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Do trait responses to simulated browsing in *Quercus robur* saplings affect their attractiveness to *Capreolus capreolus* the following year?

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Abstract

With the rise of large herbivore populations in most northern hemisphere forests, browsing is becoming an increasingly important driver of forest regeneration dynamics. Among other processes affecting the regeneration, the concept of plant-herbivore feedback loops holds that browsed saplings are more subject to subsequent herbivory. This phenomenon is interpreted as a consequence of compensatory growth following browsing since fast growth is generally associated with higher digestibility and lower defense against herbivores. However, studies linking browsing-induced trait variations to subsequent attractiveness to herbivores are still lacking, especially in the forest context. In this study, we experimentally examine the existence of a feedback loop between oak (*Quercus robur* L.) and roe deer (*Capreolus capreolus*) and investigate its underlying morphological and chemical traits.

We simulated single and repeated roe deer browsing on nursery-grown oak saplings and measured the changes in sapling height growth, lateral branching, leaf traits and winter shoot traits over two years. We conducted winter feeding trials with tame roe deer one year after the first treatment to test the effect of simulated browsing on sapling attractiveness.

Simulated browsing reduced sapling height growth but had no effect on branching. Simulated browsing had no effect on leaf traits after half a year, but decreased the phenolic content and increased the fiber content of winter shoots the following winter. Contrary to our predictions, roe deer preferentially browsed control saplings over saplings previously browsed. After two years, repeated browsing promoted fast carbon acquisition leaf traits (high chlorophyll, high specific leaf area and low fiber content), reduced leaf phenolic content and increased leaf digestibility.

We showed that a reduction in 1-year-old oak sapling height growth following browsing, combined with increased structural defense at the expense of chemical defense in winter shoots the following winter, was correlated with reduced browsing pressure, thereby challenging the feedback loop hypothesis. However, we also demonstrated that repeated browsing promoted fast carbon acquisition leaf traits in 2.5-year-old saplings, which tend to support the existence of a feedback loop on older and more intensively browsed saplings. As such, our study provides empirical evidence that morphological and physiological trait responses to browsing influence oak sapling attractiveness, but that the direction and magnitude of this effect depend on the ontogenic stage of the sapling and on the number of browsing events.

Keywords: *S*imulated browsing, feeding trial, feedback loop, functional traits, plant-herbivore interaction

1. Introduction

Over the last decades, most northern hemisphere forests have been facing an increasing herbivory pressure due to a globalized increase in wild ungulate populations (Beguin et al., 2016; Linnell and Zachos, 2011). Numerous studies show that the level of browsing damage observed on young saplings in these forests may compromise their ecological and economic value (Clasen et al., 2011; Côté et al., 2004). A better understanding of how young saplings respond to this increasing browsing pressure and of the influence of wild ungulates on forest regeneration dynamics is therefore critical to better manage these ecosystems. In temperate and boreal forests, several studies revealed that individual saplings browsed by ungulates tend to be more frequently browsed the following year (Craig, 2010; Cromsigt and Kuijper, 2011; Mathisen et al., 2017; Moore et al., 2000). This creates a positive "feedback loop" (Burkepile and Parker, 2017; Craig, 2010) between ungulates and saplings, which may decrease the number of saplings able to grow above the reach of ungulates (Bergqvist et al., 2003). In the context of feedback loops, a set of individual saplings undergo browsing almost every year and stay within the reach of cervids, whereas the remaining unbrowsed individuals should rapidly grow above browsing height. Conversely, in the absence of a feedback loop, a greater proportion of saplings are browsed at least once, which should result in a longer regeneration phase but with a higher number of individuals eventually able to grow above browsing height (Bergqvist et al., 2003). As such, feedback loops are likely to be critical for forest ecosystem dynamics, but the mechanisms governing this process remain highly understudied. In particular, it is unclear whether the variation in sapling trait in response to browsing could increase their attractiveness towards ungulates the following year.

Herbivory can induce various plant responses at the tree level, depending on the resistance strategy of the tree species (Boege and Marquis, 2005; Züst and Agrawal, 2017). Tree species that invest in defense strategies divert carbohydrate resource allocation toward structural defense (e.g. high fiber content, spines, hairs) (Barrere et al., 2019; Takada et al., 2003) and/or chemical defense (e.g. secondary metabolites like phenols) (Iason, 2005) following browsing, thereby reducing the probability of future damage (Bee et al., 2011; Stolter et al., 2005). Tree species that invest in rapid growth strategies allocate their carbohydrate resources toward fast carbon acquisition following browsing in order to compensate for the loss of biomass (Edenius et al., 1993; Palmer and Truscott, 2003). No study to date have investigated how such trait response influences the probability of subsequent browsing, but an investment in rapid growth strategies could in theory increase sapling attractiveness to ungulates via several mechanisms. First, due to the well-documented trade-off between plant growth and defense (Coley, 1988; Herms and Mattson, 1992; Züst and Agrawal, 2017), an investment in compensatory growth implies fewer anti-herbivore defensive compounds in the leaves (DuToit et al., 1990). Second, an investment in rapid growth following browsing also leads to higher values of fast carbon acquisition traits such as higher specific leaf area (SLA hereafter) (Baraza et al., 2010), or increased leaf chlorophyll content (Danell and Huss-Danell, 1985). These traits are known to positively correlate with leaf nitrogen content (Wright et al., 2004) and digestibility (Cornelissen et al., 2004), which are both important drivers of food selection by ungulates (Bee et al., 2011; Felton et al., 2018; Lloyd et al., 2010). Finally, browsing may increase attractiveness by modifying sapling stature and architecture (Hartley et al., 1997). Common morphological adjustments following browsing include a reduction in height growth and increased lateral branching (Danell et al., 1994; Drexhage and Colin, 2003), which maintains a high number of shoots at the herbivore’s optimal browsing height (Cromsigt and Kuijper, 2011).

Chemical trait responses to browsing in deciduous saplings have been detected from 2 hours (Ohse et al., 2017) to several months (Danell et al., 1994; Danell and Huss-Danell, 1985) after the browsing event, but it is not known whether the trait variations related to compensatory growth can persist after a growing season to influence subsequent attractiveness. Furthermore, although the winter shoots of deciduous saplings are more frequently browsed than their leaves (Petersson et al., 2020), studies on sapling physiological response to herbivory are generally focused on leaf traits (Danell et al., 1994; Danell and Huss-Danell, 1985; Woolery and Jacobs, 2014) and rarely on winter shoot traits (but see Ohse et al., 2017). The influence of winter browsing on the chemical composition of shoots the following year is therefore critical to understanding the mechanisms underlying feedback loops, but nevertheless remains highly understudied. Our study aims at unraveling the mechanisms underlying feedback loops by testing the effect of winter browsing on sapling morphology, leaf traits, the chemical composition of shoots the following year and sapling attractiveness the following winter. More precisely, we focused on the interaction between pedunculate oak (*Quercus robur* L., oak hereafter) and roe deer (*Capreolus capreolus*). Oak is a foundational species in European temperate forests since it defines an entire ecological community or ecosystem by virtue of its structural or functional attributes (Ellison et al., 2005). This species is of high value both for economic reasons and for the high level of biodiversity provided by oak-dominated forests (Mölder et al., 2019). Roe deer are widespread in European forests, and are known to frequently browse on oak sapling shoots (Drexhage and Colin, 2003; Löf et al., 2021), especially in winter (Petersson et al., 2020). We tested the following hypotheses:

H1: Winter browsing promotes leaf traits related to fast carbon acquisition at the expense of defense, and lateral branching over height growth. We thus expect browsed saplings to have higher SLA, chlorophyll content, nitrogen content, digestibility, and lower fiber and phenolic content, but also lower height growth and higher lateral branching than unbrowsed saplings.

