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Departamento de Biología Vegetal y Ecología

El papel de la cabra doméstica (*Capra hircus*) en la estructura y conservación del Monte Mediterráneo

Memoria que presenta el

Licenciado JUAN MANUEL MANCILLA LEYTÓN para

Optar al título de Doctor en Biología de la Conservación con Mención Europea por la Universidad de Sevilla

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ÍNDICE

0. RESUMEN

En la actualidad, los sistemas silvopastorales además de conciliar el uso de los productos y los servicios del medio natural con garantía de permanencia, buscan la estabilidad ecológica, económica y social a través de la diversificación de las estructuras y de los productos. En esta Tesis Doctoral se ha caracterizado el papel de la cabra doméstica (*Capra hircus* raza Payoya) en la estructura y conservación de la vegetación de matorral del sotobosque de una plantación de *Pinus pinea* en el Espacio Natural de Doñana (SO España). Durante cuatro años se han evaluado: i) los cambios temporales en el biovolumen, en la inflamabilidad, en la riqueza y en la diversidad de especies entre un matorral pastado por cabras y uno sin pastar; ii) las preferencias alimenticias de las cabras y la capacidad de éstas para dispersar la semillas de algunas de las especies de matorral que pastan, iii) el efecto del pastoreo en la descomposición de las acículas de pino acumuladas en el suelo, y iv) el efecto del pastoreo en producción de leche y de calidad, con especial referencia a la composición de los ácidos grasos. Las cabras pastaron una gran variedad de especies, cambiando el patrón de selección durante todo el año. El pastoreo redujo la abundancia de especies, pero no afectó a la riqueza de las mismas. Durante el pastoreo, las cabras aumentaron el consumo de especies con alto valor nutricional (contenido en Nitrógeno) reduciendo la ingesta de especies con alta cantidad de compuestos secundarios (taninos y fenoles). El pastoreo redujo la biomasa acumulada y la inflamabilidad, contribuyendo por tanto a la disminución del riesgo de incendios forestales. El ganado caprino puede ser un potencial dispersor de las semillas de algunas de las especies que pasta, ya que las semillas de las especies estudiadas fueron capaces de pasar a través del intestino y posteriormente germinar. Indirectamente, el pastoreo aumentó la velocidad de descomposición de las acículas de pino, contribuyendo a la incorporación de nitrógeno en el sistema. Por último, la leche de las cabras en pastoreo semi-extensivo mostró niveles más altos de ácidos grasos n-3 y menor ratio n6/n3 que las cabras con dieta de concentrado. Contrariamente a la creencia general de que las cabras sólo causan la degradación ambiental, los resultados encontrados en esta Tesis Doctoral evidencian múltiples efectos positivos del pastoreo sobre los ecosistemas Mediterráneos.

0. SUMMARY

At present, silvopastoral systems attempt to reconcile the use of natural products and environmental services with a guarantee of permanence, or similarly attempt to pursue ecological, economic and social sustainability through the diversification of structures and products and an efficient land use. This PhD thesis examines the effect of goat grazing on the shrub understory of a pine forest located in a Spanish protected area (Doñana Natural Park). Four specific topics of interest were investigated along four years: i) temporal changes in phytovolume, flammability, species richness and diversity between shrublands grazed by goats and ungrazed ones; ii) food preferences of goats and their seed dispersal ability; iii) effect of goat grazing in the decomposition rate of pine needle litter; and iv) effect of diet selection by goats on milk yield and quality, with specific reference to fatty acids (FA) composition. Results showed that goats released in the pine forest fed on a great variety of species, changing their diet selection pattern throughout the year. Grazing reduced shrub diversity, but did not affect species richness. During grazing, goats increased their nutritional value intake (N content) while minimizing secondary compounds (tannin and phenol), and they reduced shrub biomass and flammability, decreasing the risk of forest fires. Goats were also potentially able to disperse the seeds of some of the species they ate, since the ingested seeds passed through the goats' guts and germinate afterwards. Indirectly, goat grazing increased the decomposition rate of *Pinus pinea* needles, promoting the incorporation of Nitrogen into the system. Finally, grazing goats′ milk showed higher levels of n-3 FA and lower n6:n3 ratio than intensive grazing. Hence, contrary to the general assumption that goats only cause environmental degradation, the results of this PhD thesis evidences multiple indirect and direct positive effects of goat grazing in Mediterranean ecosystems.

CAPÍTULO 1

Introducción

1. INTRODUCCIÓN

En los ecosistemas Mediterráneos, los herbívoros domésticos -especialmente los rumiantes- han jugado un importante papel en la génesis y mantenimiento de los paisajes (Emanuelson, 2009); la cabra fue la primera especie animal en ser domesticada (7000 años, Clutton-Brock, 1999) y ha pastado el matorral desde hace milenios -en España desde hace al menos 5000 años- (Pérez Ripoll, 1980; Esteban Muñoz, 2009).

En las últimas décadas, los cambios socioeconómicos acaecidos en las sociedades industriales (éxodo rural, intensificación de la actividad agraria, baja rentabilidad de productos forestales, etc.), junto a las políticas adoptadas han favorecido la ganadería intensiva en detrimento de la extensiva, y como consecuencia se han producido cambios en la vegetación.

A pesar del innegable papel de la ganadería extensiva en el origen y mantenimiento de muchas formaciones vegetales, el efecto del pastoreo sobre la conservación del medio casi siempre ha sido percibido negativamente (Dregne ay Willis, 1983; Alados et al. 2004). La mayor parte de los efectos que contribuyen a esta percepción se deben a la mala gestión, a menudo relacionada con el sobrepastoreo (Nelson, 1988; Mace, 1991; Livingstone, 1991; Mysterud, 2006), considerado como uno de los factores más importantes de la desertización (Dregne y Willis, 1983). Recientemente se insiste en la contribución de la ganadería al cambio climático por la producción de metano (Puchala et al., 2005; Goel et al., 2008), y se le acusa de la perdida de especies (de Queiroz, 1993 a,b; Dougill y Cox, 1995; Alados et al., 2003). Sin embargo, en muchos casos la exclusión completa de la ganadería produce cambios en la riqueza y diversidad de especies (Huntly, 1991; Loucougaray et al., 2004; Caballero et al., 2011; Nikolov et al., 2011; García et al., 2013). La falta de actividad ganadera favorece el incremento de la vegetación leñosa y por lo tanto el aumento de la cantidad de material vegetal combustible (Shackleton 1993, Moreira et al., 2001; Lasanta et al., 2006), así como la modificación de ciertas interacciones interespecíficas (p.e. polinizadores, insectos herbívoros, parasitoides, etc.)(Vanbergen et al., 2006; Cipriotti y Aguiar, 2012; Rosa García et al., 2012).

Aunque las cabras son clasificadas como animales polífagos no es cierta la afirmación popular “la cabra come todo lo que encuentra”, sino que presentan preferencias y/o rechazos por determinadas especies en función de la variación estacional (Barroso et al., 1995; Dannell y Bergström, 2002; Dziba et al., 2003), hetereogeneidad de la vegetación (Hooper, 1960; Rogosic et al., 2006), disponibilidad de alimento (Genina y Pijoanb, 1993, Freeland et al., 1985; Rogosic et al., 2003), fenómenos de aprendizaje (Galef y Giraldeau, 2001; Lesley, 1994) y composición bioquímica de las plantas (Garín et al. 1996; Dannell y Bergström, 2002; Provenza et al.,2003, Rogosic et al., 2006; Baraza et al., 2009). Estas variaciones pueden dar lugar a un aumento o descenso del consumo del herbívoro entre diferentes especies, plantas de la misma especie, o incluso partes de la misma planta (Riddle et al., 1996; Alonso-Díaz et al., 2008; Baraza et al., 2009). Estas preferencias pueden ser usadas como herramientas de manejo para la conservación del monte mediterráneo (Torrano y Valderrábano, 2005), pudiendo replicar los efectos ecológicos de sus equivalentes silvestres, o de otras especies ahora ausentes o extintas.

En los últimos años, el pastoreo ha sido reconocido como una alternativa menos agresiva y/o menos cara -con respecto a las técnicas tradicionales de prevención de incendios- para controlar la invasión de arbustos (Tsiouvaras et al., 1989; Magadlela et al., 1995; Torrano y Valderrabago, 2005; Jauregui et al., 2007, Ruiz-Mirazo et al., 2011), pudiendo minimizar el riesgo de incendios forestales. Los efectos más evidentes del pastoreo sobre la comunidad vegetal son los cambios en la estructura y composición de la misma, ya que, debido a la selectividad de la cabra, algunas especies se ven favorecidas por el pastoreo y su número y/o cobertura aumentan, mientras que otras especies se encuentran en desventaja y su número y/o cobertura disminuyen (Hacker, 1984; Belsky, 1992; Milchunas et al., 1998; Briske et al., 2003).

Además del material foliar y renuevos, las flores y los frutos de matorral son consumidos por las cabras durante el pastoreo. Como consumidoras de frutos, las cabras pueden actuar como predadoras de semillas pero también como dispersoras. En este sentido, el ganado doméstico podría desempeñar un papel dispersor de semillas similar a los mamíferos herbívoros actuales o a los herbívoros extintos que han reemplazado (Janzen y Martin, 1982; Martin y Klein, 1984; Janzen, 1986; Skape, 1991; Tiffney, 2004; Hansen et al., 2008). Asimismo, el fraccionamiento por pisoteo de la hojarasca acumulada durante el pastoreo y el enriquecimiento del suelo con sus deyecciones (aumento de nitrógeno), son dos factores a considerar en la descomposición. Este efecto podría ser beneficioso en los bosques de pinos, donde sus acículas tienden a acumularse debido a su baja tasas de descomposición (Fioretto et al., 1998), y sería aun más importante en los ecosistemas con suelos pobres en nutrientes (por ejemplo, bosque mediterráneo y dehesas) donde la descomposición constituye la principal fuente de nutrientes para la producción primaria (Wang et al., 2008).

Desde el punto de vista de la producción animal, un interrogante, que se plantea en las zonas forestales, se refiere a su potencialidad para sostener la actividad ganadera. El estudio de la potencialidad de las zonas forestales para sostener la actividad ganadera requiere del conocimiento de la ingestión y del valor nutritivo de su dieta (Lachica y Aguilera, 2003). Desde un punto de vista ganadero, el monte es un gran suministrador de fibra (Rogosic et al., 2006) pero son muy escasas las explotaciones que basan la alimentación de forma extensiva, ya que la mayoría de estas explotaciones son mixtas o totalmente estabuladas (Ruiz et al., 2008). La oferta vegetal del Monte Mediterráneo junto a las distintas estrategias de aprovechamiento de la misma se puede traducir en una mayor calidad de los productos caprinos (principalmente la leche) (Boyazoglu and Morand-Fehr, 2001; Lu et al., 2010). Los paisajes ganaderos andaluces pueden generar, y generan, muchos productos alimentarios de calidad que les confieren valores adicionales a los que proporcionan su belleza natural. En este aspecto producción y conservación no tienen por que ser incompatibles sino complementarias; la conservación requiere producción y la producción debe ser obligatoriamente conservadora.

El creciente interés de la reintroducción de la cabra en los ecosistemas mediterráneos autóctonos, potenciado por la reducción del coste en la alimentación, así como herramienta de control de la invasión de arbustos, en el contexto de un sistema moderno de producción animal, requiere el conocimiento de los efectos del pastoreo sobre la vegetación y la contribución de esta fuente de forraje en la dieta de la cabra.

En este sentido esta Tesis Doctoral pretende contribuir a clarificar muchos de estos aspectos. Este conocimiento permitirá el desarrollo de una ganadería sostenible en perfecto equilibrio con el medio donde se desarrolla.

1.1. OBJETIVOS-.

En la presente memoria se caracterizar el papel de la cabra doméstica (*Capra hircus* raza Payoya) en la estructura y conservación de la vegetación de matorral del sotobosque de una plantación de *Pinus pinea* en el Espacio Natural de Doñana (SO España).

Para ello se plantean los siguientes objetivos:

1.- Caracterización del pastoreo y efecto sobre el estrato arbustivo:

1.1. Analizar las variaciones temporales en las preferencias del ganado caprino por la vegetación de matorral.

1.2. Evaluar los cambios estructurales del estrato arbustivo así como los cambios en la riqueza y diversidad de especies como consecuencia del pastoreo.

2.- Evaluación de los cambios en las propiedades de la vegetación de matorral:

2.1. Caracterizar las variaciones de la composición nutricional de las distintas especies de matorral debidos al pastoreo.

2.2. Analizar los posibles cambios en la inflamabilidad del matorral Mediterráneo como consecuencia del pastoreo con cabras.

3.- Identificación de efectos indirectos del pastoreo sobre la vegetación y el medio:

3.1. Analizar experimentalmente la capacidad de paso por el intestino de las cabras de semillas de especies arbustivas Mediterráneas y su posterior germinación.

3.2. Evaluar la función del pisoteo y el enriquecimiento en nutrientes, producido por las deyecciones de las cabras, en la velocidad de descomposición de la hojarasca acumulada de *P. pinea*.

4.- Identificación de las implicaciones en la explotación ganadera:

4.1. Evaluar el efecto del pastoreo sobre la producción y calidad de la leche, con especial insistencia en la composición de ácidos grasos.

4.2. Caracterizar aquellos aspectos del comportamiento alimenticio del ganado caprino, en régimen semi-extensivo, que pueden intervenir en el balance energético y sus variaciones estacionales.

1.2. ESTRUCTURA DE LA TESIS.-

Esta Tesis Doctoral se presenta en forma de compendio de publicaciones científicas y opta a la Mención de "Doctor Europeo". A este capítulo introductorio (Capítulo 1), le sigue el capítulo 2 donde se presentan los resultados obtenidos, los cuales han dado lugar a 8 trabajos de investigación que abordan los diferentes objetivos citados anteriormente. Estos trabajos reproducen los contenidos de los artículos publicados o en revisión en diferentes revistas científicas, por lo que se presentan en inglés. Los artículos están escritos con la finalidad de que puedan ser leídos de modo independiente, conteniendo toda la información necesaria para su comprensión. Los dos primeros trabajos versan sobre las interacciones básicas ungulado-planta: ¿qué y cuándo comen? y ¿cómo afecta el pastoreo a la estructura vegetal? En el tercer y cuarto trabajo se estudian los cambios en las características químicas e inflamabilidad de la vegetación debidos al pastoreo. El quinto y sexto trabajo tratan sobre los efectos indirectos debidos al pastoreo: supervivencia, tasa de recuperación y germinación de semillas ingeridas, y efectos en la descomposición de la hojarasca de *P. pinea* por deyecciones y pisoteo. Finalmente, los dos últimos trabajos son de investigación aplicada: cambios en la producción y calidad de la leche, y estudio del balance energético del pastoreo. Por último, en el capítulo 3 se expone la discusión general, y en el capítulo 4 las conclusiones de esta Tesis Doctoral.

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CAPÍTULO 2

Resultados

2.1. DIET SELECTION OF GOATS: EFFECTS ON THE VEGETATION OF A PROTECTED PINE FOREST IN SW SPAIN

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Summary

1. Grazing selectivity has been considered to play a major role in shaping species composition and abundance, through the control that grazing exerts over the population dynamics of plant species. But the effect of grazing animals on vegetation is not limited to defoliation. Animals also sit, lie, scratch and paw on vegetation, thus also affecting plants, especially those of lower breaking resistance.

2. We investigated the relationship between feeding selection of goats and changes in plant species abundance in the shrubby understory of a pine forest along three years. The abundance of the shrub species was measured using the point-intercept method. Goat preferences for shrub species was determined through direct observation.

3. Goats showed a selective feeding, since their browsing behaviour was not related to species abundance. *Myrtus communis* was the overall preferred species (almost half of the bites belonged to this species, despite its low abundance), throughout the monitored years and seasons. Two species were never consumed and nine others were always ingested below their abundance. Half of the species were selected some years while discarded others. Within a year, species were eaten in certain seasons but not in others. The detected changes in species abundance were not related with the feeding preferences of the goats.

4. *Synthesis*. The influence that grazing selectivity exerts on vegetation composition and abundance has long been recognized, but our study suggests that the mechanical effect of grazing herbivores on vegetation (pawing, scratching, lying), may portrait a more important role than attributed to date.

Keywords: Grazing; effect of grazing on vegetation, Doñana Natural Park.

1. INTRODUCTION

Grazing by domestic and wild ungulates is the most globally widespread land use (FAOSTAT 2004). Along with burning, grazing is the greatest vegetation disturbance activity in terms of area and biomass loss (Bond 2005), significantly influencing current vegetation composition and structure (Naveh & Whittaker, 1979)

Grazing selectivity has been considered to greatly influence species composition and abundance, through the control that it exerts over the population dynamics of plant species (Harper, 1977). Accordingly, it is accepted that goats (*Capra hircus* L) have modified the structure and composition of Mediterranean rangelands, which they have traditionally grazed, through selective feeding (e.g. Papachistou et al., 2005; Baraza et al., 2009). Goats are poliphagous animals that feed on a great variety of species, but they always select some species and refuse others (Papachistou et al., 2005; Mancilla-Leytón et al., 2012). But defoliation is not the only effect that grazing animals exert on vegetation. Animals also sit, lie, scratch and paw on vegetation, breaking the plants (Harper, 1977), and thus also affecting them, especially those of lower breaking resistance.

The aim of this study was to determine if changes in the abundance of the understory shrub species of a pine forest grazed by goats were related to diet selection of goats. In order to meet this aim we analyzed the potential relationship between the diet of a herd of goats during three years and changes in shrub species abundance along this period.

2. MATERIAL AND METHODS

2.1. Study area

The study was carried out in a 100 ha pine forest located in the Doñana Natural Park limits, SW Spain (37º 14’ N, 6º 20’ W). The climate is Mediterranean, with wet and mild winters (mean temperature is 10 ºC in December and January) and long, dry summers (mean temperature is 25 ºC in July and August). Mean annual rainfall is 540 mm. The vegetation comprises a *Pinus pinea* forest with an average density of 217 trees/ha and an average tree diameter at breast height (DBH) of 26.92 cm. There are also some cork oaks (*Quercus suber*) and holm oaks (*Quercus ilex* subsp. *ballota*) at very low densities (less than one tree per ha). The understory is dominated by shrubs (around 0.6-1.8 m tall), the more common species being *Cistus salvifolius*, *Halimium halimifolium*, *H. calycinum*, *Myrtus communis*, *Pistacia lentiscus* and *Rosmarinus officinalis*. There are some pasture patches between the shrubs. The herbaceous communities are dominated by annual species such as *Brachipodium phoenicoides* and *Ornithopus compresus.*

The study area is used for timber production and hunting (rabbit, partridge). Grazing by wild (deer) and domestic (goat) herbivores was common until 1970, when deer were removed from the private estate. Goats were excluded in 2002 and reintroduced in 2007. During this five-year period of grazing exclusion, shrub vegetation expanded and grew taller, increasing the pine forest fire risk.

2.2. Species abundance

In February 2007, before the goats were reintroduced into the area, nine 50-m x50-m quadrats (0.25-ha each) were randomly placed within the study area and fenced out in order to exclude grazers outside (9 ungrazed plots). Inside each quadrat, a permanent 25 m linear plot was established. Ten meters apart from the four edges of each quadrat, four permanent 25 m linear plots were also established parallel to each side of the quadrats (36 grazed plots). The abundance of each shrub species was estimated in each plot through the point-intercept method (Daget & Poisonet, 1971), by annotating the species in contact with a stick placed every 10 cm along the tape. The abundance of the pasture was also assessed, without species differentiation. Species were identified according to Valdés et al. (1987). Vegetation was sampled seven times along a three year grazing period: immediately before the goats entered the area (April 2007), and every six months thereafter (October 2007, April and October 2008, April and October 2009 and April 2010).

The abundance of the shrub species in each sampling date and grazing treatment was estimated as the percentage of points corresponding to each species sampled in each of the 9-ungrazed and 36-grazed permanent plots.

2.3. Goats consumption

The experimental flock comprised 350 adult female *Payoya* goats of similar size and age (40-45 kg average weight and three years old). Goats were introduced daily into the study area by a shepherd that moved them around the whole area, daily covering one third of the available surface. The shrub species consumed by goats were determined through direct observation. We followed a modified version of the method described by Meuret et al. (1985). Every month, the goats were observed along the three consecutive days that took them to graze the whole area. Each day, 10 randomly chosen goats were followed during ten minutes, annotating the number of bites given to each species and the plant part consumed. The abundance of the shrub species in the monthly diet of goats was estimated as the percentage of bites given to each species during the three consecutive sampling dates.

2.4 Data analysis

In order to determine if the abundance of the species influenced their consumption by goats, a G test was performed to compare the shrub species abundance in the grazed area at the beginning of each year with the number of goat bites given to each shrub species throughout the corresponding year. Total annual plant consumption was estimated by adding the bites given to each shrub species during the twelve annual samplings (3 days/2 grazing treatments/2 annual surveys) and these values were compared to species abundance in the grazed area at the beginning of the year. When test results were significant, the residuals of Habermann (Habermann, 1973) were used to determine which species were consumed significantly above or below their abundance.

The Ivlev´s Selectivity index '*E*' (Ivlev, 1961) was calculated to evaluate diet selectivity variations within each year. The index relates species abundance with species consumption through the equation: $Ei = ((ri - pi)/(ri + pi))*10$, *where ri* is the proportion of species "*i*" in the diet, and *pi* the proportion of species '*i*' in thegrazing area. '*E*' varies from –10 to 0 for negative selection, and from 0 to +10 for positive selection. The index was calculated seasonally. Seasonal species consumption was estimated as the sum of the bites recorded during the three months of the season (Autumn: September, October and November. Winter: December, January and February. Spring: March, April and May. Summer: June, July and August). Species abundances changed slowly, thus seasonal surveys were not considered necessary. Instead, each April-surey was assumed as representative of that year´s spring and summer, and autumn and winter seasons were represented by October-surveys. In accordance to this, seasonal consumption was compared with plant species abundance registered in the grazed area during the previous representative April/October-survey.

The Ivlev's index was also used to determine each year´s goat grazing preferences as an indicator of the grazing pressure over each species. This annual grazing pressure was compared to the annual abundance change of each species, in order to analyze if changes in species abundances were related with different grazing pressures.

3. RESULTS

Twenty three shrub species were found in the study area. At the beginning of the experiment, before the goats entered the grazing area, two species were dominant (*Cistus salvifolius* and *Rosmarinus officinalis*) with over a 10% cover (table 1). Other four species (*Halimium halimifolium*, *H. calycinum*, *Myrtus communis* and *Pistacia lentiscus*) presented a plant cover between 5- 10% and the rest were less abundant, with a cover below 5%. The pasture, dominated by annual species, was also sparse (less than 5% cover).

We recorded 88395 goat bites along the study period. Goats browsed the leaves, stem shoots and flowers of all species except *Daphne gnidium* and *Thymus mastichina*, that were never consumed (Table 1). They also browsed the fruits of *C. salvifolius*, *H. halimifolium, M. communis, P. lentiscus* and *Quercus coccifera,* and grazed the pasture. The most consumed species was by far *M. communis* (43.8% of total bites), followed by the pasture (10.5%) and *C. salvifolius* (10.1%).

The results of the G tests showed that the goats did not consumed the species according to their abundance in any of the three years studied (G = 20578.9, 23555.0, 14875.4 for 2008, 2009 and 2010 respectively, $p < 0.000$). The analysis of the residuals showed that ten species maintained the same consumption pattern during the three years (Fig. 1). *M. communis* was always significantly consumed well above its abundance, and *Cistus ladanifer, C. libanotis, C. monspeliensis, H. calycinum, Helichrysum italicum, Lavandula stoechas and R. officinalis* were always consumed significantly below their abundance. The majority of these species were not consumed at all during the last year of study (all but *R. officinalis*). *D. gnidium* and *T. mastichina* were never consumed. Six species: *C. salvifolius, H.halimifolium, Erica scoparia, Phillyrea* *angustifolia, P. lentiscus* and *Q. coccifera*, the spiny legumes and the pasture were significantly consumed above their abundance some years and below or according to their abundance others (Fig. 1).

Table 1. Species abundance at the beginning of the study, before the goats entered in the area, measured as percentage of total cover. Goat consumption of each species measured as percentage of bites given to each species in the whole study period (2007-2010). Spiny legumes (*Genista hirsuta, G. triacanthos* and *Stauracanthus genistoides*).

Species	Initial Cover %	Total consumption %
Cistus crispus (Cc)	1.1	0.23
Cistus ladanifer (Cl)	3.0	1.19
Cistus libanotis (Cli)	3.2	0.37
Cistus monspeliensis (Cm)	0.9	0.07
Cistus salvifolius (Cs)	13.3	10.14
Halimium calycinum (Hc)	7.7	2.14
Halimium halimifolium (Hh)	9.4	7.61
Helichrysum italicum (Hi)	1.1	0.04
Lavandula stoechas (Ls)	3.5	0.19
Rosmarinus officinalis (Ro)	14.0	2.23
Thymus mastichina (Tm)	4.3	0.00
Daphne gnidium (Dg)	0.7	0.00
Erica scoparia (Es)	5.4	4.04
Myrtus communis (Mc)	9.1	43.30
Phillyrea angustifolia (Pa)	1.2	1.10
Pistacia lentiscus (Pl)	6.6	6.63
Quercus coccifera (Qc)	4.8	3.88
Spiny legumes (spn)	6.1	6.33
Grassland (Grs)	4.3	10.5

The goat's preferences also varied within the years (figure 2). Certain species tended to be less eaten in spring and summer such as *Cistaceae* (with the exception of *Cistus libanotis* and *Halimium calicynum*). Other species were only consumed in certain seasons, such as *Helycrhysum italicum* and *Rosmarinus officinalis*, that were only consumed during flowering, or the pasture, that was consumed mainly in winter and spring, when is green. Many species followed different patterns of seasonal consumption during different years. For instance, *P. lentiscus* and *Q. coccifera* were only consumed in summer during the third year of study, while *E. scoparia* was not consumed in spring and summer of this third year. The spiny legumes were consumed during spring of the first two years, and in autumn and winter of the third. *M. communis*, the most consumed species, was eaten throughout all seasons and years of study.

Vegetation cover decreased by 30% in the grazed areas from the beginning to the end of the study period (Table 2). The abundance of the majority of the species decreased: all but *Helichrysum italicum*, *Thymus mastichina*, *Lavandula stoechas*, *Phillyrea angustifolia* and the pasture. Contrastingly, plant cover slightly increased in the ungrazed area (6.2%), with most of the species (all but some *Cistaceae*) increasing in cover or remaining the same. Nine of the twenty studied species followed the same trends of abundance change during the study period in the grazed and ungrazed areas: *C. libanotis*, *C. salvifolius* and *Halimium* spp. abundance decreased, more in the grazed than in the ungrazed areas, while abundance of *H. italicum*, *L. stoechas*, *T. mastichina*, *P. angustifolia* and pasture increased.

Table 2. Percentage of change in abundance of the species between the beginning (April 2007) and the end (April 2010) of the study in the ungrazed and in the grazed area.

Species	Ungrazed	Grazed
Cistus crispus	1,1	-29,2
Cistus ladanifer	45,6	-72,2
Cistus libanotis	-5,9	-69,2
Cistus monspeliensis	0,6	-62,9
Cistus salvifolius	-65,5	-80,6
Halimium calycinum	-60,8	-87,4
Halimium halimifolium	-26,9	-91,1
Helichrysum italicum	161,1	19,2
Lavandula stoechas	24,0	17,1
Rosmarinus officinalis	66,8	-18,0
Thymus mastichina	14,9	26,6
Daphne gnidium	27,8	-29,3
Erica scoparia	25,7	-21,8
Myrtus communis	30,8	-30,8
Phillyrea angustifolia	39,7	10,4
Pistacia lentiscus	11,7	-5,9
Quercus coccifera	17,4	-34,9
Spiny legumes	20,0	-8,1
Grassland	13,3	82,0
TOTAL	6,2	-35,0

Figure 1. Percentage of cover of each species at the beginning of each year and percentage of bites given to each species each year. For the names of the species, see table 1.

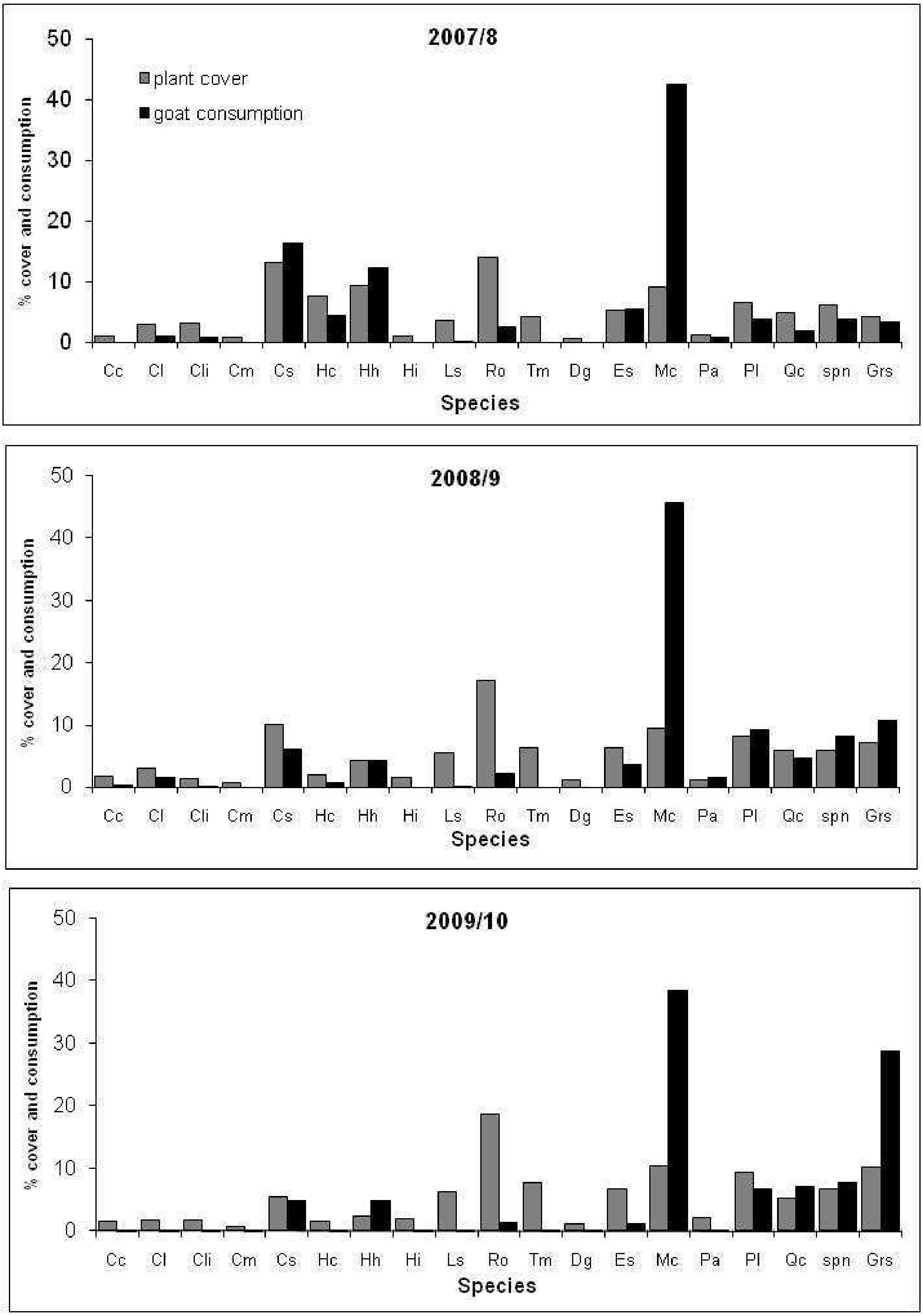
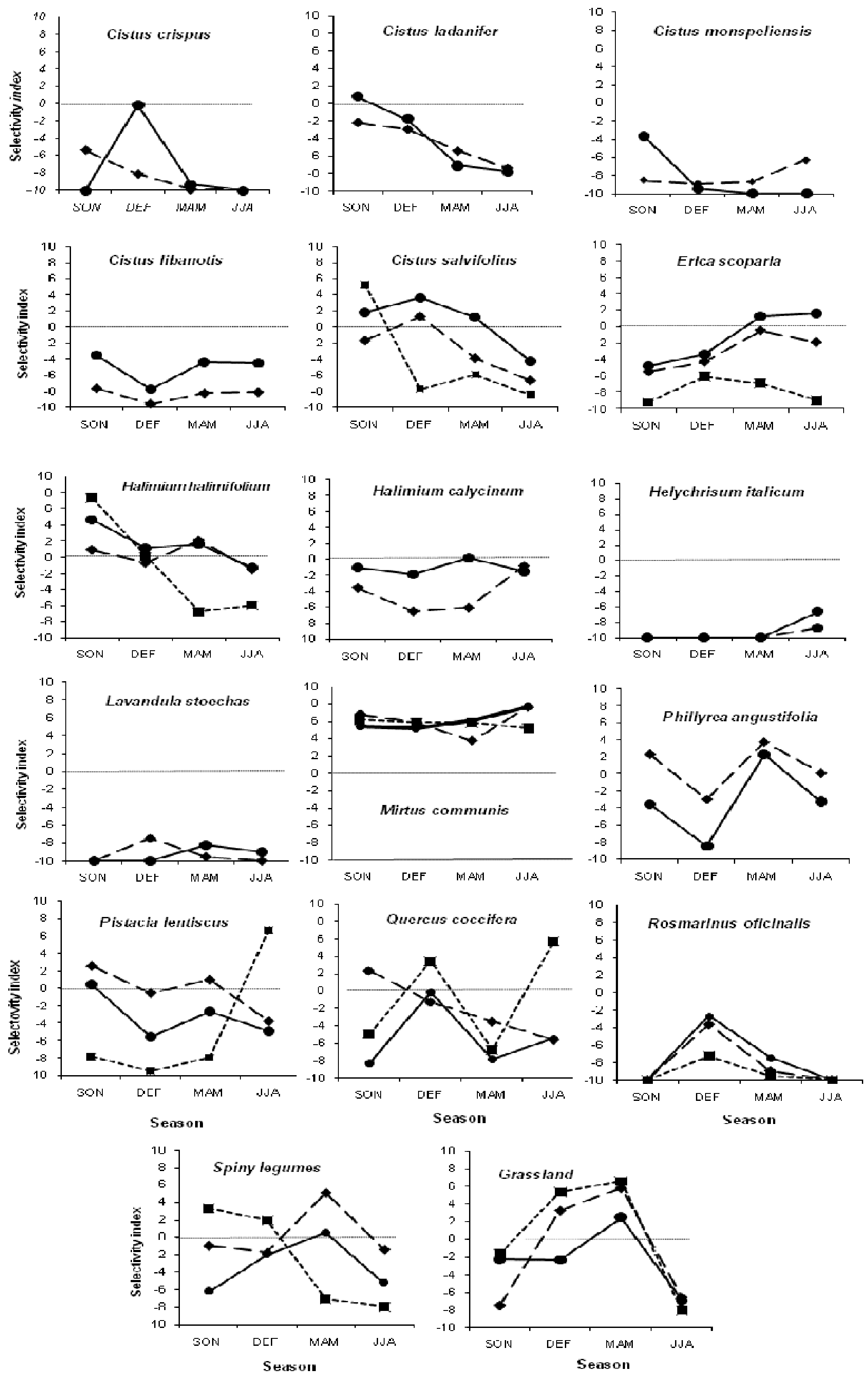
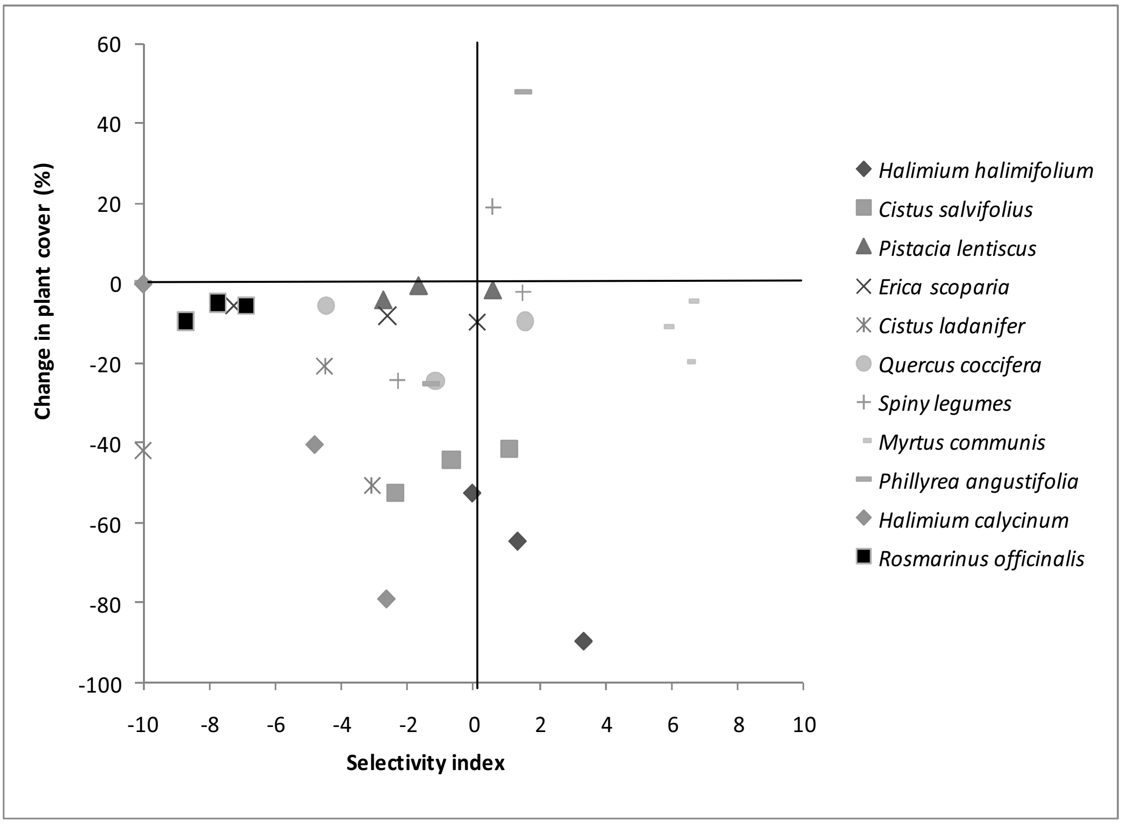


Figure 2. Seasonal species selection by goats measured with Ivlev's Index. SNO: autumn (September, October, November), DEF: winter (December, January and February). MAM: spring (March, April and May). JJA: summer (June, July and August). (●) 2007-8. (♦) 2008 -9. (■) 209-10.



