:3 n3 concentrations. *Acer pseudoplatanus* leaves had the highest C16:0 concentration, while *F. excelsior* showed the highest MUFA and C18:1 *c*9 concentrations. No differences were found for the 'Other FA' group among the species. The majority of individual FA and FA groups showed differences among the considered species during the whole trial. However, for total saturated fatty acids (SFA), C16:0, C18:3 n6, C18:3 n3, and 'Other FA', the differences among species tended to wane as DOY advanced.

Among sampling dates, FA profiles (Table 4) showed few significant variations. The concentration of C18:2 n6 was significantly ($P < 0.001$) higher at the first sampling date for all the species. Concerning *F. excelsior* and *S. caprea*, the sampling date influenced the concentration of total PUFA, which was significantly ($P < 0.05$ for both) higher at the first sampling date than at the third (*F. excelsior*) or the second and third sampling dates (*S. caprea*).

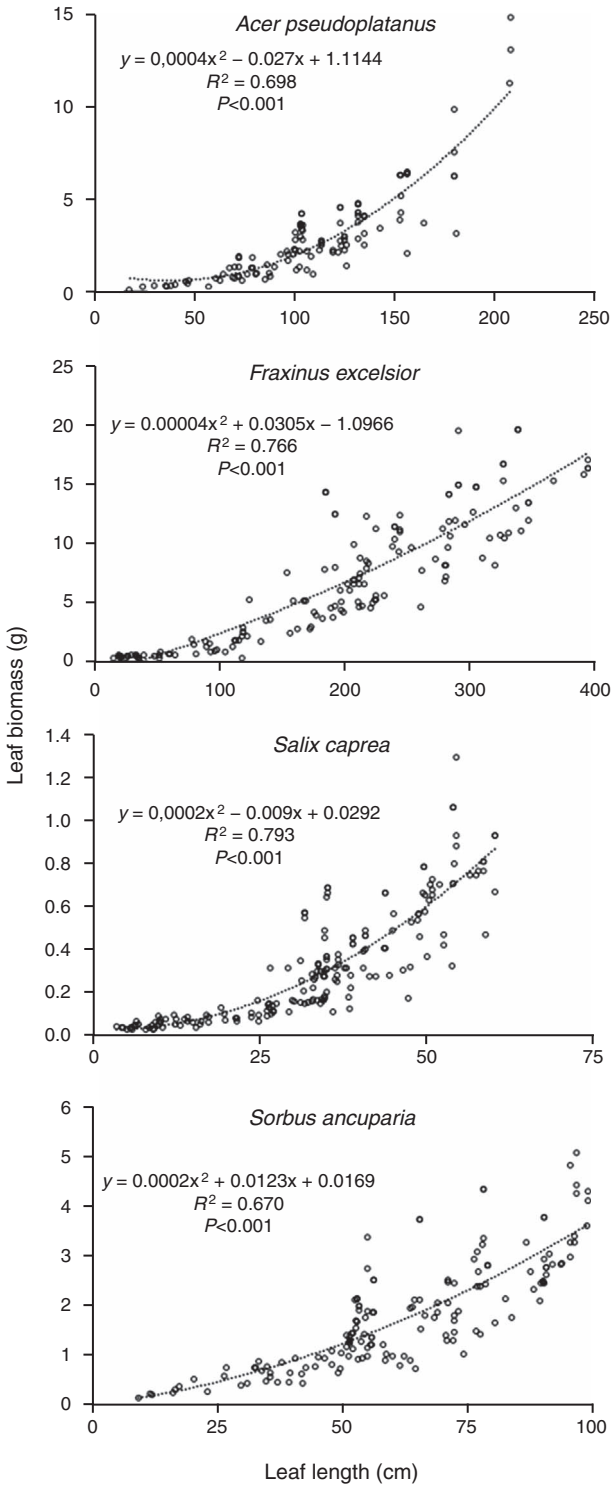

Fig. 2. Allometric equations for leaf development. Changes in cumulative leaf biomass per sprout in relation to changes in cumulative leaf length per sprout.

Phenolic compounds

On the basis of their mean phenolic compound values, the fodder tree leaves were significantly different (box plots in Fig. 5). *Acer pseudoplatanus* and *S. caprea* showed higher concentrations of TEP, TT and HT than did *F. excelsior* and *S. aucuparia*. The concentration of CT was lower in *A. pseudoplatanus* than in *S. caprea* and *S. aucuparia*. The CT were not detected in *F. excelsior*. No significant differences in NTP concentration were detected among the species. Variations in the phenolic composition among the sampling dates were not significant or negligible (Table 5).

In vitro *true dry matter digestibility*

The IVTD of leaves differed significantly among the fodder tree species (Fig. 6). On the basis of leaf mean digestibility values, *F. excelsior* and *S. aucuparia* were significantly more digestible than were *A. pseudoplatanus* and *S. caprea*.

Sampling date affected *A. pseudoplatanus* (highest value: third sampling date; lowest value: final sampling date) and *S. aucuparia* (highest values: first and final sampling dates; lowest value: third sampling date) digestibility. *Fraxinus excelsior* and *S. caprea* leaves were characterised by a more stable digestibility throughout the vegetative season (Table 6).

Discussion

The results obtained in terms of the relationships among leaf traits confirmed the leaf length as an easy recordable in-field plant trait useful to estimate leaf biomass of a tree or shrub species via allometric equations (Itô and Sumida 2017; Škėma *et al*. 2018). The use of allometric equations can, thus, represent a simple and effective tool to estimate foliage production, as it is a non-destructive and time-affordable method (Elzein *et al*. 2011; Konôpka *et al*. 2015; Pajtík and Konôpka 2015).

Concerning leaf development across the vegetative season, budburst dates for *F. excelsior* and *S. aucuparia* were consistent with those obtained in Swiss environments by Basler and Körner (2012). The same authors reported a later budburst for *A. pseudoplatanus*. As outlined in Table 2, precocity of leaf development appeared inversely proportional to final values of both leaf traits; leaves started to develop earlier (i.e. at a lower GDD) in the species with shorter and lighter leaves at the end of the vegetative season (i.e. *S. caprea* and *S. aucuparia*).