H2: Browsing modifies the chemical composition of shoots the following winter, leading to higher nitrogen content and digestibility, and lower phenolic and fiber content.

H3: Winter-browsed saplings are more attractive to roe deer the following winter than unbrowsed saplings.

To test these hypotheses, we grew oak saplings from seed in a nursery and performed a standardized simulation of roe deer browsing at the end of winter, keeping half of them as controls. Six leaf traits (leaf chlorophyll, nitrogen, fiber and phenol content, SLA, digestibility) were measured the following summer, and the plant architecture was monitored before and after the growing season (H1). The next winter, four shoot traits (nitrogen, fibers and phenol content, digestibility) were measured (H2), and feeding trials with tame roe deer were performed to estimate the effect of previous browsing on oak attractiveness to roe deer (H3).

2. Material and methods

2.1.Plant material

In March 2018 acorns collected in the Saône Valley (47°22’ N, 5°29’ E) in Autumn 2017 were sown in the experimental nursery of the French National Forest Office (ONF) in Saint-Paul-lez-Durance (43°42’ N, 5°44’ E). Acorns were stored in a cold chamber at a temperature ranging from 2°C to 4°C between collection and sowing. They were planted in 650 individual pots (1120 cm^3, 20-cm high x 56 cm^2 surface area) with a mix of blond peat (50%) and pine bark (50%), and a single application of slow-release Osmocote fertilizer (2 kg/m^3, NPK –

15:9:11) and of fast-release fertilizer (1 kg/m^3, NPK – 12:12:17). This organic growing media was selected to optimize water retention and aeration, following the study of Guehl et al. (1989). The initial nutrient supply was complemented with a fertigation system in summer 2019 (12 applications from August 6 to September 24, NPK – 12:6:8, 2g/L, 5L/m^3). Seedlings were grown in a greenhouse the first year. They were watered on a daily basis to maintain the growing media pF (log of the matric potential) between 1 and 1.5, which corresponded to a spray volume ranging between 0.5 to 15 L/m^2/day, depending on the season and the amount of precipitation. As part of the experiment, 55 additional oak saplings were also grown in the same conditions and used as a reference for the chemical analyses (see Section 2.3).

2.2. Winter browsing treatment

After one year of growth, the 650 saplings were moved outdoor and organized in two columns of plastic crates (1500 cm^2, 30 cm x 50 cm) that contained 17 pot each, resulting in an average density of 113 saplings.m^{-2} (see Supplementary Material, Appendix B, Figure B.1.b). In March 2019, the crates were assigning to the control or the "browsed" groups, so that crates of the same group were not adjacent to each other (Supplementary Material, Appendix B). The control group (C hereafter, n = 340, split in 20 crates of 17 saplings) did not undergo any treatment, and the "browsed" group (Br hereafter, n = 310, split into 18 crates of 17 saplings and 1 crate of 4 saplings) received a browsing treatment (Fig. 1.c). Browsing was simulated by clipping a 5cm long piece of the apical shoot with scissors and applying 10 µL of roe deer saliva on the clipped section with a pipette (Ohse et al., 2017) (Fig. 1.c; and see Supplementary Material, Appendix A, for details on saliva collection).

After two year of growth, in March 2020, the simulated browsing treatment was repeated according to the following design: 30 BrBr saplings underwent the browsing treatment in both 2019 and 2020, 30 CBr saplings underwent the treatment only in 2020, and 30 CC saplings did not undergo any browsing treatment (Fig. 1.c). The 30 saplings of each treatment were split into one crate of 17 saplings and one crate of 13 saplings (see Supplementary Material, Appendix B, Figure B.1.b).

2.2.Trait measurements

Sapling architecture

To determine the influence of the browsing treatment on sapling height growth and lateral branching (H1), we measured sapling height (distance between the collar and the highest living bud), root-collar diameter (hereafter referred to as "diameter") and the number of ramifications before the beginning of (March, n=650) and after (December, n=590) the growing season in 2019. All living shoots longer than 1 cm were considered to be ramifications, regardless of whether they stemmed from the apical shoot or from other shoots (Fig. 1.a).

Leaf traits

Six leaf traits were measured on 30 individual per treatment in August 2019 (treatments C and Br), and in August 2020 (treatments CC, CBr and BrBr) to test the influence of the browsing treatment on leaf traits the following summer over two years (H1) (Fig. 1.c).

Three large mature leaves exposed to direct solar radiation and located in the top fourth of the plant were selected per sapling to measure their chlorophyll content (LChl hereafter) and specific leaf area (SLA hereafter). LChl were obtained using a CCM-200 chlorophyll meter (Opti-Sciences, Tyngsboro, MA, USA) and the equation presented in Bagard et al. (2008). The sapling LChl value is the average of six points, made on the upper side of the three leaves, one on each side of the midrib. The leaves were scanned to measure their projected area (using ImageJ software version 1.52a (Rasband, 2012)) and oven-dried to measure their dry mass. The saplings SLA value is the average the ratios between the leaf area and their dry mass (mm^2/mg) (Pérez-Harguindeguy et al., 2013).

Leaf biochemical composition was estimated using near-infrared spectroscopy (NIRS). All leaves from each individual sapling were harvested and pooled with the three leaves selected for LChl and SLA measurements. They were then oven-dried (60°C for 24 h) and ground in a cutting mill (Retsch SM100, Retsch GmbH, Hann, Germany) equipped with a 1-mm sieve. A reflectance monochromator (FOSS NIRsystems 6500, Laurel, MD, USA) was used to scan the samples placed in small circular cups (50 mm) with a quartz glass cover. Spectral data were collected every 2 nm between 400 nm and 2500 nm. Each sample was scanned twice (with different cup fillings) and the two values were averaged for each sapling. We predicted the values of leaf fiber content (Neutral Detergent Fibers, LNDF hereafter), nitrogen content (LNC hereafter), dry matter digestibility (LDMD hereafter) and total phenols (LPhenol hereafter) (Singleton et al., 1999) from these NIRS spectra (table 1). We used an existing calibration from CIRAD (Center for International Cooperation in Agronomic Research), combined with direct measurements on 55 additional oak saplings grown in the nursery (calibration R^2 ranging from 0.91 to 0.94 for the 4 traits measured). Calibration was performed using modified partial least square regression with WINISI software (version 4, Infrasoft International, Port Matilda, PA, USA). The oak leaf used for the calibration were oven-dried (60°C for 24 h) and ground. We used a Kjeldahl procedure to measure nitrogen, the Folin Ciocalteu colorimetric method (Singleton et al., 1999) for total phenols, the pepsin-cellulase method (Aufrère et al., 2007) for dry matter digestibility, and sequential fractionation (Van Soest et al., 1991) for fiber contents. Some studies reported that air-drying underestimates some phenolic compounds (Eichenberg et al., 2014; Julkunen-Tiitto and Sorsa, 2001), whereas other studies did not reveal any biases (Couture et al., 2016; Makkar and Singh, 1991), but it has not been shown to affect the hierarchy between the different individuals sampled in either cases.