There was not a clear relationship between changes in species abundance and grazing pressure over the species, measured through the selectivity index (Fig. 3). The most preferred species, *M. communis*, annually decreased less than 20% in cover, the same trend registered for other species such as *R. officinalis*, *E. scoparia* or *P. lentiscus*, which were consumed below their abundance. The *Cistaceae* species showed the greatest plant cover decrease, also decreasing in the ungrazed areas. The only species that showed a higher cover reduction when more heavily eaten were *Halimium* spp. The others did not show any trend of change: their abundance was not affected by consumption intensity.

Figure 3. Relationship between selectivity index each year and changes in plant cover this year for each species and year. Only the species that received more than 1% of the bites of the goats at least in one year have been represented.



4. DISCUSSION

Goats showed a selective feeding, since they did not browse species according to their abundance. A variety of other factors could be supporting this selective behaviour such as chemical characteristics of plants (nutrients, secondary compounds, taste, etc.) or vegetation heterogeneity (Lu, 1988; Papachistou et al 2005; Baraza et al., 2009). Secondary compounds could explain the low consumption of aromatic species such as *L. stoechas*, *R. officinalis*, *T. mastichina* and *H. italicum* (Rogosic et al., 2009; Estevez et al., 2010; Vasta & Luciano, 2011), species of the *Cistaceae* family (*C. ladanifer, C. libanotis, C. montpeliensis, Halimium calycinum*), which are rich in tannins (Vasta et al. 2009; Jerónimo et al. 2010), and *Daphne gnidium*, a toxic species (Benítez et al., 2011). Moreover, Changes in vegetation heterogeneity as plant cover decreases could be behind the interannual changing preferences for species. A dense understory provides limited amounts of usable forage because it is difficult to penetrate in comparison to a more open shrubland. When the shrubland is thinned, the accessibility to different species changes, perhaps also changing the preferences of the goats. This could explain why *Q. coccifera* and the spiny legumes were consumed below their abundance at the beginning of the study and above it at the end. Furthermore, the induction of secondary compounds in the plants by herbivory (Bennet & Wallsgrove, 1994; Mithöfer & Boland, 2012) could explain the interannual changing preferences for species such as *C. salvifolius, H. halimifolium, P. angustifolia* and *P. lentiscus*, which were selected one year, refused the next year, and selected once again. These induced secondary compounds are produced after plant damage, ceasing their production when damage diminishes. The increase in pasture consumption as its cover increases could be related with a selectivity behaviour according to abundance. Goats are not exclusively browsers, they are also grazers, preferring grasses over shrubs when they are green and abundant (Lu, 1988). As available pasture increased over time facilitated by the thinning of the shrubland and the fertilizing effect of goat depositions, goats began to consume it when it was green (winter and spring) over its abundance.

Changes in species abundance were poorly related with the preferences of the goats. Species that were consumed significantly below their abundance such as *C. ladanifer, C. libanotis* or *C. Monspeliensis* notably decreased in the grazed areas and not in the ungrazed ones, while the abundance of the most preferred species, *M.*

communis, decreased much less. Even *D. gnidium*, which was not consumed at all, reduced its cover in the grazed areas but not in the ungrazed ones. The decline of these species could be related to a special sensibility to damage caused by pawing and scratching of goats.

In summary, the goats released into the pine forest fed on a great variety of species, browsing nearly all species available in the understory with different intensities, and also grazing the pasture. But the changes detected in species abundance were not related with their feeding preferences, suggesting that other effects such as pawing or scratching could also be shaping species abundance.

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2.2. DO GOATS PRESERVE THE FOREST? EVALUATING THE EFFECTS OF GRAZING GOATS ON COMBUSTIBLE MEDITERRANEAN SCRUB

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Do goats preserve the forest? Evaluating the effects of grazing goats on combustible Mediterranean scrub

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Keywords
Doñana; Flammability; Forest fires; Phytovolume; Silvopastoral systems

Nomenclature
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Abstract

Question: Can grazing by goats eliminate potentially combustible plant biomass and thereby change the flammability of mediterranean vegetation?

Location: Doñana Natural Park, SW Spain.

Methods: The effect of goat grazing was evaluated, over a period of 42 mo, in 100 ha of pine forest understorey with an average density of 217 trees·ha^{-1}. Grazing by large herbivores was halted temporarily in the study area, with wild deer excluded from 1970, and domestic goats excluded from 2002. However, following the creation of grazing exclusion plots and sampling of the vegetation within as a control, adult female *Payoya* goats were introduced to the area in spring 2007. Vegetation was sampled twice yearly using the point-intercept method, and data of frequency, cover and phytovolume obtained. The study was completed with an analysis of change in the flammability of the study area (determined using data on the phytovolume and flammability of species). Changes in species richness and species diversity were determined.

Results: After 42 mo, the phytovolume of the ungrazed vegetation had increased significantly by 32%, while bare soil had decreased by 5%. This gave rise to a significant increase in flammability of 25%. Within the grazed area, species phytovolume decreased significantly by 34%, leading to a significant increase in bare soil of 51%, while the flammability of the area decreased by 22%. The number of species remained constant throughout the study at 20 species. Significant differences in species diversity were observed between grazed and ungrazed areas after 42 mo of grazing (species diversity index after 42 mo of exclusion = 1.59 ± 0.17, species diversity index after 42 mo of grazing = 0.95 ± 0.13).

Conclusions: Monitoring of the scrub understorey has shown the positive impact of grazing goats: the resulting decrease in the quantity of total phytovolume (easily combustible vegetation) induces changes in species diversity without lowering species richness and reduces the risk of fire.

Introduction

Wildfires have increased dramatically over the last 50 yr in Mediterranean Europe (EFFIS 2010). Over the last decade in Spain, for example, wildfire events have reached an annual average of 20 000 (Moreno et al. 1998; Vélez 2000; Pausas 2004; Röder et al. 2008). This increase can be related to changes in traditional social systems (rural depopulation, decline of agriculture, low profitability of forest products, etc.; Antrop 1993) and to policies adopted in recent decades regarding the exclusion of livestock from rangelands (Pausas 2004). These policies resulted in the extensive recovery of the maquis (a Mediterranean ecosystem dominated by semi-deciduous shrubs such as *Rosmarinus officinalis*, *Cistus* spp. and *Thymus* spp.) but also caused the closing-up of open grasslands and shrublands, modifying the composition of plant and animal communities. Both of these processes have favoured the spread of woody vegetation and hence increased the amount of combustible plant material. This process of rural abandonment has resulted in an expansion of homogeneous fire-prone vegetation communities having increased fuel loads (Moreira

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et al. 2001; Lasanta et al. 2006). At present, widespread forest fires are perceived publicly as a leading environmental concern (IESA/CSIC 2008) and their prevention, detection and suppression attract a substantial portion of the forest management budget in Mediterranean countries (Vélez 2009). Management strategies to reduce the current coverage of highly flammable communities are a priority in vast areas of the western Mediterranean region (Baeza & Vallejo 2008).

Several management practices, such as the use of herbicides and cutting with manual or heavy equipment, have been used to reduce the presence of flammable species (Etienne et al. 1996; Henkin et al. 1998; Wagner et al. 1998). In recent decades, wildfire prevention efforts have focused on the creation of a network of firebreaks designed to contain the spread of fires and contribute to the success of fire fighting efforts (Agee et al. 2000). However, this strategy is costly: Varela Redondo et al. (2007) conducted an economic study and found that application of domestic grazing for wildfire prevention costs only an average of 23% of the alternative manual clearing of firebreaks. A feasible and cost-effective alternative with which to improve fire prevention strategy is, therefore, the incorporation of grazing livestock into fire prevention programmes (Franca 2001; Rigueiro-Rodríguez et al. 2005; Launchbaugh et al. 2008; Ruiz-Mirazo et al. 2011). The potential offered by goats, with their ability to survive in disadvantaged areas, is broadly recognized at national and international level (Gall 1981; Devendra & McLeroy 1987; Rigueiro-Rodríguez et al. 2005; Torrano & Valderrábano 2005; Mosquera-Losada et al. 2006), but their use within forest areas has, until relatively recently, been a subject of controversy (Martín & Huss, 1981). The effect of livestock grazing on environmental conservation has historically been perceived as poor. Accused of causing overgrazing (Nelson 1988; Livingstone 1991; Mace 1991) and desertification (Dregne & Willis 1983), livestock grazing has more recently been seen as responsible for methane emissions (Puchala et al. 2005; Goel et al. 2008) and irreversible decline in species diversity (De Queiroz 1993a,b; Dougill & Cox 1995; Alados et al. 2003). However, grazing does not necessarily preclude ecosystem resilience, and adoption of the practice of conservation grazing can in fact maintain natural or cultural landscape processes (Concepción et al. 2008; Reinhardt et al. 2008). With this practice, grazing livestock are utilized appropriately in the management of their habitats, often to replicate the ecological effects of the wild relatives of livestock, or those of other species now absent or extinct. This form of extensive livestock management contributes to a more diversified and heterogeneous landscape and can play a role in fire prevention when intensified locally along the length of firebreaks. The capacity of livestock to effectively control shrub growth is supported by scientific evidence (e.g. Magadlela et al. 1995; Torrano & Valderrábano, 2005; Jáuregui et al. 2007; Celaya et al. 2007), and such targeted grazing could therefore be expected to successfully reduce the combustible material loads of the areas concerned.

Goats are well adapted to the consumption of shrubs inhabiting the Mediterranean forest understorey, probably due to their physiological adaptations (Green & Newell 1982; Perevolotsky & Seligman 1998). They are able to incorporate a greater variety of scrub species into their diet than other domestic ruminants, and have the ability to consume different plants or groups of plants according to the time of year, thereby consuming virtually all species growing within the Mediterranean forests (Morand-Fehr et al. 1983; Mitchell 1991). In this regard, goats hinder plant growth and the formation of dense and closed patches of vegetation. The most obvious effects of grazing disturbance in native plant communities include changes to vegetation structure and composition, as certain species are favoured by grazing and their numbers and/or cover increase, while other species are disadvantaged and reduce in number and/or cover (Hacker 1984; Belsky 1992; Milchunas et al. 1998; Briske et al. 2003).

This paper describes changes to the vegetation caused by goat grazing in the understorey of a pine forest in Doñana Natural Park in Spain. The objectives of the study were: (1) evaluation of changes in the phytovolume of different groups of plant species important to ecosystem functioning; (2) determination of whether grazing by goats causes changes in species richness and species diversity between remnants exposed to and excluded from grazing; and (3) analysis of change in the flammability of the understorey as a result of grazing.

Methods

Study area

The study was carried out in a 100-ha fenced pine forest situated in Doñana Natural Park in SW Spain (37°14′ N; 6° 20′ W). The climate is mediterranean, typified by wet and mild winters and long, dry summers. Mean annual rainfall is 540 mm, with 80% of precipitation occurring from October to March. Summer drought is severe, with no precipitation from July to August. Winter is mild, with a mean temperature of 10 °C in December and January, the coldest months, while the summer is hot, with a mean temperature of 25 °C in July and August, the hottest months (Fig. 1).

The geological substratum is quarcitic sand, with an undulated relief. Soils are shallow, acidic and poor in nutrients. The vegetation is a *Pinus pinea* forest, with an average density of 217 trees·ha^{-1} and an average tree diameter at

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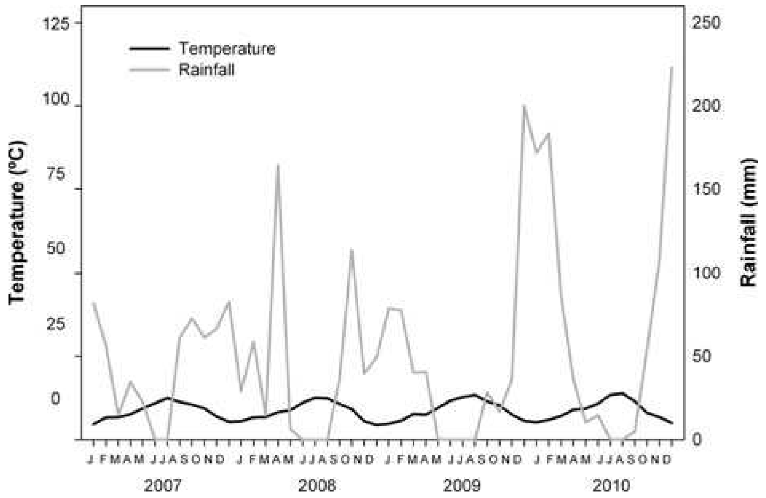

Fig. 1. Temperature and rainfall, monthly averages, from 2007 to 2010 in Doñana Natural Park (SW Spain).

breast height (DBH) of 26.92 cm. There are also some cork (*Quercus suber*) and Holm oaks (*Quercus ilex* subsp. *ballota*) at very low densities (<1 ha^{-1}). The understorey is dominated by shrubs (around 0.6–1.8-m tall), including *Cistus salvifolius*, *Halimium halimifolium*, *H. calycinum*, *Myrtus communis*, *Pistacia lentiscus* and *Rosmarinus officinalis*, as the most common species.

The study area is used for timber production, hunting (rabbit, partridge) and grazing. However, wild herbivores (deer) were eliminated in 1970, and domestic goats excluded from 2002, and grazing was therefore completely excluded from the study area for a period of 5 yr prior to the reintroduction of goats. During this 5-yr period, the natural vegetation of the study area was not subjected to any form of management, and consequently it grew and accumulated rapidly. So, in the spring of 2007, a herd of adult female *Payoya* goats (average weight 40 kg) was introduced into the area at a stocking rate of 2.7 goats $ha^{-1}·yr^{-1}$ (characterized as moderate grazing). In order to exploit the 100 ha in a uniform manner, goats are closely controlled and moved by a shepherd. These goats browse the entire area over three consecutive days and the fourth day is spent grazing outside the area (280 $d·yr^{-1}$). The goats were also fed with 450 g of fodder when milked.

Vegetation sampling

In February 2007, nine 50-m × 50-m plots (0.25-ha each) were chosen at random within the study area and fenced in order to exclude them from grazing by goats. A 25-m fixed linear transect was placed diagonally within the fenced-off exclusion plots in order to avoid edge effects. The transect was delimited and all species growing along the length of it were identified. Four transects (25 m each), were placed at 10-m distance from the four edges of each square plot, extending outwards to the cardinal points N, S, E and W. Measurements from the four transects outside the plot at each site were averaged, in order to have a single measurement comparable to the transect within the plot, thereby producing paired sampling within each of the nine sites, which were each considered as individual experimental units.

All transects were sampled eight times throughout the study period: once before the goats entered the pine forest (April 2007), and then every 6 mo over 42 mo of grazing (October 2007, April and October 2008, April and October 2009, and April and October 2010). In each plot, individual species cover was estimated by placing a metal rod of diameter 20 mm at 250 points (at regular intervals of 10 cm) along each transect. At each point, all species in contact with the rod were recorded (point-intercept method; Daget & Poissonet 1971). Species were identified according to Valdés et al. (1987). In addition, the height of each species at each point was recorded.

In order that the calculation of these variables incorporate the relative importance of each individual in the stratum, individual plant heights were weighted by the length they occupied on the transect. Shrub cover was calculated as the percentage of the transect length occupied by a shrub. Bare soil was also recorded as a variable since these data are of importance to the evaluation of changes in spatial continuity of vegetation. The bare soil cover results were grouped using classes (<1, 1–2, 2–3, 3–4, 4–5 and >5 m). Shrub phytovolume ($m^3·ha^{-1}$) was calculated as the product of the percentage cover and the weighted height (Frandsen 1983). Species richness was calculated for each plot and date using the Shannon–Wiener diversity

Table 1. Height, cover and flammability index (IF) of shrub species found in the understorey of a Mediterranean pine forest in Doñana Natural Park, SW Spain. Initial values (mean ± SE from 18 experimental units, April 2007) prior to the experimental entry of goats.

Group	Species	IF*	Height (cm)	Cover (%)
Cistaceae	*Cistus crispus*	(2)	113.83 ± 4.38	1.40 ± 0.65
	Cistus ladanifer	(4)	550.81 ± 7.30	3.67 ± 0.72
	Cistus libanotis	(2)	223.85 ± 8.57	3.90 ± 1.05
	Cistus monspeliensis	(2)	117.54 ± 5.15	1.05 ± 0.39
	Cistus salvifolius	(2)	339.37 ± 7.66	16.20 ± 0.64
	Halimium calycinum	(1)	225.68 ± 6.59	9.42 ± 4.05
	Halimium halimifolium	(1)	446.18 ± 12.40	11.52 ± 6.53
Aromatic	*Helichrysum italicum*	(3)	118.41 ± 7.13	1.30 ± 0.49
	Lavandula stoechas subsp. *pedunculata*	(3)	228.27 ± 3.63	4.30 ± 0.89
	Rosmarinus officinalis	(3)	552.12 ± 12.69	17.13 ± 5.86
	Thymus mastichina	(4)	333.29 ± 4.51	5.33 ± 1.75
Sclerophyllous	*Daphne gnidium*	(1)	335.55 ± *1.64*	0.95 ± 0.18
	Erica scoparia	(4)	1108.65 ± 23.34	6.58 ± 3.50
	Myrtus communis	(2)	557.63 ± 15.82	11.16 ± 4.23
	Phillyrea angustifolia	(4)	991.27 ± 19.09	1.5 ± 0.07
	Pistacia lentiscus	(1)	668.97 ± 9.21	8.15 ± 3.32
	Quercus coccifera	(3)	338.34 ± 23.09	5.9 ± 2.62
Resprouters Aphyllous	*Genista hirsuta*	(4)	444.00 ± 10.38	2.98 ± 0.45
	Genista triacanthos	(4)	448.00 ± 8.42	3.01 ± 0.62
	Stauracanthus genistoides	(4)	446.05 ± 15.59	1.53 ± 1.73

*Flammability index: 1 = Non-flammable; 2 = Slightly flammable; 3 = Flammable; 4 = Highly flammable. (Elvira and Hernando 1989; Vélez 1990; Villalón et al. 2001; Valette 1997).

index H′ (Magurran 1988): $H' = -\sum p_i \ln p_i$; where p_i = contacts of species i in a given plot and date/contacts of all the species in that plot on that date. The study was completed with an analysis of change in the flammability of the study area. The following formula was applied: Δ Flammability (time X) = $\sum$ (% B_i * I_i), where B_i is the phytovolume of species i and I_i is the flammability of species i. Mean flammability data for each species were obtained from the data of Elvira & Hernando (1989), Vélez (1990), Villalón et al. (2001) and Valette (1997) (Table 1). In these studies, flammability experiments were performed on branches of every species (not more than 8 cm in length), in different seasons, following the standard UNE-23-721 and using a standard epiradiator of 500 W constant nominal power.

Data were collected regarding plant species; however, unequal distribution of these species in the plots produced too many zero values, therefore data were grouped for data analysis into Cistaceae, aromatic, aphyllous and sclerophyllous resprouters (Table 1). Similarly, for the flammability analysis, species were classified into non-flammable, slightly flammable, flammable and highly flammable.

Data analysis

An ANOVA model for repeated measures was fitted for each dependent variable (phytovolume, flammability, bare ground and diversity). The model includes two within-unit factors: treatment, with two levels (grazed areas and ungrazed areas), and time, with eight levels, the first is the initial time, while the other seven levels represent the 6-mo periods. We considered area type as a within-unit factor because the sampling units are paired (transects inside and outside the exclusion plots). The ANOVA model included treatment, time and interaction terms. We performed a univariate repeated measures analysis of variance with SPSS v18.0 (SPSS Inc., Chicago, IL, US) and the library car of the R system (R Foundation for Statistical Computing, Vienna, AT). The compound symmetry structure of the covariance matrix was tested with the Mauchly procedure, rejecting the null hypothesis for all models. We therefore used the adjusted procedures based on Greenhouse–Geisser and Huynh–Feldt corrections for departure from sphericity. Both corrections agreed, therefore we only present results according to the Huynh–Feldt corrections. It is noteworthy that the results obtained assuming sphericity were coincident with those arising from these last corrections, thus solving a possible disagreement between the two approaches. Finally, the relationships between phytovolume, flammability, diversity and bare ground were explored using Pearson correlation.

Results

The characteristics of the species prevalent in the study area prior to introduction of the goats, and the groups

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formed by these species, are detailed in Table 1. Goat grazing decreased the total phytovolume of the shrubs (Fig. 2). The decrease in total phytovolume from the initial values was progressive, being 7%, 9%, 21%, 23%, 30%, 29% and 32%, at 6, 12, 18, 24, 30, 36 and 42 mo of grazing, respectively. In contrast, the total phytovolume of the species in the exclusion plots increased by 14%, 33%, 25%, 33%, 24%, 28% and 36% at 6, 12, 18, 24, 30, 36 and 42 mo, respectively (Fig. 2). This interaction for the total phytovolume of the species was statistically significant ($P = 0.014$; Table 2). The aphyllous and sclerophyllous resprouters group presented a significant interaction between time and treatment, as can be seen in Table 2 ($P = 0.021$ and $P = 0.031$, respectively). This interaction is displayed in Fig. 2, where there is a clear difference, over time, between the profiles of grazed and ungrazed areas for these groups. However, not all of the plant groups responded in the same way to the grazing treatment.

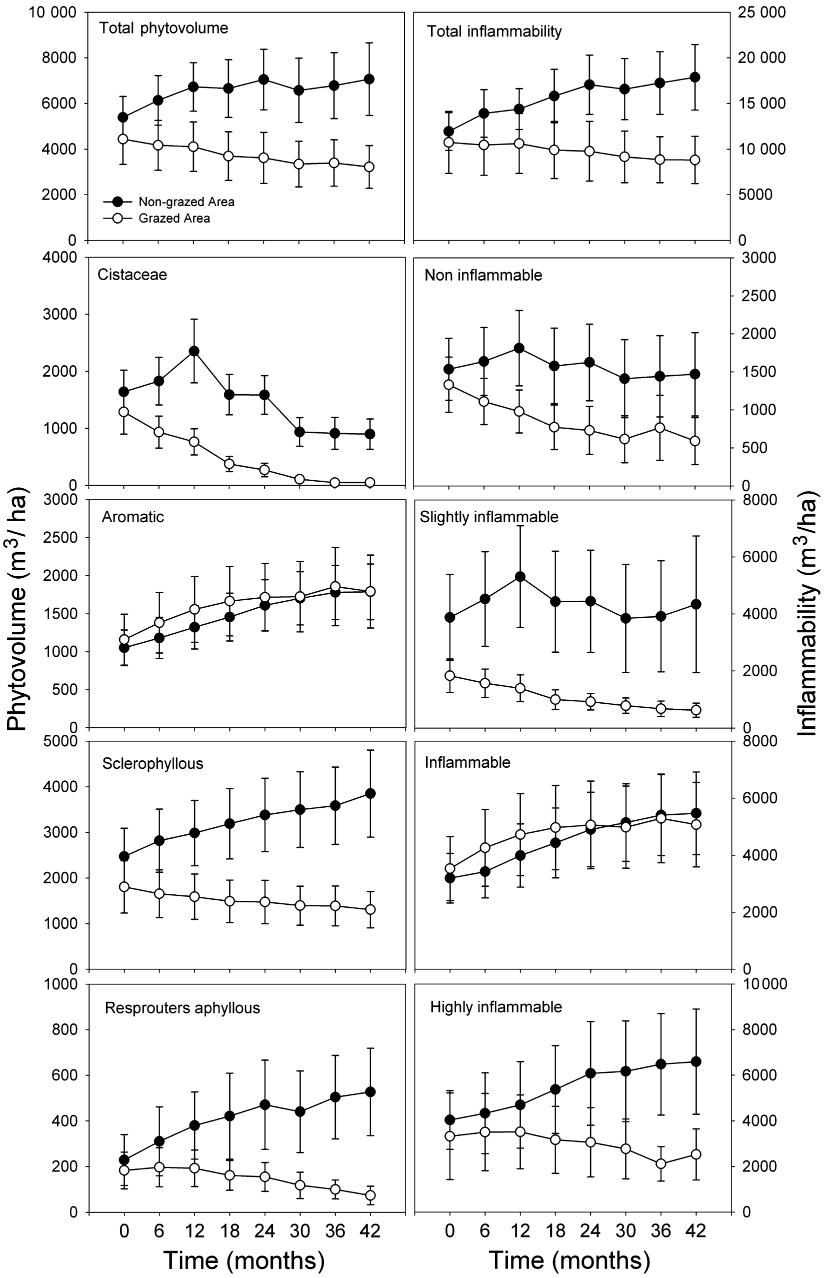

Fig. 2. Changes in phytovolume and flammability in the understorey of a Mediterranean pine forest in Doñana Natural Park (SW Spain) over 42 mo with or without goat grazing (mean ± SE, $n = 9$).

Table 2. Overall (averaged over time) least squares means for each grazing treatment (grazed and ungrazed), Standard error of the mean (SEM) and *P*-values for the hypothesis of the main effects (treatment, time and interaction) found in the understorey of a Mediterranean pine forest in Doñana Natural Park (SW Spain) over 42 mo under goat grazing or no grazing.

Variable	Means		SEM	*P*-values		
	Ungrazed	Grazed		Treatment	Time	Interaction
Total phytovolume ($m^3 \cdot ha^{-1}$)	6547.22	3744.87	513.37	0.006	0.417	0.014
Cistaceae ($m^3 \cdot ha^{-1}$)	1467.72	478.52	250.03	0.027	0.001	0.086
Aromatic ($m^3 \cdot ha^{-1}$)	1486.38	1606.16	311.44	0.793	0.001	0.451
Sclerophyllous ($m^3 \cdot ha^{-1}$)	3222.16	1512.75	766.80	0.159	0.138	0.031
Resprouters aphyllous ($m^3 \cdot ha^{-1}$)	411.57	147.45	111.63	0.179	0.060	0.021
Total flammability ($m^3 \cdot ha^{-1}$)	15594.97	9781.01	747.43	0.001	0.024	0.017
Non-flammable ($m^3 \cdot ha^{-1}$)	1562.84	860.86	325.96	0.172	0.225	0.296
Slightly flammable ($m^3 \cdot ha^{-1}$)	4334.31	1097.83	1282.90	0.118	0.116	0.267
Flammable ($m^3 \cdot ha^{-1}$)	4493.34	4734.76	777.34	0.832	0.004	0.201
Highly flammable ($m^3 \cdot ha^{-1}$)	5204.47	3087.57	586.17	0.038	0.069	0.043
Bare ground (%)	35.30	58.78	6.01	0.033	0.000	0.035
Diversity index	1.47	1.33	0.16	0.257	0.000	0.001

In the case of the Cistaceae group, a significant difference was observed between the grazed and ungrazed treatment ($P = 0.027$; Table 2), but there was no significant interaction between treatment and time ($P = 0.086$; Table 2). Finally, the aromatic group did not present significant differences between the grazed and ungrazed areas, but a significant increase of the aromatic group across time was observed ($P = 0.001$; Table 2).

Through the use of transects, it was possible to measure changes in the linear cover of the studied species (from the data regarding patches of bare ground). Table 2 shows a significant interaction between time and treatment for bare ground ($P = 0.035$). After 42 mo of study, the spatial continuity of the vegetation had increased within the exclusion plots: the number of patches of bare ground <1 m had increased, reducing the number of patches of 1 –2 m (Fig. 3). This led to a reduction of 5% in the quantity of bare ground within the ungrazed area (from an initial 37% bare soil to 35% after 42 mo of enclosure). The opposite was true of the grazed area, where the number of <1 and 1–2 m patches of bare soil decreased in favour of an increase in bare soil patches of 2–3, 3–4, 4–5 and >5 m. This led to an increase in bare soil of 51% (45% of bare soil initially, increasing to 67% after 42 mo of grazing).

The number of species was unaltered throughout the experimental period in the grazed treatment, and remained constant at 20 species. In terms of the ungrazed vegetation, there were no significant differences in the diversity index of these species over the entire study period. However, significant differences in the diversity index of these species were observed across time ($P < 0.001$; Table 2). Diversity index showed a significant interaction between time and treatment ($P = 0.001$; Table 2), with profiles differing after the three first time periods (Fig. 4; Diversity index before grazing = 1.51 ± 0.12; after 42 mo of exclusion = 1.59 ± 0.17, after 42 mo of grazing = 0.95 ± 0.13).

A significant interaction was also observed for total flammability ($P = 0.017$; Table 2). This variable produced a clear growth in the exclusion group throughout the study period (increase of 25% after 42 mo of exclusion), while in the grazed area, flammability was reduced (decrease of 22% after 42 mo of grazing; Fig. 2). The

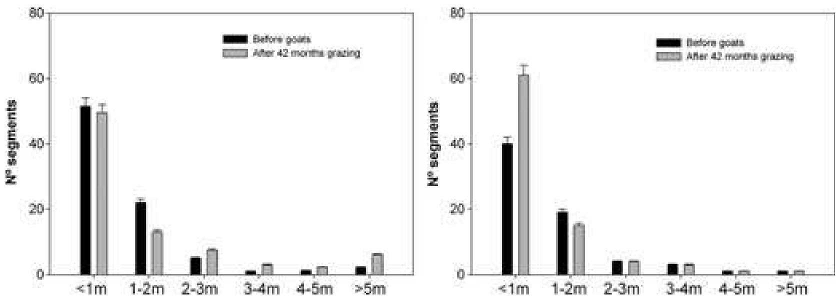

Fig. 3. Changes in number of patches of bare ground from number of segments shown per 25-m transect in the understorey of a Mediterranean pine forest in Doñana Natural Park (SW Spain) over 42 mo of exposure to or exclusion of goat grazing (mean ± SE, $n = 9$).