These considerations concerning variations in leaf length and biomass across the vegetative season can be used as proficient tools for fodder tree management, since productivity is one of the basic criteria for using a particular tree species as feeding resource (Papanastasis *et al*. 1998). Indeed, in European alpine regions, *F. excelsior* trees growing close to farm households were often used to forage ruminants (Gómez García and Fillat 1984). Trees were managed with pollard practice and yearling sprouts were given to animals either as fresh (in summer) or conserved (in winter) forage (Thiebault 2005).

Concerning the proximate composition, the results obtained for DM and EE were consistent with those already reported by other authors (Ricklefs and Matthew 1982; Kökten *et al*. 2012; Emile *et al*. 2016). The CP contents of the four species were higher than the minimum level of 70–80 g/kg DM required for optimum rumen function and feed intake in goats (Van Soest 1994), with *A. pseudoplatanus* showing the highest values. The range of CP content in tree foliage was comparable to the one of

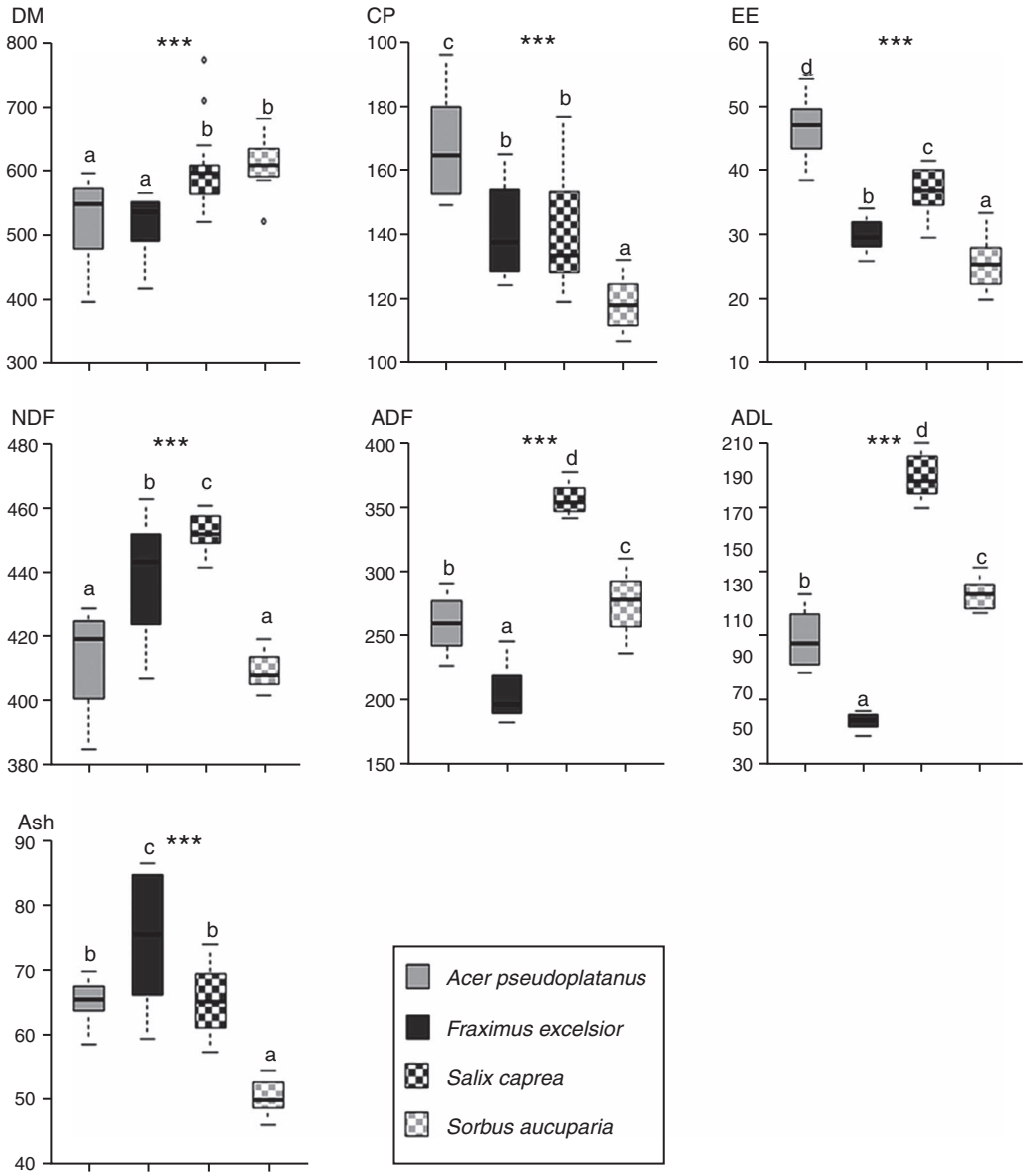

Fig. 3. Proximate composition of the four tree species: overall ranges. Provided data are expressed as g/kg dry matter (DM), except for DM, which is expressed in g/kg fresh matter. CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin. Error bars represent the s.e. of the means, while different letters indicate significant differences among the tree species. ***, $P < 0.001$.

herbage growing in similar alpine environments across the vegetative season (Revello-Chion *et al*. 2011; Luske and van Eekeren 2015; Ravetto Enri *et al*. 2017). Conversely, in dry to arid environments, other authors reported higher CP contents in the foliage of deciduous fodder tree species than in local grass or hay (Yayneshet *et al*. 2009; Gardiner *et al*. 2013). Lu *et al*. (2005) showed that ADF concentrations of 180–200 g/kg DM or NDF concentrations of 410 g/kg DM meet the nutritional requirements for dairy goats. Other research highlighted that increasing the ADF or NDF contents over 180–200 g/kg DM and 600 g/kg DM respectively, may limit feed intake due to rumen fill (Santini *et al*. 1992; Mertens 1994; Riaz *et al*. 2014). Therefore, all the studied species showed adequate average NDF amounts according to these authors. However, if the leaves of the four species were given as sole feed, *A. pseudoplatanus*, *S. aucuparia*, and (with a stronger effect) *S. caprea*, may reduce the feed intake, due their high average ADF contents (Santini *et al*. 1992; Lu *et al*. 2005). In this respect, *F. excelsior* may exert the mildest effect, being slightly over the ADF limit. Other studies provided results sometimes different for the proximate composition, even if leaf samples were collected from the same tree species, in similar environments, and at comparable dates. Regarding *F. excelsior*,