Winter shoot traits

In March 2020, the shoots clipped after the second browsing treatment (applied on 30 C and 30 Br saplings, Fig. 1.c) were oven-dried and ground following the same methodology as that was used for the leaves (H2). For methodological requirements of the NIRS methods, the shoots of three saplings from the same treatment were pooled obtain ten samples of shoots per treatment. Shoot fiber content (SNDF), nitrogen content (SNC), dry matter digestibility (SDMD) and total phenolic content (SPhenol) were estimated using the same procedures as described for the leaves, and with the same calibration spectra.

2.3.Feeding trials

In December 2019, 500 saplings (250 C and 250 Br) not used for any leaf and shoot trait measurement were transferred to the experimental unit of the French National Institute for Agricultural and Environment Research (INRAE) in Gardouch (43°22’ N, 1°40’ E) to test the influence of the treatment on oak sapling attractiveness to tame roe deer (H3) (Fig. 1.c). Feeding trials were conducted at the end of winter (February 2020), on leafless saplings before budburst. The tests took place in three enclosures of approximately 0.5 ha that each hosted a birth cohort of two or three adult female roe deer. The captive roe deer were fed with pelleted food *ad libitum* and had access to the natural vegetation available in their enclosure (mainly grasses and forbs). The deer were used to feeding on browse as they were regularly given fresh branches of deciduous tree species to supplement their diet (i.e., *Prunus domesticus*, *Quercus sp*, *Cornus sangineus*, *Ulmus carpinifolia*, *Crataegus monogyna*, and *Acer campestre*).

For each feeding trial, 24 saplings were placed in the center of the enclosure, in pre-dug holes in the soil, to form two groups of 12 saplings per treatment (Br vs C) (Fig. 1b). The aggregation of saplings of the same treatments helps the animal to detect differences between the treatments (see Verheyden-Tixier and Duncan, 2000; Verheyden et al., 1998 or Rea et al., 2017 for similar approaches). Saplings were spaced by 50 cm, which is sufficiently large to enable the animal to move between saplings, but sufficiently small so that it can easily switch from one group of saplings (i.e. treatment) to the other. (H. Verheyden, pers. obs.). The position of the groups (C and Br) was systematically switched between two consecutive tests to avoid any bias due to the positioning of the sapling groups. During the three weeks preceding the feeding trials, the animals were acclimated to the experiment by placing 24 oak saplings with morphological characteristics similar to the saplings in the experiment in each enclosure one day per week. All the tests began between 8.45 and 9 a.m. A minimum period of two days was left between two tests in the same enclosure. Two types of tests were conducted over a period of four weeks from the 3rd to the 26th of February 2020.

(1) The immediate selection patterns were tested using ≤ 1 hour feeding trials ("1-h tests" hereafter). Eight tests consisted in filming three roe deer (consuming the plant or not) until half of the sapling were browsed at least once using a Canon SX60HS Power Shot camera (Fig. 1.b). We used this method to avoid a bias due to resource depletion (i.e., if all the saplings were browsed down, it might have altered the deer's initial food choice). The number of saplings browsed in each treatment was recorded. The shoot length that has been consumed was also estimated as the difference between total shoot lengths (including the plant height and all ramifications) measured before and after the test. The video recordings were analyzed with version 6.3 of the Behavioral Observation Research Interactive Software (Friard and Gamba, 2016). In the video

analysis, the total amount of time the roe deer spent sniffing and consuming the saplings of each treatment was counted.

(2) Eleven longer feeding trials ("24-h tests" hereafter) were performed to assess consumption preferences in two other enclosures containing two and three deer, respectively. For each test, the saplings were left in place for 24 hours under the surveillance of two camera traps, one facing the C saplings and the other facing the Br saplings, to ensure that the animals consumed the saplings within the 24 h period. Again, we counted the number of browsed saplings and the shoot length that has been consumed after each test.

2.4.Data analyses

Effect of simulated browsing on sapling height growth, lateral branching and leaf traits (H1)

To compare height growth between treatments, we estimated a standardized measurement of height increment between March and December 2019 that accounts for sapling root-collar diameter. Indeed, both initial height and diameter accounted for a significant part of the variability in final height (see Supplementary Material, Appendix C). Therefore, the standardized height growth is the residuals of a linear model that predicted the final height from initial height, initial diameter and their interaction. Differences between standardized height growth were tested using Student's t-test. Similarly, a standardized branching was computed as the residuals of a generalized linear model with a Poisson error distribution that predicted the number of ramifications in December 2019 as a function of the number of ramifications and diameter in March 2019. These two models are presented in detail in the Supplementary Material, Appendix C.

The effect of the treatment on leaf traits was tested using a redundancy analysis (RDA). Although describing different leaf functions and properties, the selected traits are often found to be correlated (see Coley 1988; Wright *et al.,* 2004; Sterck *et al.,* 2006; Díaz *et al.,* 2016) (Fig. 3.b). This analysis shows the major axes of differentiation in leaf traits between treatments in a multivariate space accounting for the correlation structure between traits. We constructed a unique multivariate space using traits values from both years (2019 and 2020) and the coordinates of individual saplings on the first RDA axis were compared between treatments using an ANOVA, followed by Tukey's post-hoc test, for each year separately. All traits were centered and scaled prior the analysis and LChl was log-transformed to fulfill normality assumptions. Univariate analyses were also performed for each trait separately and are presented in detail in the Supplementary Material, Appendix D.

Effect of simulated browsing on winter shoot traits (H2)

The effect of the simulated browsing was tested on all shoot traits simultaneously (SNC, SDMD, SPhenol and SNDF) using a RDA. All shoot traits were centered and scaled prior to the analysis. The coordinates on the first RDA axis of C and Br individuals were compared using an ANOVA, followed by Tukey's post-hoc test. Univariate analyses were also performed for shoot traits and are presented in detail in the Supplementary Material, Appendix D.

Effect of simulated browsing on attractiveness for roe deer (H3)

For each test type separately (1 h or 24 h), paired Wilcoxon signed-rank tests were used to analyze the effect of the simulated browsing on the number of saplings browsed and on the shoot length consumed per treatment. In the 1-h tests, it was found that one individual roe deer accounted for more than 90% of the total time spent both consuming and sniffing saplings in all of the tests, the statistical analyses of these two variables was restricted to this individual only. Paired Wilcoxon signed-rank tests were used to compare the time spent by the animal consuming and sniffing C and Br saplings in each 1-h test.