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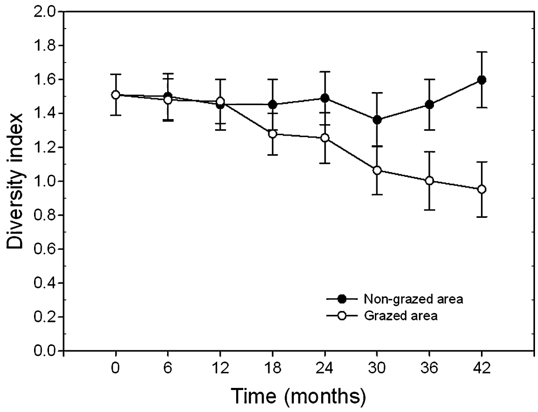

Fig. 4. Change in Shannon–Wiener diversity index in the understorey of a Mediterranean pine forest in Doñana Natural Park (SW Spain) over 42 mo with or without goat grazing (mean ± SE, $n = 9$).

marked decrease in phytovolume of the highly flammable species group (*Cistus ladanifer*, *Erica scoparia*, *Genista* spp., *Stauracanthus genistoides*; Table 1), produced an overall reduction in total flammability of the grazed area, with a profile significantly different to the profile in the ungrazed area ($P = 0.043$; Table 2).

Changes in total phytovolume were negatively correlated with bare ground ($r = -0.79$, $P < 0.001$). However, total phytovolume was positively correlated with diversity index ($r = 0.47$, $P < 0.001$) and total flammability ($r = 0.94$, $P < 0.001$). This last correlation seems quite natural since they are interrelated variables. Changes in total flammability were positively correlated with changes in diversity index ($r = 0.56$, $P < 0.001$) and negatively correlated with bare ground ($r = -0.76$, $P < 0.001$). Diversity index and bare ground were negatively correlated ($r = -0.39$, $P < 0.001$).

Discussion

Despite the important loss of phytovolume in certain species in this study, moderate grazing was insufficient to remove these species completely, although the response of each species or vegetation group to grazing differed. In the case of the Cistaceae group, a significant difference in phytovolume was observed between the grazed and ungrazed treatment, but this difference was not observed in the aromatic group. Mediterranean scrubland is an ecosystem with a long history of grazing by domestic animals (Emanuelsson 2009); the study area has been heavily influenced by both fire and grazing (Granados et al. 1988). Light grazing has no profound effects on vegetation and soils, while moderate grazing is considered to be essential for maintaining species diversity and ecosystem stability (McNaughton 1983; Noy-Meir 1998).

Within the exclusion plots, we found a progressive increase in total living phytovolume of the species corresponding incrementally to the time of exclosure. However, in some species of the Cistaceae, live phytovolume decreased after 18 mo of exclusion as a result of the death of several individuals (senescence). It appears that the demographic characteristics of *Cistus* spp. (recruitment limited to the 5 yr immediately following a fire, light inhibition of germination and short longevity) lead to stand decline, which begins at around 15 yr after a fire event (Roy & Sonié 1992). In addition, Juhren (1966) established the period of senescence for *Cistus monspeliensis* in the Mediterranean basin at between 7–8 yr. Similarly, the decline in *Cistus* and *Halimium* spp. observed in the summers of 2008 and 2009 might have been aggravated by the drought years that have prevailed in Southern Spain since 2003–2005, with annual rainfall well below average for the area, at 200 and 382 mm, respectively (data from the Natural Processes Monitoring Team of Doñana Biological Station). On the other hand, the aromatic group experienced an increase in phytovolume in both areas across time (grazed and ungrazed). Non-grazing or low consumption of aromatic plants can be attributed to their high oil content (Guillén et al. 1996), a feature which reduces their palatability. In this area, *Thymus mastichina* and *Lavandula stoechas* are never consumed by goats, while *Helichrysum italicum* and *Rosmarinus officinalis* have a seasonal consumption that occurs only while they are in flower (Martín et al. 2011). This result supports that of Valderrábano & Torrano (2000), who observed that goat browsing also has a remarkable effect on both regrowth and flowering density in *Genistas scorpius* and, consequently, on short- and medium-term survival rate, although the response of the plant clearly differs depending on the time of grazing.

The other two groups (aphyllous and sclerophyllous resprouters) showed significant differences in terms of phytovolume development. The constant and intense consumption of *Erica scoparia*, *Myrtus communis*, *Pistacia lentiscus* and *Phillyrea angustifolia* (sclerophyllous resprouter species) throughout the year, as well as the intense consumption, while in leaf or flowering/fruiting, of *Genista* spp. and *Stauracanthus genistoides* (aphyllous resprouter species), caused a considerable decrease in their phytovolume (Martín et al. 2011), and led to differential development between grazed and ungrazed individuals.

As described above, grazing was insufficient to eliminate any species completely, although total phytovolume, and that of different species groups, was significantly reduced in the grazed area compared to the ungrazed area, in which values remained similar to those recorded at the beginning of the study. Grazing directly affects the demography of plants by changing establishment, growth and survivorship rates (Huntly 1991). However, heavy grazing of specific species can benefit the ungrazed or seasonally consumed species, by reducing competition for resources (Harper 1969; Milchunas et al. 1992; Perevolotsky et al. 1998). The reduced diversity (Shannon–Wiener diversity index, Fig. 4) found in the grazed vs ungrazed treatment was a consequence of reduced evenness (increased dominance), since species richness remained constant. There may be other non-exclusive mechanisms through which grazing animals can trigger shrub dominance cover, including changes in resource availability, demographic changes (e.g. changes in seed availability, establishment and survivorship) and changes in biotic interactions (e.g. changes in the competitive environment) (Facelli & Springbett 2009).

Mediterranean vegetation is able to survive various disturbances due to regeneration of lost organs in the case of resprouting species, and to the soil seed bank that ensures the presence of seeding species, such as those of the Cistaceae, in the next generation (Keeley 1986). The resprouting capacity of many Mediterranean woody species has allowed them to withstand significant periodic disturbances, sprouting vigorously from roots, rhizomes or stocks, in which they have abundant carbohydrate reserves. Examples of species of this type include *Erica scoparia, Myrtus communis*, *Pistacia lentiscus, Phillyrea angustifolia, Genista* spp. and *Stauracanthus genistoides* (Ojeda 2001; Vallejo et al. 2006).

The changes found in the flammability of the area were significantly and positively correlated to changes in the phytovolume of the species. The flammability of a forest, which also considers other variables, is determined mainly by the pyrophytism, or characteristic flammability, presented by the understorey shrub species (Valette 1990). Thus, medium-high density pine forests (190–390 trees·ha^{-1} and 50–60 yr) with tall, thick undergrowth are more flammable (Montero et al. 2004; Villalón et al. 2001): these characteristics are relatively similar to those found in our study area. The reduction of potentially combustible understorey plant biomass found in this study translates into a reduced fire risk, as many of these species (e.g. *Cistus ladanifer, Erica scoparia, Genista* spp., *Phillyrea angustifolia* and *Thymus mastichina*: Table 1) are highly flammable, not only in summer, but throughout the year (Elvira & Hernando 1989; Valette 1997).

It should also be noted that fire can have difficulty starting and spreading where there is discontinuity in the vegetation; under this scenario, even highly flammable species may not present a great danger (Morvan & Dupuy 2004). In this sense, and further to decline of the phytovolume of the species, a significant reduction of horizontal vegetation cover, or the increase of bare ground found after 42 mo of grazing (51% relative to the initial value), can all contribute to a progressive reduction in fire risk. This effect of grazing on vegetation at light to moderate intensities (creation of 'regeneration gaps') controls the combustible biomass, reducing the fire risk, and permits the co-existence of species that were previously suppressed by the dense sward of the dominant species (Grime 1979; Noy-Meir 1995). The creation of open canopy environments, when seeds exist in the soil or are introduced by animals (Ramos et al. 2006; Mancilla-Leytón et al. 2011), can lead to improved pastures with a consequent increase in the availability of food for small wild herbivores (e.g. rabbit; *Oryctolagus cuniculus*) that feed on grass. A dominance of shrubs causes the absence of grass, which therefore has a considerable impact on herbivore populations. Rabbits form an important part of the ecology of the study area: they constitute prey for many endangered species that breed in the Doñana Natural Park, such as the Iberian lynx (*Lynx pardinus*), Iberian imperial eagle (*Aquila adalberti*) and the Eurasian eagle-owl (*Bubo bubo*). When the above factors are all taken into account, instead of provoking a competition for resources between domestic and wild animals, goat grazing can become an important factor enabling an overall increase in total (plant and animal) species richness in an area.

This combination of agricultural land use, forestry and livestock production, also known as agro-silvo-pastoralism, is a traditional land use in the Mediterranean region and is already successfully applied in several temperate regions of the world. In the Mediterranean basin, the flora has co-evolved with large gregarious herbivores that heavily graze vegetation. Domestic herbivores have been used for economic purposes for thousands of years (production of meat and milk is still the primary economic activity in many rural areas). While increasing the income from the land, grazing has also acquired an environmental dimension in the last decade: control of the understorey vegetation, thus reducing fire risk and increasing forest structural diversity and biodiversity.

The results of this study demonstrate that in our study area, which is representative of Mediterranean ecosystems, adoption of the practice of conservation grazing can be an effective and economically sound method through which to both promote the growth of native vegetation and minimize the risk of its loss by reducing the likelihood and potential destructivity of forest fires in the region.

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2.3. EFFECT OF GRAZING AND SEASONS ON THE CHEMICAL COMPOSITION OF MEDITERRANEAN SHRUB SPECIES IN DOÑANA NATURAL PARK

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Abstract

Chemical composition and its variability influence the probability of a plant being eaten augmenting or diminishing herbivore consumption between different species. The objective of this study was to assess the interaction between seasonal changes and grazing pressure on chemical composition in six Mediterranean shrub species (*Cistus salvifolius, Halimium halimifolium, Myrtus communis, Phillyrea angustifolia, Pistacia lentiscus* and *Rosmarinus officinalis*). In each season, samples of forty-five individuals of each species were collected from grazed areas and protected areas for grazing. Condensed tannin, total phenol, N and C concentrations were obtained by NIRS spectroscopy. The values predicted by NIR (R^2> 0.92) showed changes in the secondary components along the different seasons and treatments. In the case of nitrogen, tannins and phenols content, all species presented a significant relationship between time and treatment. Carbon content did not show significant differences between the grazed and non-grazed areas, but significant changes of the carbon content across time was observed. Nitrogen content was higher in vegetative growth periods and fell dramatically in summer. Species-specific responses of analyzed compounds were detected: in grazed plants *H. halimifolium* and *M. communis*, unlike the rest of the species, tannins and phenols concentration was lower. The results of multiple regression models showed a significant relationship between the consumption of the species with carbon and tannin contents (grazing is greater when species decrease their carbon and tannin contents). This study suggests that, contrary to popular belief, goats are selective mix grazers; goat grazing is not random. During grazing, goats increase their nutritional value intake (N content) while minimizing secondary compounds (tannin and phenol).

Keywords: shrubland; NIRS; goat grazing; tannin; phenol; Doñana

1. INTRODUCTION

Wild or domestic livestock grazing has been described as an important biological factor that determined the composition and structure of Mediterranean ecosystems (Carrión et al. 2003). Goats are polyphagous animals that feed on a great variety of species, but they always prefer some species and reject others (Papachistou et al. 2005; Mancilla-Leytón et al. 2012). This selective behaviour may be governed by a series of factors: plant abundance, chemical characteristics of plants (nutrients, secondary compounds, taste, etc.) and vegetation heterogeneity (Lu, 1988; Papachistou et al. 2005; Baraza et al. 2009). According to Merrill and Taylor (1981) diet selection by goats is primarily determined by the variety of plant species and the relative abundance of each. Also, depending on the nutritional quality or concentration of chemical defenses, selection by goats can be from diverse plant species or between diverse individuals of the same plant species (Riddle et al. 1996; Barroso et al. 2001; Provenza et al. 2005). So, their nutritional quality may differ between different species, or individuals or branches of the same species (Orians and Jones 2001). These variations can be measured as differences in nutritional value, as well as morphological characteristics of twigs and tissues (Hartley and Jones, 1997), augmenting or diminishing herbivore consumption between different species, plants of the same species, or even parts of the same plant (Riddle et al. 1996; Alonso-Díaz et al. 2008; Baraza et al. 2009).

As already mentioned, the chemical composition (as secondary compounds) is an important factor for the consumption of a species. Antiherbivory defense system chemicals (secondary compounds) could be native to the plant (Distel and Provenza 1991; Alonso-Díaz et al. 2008) or may appear as a result of herbivory (Barroso et al. 1995; Baraza et al. 2009). So, their diet not only depends heavily on plant availability but also on secondary compounds (some species are positively selected, while others are partially or totally rejected) (Ngwa et al. 2000; Dziba et al. 2003; Alonso-Díaz et al. 2008).

In these times it may be important to know these changes of grazing over time (how they select their diet and the factors that influence this diet), as this may lead the appropriate management of the shrublands they occupy. Changes in traditional social systems (rural depopulation, decline of agriculture, exclusion of livestock from rangelands, low profitability of forest products, etc.) caused the closing-up of open grasslands and shrublands (semideciduous and resprouting species), modifying the composition of plant and animal communities. These processes have favoured the spread of woody vegetation and hence increased the amount of combustible plant material. At present, livestock is again important in forest systems; the incorporation of grazing livestock into fire prevention programs is a feasible and cost effective alternative with which to improve fire prevention strategies (Ruiz-Mirazo et al. 2011).

So, the objectives of this study were: i) to explore the time-course of the chemical composition of six Mediterranean shrub species under long-term utilization by large herbivores, ii) to relate variation in browsing intensity and chemical composition particularly with condensed tannin, total phenol, N and C concentrations. We assumed that the change in leaf composition content due to grazing was effectively related to the change in its biochemical composition and therefore in its spectral properties. We presume that the measured chemical traits are responses to grazing, but we too admit that the chemical characteristics and phenology state of the species could be significant.

2. MATERIAL AND METHODS

2.1. Data sets

Samples were collected from the understory of a pine forest (*Pinus pinea*) located in Doñana Natural Park (37º14'N, 6º20'W, SW Spain). The climate is Mediterranean, with a mild and rainy winter (monthly average temperature is 10 ºC in December and January), and a long dry summer (mean temperature of 25 ºC in July and August). Mean annual rainfall is around 540 mm, with 80% of precipitation occurring from October to March. The geological substratum is quartztic sand, with an undulated relief. Soils are shallow, acidic and poor in nutrients (<5% MO).

The study area is used for timber production, hunting (rabbit, partridge) and grazing. However, wild herbivores (deer) were excluded in 1970 and domestic goats in 2002, so the study area remained ungrazed for a period of five years prior to the reintroduction of goats (2007). During this five-year period of livestock exclusion, the natural vegetation was not subject to any form of management and consequently grew and expanded rapidly. In 2007, before introducing the goats for the first time, nine fenced exclusion plots of 0.25 ha were established in the area. Afterwards, in the spring of 2007, a herd of adult female Payoya goats (average weight of 40-45 kg) was introduced in the study area at a stocking rate of 2.7 goats ha-1 yr-1 (characterized as moderate grazing). The livestock management may be considered to be semi-extensive, although in order to exploit the 100 ha in a uniform manner, the goats were closely controlled and moved around by a shepherd (Mancilla-Leytón et al. 2012).

The number of plant species used in the study was restricted to six common Mediterranean shrub species: *Cistus salvifolius* L. and *Halimium halimifolium* (Cistaceae), *Myrtus communis* (Mirtaceae), *Pistacia lentiscus* (Anacardiaceae) *Phillyrea angustifolia* (Oleaceae) and *Rosmarinus officinalis* (Lamiaceae). The species selected were among the most abundant and evenly distributed in the study area (semideciduous, resprouter and aromatic species; representing 80% of the understory cover), so these species are high grazing by goats (Mancilla- Leytón et al. 2012).

Current year foliage samples from each species were collected once every season during 2011 and 2012 (spring, summer, autumn and winter). For each species, samples of terminal twigs with their leaves were cut in a sufficient quantity (simulating a bite) from 45 different individual plants in each area (9 groups of 5 individual plants inside the exclusion plots [not-grazed area] and 9 groups outside [grazed area]). Samples were cut at midday, brought back to the laboratory and weighed; they were then oven-dried at 60º C for 48 h. Measurements from the 5 individuals were averaged, thereby producing paired sampling within each of the sites (ungrazed and grazed area), which were each considered as individual experimental units.

2.2. NIRS analysis

All these dried samples (2160 samples) were ground in a cyclone mill through a mesh size of 1 mm, and scanned using a NIR spectrophotometer (NIRSystems 6500, Silver Spring, MD, USA) in Centre d'Écologie Fonctionnelle et Évolutive (CNRS Montpellier, France). For each measurement, 32 scans were made, at 2 nm intervals over a range from 400 to 2500 nm, to produce a mean spectrum with 1050 data points. Using ceramic standards, the internal software evaluated the spectrum of the apparent reflectance (R). Then the spectral data recorded were processed and stored in absorbance units (A) equal to log (1/R). Data analysis was conducted using the ISI software system (Shenk and Westerhaus 1991).

2.3. Chemical composition

After NIRS analysis, 70 representative samples were selected for analysis in the laboratory. We applied SELECT algorithm, using WinISI^{TM} III v.1.60 (Infrasoft International, LLC), to extract the 70 most representative samples in our spectral population, that included each season, species and treatment. Analyses of lignin were carried out to the calibration set by traditional wet chemistry procedures (Goering and van Soest 1970). Elemental analyses for N and C rate and phenols content were carried out according to Pella and Colombo (1973) by combustion gas-chromatography. The mean values were used for NIRS calibration and validation.

2.4. Population structuring

All sample sets were examined using the population structuring software in order to identify spectral outliers. To identify patterns in the group of spectra that contribute the most to the variation among the spectra, Principal Component Analysis (PCA) was used. An average Mahalanobis distance (Global H) was calculated and H values for individual samples were standardized by dividing by the average H value. Any sample with a spectrum above 3.0 standardized units above the mean of the sample set was regarded as a spectral outlier. An identical population structuring procedure was applied for every species.

2.5. Calibration procedures

The calibrations involved searching for predictive relationships between spectral data and chemical composition reference values. Calibration equations are mathematical transfer functions built using reference and spectral values of the calibration sample set and used to predict an unknown quantitative value *Y* from available spectroscopic measurements *X* (Martens and Naes 1989).

Partial Least Square regression method (Wold et al. 2001) uses all the spectral information, unlike stepwise regression type methods which use only a small number of wavelengths (Windham et al. 1989). In addition, the calibration was obtained after correcting the spectra by ad trending method (Barnes et al. 1989). Cross validation was used to estimate the optimal number of terms in the calibration to avoid over fitting. This consists of selecting three quarters of the samples to develop the model and one quarter for the prediction. The algorithm is repeated four times and all the residuals of the four predictions are pooled to provide a standard error of cross validation (SECV) on independent samples. The minimum SECV determines the number of terms to be used. The final model is then recalculated with all the samples to obtain the standard error of calibration (SEC) which is the standard deviation of differences between reference values and NIRS-predicted values. Different mathematical treatments, corresponding to the first and second derivative and a gap of 2, 10, 10 data points, were compared. After comparison of the results of various treatments, the calibration equation that gave the best results in terms of SECV was selected.

2.6. Data Analysis

Principal component analysis (PCA) has been performed to examine the qualitative difference between the species analyzed in this study. Before performing PCA, all of the sample spectra were preprocessed using a second derivatization algorithm to reduce baseline variations and enhance the spectral features. The full NIR reflectance spectral range was used instead of using specific spectral regions. The spectral variations based on the species are expected to present throughout whole NIR range because every species is composed of huge numbers of chemically different individual components. Only one spectral outlier were found for all samples, so 2159 samples were included in the calibration and validation sample sets.

An ANOVA model for repeated measures was fitted for values obtained from the application of the selected calibration equation (Carbon, Nitrogen, Tannin and Phenol). The model includes two within-unit factors: treatment, with two levels (grazed areas and non-grazed areas), and time, with four levels (autumn, winter, spring and summer). We have considered area type as a within-unit factor because the sampling units are paired (samples inside and outside the exclusion plots). The ANOVA model included treatment, time and interaction terms. We performed a univariate repeated measure analysis of variance with SPSS v18.0 and the library car of the R system (Fox and Weisberg 2011). The compound symmetry structure of the covariance matrix was tested with the Mauchly procedure, rejecting the null hypothesis for all the models. We therefore used the adjusted procedures based on Huynh-Feldt corrections for departure from sphericity.

Finally, to assess whether the consumption of the species is related to chemical characteristics (carbon, nitrogen, tannin and phenol) we performed a multiple regression model (All consumption values were taken from Mancilla-Leytón et al., 2012) (Table

3). So, the relationships between carbon, nitrogen, tannin and phenol were explored by Pearson correlation.

3. RESULTS

3.1. Spectrum

PCA methodology was applied to the absorbance spectra obtained for 2159 samples. Different spectral features were found, as revealed by a principal component analysis (PCA). Fig. 1 and 2 show the three dimensional (3D) score plots using first, second, and third factors. We found to account for 88.3% of the cumulate variability in total samples, 94.7% by *C. salvifolius* samples, 94.4% by *H. halimifolium*, 92.9% by *M. communis* samples, 95.8% by *P. angustifolia* samples, 98.9% by *P. lentiscus* samples and 92.7% by *R. officinalis* samples. Fig. 1 shows that PCA revealed broad patterns in the spectra that indicate an underlying structure related to species: all samples are generally classified into three groups; semideciduous (*C. salvifolius, H. halimifolium*), resprouter (*M. communis*, *P. lentiscus* and *P. angustifolia*) and aromatic species (*R. officinalis*). On the other hand, in the case of each species (Fig. 2), the samples from both treatments (not-grazed and grazed) and season studied are clearly differentiated without overlapping. This result shows that PCA can adequately discriminate samples from different species.

3.2. Calibration and prediction of chemical values in the validation sets.

Calibration accuracy was assessed on the basis of the highest R^2 and lowest SEC and SECV values. Table 1 shows the statistics of the best calibration for each variable. In Carbon, Nitrogen, Tannin and Phenol, the coefficient of determination of the regression between the reference and NIRS-predicted values is highly significant with a slope close to 1. The NIR validation also showed higher R^2 values for all components examined (> 0.92). The SECV value was relatively high for Phenol (Tab.1).

3.4. Seasonal and grazing effect on chemical composition

In orden to obtain information about the chemical components, the selected equation was applied to the remaining samples from vegetation (not included in the calibration), resulting in a set of predicted values for different variables.

Fig. 3 shows the average predicted percentages (per dry matter) of Carbon, Nitrogen, Tannin and Phenol found in the foliage of each species. The analysis showed changes in the secondary components along the different seasons and treatments. In the case of Nitrogen, Tannins and Phenols content, almost all species showed a significant interaction between time and treatment. These interactions are displayed in Fig.3, where they exhibit a clear difference, over time, between the profiles of grazed and non-grazed areas for these species.

Figure 1. The three-factor plot in PCA for all species studied.

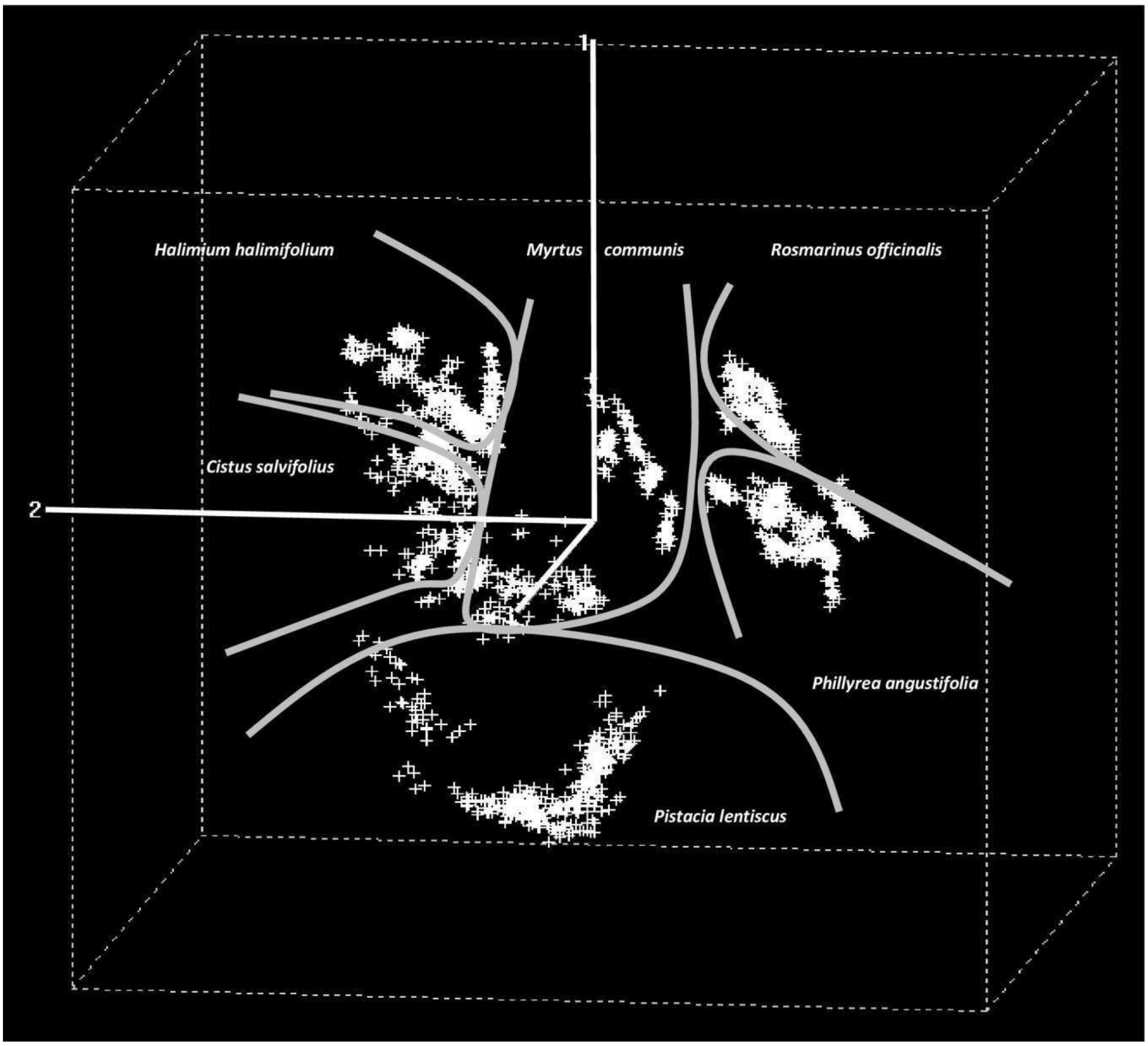
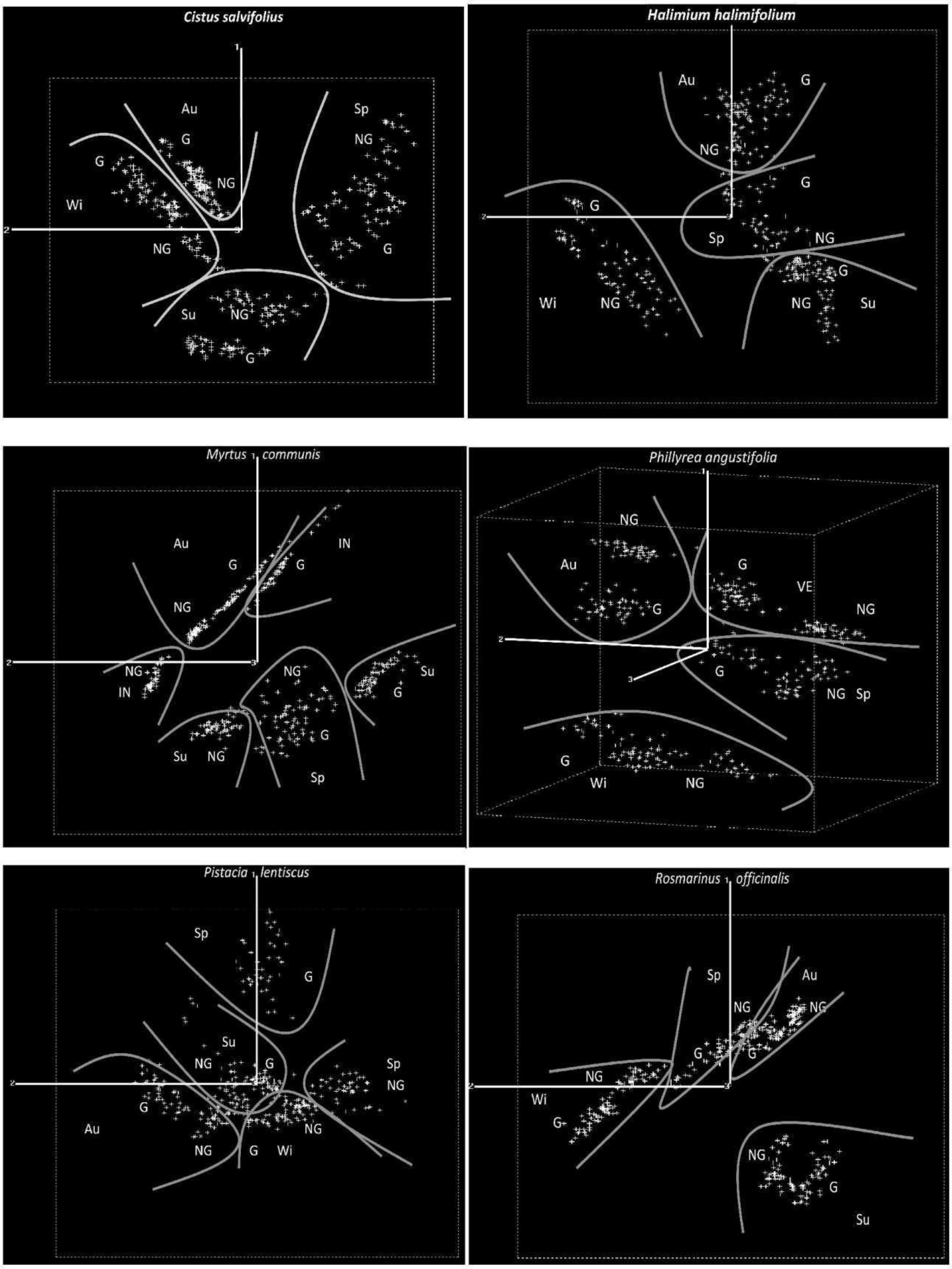


Figure 2. Spectrum all species studied. Au: Autumn; Wi: winter; Sp: Spring; Su: Summer; NG: not-grazed area; G: grazed area.



For any of the species studied, Carbon content did not present significant differences between the grazed and non-grazed areas, but in *C. salvifolius* (F= 98.13 p=0.008), *H. halimifolium* (F= 44.11 p=0.005), *M. communis* (F= 219.60 p=0.001), *P. angustifolia* (F= 97.65 p=0.009) and *R. officinalis* (F= 136.59 p=0.004) significant changes of the carbon content across time were observed (Fig. 3). For *C. salvifolius, H. halimifolium, M. communis* and *P. lentiscus* Carbon content was similar; it ranged from 44 – 47% depending on species and season. *P. angustifolia* and *R. officinalis s*howed the highest levels (48 - 52 %). In all species the carbon content decreased in winter (vegetative growth periods), except in the case of *M. communis*, which showed higher values in this period.

A significant interaction was also observed for nitrogen content in all species (Tab. 2). Nitrogen content ranged from 1.0-1.7% depending on species and season. Unlike Carbon, the nitrogen content was higher in vegetative growth periods (e.g. Autumn-Winter: *C. salvifolius, H. Halimifolium* and *R. officinalis*; Spring: *P. angustifolia* and *P. lentiscus*; Winter: *M. communis*). Despite the differences, the pattern in not-grazed and grazed areas was very similar in *C. salvifolius, H. halimifolium* and *R. officinalis*: although in these species nitrogen content fell dramatically in summer. *M. communis* showed significantly higher nitrogen content in the grazed area in summer, autumn and winter (Fig. 3).

Table 1. Calibration statistics of chemical composition using the near-infrared spectrum (400- 2500 nm). SD, standard deviation; SEC, standard error of Calibration; SECV, standard error of cross validation; 1-VR, coefficient of determination for cross validation; RPD, residual Predictive Deviation (SD/SECV).

Constituent	N	Mean	SD	SEC	R^2	SECV	1-VR	Math treatment	Nb of terms	RPD
Nitrogen	70	1,30	0,18	0,05	0,92	0,07	0,85	1 5 5 1	7	2,5
Carbon	70	48,3	2,3	0,29	0,98	0,47	0,96	2 10 10 1	7	4,8
Tannin	70	1,83	1,47	0,28	0,96	0,30	0,96	2 10 10 1	7	4,9
Phenol	70	150,9	60,2	13,3	0,95	15,0	0,94	2 10 10 1	4	4,0

Table 2. Epsilon values and P- values for the interaction between treatment (grazed, ungrazed) and time (five sampling dates), using Huynh-Feldt corrections (* significant differences p ≤ 0.05).

Variable		Epsilon-values	P- values
Cistus salvifolius	Carbon	1.646	0.070
	Nitrogen	156.95	0.003*
	Phenol	121.21.	0.001*
	Tannin	199.01	0.001*
Halimium halimifolium	Carbon	0.020	0.889
	Nitrogen	35.66	0.006*
	Phenol	133.25	0.007*
	Tannin	95.11	0.006*
Myrtus communis	Carbon	5.024	0.082
	Nitrogen	33.74	0.003*
	Phenol	100.66	0.003*
	Tannin	87311	0.002*
Phillyrea angustifolia	Carbon	6.33	0.058
	Nitrogen	360.86	0.002*
	Phenol	39.52	0.005*
	Tannin	134.05	0.006*
Pistacia lentiscus	Carbon	0.74	0.075
	Nitrogen	219.96	0.04*
	Phenol	43.86	0.001*
	Tannin	248.25	0.008*
Rosmarinus officinalis	Carbon	1.47	0.231
	Nitrogen	37.64	0.001*
	Phenol	94.51	0.003*
	Tannin	1870.5	0.005*

The tannin content showed a significant interaction between time and treatment in all species (Tab. 2). The tannin content ranged from 0.10 % DM (Rosemary: autumn) to 5.34 % DM (*H. halimifolium*: summer). In most of the study period, in the grazed area, tannin content was significantly higher in *C. salvifolius, P. angustifolia*, *P. lentiscus* and *R. officinalis*. Furthermore, this content was significantly lower in the grazed area for *M. communis* and *H. halimifolium* (Fig. 3).