Table 3. Differences among tree species and sampling dates (expressed in day of the year, DOY) in terms of proximate composition

ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; DM, dry matter; EE, ether extract; NDF, neutral detergent fibre. Uppercase letters indicate significant differences among tree species within a sampling date according to *post hoc* tests; significance levels are provided on the right side of each line. Lowercase letters indicate significant differences among sampling dates within a tree species according to *post hoc* tests; significance levels are provided on the bottom of each column. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; n.s., $P \geq 0.05$

Parameter	DOY	*Acer pseudoplatanus*	*Fraxinus excelsior*	*Salix caprea*	*Sorbus aucuparia*	Species significance
DM (g/kg fresh matter)	155	423.5 ± 16.43aA	436.2 ± 13.43aA	551.3 ± 12.27aB	590.7 ± 32.89B	***
	183	542.6 ± 20.94bA	529.9 ± 11.13bA	579.4 ± 10.51abAB	612.2 ± 12.64B	**
	210	556.3 ± 15.68bA	532.8 ± 10.72bA	668.2 ± 46.10bB	631.7 ± 15.77AB	*
	237	560.3 ± 8.67bA	552.2 ± 5.68bA	600.3 ± 3.50abB	605.0 ± 7.33B	***
	DOY significance	***	***	*	n.s.	
CP (g/kg DM)	155	189.8 ± 2.72cD	161.3 ± 1.75bB	173.1 ± 1.88cC	120.8 ± 1.94bcA	***
	183	170.8 ± 1.12bB	126.6 ± 1.46aA	134.4 ± 1.25bAB	126.4 ± 2.12cA	**
	210	152.5 ± 1.88aC	129.9 ± 2.20aB	122.8 ± 1.56aB	114.1 ± 2.22abA	***
	237	154.2 ± 2.03aD	143.4 ± 2.03abC	131.6 ± 1.91bB	110.7 ± 2.17aA	***
	DOY significance	*	**	***	***	
EE (g/kg DM)	155	47.3 ± 1.25bC	29.6 ± 0.88A	38.0 ± 0.87bB	30.3 ± 1.16bA	***
	183	39.8 ± 0.71aC	29.6 ± 1.60AB	37.6 ± 1.64bBC	25.3 ± 1.21abA	***
	210	49.5 ± 1.56bC	27.8 ± 0.85B	31.4 ± 0.94aB	22.6 ± 1.41aA	***
	237	46.8 ± 1.42bD	31.6 ± 0.78B	39.0 ± 1.24bC	22.9 ± 1.07aA	***
	DOY significance	***	n.s.	**	**	
Ash (g/kg DM)	155	63.8 ± 1.58B	64.0 ± 1.72aB	61.7 ± 1.20aB	51.5 ± 1.11A	***
	183	61.7 ± 1.41BC	67.0 ± 1.27aC	59.8 ± 1.15aB	51.3 ± 1.19A	***
	210	66.7 ± 1.25B	83.4 ± 1.29bC	68.0 ± 1.48bB	49.6 ± 1.17A	***
	237	67.4 ± 1.22B	84.9 ± 0.45bC	70.8 ± 1.41bB	48.4 ± 1.27A	***
	DOY significance	n.s.	***	***	n.s.	
NDF (g/kg DM)	155	385.5 ± 1.04aA	408.1 ± 0.78aB	442.8 ± 1.34aC	408.5 ± 3.30B	***
	183	423.5 ± 1.53cAB	459.4 ± 1.67bAB	455.9 ± 1.77bB	408.6 ± 2.12A	**
	210	413.7 ± 1.36bB	440.0 ± 1.55abC	454.7 ± 1.65bD	404.0 ± 1.84A	***
	237	423.8 ± 1.54cB	445.6 ± 1.46abC	452.5 ± 2.34bC	412.1 ± 2.24A	***
	DOY significance	***	**	**	n.s.	
ADF (g/kg DM)	155	225.5 ± 0.44aAB	194.3 ± 1.51bA	341.8 ± 1.68aB	240.0 ± 2.31aAB	**
	183	266.5 ± 2.16abB	195.8 ± 1.45bA	358.2 ± 1.80bD	278.9 ± 1.99abC	***
	210	254.3 ± 0.50abB	182.8 ± 0.98aA	346.6 ± 0.98aD	270.9 ± 2.56abC	***
	237	286.0 ± 2.03bB	239.9 ± 1.56cA	367.7 ± 2.47cD	304.7 ± 2.42bC	***
	DOY significance	**	***	***	**	
ADL (g/kg DM)	155	84.5 ± 1.69aB	53.6 ± 1.67A	190.5 ± 1.89bD	120.6 ± 2.02aC	***
	183	84.5 ± 1.99aB	54.3 ± 1.83A	183.8 ± 1.94bD	119.5 ± 2.53aC	***
	210	106.0 ± 1.66bB	48.9 ± 1.85A	175.0 ± 1.74aD	118.4 ± 2.67aC	***
	237	119.0 ± 1.86cB	56.1 ± 1.25A	206.8 ± 0.54cD	136.1 ± 1.10bC	***
	DOY significance	***	n.s.	***	***	

Emile *et al*. (2016) found lower DM and NDF and, together with Luske and van Eekeren (2015), comparable CP contents, while Masson *et al*. (1980) reported lower DM and higher ash and CP contents. Hejcman *et al*. (2016), instead, reported similar ADF but lower NDF, ADL and ash concentrations for *S. aucuparia* leaves in Iceland.

The temporal fluctuations in the proximate composition can be commonly observed in many fodder-tree species with similar trends, also in other than European environments (Papachristou and Papanastasis 1994; Yayneshet *et al*. 2009; Kökten *et al*. 2012), and in fresh grass from semi-natural pastures (Bovolenta *et al*. 2008).