All statistical analyses were conducted with R statistical software, version 3.6.3 (R Core Team, 2019). Redundancy analyses were performed with the ‘vegan’ R package (Oksanen, 2017).

3. Results

3.1.Effect of simulated browsing on sapling height growth, branching, and leaf traits (H1)

The first year of the experiment, simulated browsing significantly reduced sapling standardized height growth (Student’s t-test, $t_{582} = 3.06$, $p < 0.01$) (Fig. 2.a) – i.e., for a given initial height and diameter, C saplings grew an average of 4 cm.year^{-1} faster than Br saplings. Simulated browsing did not influence lateral branching (Student’s t-test, $t_{576} = 0.95$, $p = 0.34$) (Fig. 2.b).

The first axis (RDA1) of the RDA conducted on six leaf traits explained 15% of the differences in trait values between groups, and mainly contrasted traits related to fast carbon acquisition (i.e. LDMD and SLA) and to defense traits (i.e. LPhenol and LNDF), while LNC and LChl had weak score values on RDA1 (Fig. 3.b, and Supplementary Material, Appendix E). Simulated browsing had no effect on 1.5-year-old sapling leaf traits (ANOVA, $F_{1, 57} = 1.91$, $p = 0.17$). However, simulated browsing significantly influenced 2.5-year-old sapling leaf traits (ANOVA, $F_{2, 80} = 3.91$, $p = 0.02$): BrBr saplings had higher values of fast carbon acquisition traits, while CC saplings had higher values of defense traits (Fig. 3.a). The univariate analysis showed that simulated browsing reduced 1.5-year-old sapling SLA and increased 2.5-year-old sapling LNC and LChl (Supplementary Material, Appendix D).

3.2. *Effect of simulated browsing on winter shoot traits (H2)*

The first axis of the RDA conducted on four shoot traits (SNC, SDMD, SPhenol and SNDF) of 2-year-old saplings explained 14% of the differences in trait values between treatments, and contrasted shoots with high phenolic content to shoots with high fiber content (Fig. 4.b, and Supplementary Material, Appendix E). The simulated browsing treatment significantly influenced shoot traits (ANOVA, $F_{1, 18} = 14.26$, $p < 0.01$): Br saplings had lower SPhenol and higher SNDF compared to C saplings (Fig. 4.a). The univariate analysis also showed that simulated browsing reduced SPhenol in 2-year-old oak saplings (Supplementary Material, Appendix D).

3.3. *Effect of simulated browsing on attractiveness for roe deer (H3)*

During the 1-h tests, simulated browsing significantly reduced sapling attractiveness: roe deer browsed an average of 7.88 C saplings compared to 4.38 Br saplings out of 12 saplings per treatment, but the trend was not significant (Wilcoxon signed rank, $V_7 = 24$, $p = 0.12$). The same trend was significant for the 24-h tests: 9.91 saplings browsed on average for C, vs. 6.27 for Br (Wilcoxon signed rank, $V_{10} = 45$, $p < 0.01$) (Fig. 5.a). Similarly, simulated browsing reduced the shoot length browsed by roe deer (Fig. 5.b) both in the 1-h tests (Wilcoxon signed rank, $V_7 = 31$, $p = 0.078$) and in the 24-h tests (Wilcoxon signed rank, $V_{10} = 24$, $p < 0.01$), but the trend was only significant for the 24-h tests. During the 1-h tests, high inter-individual variability was observed among the three female roe deer present in the enclosure: one individual was responsible for 93% of the total time spent consuming. In six out of the eight 1-h tests, this individual spent more time consuming C than Br saplings, which resulted in a significant negative effect of the simulated browsing treatment on the time spent consuming (Wilcoxon signed rank, $V_7 = 34$, $p = 0.02$) (Fig. 5.c). However, the treatment did not influence the time spent sniffing the saplings (Wilcoxon signed rank, $V_7 = 5$, $p = 0.42$) (Fig. 5.c).

4. Discussion

4.1. Effect of simulated browsing on sapling height growth, lateral branching and leaf traits (H1)

Simulating apical bud browsing in winter was found to be sufficient to reduce oak sapling height growth. While other species like beech (*Fagus sylvatica*) (Frank et al., 2019) or Scots pine (*Pinus sylvestris*) (Edenius et al., 1993) have been found to compensate for the loss of the apical bud by increasing their annual growth rate, our results indicate that one-year-old oak saplings are not capable of such a response. A reduction in sapling height growth following browsing of the apical shoot was found in similar studies conducted on sessile oak (*Quercus petraea* (Matt.) Liebl.) saplings, but this decrease in height growth was compensated for by an increase in the number of ramifications (Chaar et al., 1997; Drexhage and Colin, 2003). This response is interpreted as a biomass allocation strategy to promote a plurality of competitive growth axes, which could increase resilience in the eventuality of future browsing events (Drexhage and Colin, 2003). In our study, simulated browsing did not influence the production of new ramifications. The ability of saplings to develop more ramifications following browsing has been shown to increase with the number of shoots browsed (Bergström and Danell, 1987; Frank et al., 2019), and to increase with sapling age (Hódar et al., 2008). Thus, the absence of response to browsing in our study is probably likely due to the young age of the saplings (i.e., browsed at one year of age), and to the fact that only one shoot was browsed. Alternatively, it cannot be excluded that the saplings in our study responded to browsing by allocating their biomass to plant parts that we did not measure. For instance, birch saplings (*Betula pubescens* and *B. pendula*) have been shown to increase the growth of their existing lateral shoots following browsing (Bergström and Danell, 1987), which tends to prevent large herbivores from accessing the internal parts of the sapling (Archibald and Bond, 2003). Similarly, Hódar *et al.* (2008) found that Scots pine saplings responded to browsing by allocating more biomass to roots, which protect carbon resources from aboveground herbivores. Overall, our results indicate that young oak saplings do not seem to show any morphological plasticity in response to browsing.

Browsed and unbrowsed 1.5-year-old saplings had similar values of leaf traits, whereas we expected browsed saplings to have higher values of traits related to fast carbon acquisition (LNC, SLA, LChl) and digestibility (LDMD), and lower values of defense traits (LPhenol and LNDF). At this ontogenic stage, saplings have a very limited amount of stored reserves and rely predominantly on carbon acquisition via photosynthesis to grow (Boege and Marquis, 2005). As a result, if saplings do not respond to herbivory by investing in traits related to fast carbon acquisition, the removal of photosynthetic organs by herbivores will necessarily result in lower carbon acquisition and, therefore, in reduced growth. Our observation that browsing reduces height growth and does not influence branching tends to support this, although a measurement of the plant biomass would be necessary to draw any conclusion on the effect of browsing on primary productivity. A possible explanation for the absence of browsing-induced leaf trait variation could be an insufficient intensity or frequency of the simulated browsing. In this study, we only removed 5 cm of the apical shoot, whereas other studies found that several winter browsing events within a year were necessary to induce changes in leaf nutrient content of *Betula pubescens* saplings (Danell et al., 1994). Nosko and Embury

(2018) even found a threshold of biomass removal in early summer that induced changes in the phenolic content of balsam fir (*Abies balsamea*) sapling leaves.