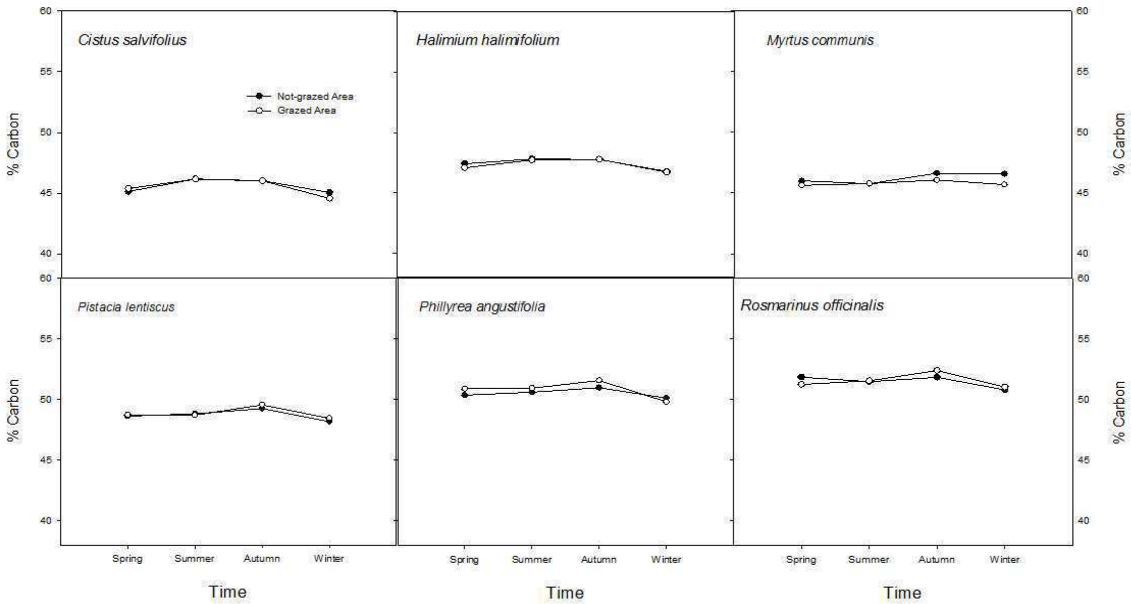
Finally, as for nitrogen and tannins, phenol content showed a significant interaction between time and treatment in all species (Table 2). The phenols content ranged from 48 mg/g (Rosemary: Autumn) to 276 mg/g (*C. salvifolius*: summer). *C. salvifolius*, *H. halimifolium* and *P. lentiscus* showed a phenols content higher than other species. The phenols content suffered a significant drop in autumn in all species, except in *M. communis,* which remained almost constant during the study period. In the grazed area, one or several seasons, *C. salvifolius*, *H. halimifolium* and *R. officinalis* values were significantly higher, and were significantly lower than in the not-grazed area in *P. angustifolia* and *M. communis.*

Table 3. Percentages of the total bites/species/season obtained by the direct observation of goats grazing on the understory of a Mediterranean pine forest in Doñana Natural Park (SW Spain) (Values from Mancilla-Leytón et al., 2012).

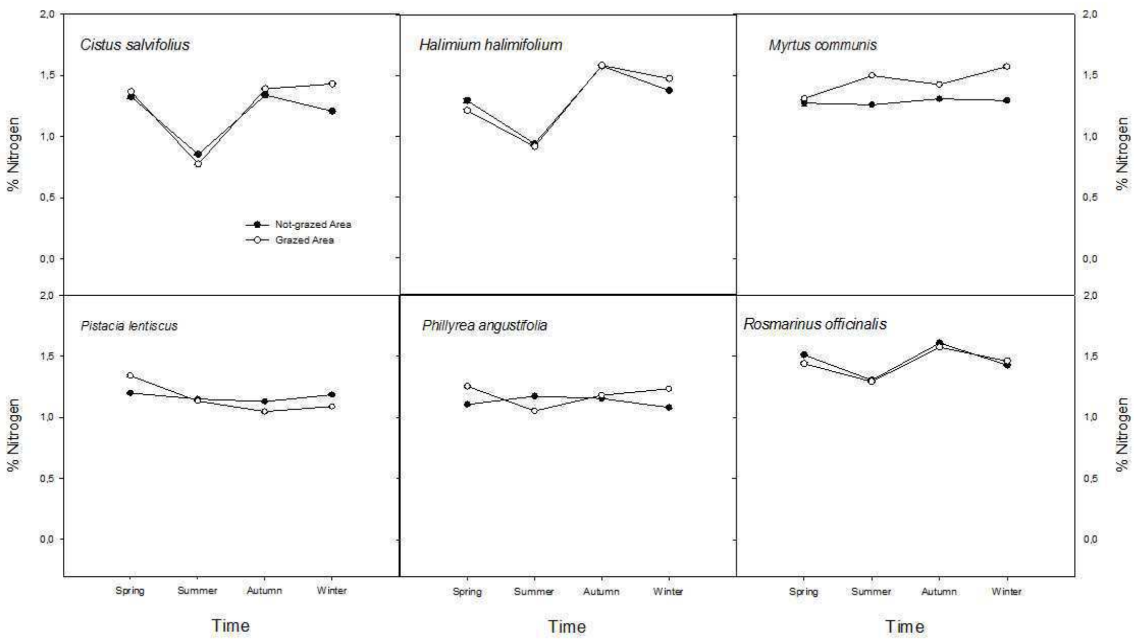
Species	Autumn	Winter	Spring	Summer
Cistus salvifolius	18.7	27.5	15.9	5.0
Halimium halimifolium	25.2	11.6	10.4	5.7
Myrtus communis	30.1	27.7	36.4	67.6
Phillyrea angustifolia	0.6	0.1	2.1	0.7
Pistacia lentiscus	7.3	1.9	4.2	4.1
Rosmarinus officinalis	0.0	7.9	2.3	0.0

Figure 3. Summary of averaged predicted chemical characteristic (A, Carbon; B, Nitrogen; C, Tannin; D, Phenol) for species studied.

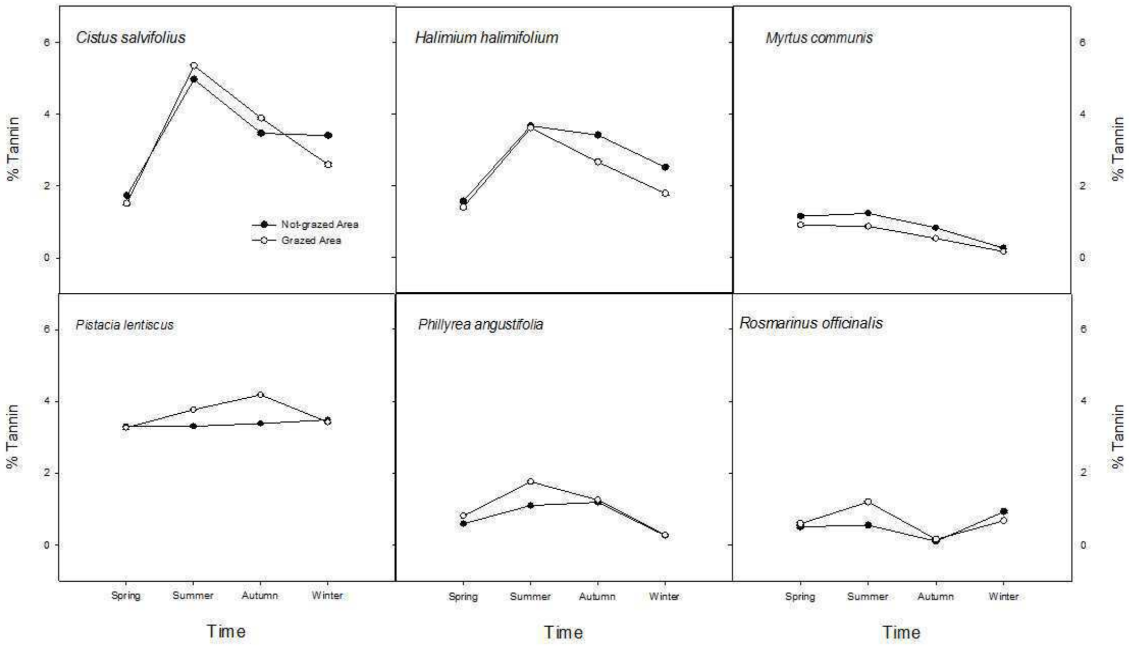
A



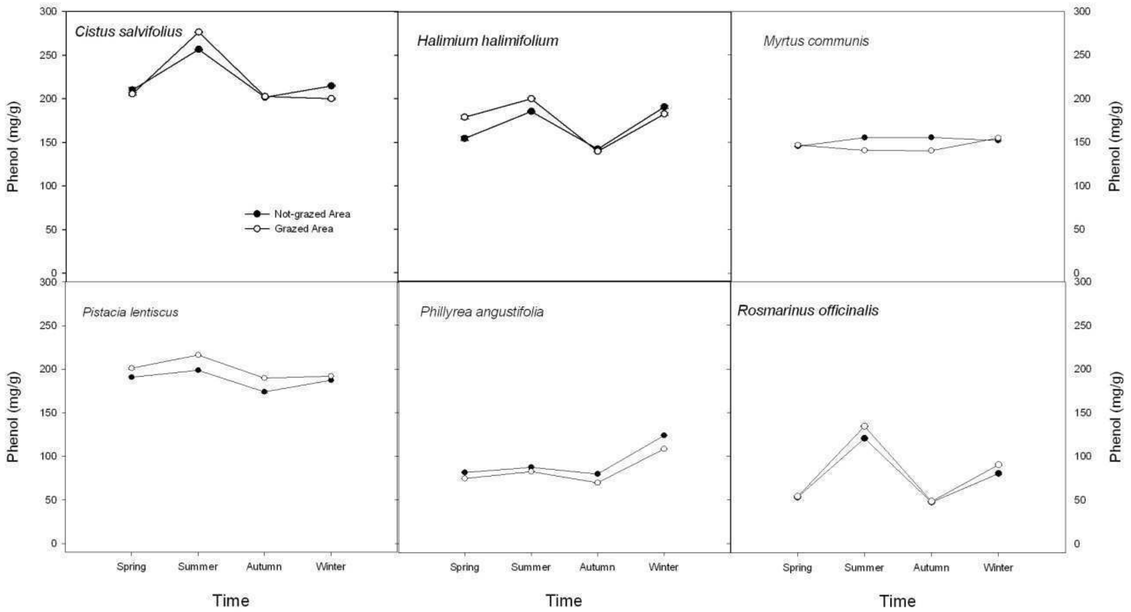
B



C



D



Finally, Tannin content was negatively correlated with Nitrogen (r= -0.41, p<0.001) and Carbon (r= -0.70, p<0.001) and was positively correlated with phenol (r= 0.77, p<0.001). Phenol was negatively correlated with Nitrogen (r= -0.63, p<0.001). After verifying the normality, homoscedasticity and absence of autocorrelation, the results of multiple regression model showed the existence of a significant relationship between the consumption of the species with the carbon content and tannin content (r^2 =

0.71, F=22.86; p ≤ 0.003) described by the following equation: % consumption = 417.09 –7.77* carbon – 0.19*Tannin.

4. DISCUSSION

We found that the measured chemical traits not only responded to seasonal changes (phenophases) but also to herbivores browsing. Species-specific responses of the analyzed compounds were detected: in grazed plants *H. halimifolium* and *M. communis*, unlike the rest of the species, tannins and phenols concentrations were lower. One reason given for positive feedback is favored distribution of carbon toward growth (as new shoots) somewhat rather than carbon based secondary compounds (Fornara and du Toit 2007; Scogings et al. 2011). It is known that defoliation induces chemical defenses in woody plants (Ward and Young 2002; Wessels et al. 2007); however, severe grazing can produce a opposite effect, by causing either augmented N concentrations or reduced phenol and tannin concentrations in individual plant species (du Toit et al. 1990; Danell et al. 2003). In the present study, grazing was negatively related to the rate of carbon and tannins. In Mediterranean ecosystems, tannins and phenols, among other chemical compounds, strongly influence livestock grazing, augmenting or diminishing diverse species consumption (Alonso-Díaz et al., 2008). Tannins in plants, due to their high astringency, may perhaps cause a reduction in intake due to a pre or post- ingestive effect (Ben Salem et al. 2005; Guimaraes-Beelen et al. 2006; Moujahed et al. 2005; Rogosic et al. 2008)., Plants, from a herbivore's viewpoint, turn out to be potentially more grazed as reflected by augmented N: Tannin (Cronin and Hai, 1996). In Mediterranean ecosystems, nitrogen is a limiting element, and consequently N content in plant species plays a key role in herbivore diet selection (Mattson 1980; Tipler et al. 2002).

Goats like to alter their diet and select a great variety of foods. In general terms, herbivores avoid plant species with low nutrient content or high toxin levels and prefer nutritious plant species (Bryant et al. 1991; Hódar and Palo, 1997). Livestock have accurate spatial memories and appear to have sufficient cognitive abilities to select patches and feeding sites based on travel costs and the quality and quantity of forage expected to be there (Bailey et al. 1998). In a previous study (Mancilla-Leyton et al. 2012), we found in their daily routes that the goats selected those species that pleased the most; that is, consumptions were not random. The time variations of food selection seem to be greatly related to the phenology of the species: in most of the study species, the probability of being consumed increases in the flowering and fructification stages; thereby we could speak of seasonal consumption. This seasonal consumption must be related not only to the phenophases, but must also be related to biochemical changes. In this study, we found that goats consumed most of the studied species throughout the year (except rosemary). However, for each species, consumption peaked in a particular season, coinciding with a decline in rates of tannin and phenols, and increased nitrogen concentration (vegetative growth period, leaf shoot) (eg *C. salvifolius* and *R. officinalis* in winter; *H. halifolium* in autumn; *P. angustifolia* and *P. lentiscus* in spring; *M. communis* in summer) (Tab. 3). Most studies on browsing habits of goats have demonstrated a significant correlation between species selection as well as chemical composition of the diet and season of use (Taylor and Kotmann 1990). Also plants in the early flowering to seed formation are most susceptible to grazing pressure (Rinnella 2011), this happened in the case of *R. officinalis.* The negative relationship between browsing intensity and both tannin and phenol content support observations by Rooke and Bergström (2007) and corroborate the above hypothesis.

Unlike in classical experiment consumption, free-ranging goats have access to diverse plant communities (mixed diet), and can have a wide array of biochemically diverse forage species that create a multidimensional feeding environment. During grazing, animals learn to combine plants that contain different types of nutrients and secondary plant substances so that the use of all the plants with different chemical characteristics is more uniform (Provenza et al. 2003; Papachristou et al. 2007). Different studies have shown that domestic goat has a strong capacity to adapt its grazing behavior to diverse plant species with important different chemical characteristics (du Toit et al. 1991; Provenza et al. 1992; Villalba and Provenza 2002). This diversity is very interesting, since these interactions between chemicals, lead to complementary relationships among plant species when a grouping of plant species exceeds the advantage of grazing the plant species in isolation (Lyman et al., 2008). This explains that species with high contents of tannins (> 5 % DM) and phenols (> 200 g /mg) are ungrazed (e.g. *C. salvifolius* and *H. halimifolium*).

In conclusion, the result of our study suggests that goats are not generalist herbivores, their grazing is not random and that they are selective mix grazers. The goat food preferences are a combination of aiming to increase nutritional value intake (N content) while minimizing the ingestion of secondary compounds (tannin and phenol). Their diet is based on gathering an amount of digestive organic matter of different parts of a single plant species or diverse plant species as they avoid the intake of secondary compounds.

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2.4. CAN SHRUB FLAMMABILITY BE AFFECTED BY DOMESTIC GOATS? FLAMMABILITY PARAMETERS OF FIVE MEDITERRANEAN SHRUB SPECIES UNDER GRAZING

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Abstract

Context In the last years, livestock grazing is a less aggressive and/or expensive alternative to control shrub encroachment (reducing the risk of forest fires) than wildfire prevention methods. However, the response of plants to herbivory can cause changes in their modular structure and development, nutritional values, antinutritional compounds, etc. Likewise, these effects could cause changes in their flammability.

Aims We evaluated the potential flammability changes in five Mediterranean shrub species (*Cistus salvifolius, Halimium halimifolium, Myrtus communis, Pistacia lentiscus* and *Rosmarinus officinalis*) of a pine forest (*Pinus pinea*) in Doñana Natural Park, as a result of goat grazing.

Methods At beginning of the study, in order to characterize the structure of the selected species, measures of plant height, total biomass, fine fuel biomass and leaves/wood ratio were taken of individuals per species in grazed and non-grazed areas. After, Moisture content and the flammability of the live fuel were determited according to Valette's protocol. Finally, Gross Heat of Combustion of the fine live fuel was determined following Spanish Standard UNE 164001 EX.

Results: The results of this experiment have shown grazing affected flammability characteristics of studied shrub species (Mean Time of Ignition and Mean Time of Combustion), however, this change was not enough to modify their flammability index, except in the case of *M. communis*, whom grazed individuals had a lower flammability index than non-grazed ones. Temporal changes in FMC were negatively correlated with TC and positively with TI. According to Valette's classification, *C. salvifolius, H. Halimifolium* and *P. lentiscus* were classified as flammable species; *R. officinalis* as flammable - high flammable species; and *M. communis* as non-flammable. The Gross Heat of Combustion values obtained were generally "intermediate" (18.81 – 20.90 MJ kg-1), except for *Rosmarinus officinalis*.

Conclusions: Grazing itself did not modify the flammability of the studied species but did change the structure of individuals and decrease their leaves and fine twigs biomass, thus altering their physical characteristics, and perhaps modifying the fire risk of shrublands.

Keywords: Goats; *Rosmarinus officinalis*; *Myrtus communis*; Phytovolume; Wildfire; Doñana Natural Park.

1. INTRODUCTION

Ever since the Neolithic, fire has been used by man as the main vegetation management tool (Naveh and Dan 1973; Singh et al. 1981) and from then onwards, the frequency of wildfires has increased progressively and in parallel with the expansion of human populations (Naveh 1975). Currently, wildfires are catastrophic in the Mediterranean basin, as they not only affect forests and natural ecosystems, but cultivated land and human settlements, frequently implying evacuation needs.During the last decades, land use change dynamics related to the abandonment of rural areas and agricultural activities, the limited priority on the political agenda concerning forest management in general and wildfire preventive activities in particular, the policies of livestock exclusion from forests, the prolonged protection of forest lands (and consequently fire suppression from ecosystems) and the expansion of wildland-urban interface areas have aggravated fire hazards and disaster potential in the Mediterranean countries (Galiana et al. 2011 ; Vélez 2002; Vilar del Hoyo et al. 2008). In fact, the number of wildfires in many European countries has increased (Giovando et al, 2010) in relation to previous decades, reaching an annual average of 17,127 wildfires in Spain (2001-2010) and affecting a total average of 113.847 ha (ADCIF 2012).

One of the main wildfire prevention methods consists of reducing fuel load and continuity of forest stands (shrub clearing, pruning or thinning) by mechanical means or prescribed burning (Agee and Skinner, 2005). Livestock grazing is another less aggressive and/or expensive alternative to control shrub encroachment, reducing the risk of forest fires (Tsiouvaras et al. 1989; Magadlela et al. 1995; Ferrer et al, 1997; Torrano and Valderrabago 2005; Jauregui et al. 2007; Ruiz-Mirazo et al. 2011). The maintenance of fuel breaks by controlled grazing is being used in some parts of the world (Rigolot 1995; Hadar et al. 2009; Mavsar et al. 2007; Carrasco et al. 2007; Keane et al. 2009; Diamond 2010; Ruiz-Mirazo 2009). In fact, practices to prevent fuel accumulation such as grazing combined with prescribed burning and/or mechanical treatments (thinning and shrub clearance/removal), are beginning to show excellent results, with the added value of producing several positive externalities on rural life and the environment, contributing to a sustainable rural development (Mosquera-Losada et al. 2008).

Goats are suitable for this purpose due to their browsing ability, and it has been shown they can reduce woody biomass with adequate stocking rates (Celaya et al. 2007; Mancilla-Leytón et al. 2013). However, the response of plants to herbivory can greatly vary. The net effect of single or repeated grazing events on the cumulative growth of plants may thus be zero, negative, or positive, depending on availability of leaf biomass, meristems, stored nutrients, and soil resources, and on the frequency and intensity of defoliation (Alward and Joern 1993; Noy Meir 1993). Plants are preadapted to compensate losses due to grazing, up to a certain point, by virtue of their modular structure and development (Noy Meir 1993), nutritional values and antinutritional compounds (Barroso et al. 2001). This evolution of nutritional, chemical content and phenological defences in grazing plants has been studied in detail (Noy Meir 1993; Kimball 1998; Barroso 2001; Baraza et al. 2009).

Plant flammability (the ability of a species to ignite and sustain fire) is a complex phenomenon whose direct measurement under laboratory conditions is both difficult and dubious, due to the lack of standard methodology and the complexity of the involved parameters (e.g. Anderson 1970; Mak 1988; Dimitrakopoulos 2001; Marino 2010; Madrigal et al. 2011, 2013). There are many studies on this matter, following several methods and for various purposes, but most of the methods are based on measuring the time to ignition of a plant sample. However, there are few or no studies regarding changes in the flammability of the grazed species. The aim of this study was to evaluate the potential flammability changes in different Mediterranean shrub species of a pine forest in the South of Spain, as a result of goat grazing.

2. MATERIALS AND METHODS

The experimental site was established in Doñana Natural Park (37º14’N, 6º20’W, SW Spain). Doñana is one of the most important Nature Reserves of Europe due to its high biological diversity and its strategic location between Europe and Africa, and the Atlantic Ocean and the Mediterranean Sea. Its ecosystems are highly diverse and well preserved, and have been broadly studied (García Novo et al. 2007). Human activities are restricted to traditional uses, such as extensive cattle grazing or pinecone harvesting.

The studied forest stand is dominated by *Pinus pinea* (100 ha), with an average density of 217 trees/ha and an average diameter at breast height (dbh) of 26.92 cm. The climate is Mediterranean, with a mild and rainy winter (monthly average temperature is 10 ºC in December and January), and a long dry summer, (mean temperature of 25 ºC in July and August). Mean annual rainfall is around 540 mm, with 80% of precipitation occurring from October to March.

The study area is used for timber production, hunting (rabbit, partridge) and grazing (domestic ungulates). In the *90s*, five plots (2-3 ha each) were chosen at random within the study area and fenced to exclude them from goat grazing. As a result, the study area comprises these 5 fenced plots established over 15 years ago (ungrazed area, 5.5 Tn/ha potentially biomass grazing) and the rest of the unfenced area which continues to be grazed (3.2 Tn/ha potentially biomass grazing) by a herd of adult domestic goats (average weight of 40-45 kg) at a stocking rate of 2.7 ungulates ha^{-1} yr^{-1} (characterized as moderate grazing) (Mancilla-Leytón et al. 2013). The livestock management may be considered to be semi-extensive, although in order to exploit the 100 ha in a uniform manner, the goats were closely controlled and moved around by a shepherd.

A total of five understory species were used to study various flammability parameters. These species were *Cistus salvifolius, Halimium halimifolium, Myrtus communis, Pistacia lentiscus* and *Rosmarinus officinalis,* which were chosen based on their abundance in the study area (representing 80% of the understory cover) and high consumption by goats (Mancilla- Leytón et al. 2012).

2.2. Previous characterization

At the beginning of the experiment, and in order to characterize the structure of the selected species, measures of plant height, total biomass, fine fuel biomass (leaves and twigs < 6 mm) and leaves / wood ratio were taken of 30 individuals per species (15 grazed and 15 non-grazed area). Selected variables were considered relevant due to their potential influence on species flammability.

2.3 Field sampling

In June 2011, 5 individuals of each species growing under similar microclimatic conditions (mean daily temperature and light intensity) were randomly chosen and marked in each plot in order to enable the collection of data from the same individuals at every sampling session. Outside each fenced plot, 5 other individuals of each species with similar characteristics to those of the adjacent ungrazed plot were randomly chosen and marked in the grazed area. Measurements from the 5 individuals of each species at each plot were averaged, and each pair of inside/outside plots were considered as individual grazed/ungrazed experimental units, thereby producing a total of 5 paired samplings for each species within the study area. To characterize the flammability of the live fuel according to Valette's protocol (1990), samples of terminal twigs (10 cm) with their leaves from mature plants of each species and area was collected on a homogeneous way and immediately put into large sealed plastic bags and stored in a cooler to be transported to the lab. Vegetation sampling took place over a long period during the fire risk season, between June and September 2011 (beginning, middle and end of summer).

Once the samples were in the lab, and in order to estimate the flammability of the live fresh fuel, a quantity of about 160 g was immediately drawn and separated into three sets of 50 one-g samples. The first set of samples was used for the flammability test. Samples from the second one were used for moisture content determination purpose. This samples were put in the oven at 100±5°C during 48 h after which moisture content was expressed as the percentage of oven-dry weight (% O.D.W.), and its average calculated on the basis of three replicates. The third set of samples was used for Gross Heat Content tests.

2.4. Description of laboratory procedures

2.4.1 Flammability Testing Method

Valette (1990) defines 'inflammability' as 'the ability of a fuel to ignite after having been submitted to calorific energy'. This term coincides with the term 'ignitability' in American literature (Anderson 1970). In accordance with this definition, the laboratory flammability test was performed using an electric radiator, an ignition apparatus with an electric heating resistance (500 watt of heat capacity) beneath a 10 cm diameter ceramic plate located 4 cm below a pilot flame (Valette, 1990; Hernando, 2009). The flammability test method described by Valette (1990) was applied as follows: once the electric radiator reached the heating required, we placed one gram species sample on the heated ceramic surface and simultaneously started a chronometer to measure the following parameters: time to ignition (TI) in seconds, time of combustion (TC) in seconds. For each species, treatment and sampling time, 50 flammability tests were performed and used to calculate the means of time to ignition (MTI) and time of combustion (MTC). The total number of positive ignition tests was used to calculate the ignition frequency (IF) in percentage (Valette 1990; Lara et al, 2004; Hernando 2009).

2.4.2. Gross Heat of Combustion Testing Method

Gross Heat of Combustion (GHC), also known as high calorific value, of the fine live fuel (leaves and particles < 6 mm in diameter) was determined following Spanish Standard UNE 164001 EX (Asociación Española de Normalización y Certificación 2005). For each species, treatment and sampling time, a fuel sample was ground in a mill. From the ground material, pellets of about 1 g were prepared using a hand press, oven-dried at 100±5°C for 24 h and then weighed. The measurements were carried out with an adiabatic bomb calorimeter equipped with a platinum resistance sensor (PT 100). The calorific value of benzoic acid (26.44 kJ. kg^{-1}) was used to calibrate the calorimeter. Two or three measurements of the calorific value of each sample were made; values differing by more than 2% from other values obtained with the same sample were eliminated.

2.5. Data Analysis

The data obtained from flammability tests were statistically analyzed. An ANOVA model for repeated measures was fitted for each dependent variable (flammability (MTI and MTC), gross heat of combustion and fuel moisture content). The model included two within-unit factors: treatment (grazed and non-grazed), and time (three sampling dates; beginning, middle and end of summer). We have considered treatment type as a within-unit factor because the sampling units are paired (plants inside and outside each exclusion plot). The ANOVA model included treatment, time and interaction terms. The compound symmetry structure of the covariance matrix was tested with the Mauchly procedure, rejecting the null hypothesis for all the models. We therefore used the adjusted procedures based on Huynh-Feldt corrections for departure from sphericity. Results are presented according to the Huynh-Feldt corrections. IBM SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA) was used in all statistical analyses. Finally, the relationships between moisture content, time to ignition, and time of combustion were explored through Pearson correlation.

3. RESULTS

3.1. Previous fuel characterization

During four years, goat grazing continuously decreased the total phytovolume of the monitored shrubs while the response of each species to grazing was different. Table 1 shows the different characteristics of the species in the grazed and non-grazed areas.

Trends were significantly different among individuals situated in grazed and ungrazed areas in almost all species; total biomass accumulated, the ratio leaf / wood and vertical structure (height) were significantly lower in grazed individuals than in non-grazed ones ($P \leq 0.05$; Table 1). Highly flammable fuel (leaves and twigs <6 mm) was also significantly lower in the grazed shrubs. Only in the case of Rosemary (*Rosmarinus officinalis*) were the values similar. The most affected species by the presence of cattle was *Myrtus communis* (Table 1).

3.2. Flammability test parameters

As mentioned, sampling took place thrice during the summer season: at early summer (average max. temperature = 32.37 º C, mean precipitation = 6.41 mm), midsummer (average max. temperature = 34.10 º C, mean precipitation = 0 mm) and late summer (average max. temperature = 31.97 ° C, mean precipitation = 17.01 mm) (Figure 1). These weather differences were reflected in the samples fuel moisture content (FMC) (Table 2). FMC did not show a significant interaction between treatments and time in all species, but a significant interaction with time was found, the values for early and late summer being significantly higher than for mid-summer (FMC ranged between 70 - 167 %)(Tables 2 and 3). Except for *C. salvifolius*, significant differences were found in FMC for all species between grazed and non-grazed individuals; FMC significantly decreased in grazed individuals (Tables 2 and 3).

Means of time to ignition (MTI), time of combustion (MTC), and flammability index (FI) values, measured via the laboratory flammability tests, are shown in Table 2. To understand the moisture effect on the remaining variables, and be able to estimate the natural experimental variability, maximum and minimum values are also included in this table. The corresponding least square means for each grazing treatment, standard error of the mean and p-value, according to the ANOVA results, are shown in table 3.

The shortest MTI was registered for *P. lentiscus*, while *M. communis* showed a short MTC that increased as the summer progressed, especially in non-grazed individuals (Table 2). As for MTI, all species, except for *C. salvifolius*, showed significant interactions between treatments and sampling time. In the presence of goats, MTI significantly decreased in *C. salvifolius*, *H. halimifolium* and *R. officinalis* ($p = 0.002$; $p = 0.005$; $p = 0.001$, respectively, Table 3), and tended to significantly increase in *M. communis* and *P. lentiscus* ($p = 0.001$; $p = 0.006$ respectively). All species showed a significant interaction with time; the lowest MTI registered in the second sampling event (midsummer) (Table 2 and 3).

With respect to MTC, results showed a significant interaction between treatments and time in all species (Table 3). When grazed by goats, MTC significantly increased in all species (this difference was very pronounced in *M. communis*), except in the case of *P. lentiscus* whom did not show significant differences between treatments (Tables 2 and 3). As for MTC, all species showed a significant interaction with time; the highest values registered in the second sampling event (midsummer) (Tables 2 and 3).

Based on the flammability index classification proposed by Valette (1990), almost all the tested species in this study were found to be flammable (FI = 3) (Table 2). As it may be noted, *C. salvifolius*, *H. halimifolium*, *P. lentiscus* and *R. officinalis* showed flammability index values equal or greater to 3. Overall, the results showed that herbivory by goats did not affect the flammability of any studied species, except for *M. communis* (Table 2). This species flammability remained constant during the study period in grazed individuals (index = 0), while increasing over time in non-grazed plants (from index = 1 to index = 3) (Table 2).

Results of Pearson correlations showed changes in moisture content were negatively correlated with time of combustion ($r = -0.69$, $p \leq 0.05$) and positively correlated with time to ignition ($r = 0.44$, $p \leq 0.05$).

Table 1. Characterization of five individuals of *Cistus salvifolius* (CS), *Halimium halimifolium* (HH), *Myrtus communis* (MC), *Pistacia lentiscus* (PL) and *Rosmarinus officinalis* (RO) present in the grazed (G.A.) and non-grazed area (N.G.A.) of a Mediterranean pine forest understory in Doñana Natural Park, SW Spain, prior to beginning of experiments, June10th 2011 (Mean, n = 15).

		Total biomass (g/m^2)	Foliar biomass (g/m^2)	Leaves + twigs < 6 mm (g/m^2)	Leaves /Wood	Height (cm)	Browse pressure*
C.S.	N.G..A.	712.56	155.48	334.25	0.44	97.01	--
	G..A.	517.84	46.61	150.79	0.08	47.32	High
H.H	N.G..A.	896.99	126.51	536.25	0.34	106.40	--
	G..A.	611.45	3.16	225.07	0.14	88.67	High
M. C.	N.G..A.	1374.29	269.74	717.96	0.37	126.42	--
	G..A.	1004.74	49.94	305.54	0.04	84.28	Very high
P. L.	N.G..A.	2049.88	485.35	500.65	0.46	193.19	--
	G..A.	1753.55	196.23	264.74	0.14	148.61	Medium
R. O.	N.G..A.	1100.31	333.07	483.36	0.62	98.57	--
	G..A.	1152.38	321.87	390.34	0.49	82.14	Low

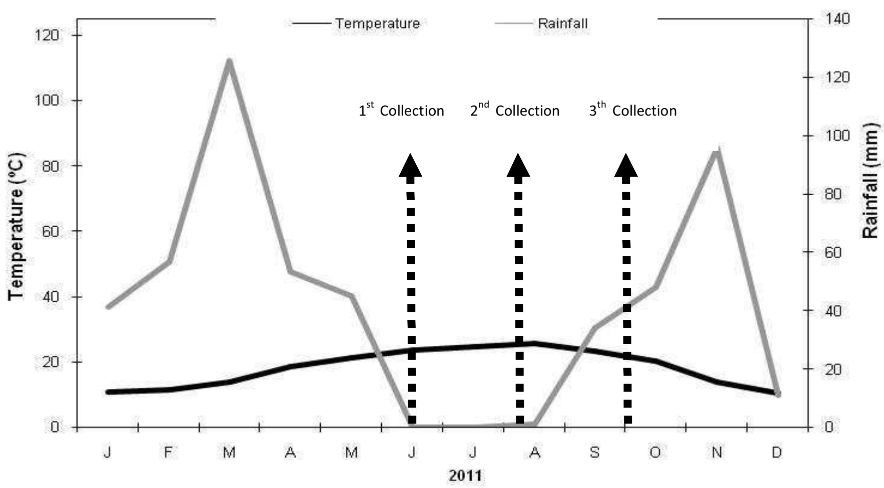
*Values obtained from Mancilla- Leytón et al. 2012

Based on the flammability index classification proposed by Valette (1990), almost all the tested species in this study were found to be flammable (FI = 3) (Table 2). As it may be noted, *C. salvifolius*, *H. halimifolium*, *P. lentiscus* and *R. officinalis* showed flammability index values equal or greater to 3. Overall, the results showed that herbivory by goats did not affect the flammability of any studied species, except for *M. communis* (Table 2). This species flammability remained constant during the study period in grazed individuals (index = 0), while increasing over time in non-grazed plants (from index = 1 to index = 3) (Table 2).