The concentrations of the main FA were consistent with the findings for other fodder tree species and with grassland fodder values, also from environments other than the alpine ones (Ndondo *et al*. 2004; Uysal *et al*. 2015; Ravetto Enri *et al*. 2017). However, the concentration of C18:3 n3 in *S. aucuparia* leaves was lower than that observed in herbage collected in the same environment (Iussig *et al*. 2015*a*). Rosenqvist and Laakso (1991) reported a FA profile and main FA percentages comparable to those of the present study for *S. caprea* in northern Finland. Concerning *A. pseudoplatanus*, *F. excelsior* and *S. aucuparia*, no data on FA profile of leaves are currently available in literature. Other studies conducted in dryer environments have found amounts of some individual FA and FA groups similar to those of the fodder-tree species studied, while higher SFA and lower PUFA contents have been reported (Ndondo *et al*. 2004; Uysal *et al*. 2015). According to the obtained results on FA profile, *A. pseudoplatanus*, *F. excelsior* and *S. caprea* leaves could be used as feedstuff for goat nutrition, also in late summer, when quality (and particularly C18:3 n3 concentration) of grassland species decreases (Revello-Chion *et al*. 2011; Uysal *et al*. 2015; Ravetto Enri *et al*. 2017). Indeed, the lipid metabolism in the

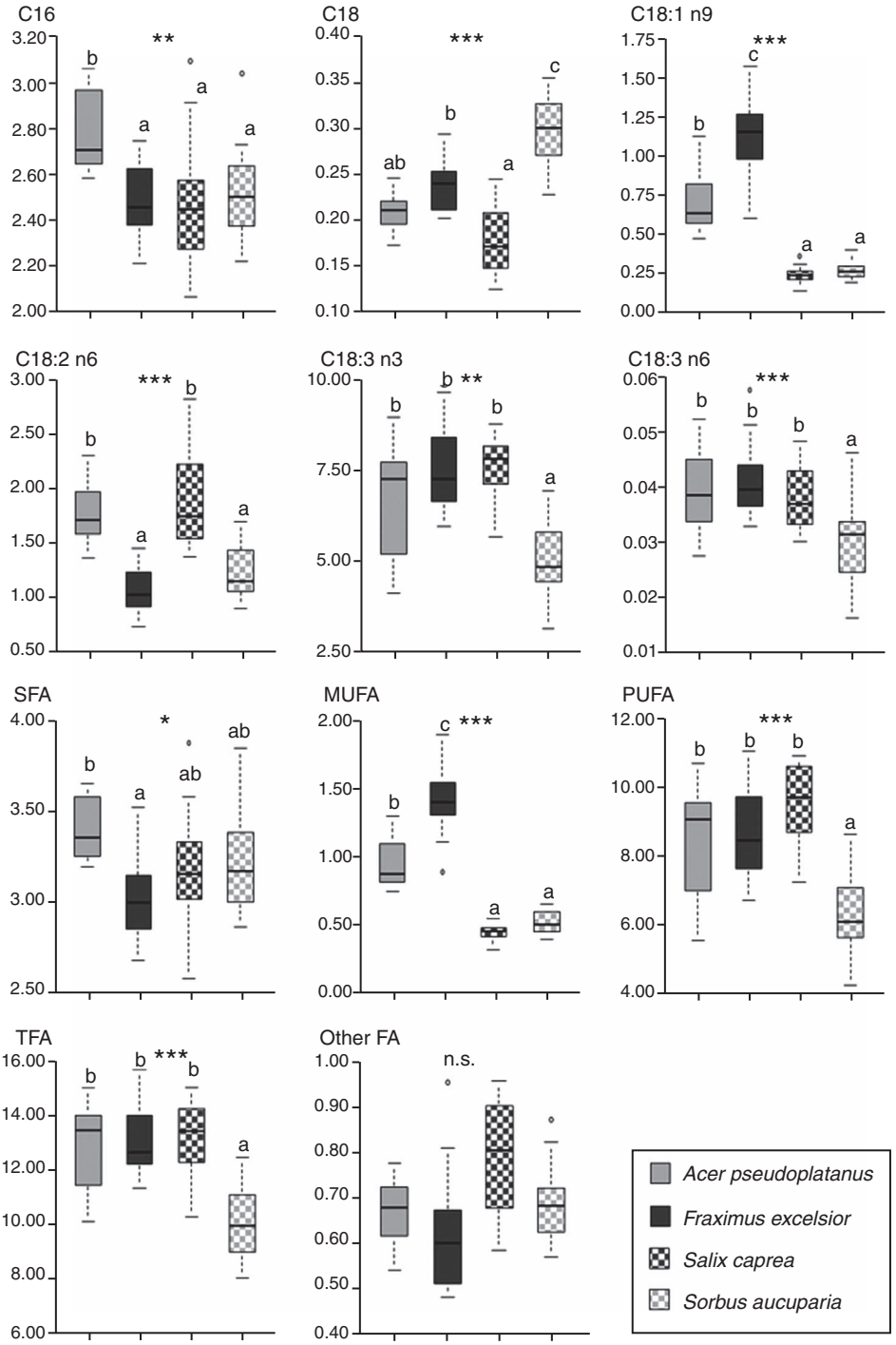

Fig. 4. Fatty acid profile of the four tree species: overall ranges. Provided data are expressed in g/kg dry matter. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, total fatty acids; Other FA, C12:0 + C14:0 + C15:0 + C16:1 n9 + C18:1 *c*11 + C20:0 + C20:1 *c*11 + C22:0 + C20:4 n6. Error bars represent the s.e. of the means, while different letters indicate significant differences among the tree species. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; n.s., $P \geq 0.05$.

rumen and in the mammary gland can be affected by such decrease in the quality of grassland species, usually resulting in higher concentrations of hypercholesterolaemic SFA and lower concentrations of beneficial FA (i.e. vaccenic acid, rumenic acid and n3 FA) in the derived dairy and meat products (Ferlay *et al*. 2017).