Interestingly, 2.5-year-old oak saplings responded to the treatment: compared to unbrowsed saplings, saplings browsed for two consecutive years had higher values of SLA and LDMD, and lower values of LPhenol and LNDF, two traits related to chemical and structural defense, respectively. Although LDMD is known to be an important driver of food selection by large herbivores (Bee et al., 2011; Felton et al., 2018; Lloyd et al., 2010), studies on the influence of browsing on this trait are surprisingly scarce (Danell and Huss-Danell, 1985). Digestibility tends to be positively related to fast carbon acquisition traits such as SLA or chlorophyll content (Cornelissen et al., 2004): an investment in fast carbon acquisition in response to browsing should therefore also result in increased LDMD. Our study confirms this hypothesis, thereby supporting the assumption that browsing can promote traits that increase further attractiveness. While defense is often seen as the only mechanism used by woody species to deal with herbivory, our study corroborates the less documented assumption that woody species may also invest in growth rather than in defense to compensate for the loss of tissues to herbivores (Haukioja and Koricheva, 2000). It is noteworthy that these changes only occurred for 2.5-year-old saplings and not for 1.5-year-old saplings. This finding provides strong empirical support for the theory that the ability of saplings to change their resource allocation in response to herbivory depends on their ontogenic stage (Barton, 2013; Elger et al., 2009). In particular, this theory predicts that the root:shoot ratio decreases throughout the sapling stage, and that plants acquire an increasing amount of resources that can be either stored, or allocated to resist herbivory via compensatory growth or defense (Boege and Marquis, 2005).

4.2.Influence of the simulated browsing treatment on winter shoot traits (H2)

In our study, one year after the application of the simulated browsing treatment (at the end of winter and before budburst), the shoots of the browsed saplings had a lower phenolic content and a higher fiber content than the controls. To our knowledge, this is the first evidence that a winter browsing event modifies shoot chemical composition the following winter. These results suggest a trade-off between the investment in different types of defense, notably chemical vs. physical defense. Palo et al., (1992) showed that as the thickness of birch winter shoots increased, their phenolic content decreased and their fiber content increased, which is interpreted as a positive correlation between the thickness of cell walls and shoot diameter. Under this perspective, winter browsing would have induced an increase in shoot thickness at the expense of their phenolic content, which would also be consistent with the findings of Bergström and Danell (1987) who showed that simulated browsing enhanced the radial growth of birch sapling lateral shoots.

Although both phenolic and fiber content tend to decrease attractiveness by reducing the nutritional value of plant tissues (Iason, 2005), their relative efficiency depends on the herbivore species. In the case of cervids, high fiber content is known to deter herbivory (Bee et al., 2011), but a few species, including roe deer, digest tannins relatively well since some of the proteins contained in their saliva are able to bind with tannins. Roe deer are even known to select for a certain level of hydrolysable tannin in their diet (Verheyden-Tixier and Duncan, 2000). Although hydrolysable tannins are not the only component of total phenols, it could be expected that phenol-rich shoots of unbrowsed seedlings are more palatable for roe deer than fiber-rich shoots of previously browsed saplings.

In this study, leaves and winter shoots did not respond similarly to browsing, which is consistent with the recent findings of Ohse et al., (2017) who reported that simulated browsing decreased phenolic acid content in beech sapling buds but not in their leaves. These differential responses could be due to different constrains in the functioning of these organs. In leaves, investment in defense trade-offs with the capacity of carbon acquisition (Herms and Mattson, 1992; Züst and Agrawal, 2017), and different browsing-induced chemical changes are found across species, ranging from low defense associated with high carbon acquisition traits (e.g., oak in this study, birch in Danell and Huss-Danell, 1985) to high chemical defense but low carbon acquisition rate (e.g., *Acer pseudoplatanus* in Ohse et al., 2017 and *Pinus strobus* in Champagne et al., 2021). Conversely, no function clearly trade-offs with defense in winter shoots, which likely explains the consistent response to browsing across tree species (see Ohse et al., 2017 for beech, Danell and Huss-Danell, 1985 for birch or Keefover-Ring et al., 2016 for *Populus tremuloides*).

4.3.Influence of the simulated browsing treatment on roe deer feeding preference (H3)

In the feeding trials conducted on 2-year-old saplings, a consistent trend in roe deer preference appeared in both type of tests, although the results of the 1h-test must be cautiously interpreted since they rely on the decision of only one individual. While it was expected that roe deer would prefer saplings browsed the previous winter, the animals preferentially fed on unbrowsed oak saplings. Whereas similar findings have been found in other feeding trials conducted with red deer (*Cervus elaphus*) (A. J. Duncan et al., 1998) and moose (Edenius, 1993), most studies conducted in field conditions found the opposite: cervids tend to prefer previously browsed saplings (Bergqvist et al., 2003; Makhabu and Skarpe, 2006; Mathisen et al., 2017; Moore et al., 2000). The preferences for unbrowsed individuals in our study could be the consequence of the lower fiber content in their winter shoots, which would be consistent with previous observations that roe deer tend to select plants with low fiber content despite high levels of hydrolysable tannins (Verheyden-Tixier and Duncan, 2000).

The preference of roe deer for unbrowsed saplings could also be linked to differences in architecture since these saplings were generally higher and more ramified compared to previously browsed saplings (Supplementary Material, Appendix F). Several studies have shown that sapling morphology, particularly the distribution of biomass in 3D space, influences attractiveness to deer (Hartley et al., 1997; Renaud et al., 2003). According to the optimal foraging theory, deer make their foraging decisions to maximize their net energy gain (Stephens and Krebs, 1986). The animal's energy gain depends on its feeding rate – which is the product of bite size (biomass available at each bite) and bite rate – and on plant chemical composition (Charles-Dominique et al., 2020). It has been shown that the production of a high number of ramifications leads to the formation of a 'cage-like' structure that decreases the feeding rate (Charles-Dominique et al., 2020), and, consequently, saplings attractiveness to large herbivores (Archibald and Bond, 2003). Despite the fact that sapling height has a rather weak influence on feeding rate (Renaud et al., 2003), studies show that deer preferentially browse near their shoulder height (P. Duncan et al., 1998; Nichols et al., 2015). As a consequence, deer browsing often prevents saplings from growing above their maximum browsing height, thus maintaining a high density of saplings at their optimal browsing height – around 75 cm for roe deer (P. Duncan et al., 1998). Cromsigt & Kuijper (2011) showed that palatable trees were maintained at a constant height of 80 cm, which corresponds to the optimal browsing height for red deer (Nichols et al., 2015). In that regard, our findings support the assumption that height, rather than the number of ramifications, is the main morphological factor undermining roe deer preferences.