3.3. *Gross Heat of Combustion*

Table 4 shows the results of the gross heat of combustion (GHC) values of the studied species in the different sampling areas (grazed and non-grazed) and events. For all species, excluding *H. halimifolium*, results showed a significant interaction between

Figure 1. Monthly temperature and rainfall averagesof 2011 in Doñana Natural Park (SW, Spain). The graph highlights the different sampling times of vegetation.



treatments and sampling events (Table 3). *R. officinalis* presented the highest GHC value (F=39.433, p≤0.01) (Tabla 3). Only *P. lentiscus* and *R. officinalis* showed significant differences between treatments; calorific values were significantly lower in the ungrazed samples (p= 0.010, p=0.009, respectively) than in the grazed ones (Table 4). Finally, all species showed a significant interaction with time; in general, the calorific values of the species increased as the summer progressed (maximum values in late summer, Tables 3 and 4).

4. DISCUSSION

Vegetation, as fuel for fire, is extremely heterogeneous, and species differences in plant properties affecting flammability can make some plant communities more fire-prone than others (Bond and Van Wilgen 1996; Dimitrakopoulos 2001). Any change, such as grazing, may modify these properties. The results of this experiment have shown grazing affected flammability characteristics of studied shrub species (MTI and MTC). However, this change was not enough to modify their flammability index, except in the case of *M. communis,* whom grazed individuals, had a lower flammability index than non-grazed ones. Therefore, nothing suggests that species changed their flammability characteristics in response to herbivore, but maintained their own characteristics. According to Valette's classification, which takes into account ignition frequency and time to ignition, the studied species were ranked from 0 to 4 depending on timing and the grazing (Table 2). *C. salvifolius, H. halimifolium* and *P. lentiscus*

Table 2. Mean values of moisture content and flammability test parameters of forest fuel samples collected at different surveying times and areas (grazed (G.A.) and non-grazed area (N.G.A.)) of a Mediterranean pine forest understory in Doñana Natural Park (SW, Spain).

Species	SamplingTime	Treatment	Moisture conten [%]	Mean time to ignition [s]			Mean time of combustión [s]			F. I. *
				Min.	Mean	Max.	Min.	Mean	Max.	
Cistus salvifolius	Early	N.G..A.	130	13	**25**	36	2	**12**	21	3
		G..A.	136	20	**23**	25	9	**11**	14	3
	Mid	N.G..A.	88	16	**20**	24	10	**15**	18	3
		G..A.	89	16	**18**	22	13	**16**	18	3
	Late	N.G..A.	160	22	**27**	34	6	**9**	13	3
		G..A.	155	22	**26**	32	9	**13**	17	3
Halimium halimifolium	Early	N.G..A.	148	22	**27**	31	6	**9**	12	3
		G..A.	146	21	**24**	27	8	**10**	12	3
	Mid	N.G..A.	104	16	**19**	22	12	**15**	20	3
		G..A.	97	12	**16**	21	11	**17**	22	3
	Late	N.G..A.	150	17	**20**	24	9	**12**	15	3
		G..A.	138	17	**20**	25	8	**11**	14	3
Myrtus communis	Early	N.G..A.	167	22	**32**	45	1	**3**	9	1
		G..A.	103	14	**26**	51	2	**24**	65	0
	Mid	N.G..A.	133	13	**22**	36	3	**9**	14	1
		G..A.	79	11	**23**	44	11	**38**	57	0
	Late	N.G..A.	125	12	**19**	28	7	**12**	21	3
		G..A.	97	15	**29**	53	3	**24**	58	0
Pistacia lentiscus	Early	N.G..A.	144	10	**17**	29	5	**9**	14	1
		G..A.	129	11	**16**	32	5	**9**	17	1
	Mid	N.G..A.	122	5	**10**	16	9	**15**	23	3
		G..A.	103	5	**12**	21	9	**16**	25	3
	Late	N.G..A.	141	9	**16**	34	6	**11**	21	3
		G..A.	100	9	**17**	28	5	**11**	21	3
Rosmarinus officinalis	Early	N.G..A.	140	19	**25**	33	3	**8**	15	3
		G..A.	107	16	**24**	33	5	**10**	17	3
	Mid	N.G..A.	93	9	**16**	22	7	**12**	17	4
		G..A.	70	11	**15**	19	6	**12**	20	4
	Late	N.G..A.	149	20	**26**	35	5	**7**	14	3
		G..A.	135	20	**24**	28	5	**10**	17	3

Table 3. Overall (averaged over time) least squares means for each grazing treatment (grazed and non-grazed), Standard Error of the Mean (SEM) and p-values for the hypothesis of the main effects (treatment, time and interaction) found in the understory of a Mediterranean pine forest in Doñana Natural Park (SW Spain) over summer 2011 under goat grazing or ungrazed.

Variable	Means		SEM	P-values		
	Non-Grazed	Grazed		Treatment	Time	Interaction
			C. salvifolius			
Moisture conten (%)	126.40	126.74	2.20	0.897	0.002	0.126
MTI (s)	24.16	22.36	0.45	0.002	0.001	0.221
MTC (s)	11.71	13.30	0.40	0.001	0.002	0.001
Calorific values (MJ/kg)	19.28	19.21	0.11	0.093	0.002	0.007
			H. halimifolium			
Moisture conten (%)	135.49	125.35	2.35	0.034	0.004	0.707
MTI (s)	22.11	20.24	0.42	0.005	0.006	0.001
MTC (s)	11.98	12.74	0.36	0.007	0.001	0.004
Calorific values (MJ/kg)	19.98	19.87	0.17	0.087	0.001	0.360
			M. communis			
Moisture conten (%)	143.07	93.52	3.58	0.001	0.002	0.107
MTI (s)	24.17	26.03	0.71	0.001	0.031	0.003
MTC (s)	7.98	28.47	3.14	0.012	0.008	0.001
Calorific values (MJ/kg)	19.27	19.25	0.10	0.164	0.005	0.019
			P. lentiscus			
Moisture conten (%)	125.33	108.16	2.31	0.001	0.048	0.523
MTI (s)	14.11	15.17	0.48	0.006	0.004	0.020
MTC (s)	11.54	11.89	0.31	0.057	0.017	0.001
Calorific values (MJ/kg)	20.21	20.38	0.05	0.010	0.024	0.030
			R. officinalis			
Moisture conten (%)	127.77	104.37	4.20	0.032	0.001	0.270
MTI (s)	22.28	20.68	0.58	0.001	0.002	0.041
MTC (s)	9.03	10.63	0.51	0.004	0.001	0.006
Calorific values (MJ/kg)	23.14	23.48	0.11	0.009	0.004	0.001

Table 4. Mean calorific values (MJ/kg) of forest fuel samples collected from different areas (grazed (G.A.) and non-grazed area (N.G.A.)) of a Mediterranean pine forest understory in Doñana Natural Park (SW, Spain) (n = 3).

	C. salvifolius		*H. halimifolium*		*M. communis*		*P. lentiscus*		*R. officinalis*	
	N.G.A	G.A.	N.G.A	G.A.	N.G.A	G.A.	N.G.A	G.A.	N.G.A	G.A.
Early	18.84	19.01	19.76	19.72	19.18	19.34	20.13	20.22	23.03	23.11
Mid	19.38	19.18	10.89	19.76	19.09	19.26	20.26	20.52	23.70	23.34
Late	19.64	19.68	20.35	20.22	19.60	19.18	20.26	20.47	22.78	23.48

were classified as flammable species (Dimitrakopoulos 2001; Hernando 2009); *R. officinalis* as flammable - high flammable species (Elvira and Hernando 1989); and *M. communis* as non-flammable.

The pyric properties of each plant species are considered major components of its flammability (Anderson 1970; White and Zipperer (2010). Physical structure and components (e.g. canopy architecture, fine fuel biomass, leaf size and shape and retention of dead material) and physiological or cellular elements (e.g. volatile oils and resins, moisture content, mineral content, lignin and waxes), usually affect species flammability characteristics (Alessio et al. 2008; Ormeño et al. 2009).

From a physical perspective, surface area-to-volume ratio of fuel particles is often considered a significant factor in flammability (Fernandes and Rego 1998). However, for whole plants, flammability mainly depends on the physical arrangement of the plant biomass (Doran et al. 2004). Fine particles ignite more readily and release their heat quicker than thicker particles of an equivalent total weight do. The long-term absence of disturbance in shrublands results in the accumulation of large amounts of dead plant material (Lunt et al. 2012). Grazing animals have been thought to inhibit the accumulation of dead biomass through foliage consumption (Whalley 2005). Where grazing pressure is sufficient to develop grazing lawns, plants are maintained in a state of continuous regeneration (McNaughton 2009) and the proportion of accumulated dead material may be very low, reducing fire risk (Marino et al. 2011). In the study area, continuous and moderate goat grazing exerted significant effects on the phytovolume and height of studied shrubs. A previous work by Mancilla-Leytón et al. (2013) showed that, after 42 months, goat grazing significantly decreased species phytovolume by 34%, increased bare soil by 51%, and decreased the flammability of the area by 22%. All studied species, except *R. officinalis*, significantly decreased their phytovolume and height when grazed, while the percentage of fine fuel was also significantly reduced. The degree to which grazing reduces fuel loads is determined by the density of grazers, their rate of food intake and plant growth rates (Noy-Meir 1975; Leonard et al. 2010). The impact of grazing on shrubland fuel loads varies between components of the vegetation due to variation in feeding preferences and the behavioral, morphological and physiological traits that influence food intake (Mancilla-Leytón et al. 2012). Thus, the effect of grazing was most evident in *M. communis* (highly grazed) and less in *R. officinalis* (very ligthly grazed) (Table 1). This effect of light to moderate intensities of grazing on vegetation (breaking the continuity between shrubs and creating 'regeneration gaps') controls the combustible biomass, reducing the fire risk, and allows the co-existence of species that were previously suppressed by the densely packed dominant species.

From a physiological perspective, volatile organic compounds (VOCs) are produced by many Mediterranean plant species and are related to secondary metabolism processes (Barboni et al. 2011). These compounds have a low ignition temperature, and when ambient temperature increases due to sun exposure or radiation from a flame front, they create a flammable gas mixture. The gross heat of combustion of the tested species was generally lower than that of common Mediterranean forest fuels (Madrigal et al., 2011). According to the classification proposed by Elvira and Hernando (1989), the GHC values obtained were generally "intermediate" (18.81 – 20.90 MJ kg^{-1}), except for *Rosmarinus officinalis* (Table 4). The calorific value of plants increases as stems are lignified (due to the higher heating value of lignin in comparison to cellulose). The most lignified species and those with higher volatile organic compounds content (resins, terpens and essential oils) have a high calorific value (e.g. *Rosmarinus officinalis*). The increase found in the calorific value of grazed species is attributed to the fact that goats remove tender shoots, thus the most lignified plant parts remain.

In conclusion both physical and physiological factors are time dependent. These properties may change within the same species due to changes in the plant status (in bloom, with regrowth, woody, etc.) or environmental conditions (temperature, moisture, rainfall, etc.). This means that their flammability characteristics (MTI, MTC, etc.) may change depending on the state of the same, which in turn is determined by the time of the season (Elvira and Hernando, 1989). For instances, the fire prone season is dictated by the coincidence of high ignition values and low fuel moisture (Bond and Keeley 2005). This is usually the driest time of the year, which varies with regional climate. In Mediterranean-type shrublands, summer is the season of higher fire risk (ADCIF 2012, Spain). Although we have only evaluated one season, FMC values differed during the three summer periods examined; changes in temperature and precipitation were reflected in FMC values (Tables 1 and 2). Furthermore, these changes in FMC were negatively correlated with MTC and positively with MTI. Therefore, temporal changes could be playing a more relevant role in the analyzed flammability characteristics than goat grazing.

The role of native and introduced domestic herbivores in reducing fire risk, as consumers of fine fuels, is well documented (Bond and Keeley 2005; Ruiz- Mirazo et al. 2011). Herbivores may select plants with particular chemical or morphological traits, alter competitive hierarchies and directly modify vegetation structure in ways that either promote or diminish potential wildfire activity (Holmgren 2002; Bond and Keeley 2005). White and Zipperer (2010) showed that flammability characteristics of a particular species were influenced not only by the species itself but also by the context of its environment. It should also be noted that a catching and spreading of fire can be difficult where there is discontinuity in the vegetation; under this scenario, even highly flammable species may not present a great danger (Morvan and Dupuy 2004). It can also be quite the opposite, that less flammable species, but arranged in horizontal and / or vertical continuity, represent greater risk by complicating fire attack and suppression conditions/practices.

Nonetheless, specific characteristics (i.e. ecological, economic and/or social aspects) of each forest area should serve as the basis for selecting the most appropriate fuel treatment in each case (mechanical treatments, prescribed burning or controlled grazing). However, there is no single ideal technique for wildfire prevention, and most commonly, fuel management plans that combine different practices are highly desirable (Fernandes and Botelho 2003). Understanding the benefits and limitations of each fuel manipulation method is important so that they can be correctly applied and their potential for achieving management objectives materialized (Reinhart et al. 2008). Several studies on the pre-fire and post-fire effects of these introduced herbivores conducted across a broad range of forest and shrubland types have shown that herbivore pressure: (1) impedes regeneration of the obligate-seeding tall tree species (Tercero-Bucardo et al. 2007); (2) results in reduced phytovolume and height growth of shrub and tree seedlings so that the development of a closed canopy is unlikely (Mancilla-Leyton et al. 2013), and (3) favours the invasion and spread of forbs (Mancilla-Leyton et al, 2011). As a result, controlled grazing can add economic and social value to fuel management for wildfire prevention, especially through quality recognition to the animal products obtained when Protected Origin status is awarded.

5. CONCLUSIONS

From the present results, it is noticeable that flammability parameters do not show a very marked trend with relation to grazing, especially when compared to other fire hazard components or to sampling period effect. However, from a physical perspective, goat grazing reduced the species phytovolume, significantly decreased horizontal and vertical (height) plant cover, and increased bare ground surface, thus potentially contributing to a progressive reduction in the fire risk of shrublands (catch and spread of fire).

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2.5. PLANT-UNGULATE INTERACTION: GOAT GUT PASSAGE EFFECT ON SURVIVAL AND GERMINATION OF MEDITERRANEAN SHRUBS SEEDS

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Plant–ungulate interaction: goat gut passage effect on survival and germination of Mediterranean shrub seeds

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Keywords
Cistaceae; Doñana; Endozoochorous; *Myrtus*; *Pistacia*.

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Abstract

Questions: (i) How do large proportions of seeds pass through the guts of goats without damage? (ii) What is the temporal pattern of seed defecation? (iii) Does ingestion by goats enhance or depress seed germination?

Location: Doñana Natural Park, SW Spain.

Methods: Six female goats of similar size and age were fed with 1000 seeds of four common Mediterranean shrub species (*Cistus salvifolius*, *Halimium halimifolium*, *Myrtus communis*, *Pistacia lentiscus*), which were retrieved from the goat's dung 96 h after ingestion. The seeds retrieved were tested for germination and viability, along with seeds not eaten by the goats.

Results: Less than 30% of the seeds eaten were retrieved from the dung, with significant differences between species. No seeds of *P. lentiscus* were retrieved. The major part of the seeds was retrieved between 48 and 72 h after ingestion in all other species. The passage through the goat gut significantly increased seed germination in *C. salvifolius* and *H. halimifolium*, and depressed it in *M. communis*. Viability was significantly lower in the eaten seeds of *M. communis* than in the uneaten ones, while there were no differences in *C. salvifolius* and *H. halimifolium*.

Conclusions: Goats can potentially disperse seeds of the plants that they eat. This should be taken into account when designing management plans in order to prevent shrub invasion in undesired areas. It could also be used as a management tool for spreading populations of desireable shrub species.

Introduction

Endozoochorous seed dispersal by animals has attracted scientific attention for a long time, and attention has increased in recent years. Traditionally, research focused on the fate of fleshy fruits, dispersed mainly by birds and mammals (Debussche & Isenmann 1989; Herrera 1989; Willson 1993; López-Bao & González-Varo 2011), but recently more attention has been paid to the role of herbivorous mammals as endozoochorous seed dispersers (Willson 1993; Pakeman et al. 2002; Myers et al. 2004). Several studies have shown the presence of seeds in the dung of wild and domestic large herbivores (Malo & Suárez 1998; Sánchez & Peco 2002; Manzano et al. 2005; Kuiters & Huiskes 2010; De la Vega & Godinez-Alvarez 2010). Since herbivorous mammals have long gut passage time for seeds (24–72 h; Olson & Wallander 2002) and can travel long distances (Cory 1972; Klein 1981), they may promote the rapid spread of plant populations.

Domestic goats have grazed in Mediterranean shrublands for millennia. Goats were the first animal species to be domesticated (7000 years ago; Clutton-Brock 1999) and have been reared in Spain for 5000 years (Pérez Ripoll 1980; Esteban Muñoz 2009). The feeding habits and forage preferences of domestic and wild goats are similar (Aldezabal & Garin 2000). Goats are mainly browsers; they consume the leaves, flowers and fruits of shrubs and trees. As fruit eaters they can behave as seed predators, but also as seed dispersers. Domestic livestock could play a similar seed disperser role to the contemporary and extinct herbivorous mammals that they have

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replaced (Janzen & Martin 1982; Martin & Klein 1984; Janzen 1986; Skape 1991; Tiffney 2004; Hansen et al. 2008). Despite the paramount importance of domestic goats as browsers in Mediterranean shrublands – around 1,090,000 head grazed in the shrublands of southern Spain in the 19th century (SIGGAN 2008) – little attention has been paid to their role as seed dispersers of the shrub species on which they browse. It has been documented that goats can disperse the seeds of thorny shrubs in Mexico (Baraza & Valiente-Banuet 2008) and legume shrubs in Spain (Robles et al. 2005), but there is no information about the common shrubs that they eat in Mediterranean shrublands.

The aim of this study was to investigate whether goats can potentially disperse the seeds of the shrub species on which they browse by quantifying the number of seeds that pass through the goat's gut and then germinate afterwards. Four shrub species that are very common in Mediterranean shrublands and browsed by goats were studied: *Cistus salvifolius* L., *Halimium halimifolium* (L.) Willk., *Myrtus communis* L. and *Pistacia lentiscus* L. We addressed three specific questions: (i) How do large proportions of seeds pass through the goat gut without damage? (ii) What is the temporal pattern of seed defecation? (iii) Does ingestion by goats enhance or depress seed germination?

Methods

Seed collection

Fruits were collected in a rangeland located in Doñana Natural Park, SW Spain (37°14′52″N, 6°20′35″W), where the four species are very common and largely consumed by goats (Mancilla-Leytón et al. 2009).

Ripe fruits of the four chosen species were randomly collected from 25 different plants of each species. Fruits of *Cistus salvifolius* and *Halimium halimifolium* were collected in summer and fruits of *Myrtus communis* and *Pistacia lentiscus* in autumn. The fruits collected from each species were mixed together and stored in the laboratory at room temperature in dry and dark conditions until the beginning of the experiments.

Fruit and seed characteristics

In order to determine average fruit and seed length and weight, 100 fruits and 1000 seeds of each species were dried at 80 °C for 2 days. Fruits were individually weighed, and seeds were weighed in 100 groups of ten seeds. The seed length was measured in 100 fruits and 100 seeds with vernier calipers. In order to determine the number of seeds ingested by goats, the mean number of seeds per fruit was estimated by counting the seeds in 100 fruits of each species.

Seeds retrieved after gut passage

Six female adult goats of similar size and age (40 kg average weight and 3 years old) were used in the experiment. The goats were kept in individual metabolic pens with a collector system for faeces, where they were fed with the fruits. Fruits of each species were offered to each goat: 60 fruits of *Cistus salvifolius*, 125 fruits of *Halimium halimifolium*, 300 fruits of *Myrtus communis* and 1000 fruits of *Pistacia lentiscus*, chosen at random from the pool of collected fruits. This represented around 1000 seeds of each species given to each goat. The fruits were mixed with barley grains (250 g) to facilitate the intake. The animals also had access to alfalfa hay ad libitum and to water.

The goats were introduced in the metabolic pens in the morning. The mix of fruits and grains was offered for roughly 1 h and fully consumed by the goats. All the dung pellets produced by each goat were collected 24, 48, 72 and 96 h after ingestion, dried at room temperature and stored in the laboratory. All the pellets recorded were crushed manually, counting the number of seeds of each shrub species.

Some of the seeds retrieved were partially broken, missing part of the cotyledons but with an intact embryo. Since the number of broken seeds was very low (*Cistus salvifolius* 0.20%, *Halimium halimifolium* 0.08%, *Myrtus communis* 0.04% and *Pistacia lentiscus* 0%) these were no tested for germination. Only seeds with no evidence of apparent external damage, examined under a microscope, were used for the germination experiment.

Seed germination after gut passage

The germination of seeds retrieved from goat dung was compared to germination of seeds that were not eaten. For each species, there were three treatments, (1) Control: seeds that were not eaten; (2) 48 h: seeds retrieved between 24 and 48 h after ingestion; and (3) 72 h: seeds retrieved between 48 and 72 h after ingestion. The number of seeds retrieved in the first 24 h and after 72 h was very low and thus these were not tested. The seeds of each species eaten by different goats, for the same treatment (0–24, 24–48, 48–72 h), were mixed together for this experiment. Since we retrieved very few seeds of *Myrtus communis*, the seeds retrieved after 48 and 72 h were mixed together. Thus, we had only two treatments for this species (eaten or not eaten).

Seeds of all treatments were disinfected by immersion in a 1% sodium hypochlorite solution for 2 min, and thoroughly rinsed with sterile distilled water (10 min). Then the seeds were placed on filter paper in a 5-cm Petri dish. Each Petri dish contained 25 seeds, and there were four replicates per treatment.

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Three milliliters of distilled water were added to each dish. Dishes were wrapped with parafilm and placed in a germinator (ASL Aparatos Científicos M-92004, Madrid, Spain) for 60 days with a regime of 12 h light (25 °C, 35 μmol $m^{-2} s^{-1}$, 400–700 nm) and 12 h darkness (12 °C). This temperature regime was chosen to represent the end of autumn temperatures in the Mediterranean climate, when these species geminate. The dishes were inspected daily and germinated seeds were counted and removed. The water level was adjusted daily with distilled water. We considered that seeds had germinated after root emergence (1–2 mm).

Three parameters of germination were determined: final germination percentage, time of first germination and mean time to germination (MTG), calculated as:

$$\mathrm{MTG} = \sigma_i(n_i \times d_i)/N$$

where n is the number of seeds germinated at day i, d the incubation period in days and N the total number of seeds germinated in the treatment (Brenchley & Probert 1998).

The global effect of goat gut passage on seed germination of each species was estimated by multiplying the mean percentage of seeds retrieved by their mean germination percentage.

Viability test

The tetrazolium test was applied to three samples of 20 seeds collected from pellets and from plants (control), to determine the viability of the embryos (MacKay 1972). Seeds were kept in water for 16 h at a constant temperature of 25 °C. Seeds were then submerged in a 1% aqueous 2,3,5-triphenyl-tetrazolium chloride, pH 7, in darkness for 24 h at a constant temperature of 25 °C. Subsequently, seeds were dissected and the embryo was examined with a magnifying glass (Bradbeer 1998).

Data analysis

Differences among species in the total number of seeds retrieved from goat dung were statistically evaluated with ANOVA. The data were tested for normality with the Kolmogorov-Smirnov test. The Tukey test was used to evaluate significant differences among species.

Differences in germinability, time of first germination and mean time of germination between control seeds and seeds retrieved from dung after 24-48 and 48-72 h in each species were tested with ANOVA. The data were tested for normality with the Kolmogorov-Smirnov test. The Tukey test was used to evaluate significant differences among species.

Differences in viability between control and retrieved seeds were assessed with a t-test. The data were tested for normality with the Kolmogorov-Smirnov test. SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA) was used in all statistical analyses.

Results

The characteristics of fruits and seeds are listed in Table 1. The larger fruits were those of *Myrtus communis* and *Cistus salvifolius*, followed by *Halimium halimifolium*. *Pistacia lentiscus* had the smallest fruits, with only one seed. The number of seeds per fruit varied between species: *C. salvifolius* and .*H. halimifolium* had more seeds per fruit and the seeds were much smaller than those of *M. communis* and *P. lentiscus*.

The number of seeds retrieved from goat dung was low in all species, < 30% of the seeds eaten by the goats (Fig. 1), with significant differences between species (Tukey test, F-value = 87.24, $P < 0.01$). The highest percentages of seeds were found in *C. salvifolius*, followed by *H. halimifolium* and *M. communis*. No seeds were retrieved for *P. lentiscus*.

In all the species, the majority of the seeds were retrieved between 24 and 48 h after ingestion (*C. salvifolius* 77.19%, *H. halimifolium* 72.72% and *M. communis* 66.66% of seeds retrieved), followed by 48-72 h (*C. salvifolius* 17.54%, *H. halimifolium* 18.18% and *M. communis* 33.33% of seeds retrieved). Very few seeds were retrieved after 72 h (Fig. 1).

Passage through the goat gut significantly increased seed germination in *C. salvifolius* and *H. halimifolium* (Tukey test, F-value = 10.11 and F-value = 19.89, $P < 0.01$, respectively) (Fig. 2), especially of those that remained longer in the gut (48-72 h), which germinated significantly more than the others (Tukey test, F-value = 37.11, $P < 0.01$). In *M. communis*, in contrast,

Table 1. Mean and standard error of the length and weight of fruits and number of seeds of the four plant species studied. For more details see text.

Species	Fruit			Seed	
	Length (mm)	Mass (mg)	Seed number	Length (mm)	Mass (mg)
Cistus salvifolius	7.18 ± 0.09	91.24 ± 5.33	39.7 ± 0.88	1.55 ± 0.04	1.41 ± 0.07
Halimium halimifolium	7.64 ± 0.14	39.33 ± 1.96	18.4 ± 0.86	1.32 ± 0.03	0.72 ± 0.06
Myrtus communis	7.66 ± 0.13	122.54 ± 7.23	7.1 ± 0.4	3.54 ± 0.07	8.86 ± 0.58
Pistacia lentiscus	4.38 ± 0.04	39.08 ± 1.55	1	4.07 ± 0.04	19.48 ± 0.90

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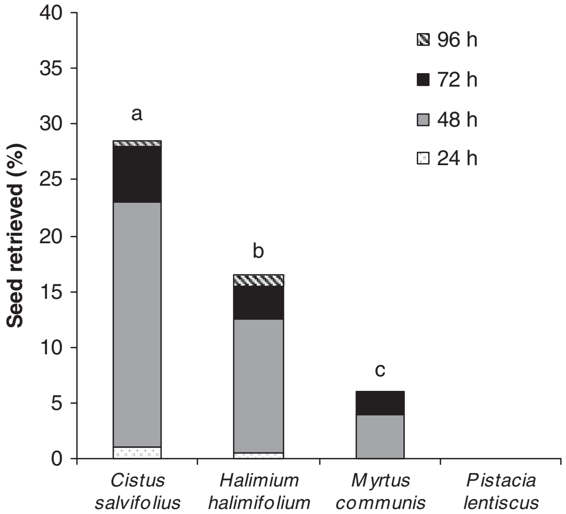

Fig. 1. Percentage of seeds retrieved from goat faeces 24, 48, 72 and 96 h after consumption in the four species studied. Different letters indicate significant differences among species in the total number of seeds retrieved (Tukey test; $P < 0.01$).

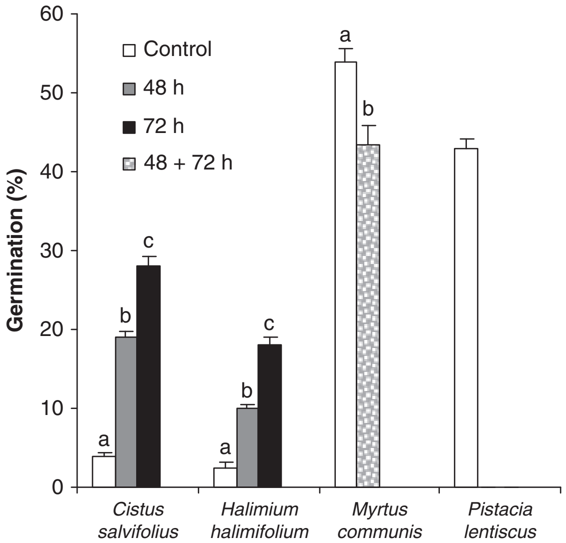

Fig. 2. Percentage of germination of control seeds and seeds retrieved from goat pellets 24-48 and 48-72 h after ingestion in the four species studied. Different letters indicate significant differences among treatments within each species (Tukey test; $P < 0.01$). The seeds of *Myrtus communis* retrieved from faeces between 24 and 72 h after ingestion were pooled due to low sample size (see text for explanation).

significantly fewer seeds eaten by the goats germinated than the control seeds (Tukey test, F-value = 14.23, $P < 0.01$).

When considering gut passage and germination together (Table 2), gut passage notably depressed seed germination in *M. communis* (54% of control seeds compared to 2.6% of retrieved seeds), but not in *C. salvifolius* and *H. halimifolium*, where retrieved seeds germinated in similar percentages to control seeds.

Table 2. Percentage of final germination in control seeds and seeds retrieved from dung pellets at different times (24-48, 48-72 h) in the four plant species studied. Figures were obtained by multiplying the percentage of seeds retrieved by their germination percentage.

Species	Control	Retrieved		
		24-48 h	48-72 h	24-72 h
Cistus salvifolius	4	4.18	1.4	5.58
Halimium halimifolium	2.5	1.2	0.54	1.74
Myrtus communis	54	0	0	2.61
Pistacia lentiscus	43	0	0	0

Passage through the goat gut significantly shortened germination time of *C. salvifolius* and had no effect in *M. communis* (Table 3). The time of first germination in *H. halimifolium* was significantly lower in seeds retrieved from goat dung, but the mean time of germination was significantly longer. This was due to the low germinability of control seeds. Only three control seeds germinated at the beginning of the experiment. The retrieved seeds began to germinate before the control seeds and continued for longer, lengthening the germination period.

The tetrazolium test showed no significant differences between control seed and seeds retrieved from goat dung in *C. salvifolius* and *H. halimifolium* (t-test, $t = 0.719$ and $t = 0.750$, $P > 0.05$). In *M. communis*, the viability of seeds retrieved was significantly lower than control seeds (t-test, $t = 5.237$, $P < 0.05$) (Fig. 3).

Discussion

Seeds from three of the four Mediterranean species studied, *Cistus salvifolius*, *Halimium halimifolium* and *Myrtus communis*, survived ingestion and gut passage through goats and germinated afterwards, and could potentially be dispersed by free-ranging goats. The seeds of the fourth species studied, *Pistacia lentiscus*, did not survived gut passage. In this case the goats behaved as seed predators.

Few seeds passed through the goat gut. In the best case, only one-third of the consumed seeds were retrieved from faeces. These low values were similar to those obtained for other grass and shrub species eaten by wild and domestic ruminants (Russi et al. 1992; Olson & Wallander 2002; Razanamandranto et al. 2004; Mouissie et al. 2005). We found significant differences between species: the small, rounded seeds of *C. salvifolius* and *H. halimifolium* passed in more quantities than the larger seeds of *M. communis*. The largest seeds, those of *P. lentiscus*, could not survive gut passage, possibly due to mastication. Other

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Table 3. Number of days to first germination and mean time-to-germination (MTG) of the control seeds and seeds retrieved from goat faeces. Values are means ± SE ($n = 4$). Different letters indicate significant differences among treatments (Tukey test; $P < 0.01$).

Species	Treatment	1st germination (d)	MTG (d)
Cistus salvifolius	Control	29.52 ± 2.32 a	34.3 ± 0.90 a
	Retrieved 24-48 h	13.50 ± 3.71 b	21.8 ± 1.98 b
	Retrieved 48-72 h	9.57 ± 2.71 b	18.1 ± 2.36 b
Halimium halimifolium	Control	7.75 ± 1.63 a	9.08 ± 2.25 a
	Retrieved 24-48 h	3.51 ± 0.75 b	16.65 ± 2.90 b
	Retrieved 48-72 h	2.97 ± 0.43 b	16.21 ± 2.13 b
Myrtus communis	Control	5.53 ± 0.65 a	10.64 ± 0.73 a
	Retrieved 24-72 h	4.11 ± 0.81 a	9.69 ± 0.61 a
Pistacia lentiscus	Control	12.50 ± 1.65	33.09 ± 2.08

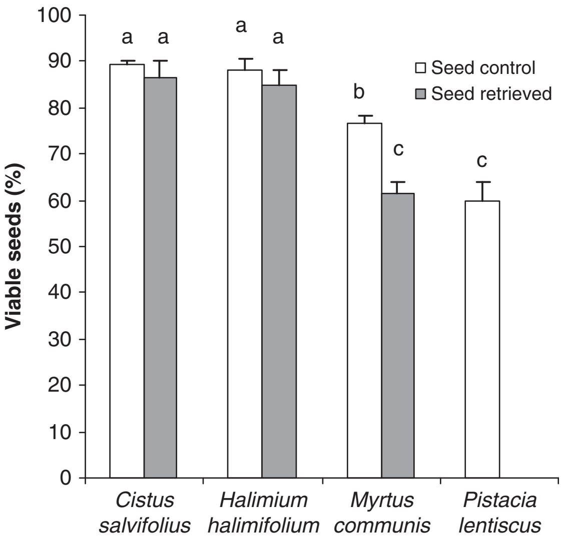

Fig. 3. Percentage of viable seeds (tetrazolium test) in the control and in seeds retrieved from dung pellets in the four plant species studied. Different letters indicate significant differences among treatments within each species.

studies have also found a negative relationship between seed size and recovery (Staniforth & Cavers 1977; Russi et al. 1992; Pakeman et al. 2002). The most retrieved seeds were not the smallest (*H. halimifolium*), as expected, but those of *C. salvifolius*, which are slightly larger. Manzano et al. (2005) also found that the tiny seeds of *Cistus ladanifer* passed in lower quantities than the slightly larger seeds of *Halimium umbellatum*, suggesting that the higher losses in the first species could be attributed to digestion. The higher surface-to-mass ratio of very small seeds limits their coat thickness, leading to a stronger effect of digestive fluids.