Table 4. Differences among tree species and sampling dates (expressed in day of the year, DOY) in terms of fatty acid profile

DM, dry matter; MUFA, monounsaturated fatty acids; Other FA, C12:0 + C14:0 + C15:0 + C16:1 n9 + C18:1 *c*11 + C20:0 + C20:1 *c*11 + C22:0 + C20:4 n6; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, total fatty acids. Uppercase letters indicate significant differences among tree species within a sampling date according to *post hoc* tests; significance levels are provided on the right side of each line. Lowercase letters indicate significant differences among sampling dates within a tree species according to *post hoc* tests; significance levels are provided on the bottom of each column. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; n.s., $P \geq 0.05$

Parameter	DOY	*Acer pseudoplatanus*	*Fraxinus excelsior*	*Salix caprea*	*Sorbus aucuparia*	Species significance
C16:0 (g/kg DM)	155	2.94 ± 0.096B	2.37 ± 0.073A	2.47 ± 0.084A	2.62 ± 0.160AB	*
	183	2.70 ± 0.062B	2.38 ± 0.045A	2.30 ± 0.101A	2.44 ± 0.088AB	*
	210	2.80 ± 0.091	2.61 ± 0.069	2.67 ± 0.200	2.56 ± 0.048	n.s.
	237	2.68 ± 0.058	2.55 ± 0.064	2.39 ± 0.058	2.41 ± 0.095	n.s.
	DOY significance	n.s.	n.s.	n.s.	n.s.	
C18:0 (g/kg DM)	155	0.22 ± 0.006B	0.23 ± 0.006B	0.17 ± 0.016A	0.31 ± 0.013C	***
	183	0.21 ± 0.007A	0.21 ± 0.003A	0.16 ± 0.018A	0.29 ± 0.014B	***
	210	0.20 ± 0.004	0.24 ± 0.016A	0.20 ± 0.018A	0.31 ± 0.018B	***
	237	0.20 ± 0.016A	0.25 ± 0.017AB	0.17 ± 0.017A	0.29 ± 0.027B	**
	DOY significance	n.s.	n.s.	n.s.	n.s.	
C18:1*c*9 (g/kg DM)	155	0.67 ± 0.051B	1.22 ± 0.057C	0.26 ± 0.031A	0.24 ± 0.016A	***
	183	0.62 ± 0.050AB	1.13 ± 0.086B	0.24 ± 0.022A	0.26 ± 0.015A	**
	210	0.82 ± 0.083B	1.19 ± 0.144B	0.24 ± 0.026A	0.32 ± 0.036A	***
	237	0.68 ± 0.149B	0.91 ± 0.141B	0.18 ± 0.021A	0.22 ± 0.028A	***
	DOY significance	n.s.	n.s.	n.s.	n.s.	
C18:2n6 (g/kg DM)	155	2.20 ± 0.035cB	1.32 ± 0.068cA	2.64 ± 0.118bC	1.56 ± 0.069bA	***
	183	1.72 ± 0.023bB	0.96 ± 0.035abA	1.74 ± 0.141aB	1.23 ± 0.074aA	***
	210	1.45 ± 0.064aB	0.81 ± 0.053aA	1.62 ± 0.064aB	1.04 ± 0.042aA	***
	237	1.66 ± 0.056bB	1.09 ± 0.083bcA	1.55 ± 0.098aB	1.03 ± 0.054aA	***
	DOY significance	***	***	***	***	
C18:3n3 (g/kg DM)	155	7.39 ± 0.289B	8.65 ± 0.509B	8.04 ± 0.147B	5.28 ± 0.565A	***
	183	6.02 ± 0.591AB	7.34 ± 0.314B	6.96 ± 0.514B	5.05 ± 0.460A	*
	210	5.93 ± 1.006AB	6.50 ± 0.245B	7.17 ± 0.265B	4.07 ± 0.474A	*
	237	7.29 ± 0.826	7.70 ± 0.771	8.10 ± 0.315	5.46 ± 0.527	n.s.
	DOY significance	n.s.	n.s.	n.s.	n.s.	
C18:3n6 (g/kg DM)	155	0.04 ± 0.002AB	0.04 ± 0.002B	0.03 ± 0.001A	0.03 ± 0.002aAB	**
	183	0.04 ± 0.003	0.04 ± 0.001	0.04 ± 0.003	0.03 ± 0.003ab	n.s.
	210	0.04 ± 0.005	0.04 ± 0.002	0.04 ± 0.003	0.02 ± 0.003a	n.s.
	237	0.04 ± 0.004	0.05 ± 0.005	0.04 ± 0.002	0.04 ± 0.003b	n.s.
	DOY significance	n.s.	n.s.	n.s.	*	
SFA (g/kg DM)	155	3.53 ± 0.099B	2.84 ± 0.067A	3.31 ± 0.097AB	3.30 ± 0.202AB	*
	183	3.29 ± 0.053B	2.91 ± 0.043A	2.94 ± 0.131A	3.13 ± 0.106AB	*
	210	3.40 ± 0.093	3.14 ± 0.110	3.34 ± 0.253	3.27 ± 0.067	n.s.
	237	3.35 ± 0.078	3.16 ± 0.118	3.09 ± 0.101	3.14 ± 0.126	n.s.
	DOY significance	n.s.	n.s.	n.s.	n.s.	
MUFA (g/kg DM)	155	0.93 ± 0.079B	1.48 ± 0.063C	0.47 ± 0.025A	0.52 ± 0.048A	***
	183	0.83 ± 0.024B	1.41 ± 0.089C	0.44 ± 0.034A	0.52 ± 0.045A	***
	210	1.05 ± 0.044B	1.50 ± 0.140C	0.47 ± 0.017A	0.57 ± 0.043A	***
	237	0.91 ± 0.129B	1.21 ± 0.140B	0.38 ± 0.031A	0.45 ± 0.029A	***
	DOY significance	n.s.	n.s.	n.s.	n.s.	
PUFA (g/kg DM)	155	9.64 ± 0.320B	10.03 ± 0.567bB	10.76 ± 0.063bB	6.89 ± 0.603A	***
	183	7.81 ± 0.591AB	8.38 ± 0.342abB	8.77 ± 0.628aB	6.33 ± 0.531A	*
	210	7.45 ± 1.051AB	7.36 ± 0.293aAB	8.87 ± 0.256aB	5.16 ± 0.508A	**
	237	9.04 ± 0.793AB	8.87 ± 0.794abAB	9.76 ± 0.399abB	6.55 ± 0.544A	*
	DOY significance	n.s.	*	*	n.s.	
TFA (g/kg DM)	155	14.10 ± 0.305B	14.36 ± 0.659B	14.54 ± 0.174bB	10.71 ± 0.701A	*
	183	11.93 ± 0.540AB	12.70 ± 0.264B	12.15 ± 0.763aAB	9.99 ± 0.629A	*
	210	11.89 ± 1.053AB	11.99 ± 0.267AB	12.68 ± 0.475abAB	9.01 ± 0.542A	*
	237	13.30 ± 0.645B	13.25 ± 0.946B	13.23 ± 0.449abB	10.14 ± 0.583A	*
	DOY significance	n.s.	n.s.	n.s.	n.s.	
Other FA (g/kg DM)	155	0.64 ± 0.026A	0.53 ± 0.037A	0.93 ± 0.015B	0.67 ± 0.071A	***
	183	0.62 ± 0.038	0.64 ± 0.069	0.72 ± 0.039	0.69 ± 0.044	n.s.
	210	0.66 ± 0.047	0.60 ± 0.046	0.74 ± 0.058	0.69 ± 0.026	n.s.
	237	0.74 ± 0.013	0.70 ± 0.087	0.78 ± 0.070	0.70 ± 0.045	n.s.
	DOY significance	n.s.	n.s.	n.s.	n.s.	