4.4. Is there any evidence of feedback loops between oak and roe deer?

The theory of feedback loops presumes that the trait response of saplings to browsing increases their subsequent attractiveness to herbivores, thereby trapping them within the reach of large herbivores through repeated browsing (Craig, 2010; Cromsigt and Kuijper, 2011; Mathisen et al., 2017). At first glance, the fact that roe deer in our study preferred unbrowsed saplings conflicts with the existence of any feedback loops between oak and roe deer. On the other hand, several results in our study also tend to support this theory. Indeed, roe deer preferred the tallest saplings, and browsing reduced sapling height growth, which support the idea that through feedback loops, roe deer actively prevent taller plants from growing beyond their reach and therefore maintain the saplings at their optimal browsing height (Cromsigt and Kuijper, 2011). In addition, we showed that repeated browsing over two years increased leaf digestibility at the expense of fiber content. Although not tested in our study, higher leaf digestibility has been shown to positively influence deer preferences (Felton et al., 2018; Lloyd et al., 2010), while leaf fiber content tends to deter herbivory by cervids (Bee et al., 2011). Finally, browsing increased the fiber content and decreased the phenolic content of winter shoots. Our results therefore show that the trait response of oak saplings to browsing modifies their subsequent attractiveness to roe deer, but that the magnitude and direction of this effect is likely to depend on the sapling phenological and ontogenic stage, but also on the browsing intensity. Our findings thus shed light on the factors driving feedback loops by showing that trait plasticity at the plant level (i.e., height growth) but also organ levels (leaf and winter shoots chemical composition) affected sapling attractiveness.

Whereas this trait plasticity in response to browsing seems consistent across species in winter shoots, as confirmed by our study, there is a high interspecific variability in leaf responses. Interestingly, the species known to exhibit a tolerance response to browsing in their leaf traits (higher digestibility, lower defense) – i.e., oak in this study, birch (Danell and Huss-Danell,

1985) or aspen (Keefover-Ring et al., 2016) – share the common feature of being relatively palatable for large herbivores (Boulanger et al., 2009; Hjältén et al., 1993). Conversely, unpalatable species such as spruce or beech tend to show limited leaf trait plasticity following browsing (Barrere et al., 2019; A. J. Duncan et al., 1998; Ohse et al., 2017), which suggest that the tree species inherent palatability could determine the strength of its plasticity in response to browsing. Testing this hypothesis with an experiment comparing the traits of browsed and unbrowsed saplings for tree species of contrasted palatability would constitute a first step towards a more general and multi-specific understanding of the relationship between browsing and subsequent attractiveness for herbivores.

5. Conclusions

We showed that (i) winter browsing reduced sapling height growth and modified the chemical composition of shoots the following winter, resulting in an increase in fiber content and a decrease in phenol content, and that (ii) these changes decreased the probability of subsequent herbivory by roe deer. This study is, to our knowledge, the first to connect browsing-induced sapling trait variations to subsequent browsing. We also showed that older and more intensively browsed saplings were able to respond to browsing by investing in fast carbon acquisition traits at the expense of defense traits, thereby supporting the assumption that browsing can promote leaf traits known to increase attractiveness (i.e., high digestibility and low phenolic content). These changes in leaf traits, along with the browsing-induced reduction in height growth and roe deer preference for higher saplings tend to support the theory that large herbivores can maintain trees at their optimal browsing height by repeated browsing. However, we suggest that further studies verify the existence of these possible feedback loops between oak and deer in field conditions, and investigate their consequences on oak dynamics at the population level to assess how they may influence forest management.

This is all the more necessary given that the regeneration of oak populations in the presence of high deer densities is an increasingly critical issue for forest management and conservation (Barrere et al., 2021; Petersson et al., 2019).

Supplementary data

Supplementary data are available online at xxx and consist of the following.

Appendix A – Protocol for roe deer saliva collection.

Appendix B – Spatial organization of the saplings at the nursery.

Appendix C – Standardization of height growth and branching.

Appendix D – Analysis of the effect of the simulated browsing treatment on each trait separately.

Appendix E –Redundancy analysis scores for leaf and shoot traits.

Appendix F – Morphology of the saplings included in the feeding trials.

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Authorship

JB, CC, SS, HV and VB designed the study. JB and VB monitored sapling morphology and collected leaf and shoot samples at the nursery. DB supervised the chemical analyses. JB, HV, HC and AB conducted the feeding trials. JB and SS collected roe deer saliva. JB and JS conducted the statistical analyses. JB led the writing of the manuscript. All authors critically contributed to the draft and approved the final version of the manuscript.

Declaration of competing interests

The authors declare that they have no conflict of interest that could have influenced the work presented in this paper.

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FIGURE CAPTIONS

Figure 1: Overview of the experimental approach: (a) morphological measurements; (b) simplified representation of a feeding trial; and (c) chronology of the experiment.

Figure 2: (a) Standardized height growth, and (b) branching (mean ± standard deviation) during the 2019 growing season for the different treatments of simulated browsing. The symbol "*" indicates that the two treatments are significantly different from each other ($\alpha = 0.05$).

Figure 3: (a) Coordinates of the individual saplings per treatment on the first component (RDA1) of the redundancy analysis conducted on six leaf traits (i.e., LPhenol, LNDF, SLA, LChl, LDMD and LNC). Treatments with no letters in common are statistically significantly different from each other ($\alpha = 0.05$). (b) Value of the correlation between each trait and RDA1.