The passage through the goat gut enhanced seed germination in *C. salvifolius* and *H. halimifolium*. Other authors have also found that passage through the gut of large herbivores enhance seed germination in some species of Cistaceae (Manzano et al. 2005; Ramos et al. 2006). Cistaceae seeds are characterized by primary seed dormancy (i.e. physical dormancy) imposed by a hard seed coat (Thanos et al. 1992; Baskin et al. 2000) and thus any factor softening the seed coat without damaging the embryo may promote germination.

The temporal distribution of seed defecation increases dispersal distance, which is especially important for species that lack specialized dispersal methods such as *C. salvifolius* and *H. halimifolium* (van der Pijl 1982; Herrera 1992; Malo & Suárez 1995; Bastida & Talavera 2002). Thus, the minimum predicted dispersal distances by goats may be much larger than those provided by abiotic dispersal mechanisms, especially for the seeds that stay in the gut for longer times (more than 48 h). Although the cost of gut passage is undoubtedly high, an appreciable number of seeds of three of the four species studied passed and germinated thereafter, 5.58% of *C. salvifolius*, 2.6% of *M. communis* and 1.l7% of *H. halimifolium*. In *Cistus* and *Halimium* the percentage germination was similar to the uneaten seeds (4% and 2.5%, respectively), which means that the goats do not seem to depress current-year germination, but enlarge the area reached by the seeds. The effect of the goats is on the seed bank, diminishing the number of seeds stored in the ground. Gut passage significantly depressed the germination of *M. communis* (54% in control and 2.6% in eaten seeds). In this case, the goats behave mainly as seed predators and only disperse a small amount of viable seeds. This species is mainly dispersed by birds (Traveset et al. 2007). If the goats defecate in other places than birds, they could facilitate the colonization of otherwise inaccessible ecosystems.

The dispersal distance attained by seeds passed through the gut depends on the management of the goats. In transhumant herds, it could be extremely high, between 30 and 90 km, according to the mean speed of transhumant herds in Spain (Manzano et al. 2005). In herds that always forage in the same area, dispersal range should be negligible. If the herds alternately graze in grasslands and shrublands, they could speed succession in grasslands by carrying the next stage seeds, i.e. the shrubland. Since the goats behave as seed predators of some species and as dispersers of others, and seed dispersal differs among species, goats could control vegetation composition in the areas they graze in the long term.

The ability of goats to disperse the seeds of shrub species should be taken into account when designing management plans for goats in order to prevent shrub invasion in undesired areas. Goats could also be used as a management tool for spreading populations of target shrub species. But all these findings require further research on the number of seeds actually dispersed by goats of a broader number of species, and on the fate of the seedlings.

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2.6. INFLUENCE OF GRAZING ON THE DECOMPOSITION OF *Pinus pinea* L. NEEDLES IN A SILVOPASTORAL SYSTEM IN DOÑANA, SPAIN

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REGULAR ARTICLE

Influence of grazing on the decomposition of *Pinus pinea* L. needles in a silvopastoral system in Doñana, Spain

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Abstract
Aims The aim of this study was to determine whether goat grazing in the understory of a pine forest at Doñana Natural Park could accelerate the decomposition of the pine needles accumulated on the soil surface and, if so, through which mechanisms. Specifically, the roles of trampling (mechanical fragmentation) and nutrient enrichment through defecation (fertilization) were evaluated in terms of their effect on pine needle decomposition rates.
Methods An experiment was conducted featuring the following 4 treatments: 1) intact needles (control), 2) trampled needles, 3) intact needles fertilized with liquid manure, and 4) trampled needles fertilized with liquid manure. Litter decomposition was determined as a function of mass loss over time, using the litter-bag method. Bags were recovered 4, 8, 16, 24 and 36 months after burial in soil, dried and weighed. Needle length, leaf mass per area and C and N concentration were also measured in the buried litter-bags.
Results Four months after burial, mass loss was greater in the trampled (23–27 %) than non-trampled (14–16 %) treatments. However, from 8 months onwards, decomposition rates in the fertilized treatments were significantly higher than those in the non-fertilized treatments (between 5 % and 15 % less mass loss). Meanwhile, fertilized treatments presented higher N content (2.1 %) than the non-fertilized ones (1.2 %), with a significantly lower C:N ratio also found in the in the fertilized treatment.
Conclusions Trampling and fertilization during grazing accelerates litter decomposition and thus promotes the incorporation of N into the system. Acceleration of decomposition reduces the accumulation of pine needles on the soil surface, reducing the risk of fire.

Keywords Decomposition rate · Leaf litter · Carbon · Nitrogen · Goat grazing · Pine needles

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Introduction

The effect of livestock grazing on environmental conservation has historically been perceived as detrimental to the environment, due to overgrazing (Mace 1991), desertification (Dregne and Willis 1983), methane emission (Goel et al. 2008) and associated biodiversity loss (Alados et al. 2003). However, grazing is not universally harmful and can actually contribute to the preservation of natural and cultural values and assets. Livestock activity has regained momentum in current forest plans; the incorporation of grazing livestock into fire prevention programs is a feasible and cost-effective method to improve fire prevention strategies (Rigueiro-Rodríguez et al. 2005; Launchbaugh et al.

2008; Ruiz-Mirazo et al. 2011). Additionally, the potential offered by goats in terms of their ability to survive in disadvantaged areas is broadly recognized at national and international level (Devendra and McLeroy 1987; Torrano and Valderrábano 2005; Mosquera-Losada et al. 2006; Celaya et al. 2007; Ruiz-Mirazo et al. 2011; Mancilla-Leytón et al. 2013). Silvopastoral systems incorporating livestock attempt to reconcile the use of natural products and services from the environment with a guarantee of permanence, or attempt to pursue ecological, economic and social stability through an efficient land use and the diversification of structures and products.

During grazing, damage by trampling and nutrient enrichment through faeces and urine deposition can indirectly accelerate litter decomposition. This process is rarely continuous; decomposition alternates between promotion and inhibition stages where chemical, physical and/or biological factors dictate the process (Swift and Anderson 1989). Many studies have evaluated foliar decomposition in relation to different climatic conditions (Silver and Miya 2001) but few have analysed the effect of grazing on foliar decomposition (Olofsson and Oksanen 2002; Garibaldi et al. 2007). Grazing may be of particular relevance in pine forests, where pine needles tend to accumulate abundantly because of their naturally low decomposition rates (Fioretto et al. 1998), and is especially important in nutrient-poor soil ecosystems (e.g. Mediterranean forests and dehesas) where it represents the main source of nutrients for new primary production (Wang and Huang 2008). At the same time, nitrogen (N) availability is widely considered to control the rate of litter decomposition, especially during the early stages in N-poor sites (Liu et al. 2011). A good correlation can often be found between the initial N concentration in litter and the decomposition rate (Dubeux et al. 2006). Furthermore, while detritivores are essential for the initial breakdown of litter (Prescott 1995), trampling during grazing can fragment the litter, thereby facilitating microbial activity and affecting the rate of litter decomposition.

The aim of this study was therefore to determine whether, goat grazing on the understory of a pine forest at Doñana Natural Park could accelerate the decomposition of the pine needles accumulated on the soil surface and to identify the relevant mechanisms. Specifically, the roles of trampling (mechanical fragmentation) and nutrient enrichment through defecation (fertilization) were evaluated in terms of their effect on pine needle decomposition rates.

Materials and methods

Study area

The study was carried out in a reforested pine forest (100 ha) on a private estate located within the Doñana Natural Park, in the southwest of the Iberian Peninsula (37º14′N, 6º20′W). The climate is Mediterranean moderated by the ocean, with wet (80 % of precipitation occurs between October and March) and mild winters (average monthly temperature of 10 °C in December and January) and very hot (average temperature of 25 °C in July and August) and dry (rarely rains in July and August) summers. Average annual rainfall in the study area is 540 mm. The substrate is mainly quartz sand and is extremely nutrient-poor, leading to poorly developed and infertile soils with low water retention capacities.

The vegetation comprises an arboreal stratum of pine trees (*Pinus pinea* L.) with a mean coverage of 38 %, an average density of 217 trees/ha, and an average tree diameter at breast height (DBH) of 26.92 cm. The understory is dominated by shrubs, with *Cistus salvifolius* L., *Halimium halimifolium* (L.) Willk., *Halimium calycinum* (L.) K., *Rosmarinus officinalis* L., *Pistacia lentiscus* L. and *Myrtus communis* L., the most common species.

The study area is used for timber production, hunting (rabbit, partridge) and grazing. However, wild herbivores (deer) were excluded in 1970 and domestic goats in 2002, with the study area remaining ungrazed for a period of 5 years prior to the reintroduction of goats (2007). During this 5-year period of livestock exclusion, vegetation was unmanaged and consequently grew and expanded rapidly. Afterwards, in spring of 2007, a herd of adult female *Payoya* goats (average weight of 40–45 kg) was introduced to the area and stocked at 2.7 goats ha^{-1} $year^{-1}$ rate (characterized as moderate grazing). This stocking rate may be considered semi-extensive, although in order to exploit the available 100 ha area in a uniform manner, a shepherd actively controlled the herd movement.

Study of litter decomposition using litter-bags

The litter-bag method was used to study the decomposition of the pine needles (Wieder and Lang 1982). In autumn of 2008 (maximum needle fall peak), a total of 45 litter traps were placed in groups of 5 below 9 pine trees (5 traps/tree), and needles were recovered after 1 week. These needles were dried at 80 °C for 48 h, and then mixed together to homogenize the sample before placing the litter into litter-bags. Two grams of the dried needles were placed in plastic mesh bags (pore size of 2 mm^2). This mesh size allows a range of detritivorous invertebrates to enter the bag, while retaining most of the fragmented needles. The needles were subject to 4 treatments: 1) intact needles (I), 2) needles trampled by goats (T), 3) intact needles fertilized with liquid manure (IF) and 4) trampled needles fertilized with liquid manure (TF). The mesh bags pertaining to the trampled treatments (T and TF) were placed into hermetically sealed plastic bags and half-buried for 5 days at the entrances and exits of the pen so that the goats could trample them. Afterwards, the bags belonging to treatments TF and IF were submerged for 24 h in a mixture of goat urine and faeces (300 g of faeces/l of urine), while the bags from treatments I and T were submerged in distilled water (also 24 h) in order to obtain a uniform moisture content in all the litter treatments.

A total of 300 bags (75 bags per treatment) were randomly distributed across a flat plot excluded from livestock, buried 3 cm under the soil (December of 2008) and covered with a uniform 2 cm layer of mixed sand and pine needles. Fifteen bags were randomly collected from each treatment after 4, 8, 16, 24 and 36 months and transported intact to the laboratory. Once opened, roots, small invertebrates and soil particles were manually removed from each bag and the needles were dried (80 °C for 48 h) and weighed.

Leaf litter decomposition (k) was determined as a function of mass loss over time (Matus and Rodríguez 1994) and calculated using the equation $y=Ao * e^{-kt}$, where y is final dry mass, Ao is initial dry mass and t is time of accumulation. From this, litter decomposition rates were calculated as: k=Ln (final mass/initial mass)/t.

Biometric and chemical characteristics of the needles

Needle length and leaf mass per area were measured after decomposition. Leaf mass per area (g/m^2) was determined by scanning the surface of the needles with image analysis software (Midebmp) (R. Ordiales, CSIC, Spain, 2000) and recording their weight. Finally, concentrations of carbon (C) and nitrogen (N) were determined from undigested dry samples using an elemental analyser (Leco CHNS-932, Spain).

Leaf litter decomposition in grazed and ungrazed areas

To determine whether the presence of livestock accelerated the decomposition of leaf litter in the pine forest, the decomposition rates of falling residues and of litter accumulated on the soil surface were calculated for both grazed and ungrazed areas. Falling residues were collected in 44 plastic bins (105 cm in height; 2,000 cm^2 capture area). These were randomly distributed, with 22 placed in areas excluded from livestock and 22 in grazed areas. The bins were placed below the crowns of the pine trees, fixed to the ground by a metal structure that prevented them from tipping over or being moved by animals. The height and resistance of the bins prevented the herbivores from consuming the tree residues contained within. Residues were collected monthly, transported to the laboratory and dried at 80 °C until reaching constant mass (48 h). Once dried, needles were separated from the other residues. Annual needle fall per unit area of arboreal canopy was estimated by summing the mass of all the needles collected within the same year and expressing this value in g/m^2. Average annual needle fall for the pine forest (L) was estimated by multiplying this production value by the canopy area (% pine tree coverage). To quantify the litter accumulated on the soil surface (H), a total of 90 plots of area 0.3 m^2 were randomly distributed, 45 across livestock excluded areas and 45 across the grazed areas. Samples (total litter accumulated on each plot) were transferred to the laboratory where they were sieved to remove soil, and dried in a fan oven at 80 °C for 48 h. Once dried, needles were separated from other fractions and the remaining litter was weighed and expressed in g/m^2 (H). This procedure was repeated 4 times; once before livestock entered the pine forest, and annually thereafter for 3 years (12, 24 and 36 months). The decomposition rate of the pine forest (k') was calculated with Olson's Model, as the ratio of needle fall production (L) to the total amount of litter accumulated on the soil (H) (k'= L/H). Finally, once the decomposition rate of the pine forest (k') was estimated, k was calculated through the equation: k=Ln (1-k') (Olson 1963; Staaf 1987).

Statistical analysis

For each variable (mass remaining (%), needle length, leaf mass per area and C and N content) a repeated measures ANOVA model was generated. The model comprised two factors: treatment (I, T, IF and TF) and time (five sampling dates). The results were adjusted according to the Huynh-Feldt correction. Finally, the linear relationship between remaining mass and leaf mass per area was calculated using a Pearson correlation. All analyses were conducted using the programme SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Leaf litter decomposition

Figure 1 shows the remaining mass (%) of the pine needles throughout the study period. Results show a significant interaction between the different treatments and time (F=39.43, $p \leq 0.01$).

Initially (at 4 months), the treatments with trampled needles (T and TF) registered a greater loss of mass (23–27 %, respectively) than those with intact needles (I and IF, 15–17 %, respectively). From 8 months onwards, the fertilized treatments (IF and TF) lost relatively more mass, with recorded differences between fertilized and non-fertilized treatments of 5–15 %.

Table 1 shows the decomposition rate (k) for each treatment and time period. The highest decomposition rates were recorded after 4 months, with the trampled needle treatments (T and TF) presenting significantly higher rates (0.93 and 0.90 year^{-1}, respectively) than the intact needle treatments (I and IF, 0.64 and 0.70 year^{-1}, respectively). From this time period onwards, however, decomposition rates in the fertilized treatments (TF and IF) were significantly higher than those in the non-fertilized treatments (I and T) (F=536.46, $p \leq 0.01$).

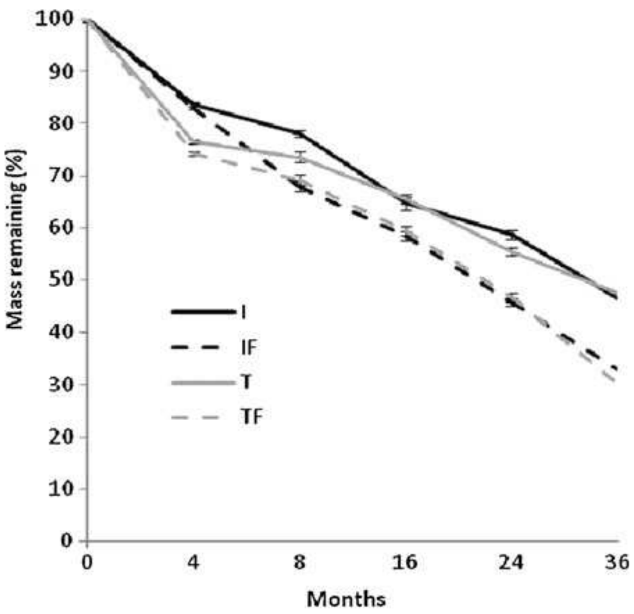

Fig. 1 *Pinus pinea* litter mass remaining over time. Intact needles (I), intact needles with liquid manure (IF), trampled needles (T) and trampled needles with liquid manure (TF). Mean ± S.E

Biometric and chemical characteristics of the pine needles

Throughout the study period, average needle length in the trampled treatments was significantly shorter (T and TF; 8.1±0.3 cm) than those in the non-trampled treatments (I and IF; 9.6±0.2 cm). These differences persisted during the whole study period (F=29.93, $p \leq 0.01$).

In terms of leaf mass per area (LMA), the results showed a significant interaction between the different treatments and time (F=4.75, $p \leq 0.01$) (Figure 2). Initially, both treatments with trampled needles (T and TF) presented significantly lower LMA values (250 and 260 g/m^2, respectively) than those with intact needles (I and IF) (300 and 310 g/m^2, respectively). From 8 months onwards, the fertilized treatments (IF and TF) presented significantly lower LMA values than the non-fertilized treatments (I and T) (F=85.23, $p \leq 0.01$). These differences (10–20 g/m^2) persisted until the end of the study period. The remaining mass of the needles and the LMA correlated positively (r=0.73, $p<0.01$).

Finally, chemical analyses showed similar initial C percentage values (approximately 50 %) in needles across all treatments (Table 2). Over time, the percentage of C decreased across all treatments, but in the fertilized treatments (IF and TF) the loss of C was greater (Table 2). As expected, the initial percentage of N was significantly greater in the treatments soaked with purines (2.1 %) compared to those that were unfertilized (1.2 %) (F=476.29, $p<0.01$). Although all treatments showed an increase in the percentage of N after 8, 16 and 24 months, by the end of the experiment (36 months), the final percentage of N was significantly lower in the treatments that had been soaked with purines (0.7 %) than in those that were unfertilized (0.9 %) (F=308.01, $p<0.01$) (Table 2). Consequently, the C:N ratio was significantly lower in the fertilized treatments (IF and TF) (F=254.47, $p<0.01$) (Table 2).

Table 1 Needle decomposition constant (k, year^{-1}) after 4, 8, 16, 24 and 36 months of burial in the soil. Intact needles (I), intact needles with liquid manure (IF), trampled needles (T) and trampled needles with liquid manure (TF). Mean ± S.E

Treatments	Months				
	4	8	16	24	36
I	0.64±0.03	0.37±0.01	0.33±0.01	0.27±0.01	0.26±0.01
IF	0.70±0.02	0.58±0.01	0.41±0.03	0.39±0.01	0.37±0.01
T	0.93±0.03	0.39±0.02	0.32±0.02	0.30±0.02	0.25±0.01
TF	0.90±0.02	0.55±0.03	0.40±0.02	0.38±0.01	0.40±0.03

Decomposition of the pine forest leaf litter

Mean annual litter accumulation (H) in the ungrazed areas was 3 times greater (442.91 g/m^2) than in the grazed area (135.83 g/m^2). The estimated mean annual needle production of the pine forest (L) was 101.87 g/m^2. The mean annual decomposition rate constant (k) of the ungrazed areas (0.26 year^{-1}) did not differ significantly from the control treatment (I) (Table 1). However, the mean annual decomposition rate of the grazed areas (1.40 year^{-1}) was much higher than that of the other treatments (Table 1).

Discussion

Large herbivores mainly affect nutrient cycling through two mechanisms: processing plants into urine and faeces

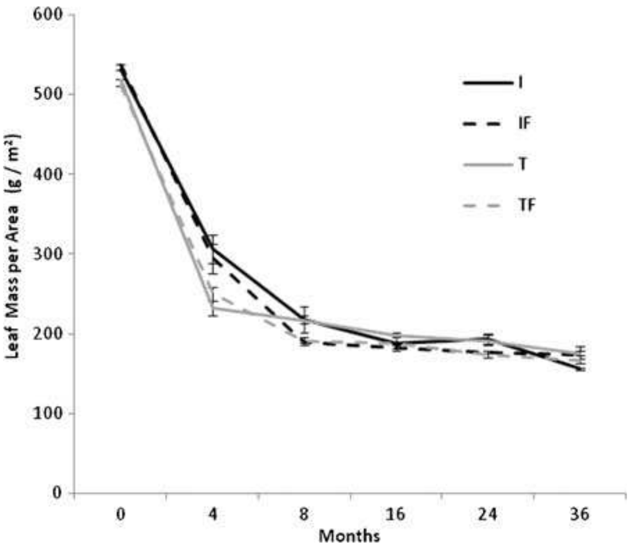

Fig. 2 Leaf mass per area (LMA) of *Pinus pinea* needles over time. Intact needles (I), intact needles with liquid manure (IF), trampled needles (T) and trampled needles with liquid manure (TF). Mean ± S.E

and influencing litter decomposition (Semmartin et al. 2008). Faeces and urine deposition by grazing animals exerts a positive influence on soil nutrient pools and microbial communities (Sankaran and Augustine 2004), thereby endorsing the use of grazers to enhance soil N availability and litter breakdown. Our results show that trampling by goats, which fragments the pine needles, significantly increases their initial decomposition rate, with nutrient enrichment (represented by soaking in purines) becoming more important with time (Table 1).

The decomposition rate of *Pinus pinea* needles in the control treatment (I) falls within the ranges found in other studies for this genus (Pausas 1997; Moro and Domingo 2000; Li et al. 2007; Harmon et al. 2009). However, the decomposition rates of the trampled and fertilized treatments were much higher. Most studies agree that the physical and chemical characteristics of plant tissues dictate their decomposition (see Prescott 2010). Some studies have shown that the macrofauna plays an important role in decomposition; fragmenters, such as earthworms, millipedes, termites, and isopods, are primarily macrofauna that can affect resource availability by modifying the physical properties of litter (Chapin et al. 2002; Yang et al. 2012). Litter fragmentation has been found to facilitate microbial attack, which in turn may produce increased decomposition rates: by enlarging the surface area available for microbial attack, litter fragmentation by small invertebrates causes a temporary increase in litter decomposition (Gillon and David 2001; Podgaiski and Rodrigues 2010; Yang et al. 2012). This study indicates that needle fragmentation during grazing can significantly increase decomposition rates (Table 1), apparently by enlarging the surface area available for attack by decomposers. However, this effect can be limited to a period of a few months following fragmentation after which rates of C mineralization may fall below the control values.

Table 2 Carbon and nitrogen content (%) 4, 8, 16, 24 and 36 months after burial in the soil. Intact needles (I), intact needles with liquid manure (IF), trampled needles (T) and trampled needles with liquid manure (TF). Mean ± S.E.

Months	0	4	8	16	24	36
I						
N	1.20±0.02	0.54±0.01	0.65±0.01	0.69±0.04	0.93±0.04	0.90±0.05
C	48.08±0.64	42.83±0.44	43.44±0.30	38.55±0.34	37.23±0.31	33.14±0.57
C/N	40.91±1.51	78.76±1.25	67.27±0.42	56.58±3.28	40.53±1.97	36.83±0.61
IF						
N	2.08±0.02	0.88±0.01	1.04±0.01	1.03±0.05	1.02±0.06	0.77±0.02
C	48.59±1.32	42.16±0.62	42.27±0.35	36.44±0.58	30.54±0.82	22.27±1.37
C/N	23.14±0.90	48.10±0.51	40.71±0.58	35.64±1.42	30.10±1.04	28.98±1.49
T						
N	1.21±0.02	0.70±0.01	0.74±0.03	0.73±0.01	0.95±0.01	0.92±0.02
C	49.01±1.08	41.95±0.44	43.53±1.17	37.49±0.45	35.64±1.42	30.57±1.29
C/N	40.94±1.15	56.74±0.84	62.59±0.92	51.63±0.96	37.40±2.19	33.44±2.27
TF						
N	2.12±0.03	0.97±0.03	1.07±0.04	1.05±0.02	1.07±0.05	0.77±0.02
C	48.89±1.10	42.32±0.55	41.36±0.52	36.19±0.61	30.86±0.79	18.90±1.29
C/N	23.49±1.94	43.63±1.74	38.59±0.80	34.67±1.17	29.41±1.05	24.82±1.64

The chemical characteristics of the litter became more relevant after 4 months of treatment (Table 2). It has been recognized for some time that mineral elements, particularly N, play an important role in controlling decomposition rates in organic material. The magnitude and even the sign of these effects are, however, not universal and the underlying mechanisms are poorly understood (Agren et al. 2001). It is not clear whether the relationship between decomposition and N content is an effect of the N itself, since changing the concentration of N promotes multiple changes in the decomposition process (such as increases in decomposer efficiency, more rapid formation of recalcitrant material and, although less pronounced, decreased decomposer growth rate) (Agren et al. 2001). For instance, Joffre et al. (2001) state that changing the N concentration has little effect on decomposition rates, while Berg and Ekbohm (1991) argue the contrary. Additionally, decomposition rates tend to decline over time with increases in the C:N ratio, the latter being accepted as a general index of quality (Seneviratne 2000). This trend is in accordance with our findings, which show a significant increase in decomposition rates from 8 months onwards in the treatments that were soaked in purines (Table 1). This increase was maintained until the end of the study (36 months).

For a few decades, the consequences of N deposition on soil nutrient renewal rates have often been attributed to microbial activity (Söderström et al. 1983; Arnebrant et al. 1996). The most likely explanation for this increase in decomposer efficiency is that the increased N promotes changes in the decomposer community, especially in N-poor systems such as Doñana Natural Park (Gallardo and Merino 1992; Allison et al. 2007; Treseder 2008; Papanikolaou et al. 2010). This mechanism is supported by numerous studies demonstrating shifts in microbial community composition after N fertilization (see Talbot and Treseder 2012), across nutrient gradients and following the addition of free N from wood ash produced by forest fires (Papanikolaou et al. 2010). We do not fully understand how this relates to the observed changes in decomposition rates, since the community of decomposers has not been analysed; however, this aspect certainly merits further study.

Gallardo and Merino (1992), in a study focusing on the decomposition of Mediterranean shrublands in Doñana, suggested that nitrogen could be imported to the litter by the microbial biomass. This may explain the N increase found as litter decomposition proceeds (Table 2). Similarly, Aber and Melillo (1982) found an inverse-linear relationship between remaining mass and N concentration in litter, this relationship being validated by a large number of litter decomposition studies.

Fixation of atmospheric N, contamination by insect frass, through fall/importation from green litter, etc., have all been proposed as possible explanations for this N increase. In this sense, Gallardo and Merino (1992) suggested that tannins could be responsible for N immobilization in leaf litter of Mediterranean shrublands, their effect being relatively more important in nutrient-poor soils with less microbial biomass (Aber and Melillo 1982). During a second step, tannins could precipitate N and immobilize it in the lignin fraction. The maximum amount of immobilized N would be limited by the size of microbial biomass (depending on the site characteristics) and the amount and quality of tannins (as a factor of litter quality) (Gallardo and Merino 1998). Therefore, since the environmental characteristics were shared across all the treatments in our study, the changes found in the N content can be attributed to quality differences in the litter itself (e.g. fertilized vs. unfertilized).

Finally, with respect to decomposition rate in the pine forest (k), the resulting k of the ungrazed areas was similar to that of the control treatment (0.26 year^{-1}), however, the decomposition rate of the grazed areas was far greater than that of the other treatments (IF, T and TF) (Table 1). The differences between these rates are attributed to the constant fertilization and trampling that occurs in the grazed areas throughout the study period (36 months) resulting in a higher decomposition rate, unlike the other treatments that were only fertilized and trampled at the beginning of the experiment. Other factors related to enclosing litter within bags with a small mesh size could have also contributed to the differences found among the different treatments (e.g. the exclusion of macroarthropods from the litter could have slowed down decomposition within the litter-bags). These factors would have been of minor effect, since decomposition in the ungrazed area was similar to that observed in the bags, but they should nevertheless be considered.

In conclusion, contrary to the general assumption that goats only cause environmental degradation, the results of this study indicate an indirect positive effect of goat grazing in the decomposition of leaf litter. Grazing increased the decomposition rate of *P. pinea* needles, promoting the incorporation of N into the system. The higher decomposition rates recorded in the grazed area could certainly contribute to changes in vegetation composition, creating a positive feedback (plant-soil) in these areas (Bardgett and Wardle 2003). In this sense, grazing could not only benefit vegetation through soil nutrient enrichment, but the acceleration of decomposition could also reduce the accumulation of pine needles on the soil surface, thereby making a valuable contribution to the mitigation of forest fire risk.

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2.7. SUMMER DIET SELECTION OF DAIRY GOATS GRAZING IN A MEDITERRANEAN SHRUBLAND AND THE QUALITY OF SECRETED FAT

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Summer diet selection of dairy goats grazing in a Mediterranean shrubland and the quality of secreted fat

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ABSTRACT

This study aims to evaluate the preferences and nutritive value of dairy goats summer diet selection in a Mediterranean shrubland and its effect on milk yield and quality, with specific reference to fatty acid (FA) composition. A commercial flock of the indigenous Payoya goat was used in the present study, carried out from August to October of 2008. The goats were divided into two groups: a free-range grazing group (goats had free access to pasture, (G) $n = 100$) and an indoor group (goats were housed in a stable, and received 1.5–2 kg/goat/day of alfalfa hay and pea straw mixture, (I) $n = 60$). A supplementary concentrate was fed to both groups at 0.5 kg/goat/d, during milking. Diet composition of the grazing goats was estimated through *in situ* observations. Chemical composition and nutritive value of selected diets, hay and concentrate samples were analyzed. Milk samples were taken from pooled milk of all animals in each group the day after diet observations, and basic chemical composition and FA profile were performed. *Myrtus communis* and *Pistacia lentiscus* were the most frequently ingested species. Milk yield and basic chemical composition were greater in group I than in group G for most of the studied parameters. The percentages of the nutritionally desirable FA (α-linolenic acid, C20:5 n-3, total n-3 PUFA) were significantly higher in milk from the G group, while the C18:2 n-6 *cis*, total n-6 PUFA and n6:n3 ratio were significantly lower than in I group. Total conjugate linoleic acid and its isomers were not affected by feeding-type group. In conclusion, the pastures selected could be considered to be of low to intermediate nutritional quality (4 MJ/kg DM of net energy for lactation). In addition, small but significant differences were found on milk yield and milk FA composition according to feeding treatment (grazing *vs.* concentrate-forage diet). Particularly, grazing goats showed higher levels of FA recognized as having beneficial effects on human health.

1. Introduction

Andalusia is the second goat milk-producing region in Europe, goat stocks comprising essentially autochthonous breeds, such as the Payoya goat (MARM, 2011). The importance of this breed has recently increased and there are actually around 15,000 Payoya goats, most of the flocks under a grazing-based management, with scrubland occupying most of the grazing area (Nahed et al., 2006; Ruiz et al., 2008). Additionally, and according to Ruiz et al. (2008), the net energy obtained from grazing to cover the animals energy requirements varies greatly between 50 and 80% from system to system. The use of indoor feeding (concentrate and forage) as a complement to grazing varies as well, and is determined by several factors such as availability of grass, productivity level of the animals and seasonal variation of pastures. Furthermore, unlike sheep and cattle, which predominantly select leafy

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material during spring, browse constitutes 50–80% of the forage selected by goats all year round (Silanikove et al., 2010). It was suggested that this behavior is an adaptive mechanism that allows goats to maintain a high intake of browse to preserve their specific superior capacity in utilizing food rich in tannins and other source of secondary metabolites (Silanikove et al., 2010). Nevertheless, the diet selection, its nutritional value, and the food intake rate of goats grazing in the Andalusian shrublands are poorly understood (Ruiz et al., 2008). An understanding of diet selection and intake rates by ruminants on rangelands can strengthen efficient management practices and profitable animal production (Ruiz et al., 2008; Mancilla-Leytón et al., 2012).

Nowadays, current research is oriented towards reducing the milk's amount of saturated fatty acids (SFA) and increasing polyunsaturated fatty acids (PUFA) which benefits human health. In particular, great attention is given to n-3 fatty acids and conjugated linoleic acid (CLA) (MacRae et al., 2005). Several works on dairy goats have highlighted the potentiality of grazing on herbaceous pasture to enhance milk and dairy products proportion of PUFA, α-linolenic acid (the main n-3 FA in milk), and/or CLA (D'Urso et al., 2008; Silanikove et al., 2010), in comparison to conventional concentrate-forage diets or low grazing systems. According to Silanikove et al. (2010) milk from goats feeding on pasture may present an overlooked "treasure trove" with respect to its health promoting lipid profile. However, there is little information on how Mediterranean forage species (particularly shrub and woody species) affect the fat composition of milk and cheese, and contradictory results have been found (Tsiplakou et al., 2006; Vasta et al., 2008).

So, the present study aims to evaluate the foraging behavior of dairy goats in summer, with focus on the preferences and nutritive value of the diet selected between shrub and woody species in a Mediterranean shrubland, and their effect on milk yield and quality, with specific reference to fatty acids composition.

2. Materials and methods

2.1. Study area, experimental farm and goats

The study was conducted in a pine forest (100 ha) of a private estate located within the Doñana Natural Park limits, in the southwestern Iberian Peninsula (37°14′N, 6°20′W). The climate is Mediterranean, with wet (80% of precipitation occurring between October and March) and mild (average monthly temperature of 10 °C in December and January) winters, and very hot (average temperature of 25 °C in July and August) and dry (rarely rains in July and August) summers. Average annual rainfall in the study area is 540 mm.

The vegetation comprises an arboreal stratum of pine trees (*Pinus pinea* L.) with 38% mean coverage, and an understory of Mediterranean scrub. The characteristics of the prevalent species in the understory are detailed in Table 1. Seventy percent of the understory was covered with shrubs, with a total of twenty species, the most common being *Rosmarinus officinalis* (17.1%), *Cistus salvifolius* (16.2%), and *Halimium halimifolium* (11.5%).