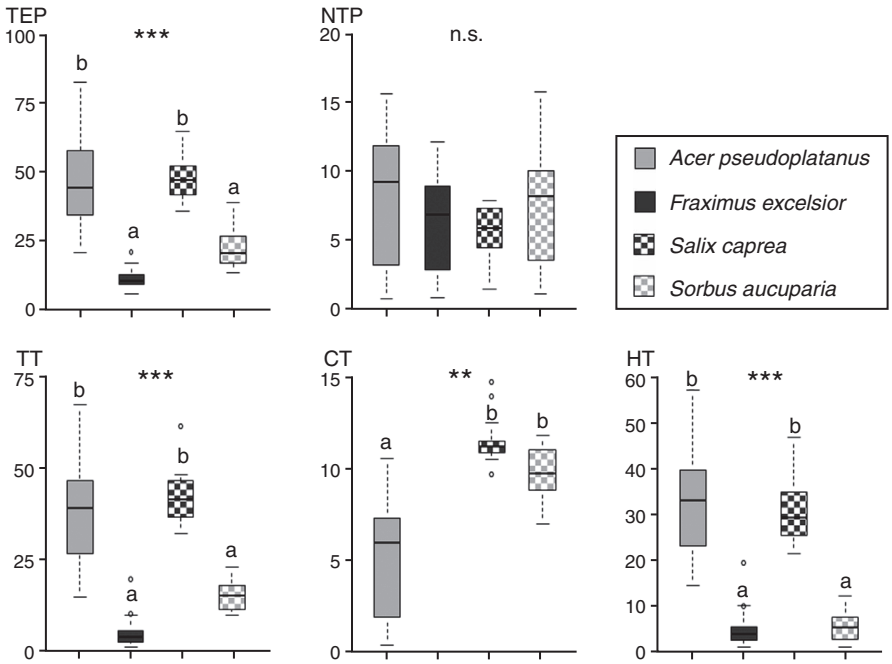

Fig. 5. Phenolic compound concentration of the four tree species: overall ranges. Provided data are expressed in g/kg dry matter, as gallic acid equivalents, except for condensed tannins (CT), which are expressed as leucocyanidin equivalents. TEP, total extractable phenols; NTP, non-tannin phenols; TT, total tannins; HT, hydrolysable tannins. *Fraxinus excelsior* values for CT were null and are not presented. Error bars represent the s.e. of the means, while different letters indicate significant differences among the tree species. ***, $P < 0.001$; **, $P < 0.01$; n.s., $P \geq 0.05$.

Table 5. Differences among tree species and sampling dates (expressed in day of the year, DOY) in terms of phenolic compound content
CT, condensed tannins; DM, dry matter; HT, hydrolysable tannins; NTP, non-tannin phenols; TEP, total extractable phenols; TT, total tannins. *Fraxinus excelsior* values for CT were null and are not presented. Uppercase letters indicate significant differences among tree species within a sampling date according to *post hoc* tests; significance levels are provided on the right side of each line. Lowercase letters indicate significant differences among sampling dates within a tree species according to *post hoc* tests; significance levels are provided on the bottom of each column. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; n.s., $P \geq 0.05$; n.d., not determined

Parameter	DOY	*Acer pseudoplatanus*	*Fraxinus excelsior*	*Salix caprea*	*Sorbus aucuparia*	Species significance
TEP (g/kg DM)	155	30.5 ± 2.81B	14.0 ± 2.40A	42.3 ± 3.26B	17.4 ± 3.16A	***
	183	55.0 ± 12.92C	9.4 ± 1.46A	46.2 ± 3.38BC	22.4 ± 1.19AB	**
	210	52.5 ± 8.18C	10.7 ± 2.14A	51.0 ± 5.77C	24.0 ± 3.99B	***
	237	45.7 ± 3.27C	10.8 ± 1.01A	49.5 ± 1.80C	25.7 ± 4.72B	***
	DOY significance	n.s.	n.s.	n.s.	n.s.	
NTP (g/kg DM)	155	5.6 ± 1.97	6.8 ± 1.95	4.1 ± 1.50	4.6 ± 2.02	n.s.
	183	10.6 ± 2.59	3.5 ± 1.25	6.6 ± 0.58	5.4 ± 2.22	n.s.
	210	6.4 ± 2.74	6.2 ± 2.22	5.5 ± 0.76	9.3 ± 1.26	n.s.
	237	8.6 ± 2.38	8.1 ± 0.56	5.2 ± 1.29	9.8 ± 1.97	n.s.
	DOY significance	n.s.	n.s.	n.s.	n.s.	
TT (g/kg DM)	155	24.9 ± 4.75AB	7.2 ± 4.13A	38.3 ± 2.46B	12.8 ± 1.21A	***
	183	44.4 ± 10.37B	5.9 ± 2.36A	39.5 ± 2.96B	17.0 ± 1.10A	***
	210	46.1 ± 8.27C	4.6 ± 0.67A	45.4 ± 6.09C	14.6 ± 2.82B	***
	237	37.1 ± 3.29C	2.7 ± 0.54A	44.3 ± 1.64C	15.8 ± 2.83B	***
	DOY significance	n.s.	n.s.	n.s.	n.s.	
CT (g/kg DM)	155	1.1 ± 0.44A	n.d.	10.7 ± 0.35aB	8.5 ± 0.89B	*
	183	6.4 ± 1.92A	n.d.	11.2 ± 0.15abB	10.3 ± 0.55AB	*
	210	6.8 ± 1.57A	n.d.	12.9 ± 0.93bB	10.0 ± 0.92AB	*
	237	6.6 ± 1.42A	n.d.	11.2 ± 0.16abB	10.0 ± 0.66AB	*
	DOY significance	n.s.		*	n.s.	
HT (g/kg DM)	155	23.8 ± 4.32B	7.2 ± 4.13A	27.6 ± 2.26B	4.3 ± 0.68A	***
	183	38.0 ± 8.48B	5.9 ± 2.36A	28.3 ± 2.89B	6.8 ± 1.38A	***
	210	39.3 ± 6.97B	4.6 ± 0.67A	32.5 ± 5.23B	4.7 ± 2.08A	***
	237	30.5 ± 2.94B	2.7 ± 0.54A	33.1 ± 1.74B	5.9 ± 2.26A	*
	DOY significance	n.s.	n.s.	n.s.	n.s.	