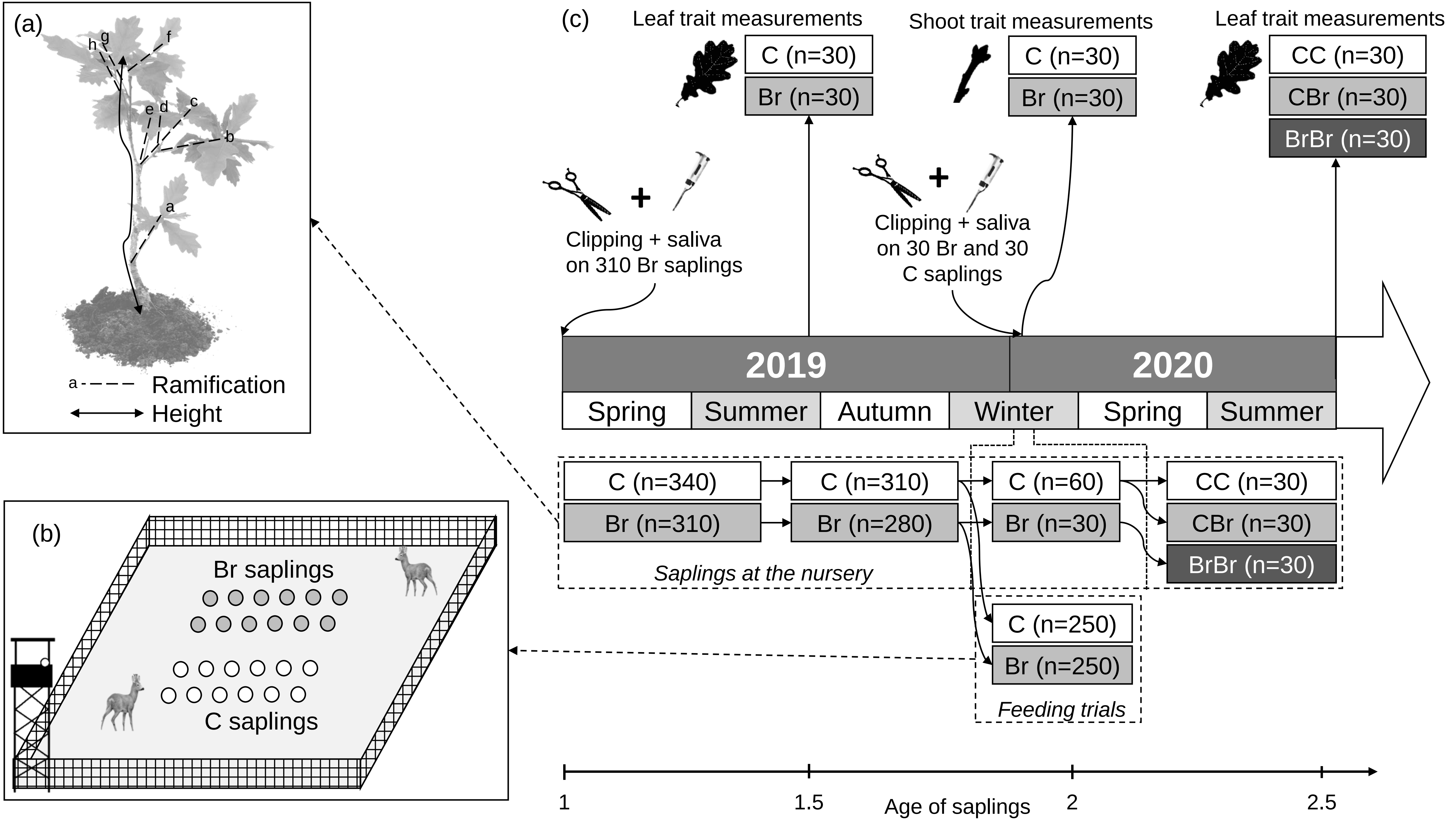
Figure 4: (a) Coordinates of the individual saplings per treatment on the first component (RDA1) of the redundancy analysis conducted on six shoot traits (i.e., SPhenol, SNDF, SDMD and SNC). Treatments with no letters in common are significantly different from each other ($\alpha = 0.05$). (b) Value of the correlation between each trait and RDA1.

Figure 5: (a) Number of saplings browsed per test and per treatment, (b) shoot length consumed, and (c) time spent by roe deer consuming and sniffing the saplings (bottom) for treatments C and Br. "*" indicates a statistically significant difference between treatments C and Br at $\alpha = 0.05$.