A commercial flock of indigenous Payoya goats was used in the present study, carried out from August to October 2008. Goats kidded between October 2007 and January 2008, and during the experiment were in late stage lactation and milked once a day. Goats were divided into two groups, homogeneous in parity and milk production at the previous lactation: a free-range grazing group (G, $n = 100$) and a control or indoor group (I, $n = 60$). The grazing group goats (G) had free access to pasture. The indoor group goats (I) were housed in a stable, and received a mixture of alfalfa hay and pea straw (in the proportion of 1 part of alfalfa to 3 parts of straw) at 1.5–2 kg/goat/day. A supplementary concentrate was fed to both groups at a rate of 0.5 kg/goat/d, during milking.

Table 1
Cover (%) of shrub species found in the understory of a Mediterranean pine forest in Doñana Natural Park, SW Spain.

Species	Cover (%)
Daphne gnidium	0.9
Cistus monspeliensis	1.1
Helichrysum italicum	1.3
Cistus crispus	1.4
Phillyrea angustifolia	1.5
Stauracanthus genistoides	1.5
Genista hirsuta	2.9
Genista triacanthos	3.1
Cistus ladanifer	3.7
Cistus libanotis	3.9
Lavandula stoechas	4.3
Thymus mastichina	5.3
Quercus coccifera	5.9
Erica scoparia	6.6
Pistacia lentiscus	8.1
Halimium calycinum	9.4
Myrtus communis	11.1
Halimium halimifolium	11.5
Cistus salvifolius	16.2
Rosmarinus officinalis	17.1

2.2. Diet selection and composition

From August to October, the grazing group entered the study area. The management may be considered to be semi-extensive, although in order to exploit the 100 ha in a uniform manner, the goats were closely controlled and moved around by a shepherd. In order to determine which shrub species are preferred by the goat herd, a method of direct observation was used. This observational surveying method is very efficient for determining the shrub species grazed, the relative preferences and variations throughout the year. Although modified, the procedure described by Meuret et al. (1985) was followed. In this experiment, surveys were performed thrice a month in the understory of the pine forest, with 10 min monitoring period per goat stretching from the beginning to the end of the grazing day. Every surveying day, 10–15 goats were monitored (30–45 goats/month). Goats were chosen at random, without repeating any individual in the same surveying. The recorded variables for each goat and monitoring period were: number of species consumed and organ of the consumed species (leave, stem, flower, or fruit), number of bites per species, consumption time per species and displacement time (from plant to plant) (Mancilla-Leytón and Martín Vicente, 2011). After every 10 min period, the monitored goat was changed.

In order to estimate the nutritional value of the shrubs ingested by goats, the amount of each species ingested in each bite (g of dry matter, DM) also needed to be estimated (Mancilla-Leytón et al., 2012). A manual simulation of grazing (handplucked) was performed (100 samples/species), from which the weight per bite was obtained. Weight per mouthful was estimated by imitating the biting behavior of goats (clipping material physically comparable to that selected by the animals) (Mancilla-Leytón et al., 2012). These samples were dried at 60 °C (48 h), their dry weight determined and their nutritive value analyzed. The Meuret et al. (1985) formula was used to calculate dry matter intake of each plant: $DMIi = (GP/OP) \times (Bi \times WBi)$; where DM*I*i is the dry matter intake of '*i*' species; GP, the grazing period; OP, the observation period; *Bi*, the bites of '*i*' species; and *WBi*, the weight of bite of '*i*' species. For each monitored period, mean grazing percentage values of each species were used to calculate the relative presence of plant species in the diet selected by goats.

2.3. Chemical analysis of concentrate and shrubs

Samples of selected diets (only G group), hay (only I group) and concentrate (both groups) were dried and ground to pass the 1-mm screen of a Willey mill before analysis. AOAC (2005) methods were used to determine dry matter (method 934.01), ash (method 942.05), ether extract (method 920.39), and N (method 968.06) content in samples of selected diets, hay and concentrate. Total N was determined by the combustion method using a CNS-2000 carbon, N, and sulfur analyzer (Leco CNS-2000, Leco Corporation, USA), and converted to crude protein (CP) by multiplying by a factor of 6.25. The analyses of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were carried out following Van Soest et al. (1991). Amylase was not used in the analysis of NDF, and both NDF and ADF were expressed as exclusive of residual ash. Acid detergent lignin (ADL) was determined through solubilization of cellulose with 72% sulfuric acid. All fiber fractions were analyzed with a Fibertec 1030 Hot Extractor (Tecator, Sweden). Fat content was measured by extraction with petroleum ether (boiling point, 40–60 °C) on a Soxtec System 1040 Extraction Unit (FOSS Tecator AB, Sweden).

Samples were analyzed for *in vitro* digestibility of dry matter (IVDMD), organic matter (IVOMD) and nitrogen (IVND) according to Tilley and Terry (1963) by using the Daisy II incubator (Ankom® Technology, New York, USA) as described by Martín García et al. (2004). The rumen inoculum was individually withdrawn from three ruminally fistulated goats 2 h after morning feeding, transferred into pre-warmed (39 °C) thermo bottles and squeezed through four layers of cheesecloth under anaerobic conditions. A pooled sample was prepared by combining equal squeezed volumes from each animal. The inoculum was a mixture (4:1, v/v) of rumen liquor and artificial saliva (McDougall, 1948). Samples (0.5 ± 0.05 g) were weighted in filter bags (Ankom® Technology, #F57) and three samples per treatment were incubated in the incubator. Two incubation runs were carried out. Every incubation run included two blanks (controls) and three standard samples for which the apparent *in vivo* DM digestibility was known. The *in vitro* DM and CP digestibilities were calculated as DM and CP disappearance, respectively, per unit of original DM and CP. Residual DM and CP were corrected for those in the blanks.

Metabolizable and net energy for lactation (ME and NEL, respectively) were calculated from chemical and digestibility analyses, according to the method of INRA (2007). The *in vivo* organic matter digestibility (OMD) of the forages and concentrate consumed were estimated from *in vitro* Tilley and Terry values (IVOMD) using a specific regression equation (Molina Alcaide et al., 1997): OMD, % = 15.9 + 0.758 × IVOMD, %. Digestibility of energy (DE) was related to OMD through different equations, in case the plant consumed by the animals of grazing group was related to following the equation for untreated and treated straw: DE = 0.985 OMD (%) − 2.949 (INRA, 2007).

Free, protein- and fiber-bound condensed tannins were sequentially extracted following the procedure described by Terril et al. (1992) and modified by Pérez Maldonado and Norton (1996). The free-CT fraction was extracted using aqueous acetone (70%), diethyl ether and ethyl acetate and partitioned into the aqueous phase to exclude pigments and small phenolic compounds. Subsequent extraction with boiling sodium dodecyl sulphate (SDS), triethanolamine and 2-mercaptoethanol yielded protein-bound tannins. The residue from this SDS extraction was washed with methanol and butanol to yield fiber-bound tannins. Analyses of the free, protein-bound and fiber-bound condensed tannins were conducted according to the butanol–HCl method of Porter et al. (1986), with the modifications proposed by Terril et al. (1992). Condensed tannins from quebracho powder (Roy Wilson Dickson Ltd., Mold, U.K.) were used as standard. Total amount of CT was calculated by adding the amounts of free, protein- and fiber-bound CT in the sample.

2.4. Milk Sample collection and basic chemical analysis

Milk samples were taken the day after observational surveys from pooled milk of all animals in each group after milking (n = 18, 9 for each feeding group). Milk composition was analyzed (fat, protein, and lactose) with an infrared spectrophotometer (Milko Skan in a Combi-Foss 5000, Foss Electric, Hillerød, Denmark).

2.5. Fatty acids composition in milk and feed samples

Feed samples and a freeze-dried aliquot of each milk sample stored at −20 °C were used for gas chromatography analyses of FA. Separation and quantification of FA methyl esters were performed with a gas chromatograph Agilent 6890N Network GS System (Agilent, Santa Clara, USA), equipped with a flame ionization detector and fitted with a HP-88 capillary column (100 m, 0.25 mm i.d., 0.2 μm film thickness). Nonanoic acid methyl ester (C9:0 ME, 4 mg/mL) was used as an internal standard. Extraction and direct methylation were performed in a single step procedure based on the method published by Sukhija and Palmquist (1998) and revised by Juárez et al. (2008) in order to minimize isomerization and epimerization in CLA. Briefly, 1 mL of n-hexane and 3 mL of freshly made 5% in wt methanolic HCl were added to freeze-dry milk samples, vortexed and heated for 90 min in a water bath at 70 °C. After the contents were cooled to room temperature, 5 mL of 6% by wt K_2CO_3 were added, followed by 2 mL of n-hexane. The contents of the tubes were vortexed, followed by centrifugation at 3500 rpm for 10 min. The upper organic phase was transferred to a culture tube and added 1 mL of $NaSO_4$ and finally dissolved in 1 mL of n-hexane for gas chromatography analysis. Individual FA were identified by comparing their retention times with those of an authenticated standard FA mix Supelco 37 (Sigma Chemical Co. Ltd., Poole, UK). Identification of the CLA isomers *cis*-9 *trans*-11, *cis*-11 *trans*-13, *trans*-10 *cis*-12 and *cis*-10 *cis*-12 was done by comparing retention times with those of another authenticated standard mix (Sigma Chemical Co. Ltd., Poole, UK). Fatty acids content was expressed as the percentage of total methyl esters identified.

After analyses, the FA composition data were grouped as: short-chain fatty acids (SCFA; C4:0–C10:0), medium-chain fatty acids (MCFA; C11:0–C17:1), long-chain fatty acids (LCFA; C18:0–C24:1), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), unsaturated fatty acids (USFA), n-3 PUFA, n-6 PUFA, and total CLA. Ratios between the different fractions, namely PUFA/SFA, USFA/SFA and n-6/n-3 were calculated. The desaturase activities were estimated indirectly as [product]/[precursor + product] ratio. Thus, activities of Δ9C14 (C14:1)/C14:1 + C14:0), Δ9C16 (C16:1)/C16:1 + C16:0) and CLA desaturase (rumenic acid, CLA/vaccenic acid, VA + CLA) indices were estimated. Finally, the atherogenicity (C12:0 + 4 × 14:0 + C16:0)/(MUFA + PUFA) and thrombogenecity (C14:0 + C16:0 + C18:0)/(0,5 × MUFA + 0.5 × n-6-PUFA + 3 × n-3-PUFA + n-3-PUFA/n-6-PUFA) indices were calculated according to Ulbricht and Southgate (1991).

2.6. Statistical analyses

To compare the number of species consumed, non-parametric tests (Kruskal–Wallis) and a posteriori U-Man-Whitney comparisons were performed. Data of milk production and milk constituents were analysed by factorial ANOVA, using the general linear model (GLM) of the SPSS 18.0 for Windows package (SPSS Inc., Chicago, USA), including the fixed effects of feeding group and month. The linear model used for each parameter was as follows: $Y_{ijk} = \mu + FG_i + M_j + (FG \times M)_{ij} + \varepsilon_{ijk}$; where Y_{ijk} = observations for dependent variables; μ = overall mean; FG_i = fixed effect of feeding group (i = grazing or indoor); M_j = fixed effect of month (j = August to October); FG × M = interactions between and among these factors, and ε_{ijk} = random effect of residual. Pairwise comparisons of means were carried out, where appropriate, using Tukey's honest significant difference tests.

3. Results

3.1. Diet selection and nutritional composition

A high variety of species comprised the diet as observed during surveys, with a maximum of six different species/goat/10 min monitoring period. There were significant differences between months; a greater consumption diversity occurred in September (4.96 ± 0.17 species) and October (5.02 ± 0.31 species), which were the periods with greater food offers, and the very opposite in August (3.96 ± 0.23 species), with the least variety of species consumed (H = 0.17, $P \leq 0.05$, K–W test).

Table 2 shows the percentage of total bites/species/season recorded through direct observation of the goats. Selection did not follow a fixed pattern: no close relationship was observed between the coverage of shrub species and their consumption degree. The most abundant species were not the most consumed ones (Tables 1 and 2). The animals tended to select the

Table 2
Forage intake (g DM and %) and phenological state (PS) of diets selected by goats grazing in summer on a Mediterranean shrublands.[a]

Shrubs	August			September			October		
	Intake			Intake			Intake		
	(g)	(%)	PS	(g)	(%)	PS	(g)	(%)	PS
Cistus ladanifer	32.0	3.3	Fr	122.4	6.3	Vg	80.8	6.3	Vg
Cistus salvifolius	84.0	8.7	Fr	98.8	5.1	Vg	41.2	3.2	Vg
Erica scoparia	11.4	1.2	Do	1.6	0.1	Vg	5.6	0.4	Vg
Genista hirsuta	62.3	6.5	Do	224.7	11.6	Vg	81.9	6.4	Vg
Halimium calicynum	29.5	3.1	Do	0.0	0.0	Do	49.5	3.9	Vg
Halimium halimifolium	178.8	18.6	Fr	40.8	2.1	Do	77.4	6.0	Vg
Mirtus communis	432.6	44.9	Fr	408.0	21.0	Fr	78.6	6.1	Fr
Pistacea lentiscus	32.4	3.4	Fr	740.4	38.1	Fr	740.4	57.8	Fr
Quercus coccifera	93.6	9.7	Fr	280.8	14.4	Fr	120.0	9.4	Vg
Stauracanthus genistoides	7.0	0.7	Do	26.6	1.4	Vg	4.9	0.4	Vg
Total	963.6			1944.1			1280.3		

[a] Results shown correspond to monthly averages of triple samples. Vg: vegetative growth; Fl: flowering; Fr: fruiting; Do: dormancy.

leaves and stem shoots in all the species, as well as the fruits of *C. salvifolius*, *Myrtus communis*, *Pistacia lentiscus*, and *Quercus coccifera*. The consumption of shrub species varied among the studied months. *M. communis* was the most consumed species in August (44.9%) and *P. lentiscus* in September and October (38.1 and 57.8%, respectively). Shrub intake varied from month to month, with smaller values in August (963.6 g DM) that increased to 1944.1 g DM in September and decreased in October (1280.3 g DM). The contribution of each species to the diet also varied during the study period (Table 2).

The average chemical composition, nutritive value and FA profile of selected pastures in each month are reported in Tables 3 and 4. Observed changes in digestibility between months are consistent with variations in the chemical composition of the selected shrubland species. The highest values in lignin and tannins and the lowest values in digestibility were registered in September and October (Table 3).

3.2. Milk yield and chemical composition

The yield and chemical composition of milk are reported in Table 5. Milk yield and milk fat and protein contents were significantly lower in milk from G group compared to milk from I group. Regarding the influence of the month, milk yield significantly decreased as a function of the sampled month, and with regards to the chemical composition, the highest values of these components were found in the last two months.

Few significant differences and trends were found in milk fat composition between the grazing and indoor

Table 3
Chemical composition and nutritive value of indoor feeds and diets selected by goats grazing in summer on a Mediterranean shrubland.

Item (g/kg DM)[a]	Forage[b]	Concentrate[c]	Pasture		
			August	September	October
Chemical composition					
Dry matter (g/kg fresh matter)	964	956			
Ash	160.8	28.5	46.7	5.08	48.6
Crude protein	158.8	127.7	68.2	69.7	68.1
Ether extract	5.6	29.3	28.6	47.1	40.6
NDF	579.4		362.1	318.7	361.9
ADF	290.7		247.2	143.3	153.1
ADL	159.7		76.0	95.8	95.3
Total CT	12.23	8.21	46.75	73.11	63.74
Free CT	1.97	1.99	19.79	27.27	26.77
Protein-bound CT	0.59	0.07	1.04	0.75	0.87
Fibre-bound CT	9.67	6.15	25.91	45.08	36.10
IVDMD (%)	42.6	83.8	49.8	49.4	45.1
IVOMD (%)	38.1	85.6	48.3	45.6	43.7
IVND (%)	60.7	95.0	35.7	24.4	15.7
ME (MJ/kg DM)	5.9	11.9	7.5	7.1	7.0
NEL (MJ/kg DM) (UFL between parenthesis)	3.2 (0.45)	7.4 (1.04)	4.2 (0.59)	4.0 (0.56)	3.9 (0.54)

[a] NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; IVDMD, IVOMD and IVND, *in vitro* digestibility of dry matter, organic matter and nitrogen, respectively; CT: condensed tannins; ME, NEL, metabolizable and net energy for lactation, respectively; UFL, unit feed for lactation.
[b] Mixture of alfalfa hay and pea straw.
[c] Supplement ingredients (%): maize grain (24.8), soybean meal (10.9), green peas (10.9), barley grain (37.1), oat grain (14.9), sunflower meal (0.5) and mineral-vitamin supplement (1). The NDF, ADF and ADL contents were not determined in the concentrate supplement.

Table 4
Fatty acids profile (% of total FA) of the indoor feeds and diets selected by goats grazing in summer on a Mediterranean shrubland.

Fatty acid[a]	Forage[b]	Concentrate	Pasture		
			August	September	October
C:8:0	0.24	0.05	0.34	0.12	0.04
C:10:0	2.58	0.41	2.69	0.89	0.15
C:12:0	1.97	0.34	1.96	1.13	0.57
C:14:0	6.47	1.89	7.24	6.19	4.97
C:15:0	0.94	0.19	0.53	0.42	0.25
C:16:0	32.23	19.38	27.78	26.13	23.59
C:16:1	2.65	0.50	0.82	0.82	0.90
C:17:0	1.18	0.22	1.04	0.79	1.06
C:17:1	0.38	0.09	0.60	0.42	0.37
C:18:0	12.98	5.72	11.73	9.26	12.63
C:18:1 n-9 *cis*	14.59	25.99	15.03	14.65	9.45
C:18:2 n-6 *trans*	0.17	0.07	0.35	1.56	0.34
C:18:2 n-6 *cis*	11.16	40.15	9.19	11.63	11.74
γ-C:18:3 n-6	0.14	0.03	0.20	0.12	0.16
C:20:0	1.51	0.41	2.69	4.50	4.35
α-C:18:3 n-3	2.34	2.35	7.76	11.69	12.87
C:20:1 n-9	0.20	0.46	0.95	1.12	1.00
C:21:0	0.07	0.03	0.08	0.09	0.18
C:20:2	0.26	0.10	0.10	0.12	0.21
C:20:3 n-6	2.08	0.26	1.86	2.02	2.14
C:20:4 n-6	0.20	0.04	0.16	0.26	0.21
C:20:3 n-3	4.92	1.19	4.67	4.11	10.41
C:20:5 n-3 (EPA)	0.32	0.05	0.24	0.29	0.63
C:22:5 n-3 (DPA)	0.23	0.02	0.20	0.22	0.33
C:22:6 n-3 (DHA)	0.18	0.03	1.77	1.48	1.45
SFA	60.17	28.66	56.08	49.51	47.80
MUFA	17.82	27.04	17.41	17.00	11.71
PUFA	22.01	44.30	26.51	33.49	40.49
n-6	13.77	40.55	11.77	15.58	14.59
n-3	7.98	3.64	14.65	17.78	25.69

[a] EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[b] Mixture of alfalfa hay and pea straw.

groups during the summer period when goats were fed concentrate and conserved forage (Table 6). Nevertheless, the percentages of C12:0 ($P < 0.001$) and C15:0 ($P < 0.01$) were significantly higher and the C16:0 ($P < 0.01$), C17:0 ($P < 0.05$) and C20:1 n-9 ($P < 0.001$) significantly lower in milk from G group when compared to milk from I group. With respect to the percentages of the nutritionally desirable FA (α-linolenic acid, $P < 0.05$; C20:5 n-3, $P < 0.05$; total

Table 5
Intake, milk yield and chemical composition of Payoya dairy goats according to feeding group (grazing Mediterranean shrubland, G; indoor, I) and month.

ITEM[a]	Feeding group (FG)		Month (M)[a]			S.E.M.[b]	Effects, P[c]		
	Grazing	Indoor	August	September	October		FG	M	FG × M
Intake (g/d)									
DM	1874	2166	1804	2294	1962				
Fat	71	23	32	64	45				
Neutral detergent fibre	477	978	663	799	720				
Acid detergent fibre	238	491	365	385	343				
Net energy (MJ/d)	9.13	8.97	8.29	10.14	8.13				
In vitro digestible protein	81	221	151	156	146				
Daily milk yield (L)	0.7	1.0	1.1a	0.8b	0.8b	0.04	*	*	ns
Fat (%)	3.72	5.54	4.02b	4.40b	5.26a	0.26	***	***	ns
Fat (g/d)	28.3	57.1	42.3a	35.6b	39.9a,b	1.04	*	*	ns
Protein (%)	3.47	3.73	3.25b	3.48b	3.94a	0.08	**	***	ns
Protein (g/d)	26.4	38.4	35.0a	29.0b	30.6b	1.03	***	***	ns
Lactose (%)	4.26	4.14	4.16b	4.12b	4.29a	0.03	**	**	ns
Total solids (%)	11.99	14.30	12.08b	12.68b	14.28a	0.37	***	***	ns

[a] Means within a row with different suffix (a or b) are significantly different ($p < 0.05$).
[b] Standard error of mean.
[c] ns: not significant, $P > 0.05$.
[*] $P < 0.05$.
[**] $P < 0.01$.
[***] $P < 0.001$.

Table 6
Fatty acids (% total FA) in milk of Payoya dairy goats according to feeding group (grazing Mediterranean shrubland, G; indoor, I).

Fatty acid[a]	Feeding group (FG)		S.E.M.[b]	Effects, P[c]		
	Grazing	Indoor		FG	M	FG × M
C4:0	1.63	1.56	0.02	ns	ns	ns
C6:0	1.66	1.61	0.03	ns	ns	ns
C8:0	2.33	1.88	0.03	ns	ns	ns
C10:0	9.18	8.56	0.40	ns	ns	ns
C11:0	0.14	0.06	0.01	ns	ns	ns
C12:0	4.11	3.08	0.15	***	ns	ns
C13:0	0.11	0.06	0.01	ns	ns	ns
C14:0	9.90	9.70	0.14	ns	ns	ns
C14:1	0.13	0.09	0.01	ns	ns	ns
C15:0	0.81	0.62	0.04	**	ns	ns
C15:1	0.08	0.06	0.00	ns	ns	ns
C16:0	28.87	31.61	0.51	**	ns	ns
C16:1 n-9	0.59	0.53	0.02	ns	*	ns
C17:0	0.61	0.74	0.02	*	**	ns
C17:1	0.24	0.27	0.01	ns	ns	ns
C18:0	14.70	15.35	0.31	ns	ns	ns
C18:1 n-9 *cis*	17.36	18.49	0.38	ns	ns	ns
C18:1 n-9 *trans*	0.28	0.36	0.02	ns	ns	ns
C18:1 *trans*-11 (VA)	0.48	0.69	0.04	ns	ns	ns
C18:2 n-6 *trans*	0.14	0.12	0.01	ns	ns	ns
C18:2 n-6 *cis*	1.37	1.64	0.04	***	ns	ns
CLA *cis*-9. *trans*-11 (RA)	0.23	0.28	0.01	ns	ns	ns
CLA *trans*-9. *trans*-11	0.04	0.02	0.00	ns	ns	ns
CLA *trans*-10. *cis*-12	0.04	0.03	0.00	ns	ns	ns
CLA *cis*-9. *cis*-11	0.04	0.02	0.00	ns	ns	ns
CLA *trans*-9. *cis*-11	0.09	0.08	0.00	ns	ns	ns
γ -C18:3 n-6	0.10	0.10	0.01	ns	ns	ns
α -C18:3 n-3	0.65	0.53	0.01	*	ns	ns
C20:0	0.31	0.37	0.02	ns	ns	ns
C20:1 n-9	0.19	0.38	0.03	***	ns	ns
C20:2	0.07	0.08	0.01	ns	ns	ns
C20:3 n-6	0.02	0.02	0.00	ns	ns	ns
C20:4 n-6 (ARA)	0.13	0.15	0.00	ns	ns	ns
C20:3 n-3	0.02	0.02	0.00	ns	ns	ns
C20:5 n-3 (EPA)	0.08	0.06	0.00	*	ns	ns
C21:0	0.09	0.10	0.00	ns	ns	ns
C22:0	0.11	0.07	0.01	ns	*	ns
C22:1 n-9	0.06	0.04	0.00	ns	ns	ns
C22:2	0.08	0.06	0.01	ns	*	ns
C22:4 n-6	0.12	0.08	0.01	ns	ns	ns
C22:5 n-3 (DPA)	0.15	0.12	0.01	ns	ns	ns
C22:6 n-3 (DHA)	0.12	0.05	0.02	ns	ns	ns
C23:0	0.08	0.07	0.01	ns	ns	ns
C24:0	0.16	0.12	0.01	ns	ns	ns
C24:1	0.12	0.07	0.01	ns	ns	ns
SFA	74.79	75.58	0.37	ns	ns	ns
MUFA	19.52	20.97	0.39	ns	ns	ns
PUFA	4.68	4.25	0.14	ns	ns	ns
UFA	24.21	25.22	0.37	ns	ns	ns
SCFA	14.79	13.61	0.58	ns	ns	ns
MCFA	45.58	46.03	0.57	ns	ns	ns
LCFA	39.62	40.36	0.56	ns	ns	ns
CLA	0.44	0.43	0.01	ns	ns	ns
n-3	1.02	0.78	0.02	*	ns	ns
n-6	1.88	2.11	0.05	*	ns	*
n6/n3	1.84	2.70	0.05	**	ns	ns
PUFA/SFA	0.06	0.06	0.00	ns	ns	ns
UFA/SFA	0.32	0.34	0.01	ns	ns	ns
Δ9C14	0.01	0.01	0.00	ns	ns	ns
Δ9C16	0.02	0.01	0.00	ns	ns	ns
Δ9C18	0.55	0.56	0.01	ns	ns	ns
CLA index	0.32	0.29	0.01	ns	ns	ns
VA/RA	2.18	2.52	0.12	ns	ns	ns
AI	3.04	2.95	0.07	ns	ns	ns
TI	2.90	3.23	0.07	*	ns	Ns

[a] CLA, conjugated linoleic acid; VA, vaccenic acid; RA, rumenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids; SCFS, short-chain fatty acids; MCFS, medium-chain fatty acids; LCFS, long-chain fatty acids; Δ9C16, Δ9C16 desaturase index; Δ9C18, Δ9C18 desaturase index; CLA index, CLA desaturase index; Δ6, Δ6 desaturase index; AI, atherogenicity index; TI, thrombogenicity index.

[b] Standard error of mean.

[c] FS, Feeding group; M, month; FGxM, feeding group × month interaction. ns: not significant, $P > 0.05$.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

n-3 PUFA, $P < 0.05$), they were significantly higher in milk from G group, while levels of C18:2 n-6 *cis* ($P < 0.01$) and total n-6 PUFA ($P < 0.05$) were significantly lower. As a result, the n6:n3 ratio was higher in milk from I group ($P < 0.05$). CLA total and CLA isomers (*cis*-9, *trans*-11; *trans*-10, *cis*-12; *trans*-9. *trans*-11; *cis*-9. *cis*-11; *trans*-9. *cis*-11) were not affected by feeding group. The thrombogenicity index was significantly lower in milk from G group ($P < 0.05$), when compared with milk from I group.

4. Discussion

4.1. Diet selection and nutritional composition

A great variety in the selected diet was observed in this study. The use of a considerable number of plants decreases the competitive advantages generated when some species are not consumed, and improves the conservation of plant biodiversity (Villalba et al., 2002). Selection and intake of shrub species did not follow a fixed pattern and the contribution of each species to the diet also varied during the study period. Therefore, along their daily routes, goats selected those species that pleased them the most, consumption not being random (Mancilla-Leytón et al., 2012). The goat, in its food selection, seems to establish a balance between swiftness and best consumption quality (Duncan and Gordon, 1999), depending on the nutritional requirements of each moment (Sánchez Rodríguez et al., 1993). The temporal variations in food selection seem to be largely related to the phenology of the species: in most of the studied species, the probability of being consumed increases during the flowering and fructification stages; thereby we could speak of seasonal consumption (Mancilla-Leytón and Martín Vicente, 2011). For instance, *M. communis* and *P. lentiscus* were highly consumed in summer (see Table 2), since the fruits of these species are an attractant for goats during this season. Contrastingly, the low consumption of Lamiaceae or Labiateae species (*e.g.* aromatic species: *R. officinalis*, *Lavandula stoechas*, *Thymus mastichina*, *etc.*) is attributed to their high oil content (Guillen and Cabo, 1996) which increases during the summer period and decreases their desirability.

The methodology adopted to build up representative samples of the selected pastures by the grazing animals has an undoubtedly subjectivity component which may lead to bias as far as feeding behavior and related parameters are concerned (García et al., 1995). Unfortunately, information on the composition and nutritive value of pastures selected by dairy goats grazing on Mediterranean shrublands is unknown. Nevertheless, these results agree with previous ones relating to single Mediterranean shrub species (Rogosic et al., 2006). The highest values in tannins could be due to *P. lentiscus* being the most consumed shrub species during those summer months, and the high tannin concentration that this species has (Rogosic et al., 2006). Observed changes in digestibility between months are consistent with variations in the chemical composition of the samples. Tannin concentrations higher than 5% adversely affect forage intake and digestibility of Mediterranean shrubs, such as *Quercus calliprinos* and *P. lentiscus* (Perevolotsky et al., 1993). Condensed tannins bind and precipitate proteins in the rumen (Jones and Mangan, 1977) reduce protein degradation and reduce absorption of amino acids reaching the small intestine, resulting in low digestibility and voluntary intake. Furthermore, lignin limits the availability of plant cell walls to ruminants and to rumen microbes (Van Soest, 1994). According to all of the above, the pastures selected in this study could be considered to be of low to intermediate nutritional quality because they contain less than 55% digestible OM and 8% CP (Leng, 1990).

4.2. Milk yield and chemical composition

In this experiment, the energy and protein requirements for maintenance and milk production of local genotype goats, with 55–60 kg of body weight (BW), were estimated in 0.0366 UFL (unit feed for lactation) and 2.32 g PDI (protein digestible in the intestine)/kg metabolic weight ($MW = BW^{0.75}$) and 0.42 UFL and 49 g PDI/kg fat-corrected milk (3.5% fat), respectively. The energy expenditure of locomotion in the grazing group was estimated in 0.10 UFL/d, according to the procedure described by Lachica et al. (1997). Though the estimated energy intake of goats was similar in both groups, the higher energy costs of walking and the lower protein supply in the G group (see Table 5), can explain the differences in the yield and chemical composition of milk (Morand-Fehr et al., 2007).

Lactational effects can explain the different results in milk yield and chemical composition in the studied months (see Table 5). With regard to this factor, goats in late stage lactation are dominant in the experimental farm of this study and the number of milking goats decreased as the months progressed. Delgado-Pertíñez et al. (2003) reported a similar tendency related to seasonality of milk production in grazing goats.

The n-3 FA are considered the most important dietary FA for human health. Present human health recommendations include an optimal dietary n-6:n-3 FA ratio of 2.0–2.5 (MacRae et al., 2005). In the present study, although the PUFA content in milk from both groups was similar, the G group displayed higher percentages of n-3 FA and a lower n-6:n-3 ratio than I group (see Table 6). These results are not surprising in view of the contrasting diets, and could be a consequence of pasture intake and FA composition (see Table 4) by G managed does. Goats fed on rangeland (herbaceous plants, leaves and shrubs) (Tsiplakou et al., 2006) and on grass pasture (D'Urso et al., 2008) have been shown to have higher n-3 FA proportions in milk fat than animals fed diets based on concentrate. Also, because of potentially increased risks of atherogenicity of C16:0, fat with a high atherogenicity index is assumed to be detrimental to human health (Ulbricht and Southgate, 1991). In this study, only the thrombogenicity index was significantly lower in milk from animals in G group, when compared to milk from I group. Values of these indexes from studies of goats under grazing–based systems are not known, but nevertheless, the atherogenicity index values for both groups were lower than those reported for milk of sheep fed Mediterranean forages (Addis et al., 2005).

In the present study, forage type had no effect on the milk content of CLA, despite large differences in the content and intake of tannins. Tsiplakou et al. (2006) observed that goats feeding on Mediterranean shrublands did not increase the milk CLA contents compared to non or low grazing animals, while Vasta et al. (2008) found that goats grazing on condensed tannins-rich grass and Mediterranean shrubs (mainly *M. communis*, *P. lentiscus*, *Quercus ilex* and *Arbutus unedo*) had a higher CLA and *trans*-vaccenic acid content in milk fat than animals grazing only grass. In addition, vaccenic acid was accumulated in *in vitro* studies, while the total CLA isomers were not affected by tannins (Vasta et al., 2010). The lack of effect on milk shown in the present work agrees with the *in vitro* studies, and could be due to the effects of tannins on ruminal biohydrogenation. Hence, future studies are necessary to determine the metabolic effects of tannins on CLA production routes.

5. Conclusions

The nutritional quality (4 MJ/kg DM of net energy for lactation; 0.56 UFL; low protein supply) of the selected pastures could be considered low to intermediate. Small but significant differences were found on milk yield and milk fatty acids composition according to feeding treatment (grazing *vs*. concentrate-forage diet). Particularly, free-range grazing goats showed higher levels of n-3 FA and lower n6:n3 ratio, which are widely recognized as having beneficial effects on human health. Nevertheless, neither total CLA content nor its isomers were influenced by feeding treatment.

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2.8. SELECTION OF BROWSE SPECIES AND ENERGY BALANCE OF GOATS GRAZING ON FOREST UNDERSTORY VEGETATION IN DOÑANA NATURAL PARK (SW SPAIN)

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Selection of browse species and energy balance of goats grazing on forest understory vegetation in Doñana Natural Park (SW Spain)

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Energy is a major limiting factor in animal production. To plan the management of a herd in extensive grazing it is essential to monitor and understand the energy balances, in order to optimize fodder supplements. The objectives of this study were to evaluate the diet selection and to quantify the energy balance of domestic goats (Payoya goat) under semi-extensive production for the shrub understory of a pine forest in Doñana Natural Park, along the year. We used direct observation for estimating the distance traveled, speed, time consuming and diet composition. These data were transformed into kcal ingested and consumed by the goats daily in different seasons. The consumption of shrub species varied throughout the year, so grazing did not follow a fixed pattern. Goats tended to select the leaves and stem shoots in all the species, as well as the fruits of some species (dry and fleshy fruits). The results showed that grazing was able to meet the needs of goats in spring and summer but not in autumn and winter. These seasonal variations in the preferences of goats, detected by direct observation, suggest the potential utility of the understory of pine forest. So, direct observation may be an efficient and economic tool for knowing and controlling the energy balances of our grazing animals at all times. This will contribute to significant improvements in models for grazing systems, being essential in herd planning (supplements and inputs).