The TEP, NTP, TT, CT and HT concentrations reported for other fodder tree species from drier regions were similar or even higher than those for the species here studied (Ammar *et al*. 2008; Kökten *et al*. 2012; Gemeda and Hassen 2015). Overall, CT concentrations were lower than the minimum threshold of 20 g/kg identified by Min *et al*. (2003) for ruminant nutrition. According to these and other authors, such CT values should have a limited negative effect on ruminal digestion, especially for goats. In fact, goats prefer tannin-rich foliage, minimising CT consumption through modification of the feed-intake pattern (Mkhise *et al*. 2018), and produce tannin-binding proteins in saliva, overcoming the negative impacts on digestibility in the rumen (Lamy *et al*. 2011).

Interesting and innovative results were shown by IVTD of the four species, which was similar to that of some Mediterranean tree and shrub species, either deciduous or evergreen (Ammar *et al*. 2005, 2008). Digestibility in rumen environment is a complex process, partially still unknown, in which several factors interact with each other (Aguerre *et al*. 2016; Seidavi *et al*. 2018). Chemical composition (i.e. mainly fibre fractions (NDF, ADF, ADL), FA profile (particularly PUFA), and phenolic compounds (above all, CT and HT) exert a crucial multi-layered influence on microbial activity (Waghorn 2008; Boufennara *et al*. 2012; Ferlay *et al*. 2017). The NDF constituents, being mainly represented by hemicellulose and cellulose, are the dominant feed fraction for grazing ruminants, supplying energy for maintenance and production. However, fibre has a slower ruminal passage rate than do other dietary elements because their chemical conformation is difficult to cleave or is even indigestible, resulting in a filling effect over time (Mertens 1994). The inverse relationship between the amount of fibre and digestibility rate reported for other fodder tree species in the Mediterranean region (Ammar *et al*. 2004; Boufennara *et al*. 2012; Gemeda and Hassen 2015) can be observed also in the present trial. This means high NDF, ADF and ADL and low IVTD in *S. caprea*, low NDF and high IVTD in *S. aucuparia*, and low ADF and ADL and high IVTD in *F. excelsior*. Interestingly, these relationships were not displayed in other cases, such as in *A. pseudoplatanus* (low NDF and low IVTD), *F. excelsior* (high NDF and high IVTD) and *S. aucuparia* (high ADF and ADL and high IVTD), as observed also in previous studies (Van Soest 1994; Riaz *et al*. 2014; Gemeda and Hassen 2015). This could be attributable to the additional influence of the other chemicals on the microbiome. The interaction of fibre with different PUFA concentrations could have also played an important role in digestibility, since PUFA can inhibit and/or alter the microbial activity and the biohydrogenation pathways in the rumen (Ferlay *et al*. 2017). For this reason, the high PUFA concentrations (especially concentrations of C18:3 n6, C18:3 n3 and C18:2 n6) in *A. pseudoplatanus* and *S. caprea* leaves, together with their high ADL contents, could have, consequently, contributed to the reduction of the degradation capacity of goat microbiome and IVTD. Indeed, ADL represents a physical barrier between digestible cell content and bacteria (Waghorn and McNabb 2003). In contrast, IVTD was promoted in *F. excelsior* because of the low amount of ADL (despite the high PUFA concentration) and in *S. aucuparia* because of the low PUFA concentration (despite the high ADL concentration). Concerning phenolic compounds, CT may have affected digestion mechanisms (i.e. by binding to dietary protein, fibre and enzymes and by reducing microbiome degradation activity; Makkar 2003; Mueller-Harvey 2006; Waghorn 2008). The chemical structure of CT can differ among tree species and can exert different binding activities, regardless of their concentration, resulting in different IVTD (Hove *et al*. 2001; McSweeney *et al*. 2001). In the present case, the lower IVTD associated with lower CT concentrations observed in *A. pseudoplatanus* leaves may be due to a higher binding activity of the CT molecules. The higher IVTD associated

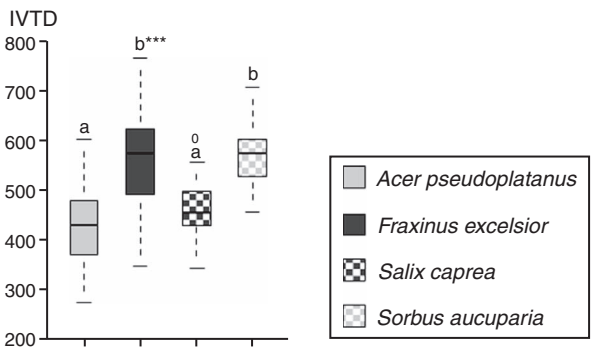

Fig. 6. *In vitro* true dry matter digestibility (IVTD) of the four tree species: overall ranges. Provided data are expressed in g/kg of dry matter. Error bars represent the s.e. of the means, while different letters indicate significant differences among the tree species. ***, $P < 0.001$.