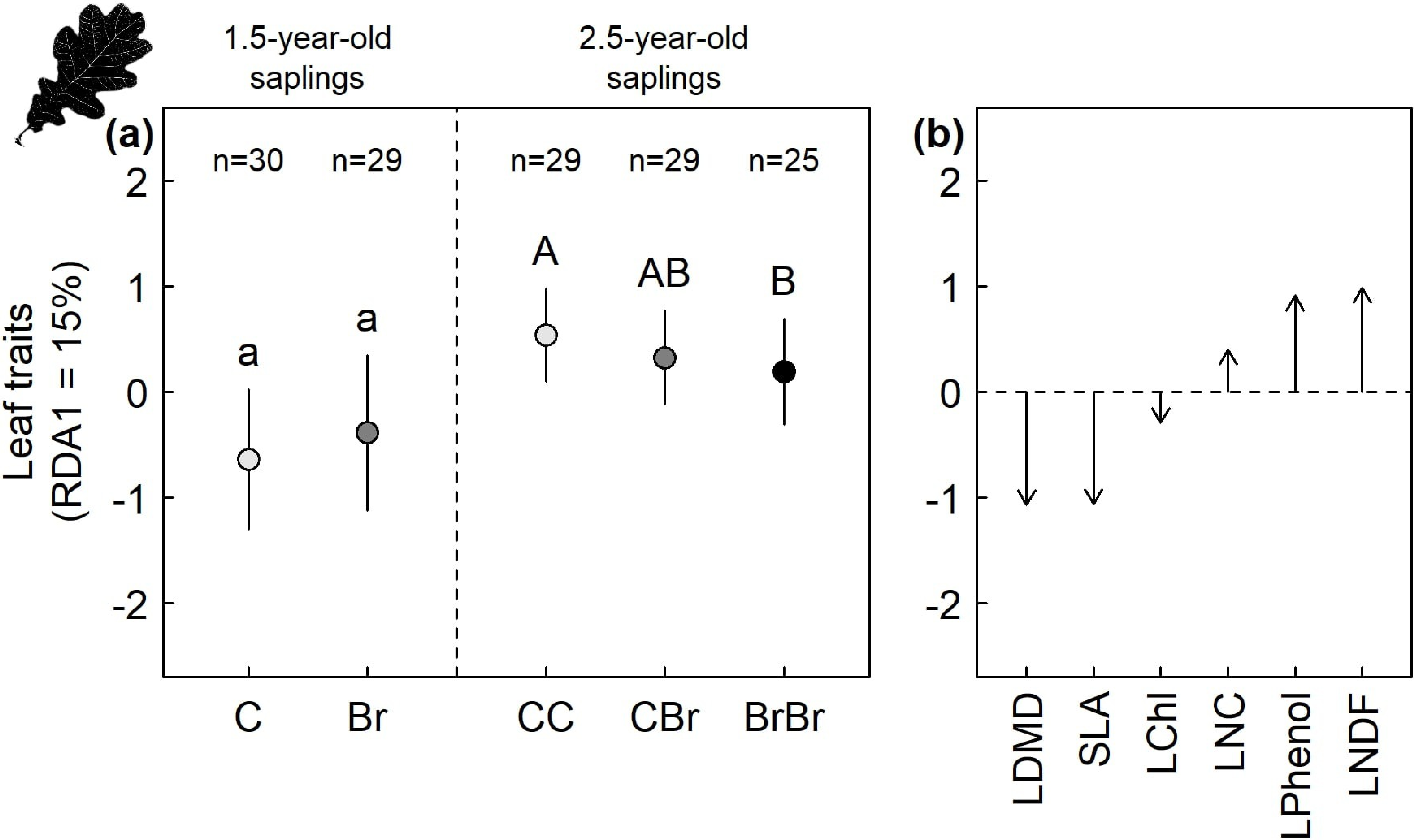
TABLES

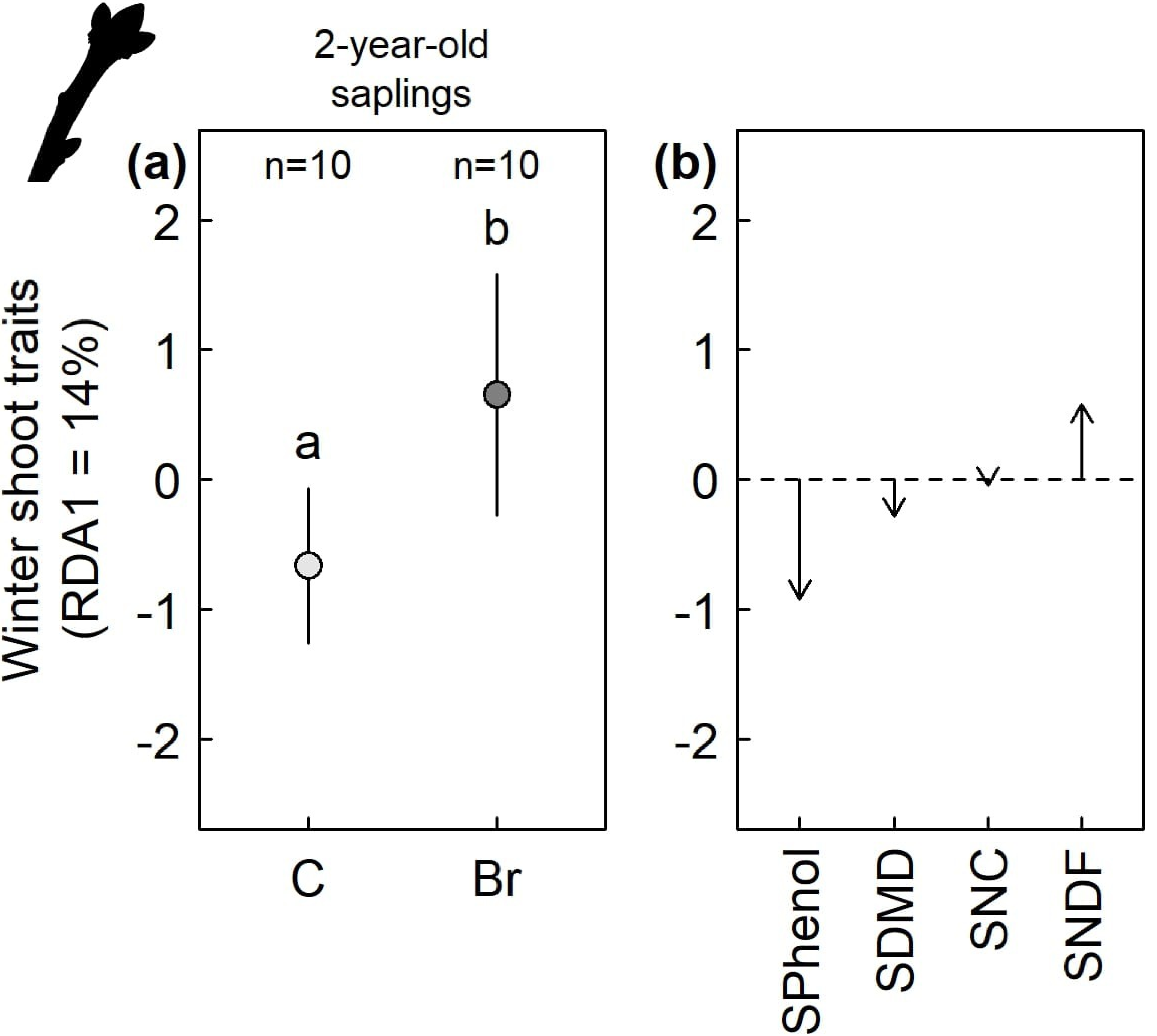
Table 1: Abbreviation, full name, organizational level, methodology used and unit of the different leaf and shoot traits measured on oak saplings.

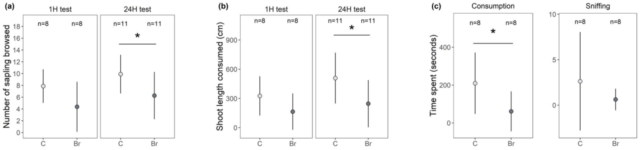
Trait	Full name	Organizational level	Method	Unit
Leaf traits				
LChl	Leaf chlorophyll content	Individual (average of three leaves per individual)	CCM-200 chlorophyll meter	$mm^2.mg^{-1}$
LNC	Leaf nitrogen content	Individual (all leaves pooled together)	NIRS	% of dry matter
LNDF	Leaf neutral detergent fiber	Individual (all leaves pooled together)	NIRS	% of dry matter
LPhenol	Leaf phenolic content	Individual (all leaves pooled together)	NIRS	% of dry matter
SLA	Specific leaf area	Individual (average of three leaves per individual)		$g.m^{-2}$
LDMD	Leaf dry matter digestibility	Individual (all leaves pooled together)	NIRS	% of dry matter
Winter shoot traits				
SNC	Shoot nitrogen content	Browsing treatment (Shoots of three individuals pooled)	NIRS	% of dry matter
SNDF	Shoot neutral detergent fiber	Browsing treatment (Shoots of three individuals pooled)	NIRS	% of dry matter
SPhenol	Shoot phenolic content	Browsing treatment (Shoots of three individuals pooled)	NIRS	% of dry matter
SDMD	Shoot dry matter digestibility	Browsing treatment (Shoots of three individuals pooled)	NIRS	% of dry matter


(a)
h
g
f
e
d
c
b
a
a
Ramification
Height
(b)
Br saplings
C saplings
(c)
Leaf trait measurements
C (n=30)
Br (n=30)
Shoot trait measurements
C (n=30)
Br (n=30)
Leaf trait measurements
CC (n=30)
CBr (n=30)
BrBr (n=30)
+
Clipping + saliva on 310 Br saplings
+
Clipping + saliva on 30 Br and 30 C saplings
2019
2020
Spring
Summer
Autumn
Winter
Spring
Summer
C (n=340)
Br (n=310)
C (n=310)
Br (n=280)
C (n=60)
Br (n=30)
CC (n=30)
CBr (n=30)
BrBr (n=30)
Saplings at the nursery
C (n=250)
Br (n=250)
Feeding trials
1
1.5
2
2.5
Age of saplings


(a)
2-year-old
saplings
Standardized height growth
20
10
0
-10
n=310
n=274
*
C
Br
(b)
2-year-old
saplings
Standardized branching
0.6
0.3
0.0
-0.3
-0.6
n=310
n=275
C
Br


1.5-year-old saplings
2.5-year-old saplings
(a)
n=30
n=29
n=29
n=29
n=25
a
a
A
AB
B
Leaf traits (RDA1 = 15%)
2
1
0
-1
-2
C
Br
CC
CBr
BrBr
(b)
2
1
0
-1
-2
LDMD
SLA
LChl
LNC
LPhenol
LNDF


2-year-old saplings
(a)
n=10
n=10
b
a
Winter shoot traits (RDA1 = 14%)
2
1
0
-1
-2
C
Br
(b)
2
1
0
-1
-2
SPhenol
SDMD
SNC
SNDF


(a)
1H test
24H test
n=8
n=8
n=11
n=11
*
Number of sapling browsed
0
2
4
6
8
10
12
14
16
18
C
Br
C
Br
(b)
1H test
24H test
n=8
n=8
n=11
n=11
*
Shoot length consumed (cm)
0
300
600
900
C
Br
C
Br
(c)
Consumption
Sniffing
n=8
n=8
n=8
n=8
*
Time spent (seconds)
0
200
400
0
5
10
C
Br
C
Br