Table 6. Differences among tree species and sampling dates (expressed in day of the year, DOY) in terms of *in vitro* true dry matter digestibility (IVTD)

DM, dry matter. Uppercase letters indicate significant differences among tree species within a sampling date according to *post hoc* tests; significance levels are provided on the right side of each line. Lowercase letters indicate significant differences among sampling dates within a tree species according to *post hoc* tests; significance levels are provided on the bottom of each column. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; n.s., $P \geq 0.05$

Parameter	DOY	*Acer pseudoplatanus*	*Fraxinus excelsior*	*Salix caprea*	*Sorbus aucuparia*	Species significance
IVTD (g/kg DM)	155	399.7 ± 32.15abA	579.8 ± 13.40B	463.7 ± 12.95A	595.1 ± 26.66bB	***
	183	461.8 ± 51.93abA	608.0 ± 26.85B	469.2 ± 13.75S	583.2 ± 1.32abB	***
	210	483.6 ± 44.31b	512.5 ± 41.34	417.9 ± 19.09	508.9 ± 19.01a	n.s.
	237	385.3 ± 61.80aA	558.3 ± 53.30B	498.5 ± 23.32B	602.9 ± 3.61bB	***
	DOY significance	*	n.s.	n.s.	**	

with the higher CT concentration in *S. aucuparia* leaves is probably due to a lower CT activity. The highest HT concentrations found in *A. pseudoplatanus* and *S. caprea* leaves (approximately 6-fold those of *F. excelsior* and *S. aucuparia*) may have negatively influenced the digestibility of these leaves, because of the potential release of compounds toxic for bacteria in the rumen (Makkar 2003; Waghorn 2008). Therefore, the high CT (with a probable high binding activity) and HT concentrations in *S. caprea* leaves could have combined and lowered the digestibility. Conversely, *F. excelsior* leaves showed a phenolic profile more suitable for ruminal digestion, since they were totally free of CT and had low HT concentrations.

The four species showed a consistent stability of IVTD throughout the vegetative season. This is in contrast with the results previously obtained for *Quercus coccifera* L. (an evergreen Mediterranean tree species), which showed a progressive decline of leaf digestibility while dry season advanced. However, it should be pointed out that evergreen species generally show lower quality at late phenological stages (Roukos 2016).

Additional research on productivity and chemical and digestibility features of other tree portions (such as sprouts) or other temperate tree species selected by goats in extensive European farming systems (e.g. *Alnus viridis* (Chaix) DC., *B. pendula*, *Corylus avellana* L., *F. sylvatica*, *Quercus* sp., *Tilia cordata* Mill., *Ulmus* spp.) would be advisable (Hejcman *et al*. 2014; Castro and Fernández Núñez 2016; Vandermeulen *et al*. 2018*a*). Moreover, the preferences by goats for the investigated tree species should be taken into consideration. Future trials should address topics such as the palatability of tree and shrub leaves and sprouts, the related goat diet selection and the effects of tree and shrub leaves and sprouts, used as sole feed or as supplement, on voluntary DM intake by goats, on digestion mechanisms (especially on protein and organic-matter digestibility and on total tract digestion), even through *in vivo* studies, and on goat milk yield, gross composition and FA profile.

Conclusions

The present study has provided original data with a comprehensive approach on leaf traits, and chemical features of four tree species widespread in Europe.

In situ, chemical, and *in vitro* measurements can be considered useful tools in initial screening studies to rank forages according to their nutritive quality. The present results have highlighted that the investigated species, due to their foliage production, proximate composition, FA profile, phenolic composition and digestibility, can represent good-quality feedstuff for goat nutrition. This is especially true in late summer when herbage quality decreases, particularly in terms of CP and the FA profile.

These species can supply goat feedstuff either for direct browsing or as fresh or dried fodder (Bestman *et al*. 2014), also depending on foliage production. Trees could be managed through copping and maintained at a maximum height of 1.80 m to facilitate direct browsing by goats. Alternatively, they could be pollarded to prevent damages from wild ungulates and their branches could be periodically cut for fresh- or dry-fodder delivery. Otherwise, new specific plantations for goat browsing could be established. In this case, the species of the stand and the relative abundance should be selected, taking into account goat feeding preferences as well as leaf nutritive values of the tree species.

The differences among the four selected species were remarkable, even if weakly related to phenological stage advancement, especially when considering the FA and phenolic composition. The high digestibility of *F. excelsior* leaves throughout the vegetative stage could be due to the positive influence of the low concentrations of ADL and phenolic compounds, irrespective of the low CP concentrations. This species could be regarded as potentially highly-nutritive feedstuff, also improving the quality of goat dairy products for human nutrition, as confirmed by its traditional use in European mountain areas (Gómez García and Fillat 1984; Thiebault 2005). Also *S. aucuparia* leaves showed similar digestibility values; however, the low CP and PUFA concentrations (especially C18:3 n3 concentration) can partially reduce the interest for this species as a feeding resource for goat dairy and meat products with healthy properties. Conversely, a lower digestibility was found in *S. caprea* samples, which was also the species with the less productive sprouts, especially at the beginning of the vegetative season, as a consequence of its high phenolic and ADL concentrations. Nevertheless, the FA profile of this species highlighted high C18:2 n6, C18:3 n6 and C18:3 n3 concentrations, generally being recognised as main lipid precursors in ruminant metabolism for the synthesis of FA considered beneficial to human health (e.g. vaccenic acid, rumenic acid and n3 FA). A modest digestibility was recorded for *A. pseudoplatanus* leaves, despite their high CP and medium–low ADL concentrations, which were probably insufficient to contrast the high concentrations of phenolics (TEP, TT and HT) and the high binding activity (CT), even if they displayed a good level of the same ‘healthy precursors’ as reported for *S. caprea* foliage.

Conflicts of interest

The authors declare no conflicts of interest.

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