1. Introduction

Knowing the relationships between phytophaga and vegetation is essential for the rational management of the natural environment and for the conservation of biodiversity and landscapes (Anderson et al., 2000). In Europe, domestic herbivores, especially ruminants, have played an important role in the genesis and maintenance of landscapes (Emanuelsson, 2009). But, in the last few years, extensive livestock farming has suffered a sharp decrease in Europe, and it is now limited to very marginal areas. In fact, it is due to their marginalization that these areas are very well conserved and have gradually become areas with very strict conservationist statuses, which complicates the management of pastoral livestock exploitation.

Aside from health and labor issues, one of the problems with extensive livestock production is the need for adjusting the energy balance between displacement and consumption, and availability of food. Energy is the main limiting factor in animal production and its availability affects the adaptation of animals to their environments, their behavior and their feeding strategy (Lachica and Aguilera, 2003). Small ruminants adapt well to environmental conditions, as they are able to obtain an adequate diet even when there is a shortage of food.

There is plenty of literature regarding the energy needs of bovid cattle, and very little with respect to the needs of goat cattle, despite the latter provides an important source of income, particularly in countries of the entire Mediterranean basin. The interest offered by the exploitation of goat cattle, due to its possibilities of surviving in disadvantaged

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areas, acknowledged internationally by wide sectors, has been the subject, until relatively recent times, of a controversy about whether or not this exploitation is convenient in forest areas. The goat is, without question, the domestic ruminant that is most specialized in foraging; thereby, when managed properly it can become a very interesting tool in the conservation of Mediterranean forests and scrublands (Torrano and Valderrábano, 2005).

The main purpose of this study is to identify and characterize those aspects related to the feeding behavior of goat cattle (Payoya goats) under semi-extensive production that may affect the energy balance and its variations throughout the four seasons of the year.

2. Material and methods

2.1. Characteristics of the experimental area

The experiment was carried out in a forest (*Pinus pinea*) of 100 ha located in Doñana Natural Park (37°14′N; 6°20′W, SW Spain). The climate is Mediterranean: humid winters with mild temperatures and long and dry summers. The geological substratum is quarcitic sand, with an undulated relief. Soils are shallow, acidic and poor in nutrients. The average annual rainfall is 540 mm. The vegetation is a *Pinus pinea* forest with an average density of 217 trees/ha and an average tree diameter at breast height (DBH) of 26.92 cm. A few cork (*Quercus suber*) and holm (*Quercus ilex* subsp. *ballota*) oaks appear intermingled with the pines.

2.2. Availability and diet selectivity

The availability of a species will be subject to its consumption, so prior to the introduction of grazing, availability of each species was sampled using the point-intercept method (Daget and Poissonet, 1971). The overall availability of each species for the whole study area was calculated by weighting the abundance of the species in each transect by the proportion of the study area occupied by the species.

A herd of 350 adult female Payoya goats (average weight of 45 kg) was introduced into the study area at a stocking rate of 2.7 goats/ha/yr. These goats browse the entire area over three consecutive days and the fourth day is spent grazing outside the area (280 d/yr). The management may be considered to be semi-extensive, although in order to exploit the 100 ha in a uniform manner, the goats are closely controlled and moved by a shepherd.

In order to determine which shrub species are preferred by goat cattle, a method of direct observation was used. Direct observation is very efficient for determining the shrub species consumed the most desired parts, the relative preferences and variation throughout the year. Although modified, the procedure described by Meuret et al. (1986) was followed. In this experiment, the samplings were performed three consecutive days per month, with 10 min monitoring per goat from the beginning to the end of the grazing day in the understory of the forest. For every day, 10–15 goats were observed (30–45 goats/month). Goats were chosen at random, without repeating any individual in the same simple. The recorded variables for each goat and monitoring time were: number of species consumed, organ of the consumed species (leave, stem, flower, and fruit), number of bites per species, consumption time per species and displacement time (from plant to plant) (Mancilla-Leytón and Martín Vicente, 2011). Finally, the ratio between the proportion of a given food item in the consumer diet (r) and the proportion of the same food item in the environment (p) was used for quantifying plant selectivity.

2.3. Energy balance of grazing

In order to estimate the nutritional values of the shrub ingested by the goats, it was necessary to estimate the amount of each species ingested in each bite (g of dry mass (DM)). A manual simulation of the grazing was performed (100 samples/species), from which the weight per bite was obtained. Weight per mouthful was estimated by manual simulation of grazing (handplucked) adapted to a goat bite by imitating the behavior of goats (clipping material physically comparable to that selected by the animals). Dry mass consumed was determined on the basis of the number of bites and the weight of DM per bite. Once the amount ingested was known for each species, determination of the compositional analysis of samples of species was carried out according to Van Soest (1964). Metabolizable energy (ME) content of dietary samples was estimated using the following equation: ME (KJ/Kg DM)$=14.41-0.144\times$ADF (acid detergent fiber) (Kirchgessner and Kellner, 1977) (All values were taken from Llorens, 2010 and Schneider, 1991) (Table 1).

The distances traveled by each goat were recorded in a Pocket PC (Airis T620) through GPS system. Then, digital aerial photography was used to calculate the distances traveled, as well as the speed, during the grazing. In order to calculate the energy expenditure in locomotion, the equation proposed by Brockway and Boyne (1980) was used, although it was modified, resulting in the following equation: locomotion energy expenditure (J/kg $\times$ m)$=2.35-0.036\times V+0.00052\times V^2$, where V is the speed (m/min)= distance traveled/displacement time. The expenditure cost was estimated for an average animal, considering the mean weight of an adult goat to be approximately 45 kg. The slope was considered null for the study area.

Finally, the metabolizable energy requirement for the maintenance (MEm) of goats was obtained according: 424.2 kJ/kg BW0.75 (NRC, 1981).

2.4. Statistical analysis

The processing of the data was performed through the analysis of variance. In the case of consumption time and distance traveled, the data were transformed to ln in order to meet the requirements of the ANOVA (normal distribution and variance homogeneity). A posteriori comparative tests were applied in order to detect significant differences among the levels of a factor (Tukey test). To compare the number of species consumed, nonparametric tests (Kruskal–Wallis) and a posteriori *U*-Man–Whitney comparisons were used. A chi square (χ^2) statistic was used to analyze the relationship between food availability and consumption. SPSS 18.0 for windows

Table 1
Cover (%), acid detergent fiber (%) and Metabolizable energy (ME) of shrub species found in the understory of a Mediterranean pine forest in Doñana Natural Park, SW Spain. Initial values prior to the experimental entry of goats.

Group	Species	Cover (%)	ADF (% DM)	ME (MJ/Kg DM)*
Cistaceae	*Cistus crispus* (*cc*)	1.4	48.7	7.4
	Cistus ladanifer (*cd*)	3.7	45.2	7.9
	Cistus libanotis (*cl*)	3.9	44.5	8.0
	Cistus monspeliensis (*cm*)	1.1	43.8	8.1
	Cistus salvifolius (*cs*)	16.2	42.4	8.3
	Halimium calycinum (*hc*)	9.4	41.7	8.4
	Halimium halimifolium (*hh*)	11.5	39.7	8.7
Aromatic	*Helichrysum italicum* (*hi*)	1.3	53.5	6.7
	Lavandula stoechas (*ls*)	4.3	59.8	5.8
	Rosmarinus officinalis (*ro*)	17.1	59.1	5.9
	Thymus mastichina (*tm*)	5.3	58.4	6.0
Sclerophyllous	*Daphne gnidium* (*dg*)	0.9	57.7	6.1
	Erica scoparia (*es*)	6.6	45.2	7.9
	Myrtus communis (*mc*)	11.1	34.1	9.5
	Phillyrea angustifolia (*ph*)	1.5	36.2	9.2
	Pistacia lentiscus (*pl*)	8.1	24.4	10.9
	Quercus coccifera (*qc*)	5.9	34.8	9.4
Resprouters Aphyllous	*Genista hirsuta* (*ge*)	2.9	45.2	7.9
	Genista triacanthos (*ge*)	3.1	48.0	7.5
	Stauracanthus genistoides (*sg*)	1.5	52.2	6.9

* From Schneider (1991) and Llorens (2010)

(SPSS, Inc., Chicago, IL, USA) was used in all statistical analyses.

3. Results and discussion

3.1. Diet selection by goats and consumption time

The characteristics of the species prevalent in the study area prior to the introduction of the goats, and the groups formed by these species, are detailed in Table 1. The understory is covered with 70% shrubs, *Rosmarinus officinalis* (17.1%), *Cistus salvifolius* (16.2%), *Halimium halimifolium* (11.5%) being the more common species (Table 1).

During the monitoring, a great variety in the diet was observed, being in some cases a maximum of ten different species/goat/10 min of monitoring. The use of a large number of plants decreases the competitive advantages generated when some plants are not consumed, and improves the conservation of vegetation biodiversity (Villalba et al., 2002). Greater consumption diversity occurred in winter (5.06 ± 0.17 species) and spring (4.86 ± 0.15 species), which are the periods with greater food offers, and all the opposite in autumn (3.87 ± 0.14 species) and summer (3.88 ± 0.17 species) with less variety of species consumed ($P = 0.20$, K–W test).

Table 2 shows the percentage of the total bites/species/season recorded by direct observation of the goats. *Myrtus communis* was the most consumed species (40%), followed by *Cistus salvifolius* (17%) and *Halimium halimifolium* (13%). Other species were registered but at a much lower frequency (1–5%) (Table 2). The animals tended to select the leaves and stem shoots in all the species, as well as the fruits of *Cistus salvifolius*, *Myrtus communis*, *Pistacia lentiscus*, *Pinus pinea*, *Quercus coccifera*, *Q. suber* and *Q. ilex* subsp. *ballota*. Selection did not follow a fixed pattern: the consumption of shrub species varied throughout the year, but there was a preferential consumption toward specific species. The goat, in its food selection, seems to establish a balance between swiftness and best consumption quality (Duncan and Gordon, 1999), depending on the nutritional requirements of each moment (Villalba and Provenza, 1999). No close relationship was observed between the coverage of shrub species and their consumption. The most abundant species are not the most consumed ones ($\chi^2 = 201.51$ $\alpha = 0.01$) (Table 1, Fig. 1). Species such as *Cistus salvifolius*, *H. halimifolium* and *Myrtus communis* showed to be consumed proportionally above their abundance (Fig. 1).

Table 2
Percentages of the total bites/species/season obtained by the direct observation of goats grazing on the understory of a Mediterranean pine forest in Doñana Natural Park (SW Spain).

Species	Autumn	Winter	Spring	Summer
Cistus crispus	0.3	0.1	0.0	0.0
Cistus ladanifer	1.9	1.6	0.9	0.4
Cistus libanotis	1.5	0.4	0.6	0.6
Cistus monspeliensis	1.9	2.2	0.7	0.1
Cistus salvifolius	18.7	27.5	15.9	5.0
Dafne gnidium	–	–	–	–
Erica scoparia	1.9	2.6	7.5	7.9
Halimium calycinum	6.2	5.2	4.1	2.9
Halimium halimifolium	25.2	11.6	10.4	5.7
Helicrisum italicum	0.0	0.0	0.0	0.3
Lavandula stoechas	0.0	0.0	0.5	0.3
Myrtus communis	30.1	27.7	36.4	67.6
Phillyrea angustifolia	0.6	0.1	2.1	0.7
Pistacia lentiscus	7.3	1.9	4.2	4.1
Quercus coccifera	0.4	4.6	0.7	1.6
Rosmarinus officinalis	0.0	7.9	2.3	0.0
Thymus mastichina	–	–	–	–
Genista spp.	1.0	3.9	6.9	1.9
Stauracanthus genistoides	0.4	0.0	0.0	0.0

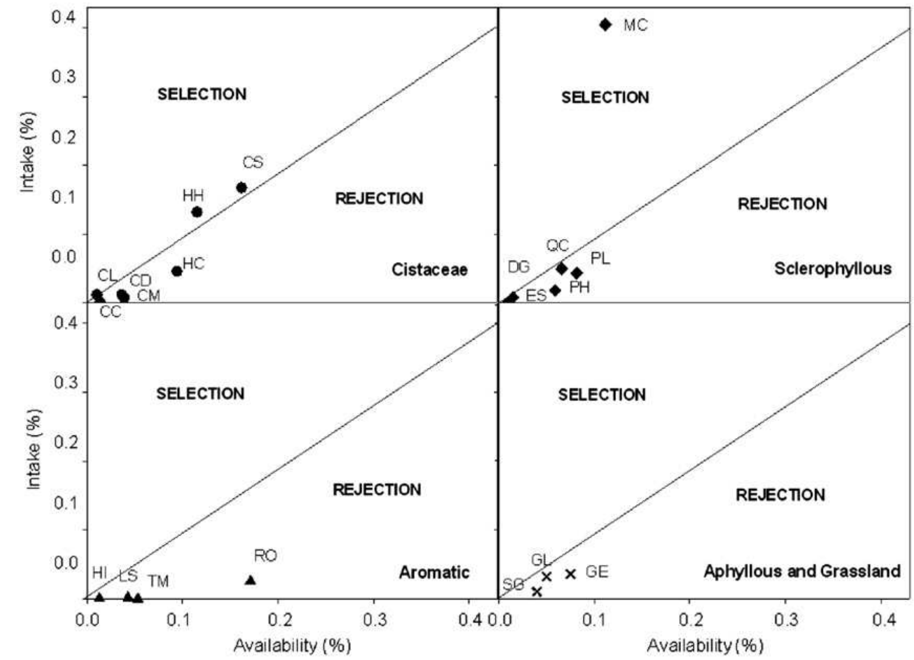

Fig. 1. Species selection *vs.* rejection on different shrubs species groups: Cistacea (*Cistus crispus* (CC), *C. libanotis* (CL), *C. ladanifer* (CD), *C. monpeliensis* (CM), *C. salvifolius* (CS), *Halimium calycinum* (HC) and *H. halimifolium* (HH)); Sclerophyllous (*Daphe gnidium* (DG), *Erica scoparia* (ES), *Myrtus communis* (MC), *Phillyrea angustifolia* (PH), *Pistacia lentiscus* (PL) and *Quercus coccifera* (QC)); Aromatic (*Helichrysum italicum* (HI), *Lavandula stoechas* (LS), *Rosmarinus officinalis* (RO) and *Thymus masticina* (TM)) and Aphyllous (*Stauracanthus genistoides* (SG) and *Genista* spp. (GE)).

Therefore, in their daily routes, the goats selected those species that pleased them most; consumption is not random (Martín Vicente et al., 2011). The time variations of food selection seem to be greatly related to the phenology of the species: in most of the study species, the probability of being consumed increases in the flowering and fructification stages; thereby we could speak of seasonal consumption (Mancilla-Leytón and Martín Vicente, 2011). For instance, *Helichrysum italicum* is consumed only in summer (Table 2), since the goats ate only its fruits and part of the scape. The consumption of *Rosmarinus officinalis* corresponds to the period of maximum flowering, between December and March (Table 2, Martín Vicente et al., 2011). The low consumption of Lamiaceae or Labiateae species (e.g. aromatic species: *Rosmarinus officnalis*, *Lavandula stoechas*, *Thymus mastichina*, etc) (Fig. 1) is assigned to their high oil content (Guillen and Cabo, 1996) which decreases their desirability; only rosemary is consumed, although solely in the period of maximum flowering. The fact that the nutritional value of thorny species decreases (*Ulex* spp., *Stauracanthus* spp. and *Genista* spp.) as the grazing season advances (Garín et al., 1996) could explain its lower consumption, coinciding its maximum consumption records in the phenophase of regrowth and flowering from January to April (Mancilla-Leytón and Martín Vicente, 2011).

3.2. Energy balance of grazing

Table 1 shows the nutritional data for the species in the study area. Among the species studied, *Pistacia lentiscus* and *Lavandula stoechas* had the highest and lowest nutritive values, respectively. The estimated metabolizable energy showed significant differences for the different seasons: the metabolizable energy intake was significantly lower in autumn and winter than in spring and summer (Fig. 2) ($F=4.26$; Tukey test $p<=0.05$).

This involves an average metabolizable energy intake, in our case (45 kg goats) of 7330.14 kJ/goat/d (NRC, 1981). Considering this parameter, we could state that the grazing met the average energy needs at 65.02% in autumn, 77.82% in winter, 112.42% in spring and 102% in summer (Fig. 2). These estimates should be useful in diet formulation as well as prediction of performance of goats. However, goats in the reports used in this study were not subjected to appreciable stress, such as environmental or nutritional. Moe et al. (1972) noted that factors such as pregnancy, nutrient imbalances, disease, tissue energy gain, environmental stress and exercise tend to increase energy expended for maintenance. Hence, application in some specific settings may require additional considerations. The data showed in this study were derived from experiments with animals at various stages of lactation and geographical locations. Animals acclimated to environments with high temperatures have lower maintenance requirements than those reared at lower temperatures (NRC, 2000; Tolkamp et al., 1994). There is increasing evidence that for goat and sheep, seasons affect MEm and feed intake differently, apart from the influence of temperature (Morand-Fehr and Doreau, 2001). Goats are well known for their adaptability to various agro-climatic conditions (low availability of vegetation in arid areas, feeds rich in fiber and low in nitrogen, lack of water, heat stress). Goats adapted to heat stress resist the negative impact of heat better than unadapted goat breeds (usually exotic breeds) by adapting their intake so as to reduce their heat production due to rumen fermentation by grazing at night and by increasing the number of short meals (Morand-Fehr, 2005). On the

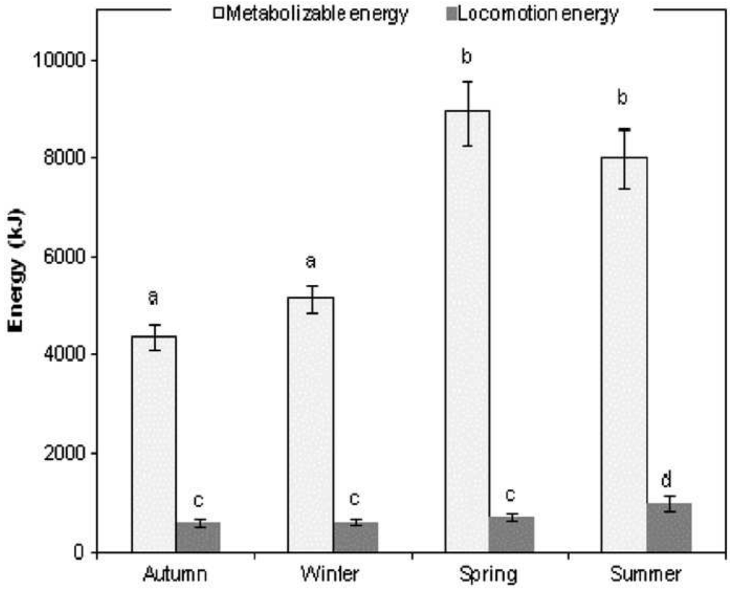

Fig. 2. Estimation of metabolizable energy and locomotion energy expenditure from direct observation of grazing in different seasons. Mean values ± SE. Different letters indicate significant differences (Tukey test, $P<0.05$).

other hand, goats well adapted to semi-arid zones do not reduce their feed intake when they are short of water for less than 48 h (Misra and Khub, 2002). Our goats (Payoya goat) are a native breed to the study area, so they are well adapted to the climatic conditions and vegetation of Mediterranean ecosystems, i.e. they are adapted to heat stress.

4. Conclusions

The increasing interest on goat cattle due to its capacity to survive and exploit vegetation motivates the study of its behavior in order to achieve the maximum use of those resources without endangering their continuity. The results obtained showed a good use of the goat in the study shrub understory. Grazing constituted an important part of the animals' diet, being able to meet their energy requirements in spring and summer. The locomotion expenditure involved an important factor to be considered, which increased in the seasons of lower food offer. Direct observation may be an efficient tool for knowing and controlling the energy balances of our grazing animals at all times, being essential in herd planning (supplements and inputs). Estimates of metabolizable energy requirement for maintenance seem useful as general describers of maintenance energy needs of goats continuously consuming diets at, near or above maintenance. Further research is necessary to develop energy requirement expressions for goats more appropriate for specific production or experimental conditions.

Conflict of interest statement

This paper is part of a research project entitled "Impact of goat grazing on the shrubland of a protected area: Doñana Natural Park", sponsored by the Council of the Environment of the region (Andalusia). We declare that we have no conflicts of interest with this organization.

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CAPÍTULO 3

Discusión General

Aunque los resultados obtenidos en esta Tesis Doctoral han sido ya discutidos parcialmente en sus apartados correspondientes, en este capítulo se identifican los principales resultados acerca de la capacidad de utilización de la vegetación por las cabras, el impacto del pastoreo sobre la vegetación y su contribución a las necesidades nutritivas de los animales a través de las siguientes preguntas.

¿Qué especies consumen las cabras?, ¿Cuándo las consumen?, ¿Por qué las consumen?

La observación directa del comportamiento animal permitió identificar el patrón de utilización de la vegetación. Las cabras mostraron una dieta muy diversa, pues consumieron, en algún momento del estudio, todas las especies de matorral presentes en el sotobosque (23 especies), excepto *Daphne gnidium* (especie tóxica, Benítez et al., 2011) y *Thymus mastichina* (especie altamente aromática, Guillén y Manzanos, 1998). No obstante, no consumieron todas las especies con la misma frecuencia y el consumo fue independiente de la abundancia, no habiéndose encontrado una relación directa consumo-abundancia; las especies más abundantes no fueron más consumidas (Aptdo. 2.1.). Las cabras mostraron ser herbívoros selectivos, consumiendo preferentemente *Myrtus communis, Cistus salvifolius, Halimium halimifolium, Pistacia lentiscus,* especies áfilas (*Genista hirsuta*, *G. triacanthos y Stauracanthus genistoides*) e hierba (fundamentalmente en primavera).

Además de la predilección por ciertas especies, los resultados pusieron de manifiesto variaciones temporales en el consumo (Aptdo. 2.1.). Estos cambios de apetencia por una especie a lo largo del año se han relacionado con la fenología de las plantas -el consumo aumenta en la floración y fructificación- (Barroso et al., 1995; Dannel y Bergström, 2002; Dziba et al., 2003). El valor nutricional y/o la concentración de defensas químicas parecen ser los factores que mejor explican las variaciones de consumo encontradas tanto entre especies como entre estaciones. Las cabras aumentaron la ingesta de especies con alto valor nutricional (contenido de N) y redujeron al mínimo la ingestión de especies con alta concentración de compuestos secundarios (tanino y fenoles) (Aptdo. 2.3).

Desde el punto de vista temporal, los resultados de los análisis nutricionales y metabolitos secundarios (Aptdo. 2.3) mostraron que, para cada especie, su consumo máximo coincidió con un descenso de la concentración de taninos y fenoles y un aumento de la concentración de nitrógeno (crecimiento vegetativo). Para *C. salvifolius* y *R. officinalis* este periodo fue el invierno, para *H. halimifolium* el otoño, para *P. angustifolia* y *P. lentiscus* la primavera y para *M. communis* el verano. Perevolotsky et al., (1993) encontraron que concentraciones de taninos superiores al 5% afectan negativamente el consumo y digestibilidad de arbustos mediterráneos. Los taninos precipitan las proteínas en el rumen (Jones y Mangan, 1977), reduciendo la degradación de la proteína y por lo tanto la absorción de los aminoácidos en el intestino delgado; esto se traduce en una baja digestibilidad y una reducción el consumo voluntario de estas especies.

La inducción de estos compuestos secundarios se produce después de los daños de la planta (Bennet y Wallsgrove, 1994; Baraza et al., 2009; Mithöfer y Boland, 2012). Esto podría explicar la apetencia cambiante interanual que se encontró en *C. salvifolius, H. halimifolium, P. angustifolia* y *P. lentiscus*, las cuales fueron muy consumidas durante el primer año, rechazadas el año siguiente, y nuevamente consumidas el tercer año.

¿Cuál es el efecto del pastoreo sobre la vegetación?

Después de cuatro años de pastoreo continuo, el biovolumen total de la vegetación así como el biovolumen de las distintas especies se redujo significativamente en comparación con el área no pastada. La diversidad se redujo debido a que la abundancia relativa de las especies cambió, pues las cabras no hicieron desaparecer ninguna especie (Aptdo. 2.2). Esta fuerte disminución de la abundancia de algunas especies no se relacionó con las preferencias que muestran las cabras por ellas; especies poco consumidas no aumentaron su abundancia en la zona pastada e incluso disminuyeron (posiblemente por pisoteo), y la abundancia de las especies muy consumidas no mostró importantes pérdidas (*M. communis*) (Aptdo. 2.1).

El papel de los herbívoros domésticos o silvestres en la reducción del riesgo de incendios ha sido bien documentado (Bond y Keeley 2005; Ruiz-Mirazo et al., 2011). Los herbívoros, al consumir plantas con caracteres morfológicos o químicos particulares, alteran las jerarquías competitivas y modifican directamente la estructura de la vegetación de manera que, o bien promueven o disminuyen el potencial riesgo de incendios forestales (Mutch 1970; Madany y West 1983; Bond y Midgley 1995, Blackmore y Vitousek 2000; Holmgren, 2002).

Los cambios encontrados en el biovolumen de las especies, principalmente de aquellas altamente inflamables como *C. ladanifer*, *Stauracanthus genistoides*, Genista spp., se correlacionaron positivamente con cambios en la inflamabilidad del matorral (Aptado. 2.2.). Tras cuatro años de pastoreo el biovolumen se redujo un 34% y el suelo desnudo aumentó un 51%, lo que implicó una reducción del 22% de la inflamabilidad del área de estudio. La propagación del fuego suele ser difícil cuando existe discontinuidad en la vegetación; bajo este escenario, incluso las especies altamente inflamables no presentan un gran peligro (Morvan y Dupuy, 2004).

En lo concerniente a la inflamabilidad, los cambios estructurales derivados del pastoreo fueron más importantes que los químicos. El efecto del pastoreo no modificó el índice de inflamabilidad de ninguna de las especies estudiadas, excepto en el caso de *M. communis,* donde los individuos pastados mostraron un índice de inflamabilidad inferior a los individuos no pastados (Aptdo. 2.4.).

Además de la reducción de la cubierta vegetal, el pastoreo puede ayudar a acelerar el ciclo de nutrientes, lo que indirectamente incrementa la producción vegetal. En las zonas pastadas, el pisoteo de las cabras y el enriquecimiento de nutrientes (deyecciones y orina) aumentaron significativamente la descomposición de la hojarasca de pino (Aptdo. 2.6). Esto es de particular importancia en los ecosistemas de suelos pobres en nutrientes, donde la disponibilidad de nutrientes limita fuertemente la producción (Wang et al., 2008). El aumento de la descomposición de las acículas reduce el riesgo de incendios forestales, ya que en los pinares, uno de los principales propagadores del fuego son las acículas acumuladas bajo el arbolado (Guijarro y Valette, 1995).

Al mismo tiempo que las deyecciones contribuyen a la fertilidad del suelo, pueden promover la dispersión de especies, ya que contienen un alto número de semillas de las especies que consumen las cabras. Las semillas de tres de las cuatro especies estudiadas, *C. salvifolius, H. halimifolium* y *M. communis*, sobrevivieron a la ingestión y el paso por el intestino de las cabras, siendo posteriormente capaces de germinar (Aptado. 2.5.). Las cabras se comportaron como depredadoras de las semillas de *M. communis* y *P. lentiscus* y como dispersoras de *C. salvifolius* y *H. halimifolium.* Recientemente se ha dado mucha importancia a la función de los mamíferos herbívoros como dispersores endozoócoros de semillas (Willson, 1993; Pakeman *et al*., 2002;

Myers *et al*., 2004). Varios estudios han demostrado la presencia de semillas en los excrementos de grandes herbívoros silvestres y domésticos (Sánchez y Peco, 2002; Manzano *et al*., 2005; Kuiters y Huiskes, 2010, De la Vega y Godínez-Álvarez, 2010). Por sus hábitos alimenticios, y particularmente por el ramoneo, las cabras domésticas pueden desempeñar la función de dispersión de semillas de manera similar a la de otros herbívoros silvestres o extintos (Janzen y Martin 1982; Martin y Klein 1984; Janzen 1986; Hansen et al. 2008) (Aptdo. 2.5.).

¿Es viable el pastoreo en extensivo?

Para poder usar el pastoreo en extensivo como una herramienta de gestión es necesario que la actividad sea "atractiva", para lo cual tiene que ser rentable. La rentabilidad se puede alcanzar por una reducción de los costos, o por un incremento de los beneficios. La reducción de los costos se puede conseguir reduciendo el costo de alimentación usando la vegetación natural, y el incremento de los beneficios incrementando la calidad del producto, en este caso de la leche.

Los resultados encontrados durante el primer año de pastoreo (Aptdo. 2.8.) pusieron de manifiesto que las cabras fueron capaces de cubrir una buena parte de sus necesidades energéticas de mantenimiento con el pastoreo. Lachica y Aguilera 2005 fijaron en 7330,14 KJ cabra^{-1} día^{-1} la ingesta de energía metabolizable que cubre las necesidades de mantenimiento del ganado caprino cualquiera que sea su situación fisiológica. A pesar que las especies presentes en el área de estudio y consumidas por las cabras son consideradas como especies de calidad nutricional baja-intermedia (contienen menos de 55% de materia orgánica digestible y 8% de proteína pura) (Leng, 1990), el pastoreo durante los meses de otoño e invierno cubrió el 60- 80 % de las necesidades energéticas y el de primavera y verano el 100% de las necesidades.

En cuanto a la producción y calidad de la leche, los resultados mostraron diferencias según el régimen de pastoreo (Aptdo. 2.7). La producción de leche de las cabras en régimen de pastoreo fue menor que la de las cabras estabuladas a base de piensos. Sin embargo, la leche de las cabras en pastoreo presentó un perfil graso más cardio-saludable (mayores niveles de ácido graso n-3 y menor ratio n6/n3). El ácido graso n-3 se considera uno de los ácidos graso más importante para la salud humana. La potencialidad de las características en la producción y la calidad de la leche derivada del aprovechamiento vegetal, supone una diferenciación en los mercados y un mayor valor añadido de la leche y los productos derivados que en la actualidad son cada vez más demandados por los consumidores.

¿Cuál es el futuro?

Durante miles de años, el ganado caprino ha sido utilizado con fines económicos. La producción de carne y leche de cabra sigue siendo la principal actividad económica en muchas zonas rurales (Rosa García et al., 2012). Sin embargo en la última década, el pastoreo también ha adquirido una nueva dimensión: la dimensión ambiental (Concepción et al. 2008; Reinhardt et al. 2008). Al contrario del pensamiento generalizado de que el efecto de las cabras es negativo, los resultados encontrados en esta Tesis Doctoral apoyan que el pastoreo, con una gestión y una carga adecuada, puede ser adoptado como práctica de conservación.

La reducción de la cubierta vegetal permite la creación de nuevos espacios de regeneración, que permiten la persistencia de las especies que habían sido suprimidas al aumentar la cobertura de especies arbustivas como consecuencia de la exclusión de grandes herbívoros (Grime, 1979; Noy-Meir, 1995). Esto provoca una mejora de pastos con el consiguiente aumento de alimento disponible para otros herbívoros como el conejo, alimento de especies amenazadas como el lince ibérico (*Lynxpardinus*), el águila imperial (*Aquila adalberti*) y el búho real (*Bubo bubo*), que crían en el área de estudio.

Hace 50 años Montserrat (1964) vaticinaba que: "*La futura ciencia agronómica será ecología y de la más compleja; una ecología de comunidades con plantas-animales en su ambiente y bajo la acción humana*". De momento parece que el futuro sigue siendo futuro. Realmente estamos como reza el cante: "*sentaito en la escalera esperando al porvenir y el porvenir que no llega*".

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CAPÍTULO 4

Conclusiones

De los resultados obtenidos durante el desarrollo de la presente Tesis Doctoral podemos extraer las siguientes conclusiones:

1. Las cabras pastaron una gran variedad de especies arbustivas, casi todas las especies presentes en el sotobosque del pinar, así como el sustrato herbáceo, pero con distinta intensidad y temporalidad.

2. Las cabras mostraron una dieta muy selectiva, el consumo no fue al azar ni estuvo relacionado con la abundancia de las especies. Durante el pastoreo, las cabras aumentaron la ingesta de especies de alto valor nutricional (contenido en Nitrógeno) y redujeron al mínimo los compuestos secundarios (tanino y fenol).

3. El pastoreo del sotobosque arbustivo disminuyó el biovolumen del área de estudio e indujo cambios en la abundancia de especies, sin disminuir la riqueza de las mismas.

4. El pastoreo modificó la estructura de los individuos: disminuyó la biomasa de hojas y ramas finas, alterando así sus características físicas, y por consiguiente modificó el riesgo de incendio.

5. Pocas semillas fueron recuperadas después de la ingestión en las cabras (30%). Sin embargo las semillas de tres de las cuatro especies estudiadas (*C. salvifolius, H. halimifolium* y *M. communis*) sobrevivieron la ingestión y paso por el intestino de las cabras, germinando posteriormente. Por lo tanto, el ganado caprino puede ser un potencial dispersor de las semillas de algunas de las especies que pasta.

6. El pastoreo aumentó la velocidad de descomposición de las acículas de *P. pinea,* promoviendo la incorporación de nitrógeno en el sistema. Las altas tasas de descomposición registradas en el área pastada pueden contribuir, sin duda, a cambios en la composición de la vegetación (favoreciendo la producción por aumento del ciclo de nutrientes).

7. Se encontraron pequeñas, pero significativas diferencias en la producción de la leche y la composición de ácidos grasos en función de la alimentación (pastoreo *vs* concentrado-forraje). La leche de las cabras en pastoreo mostró mayores niveles de ácidos grasos n-3 y menor ratio n6/n3. Estos ácidos grasos presentan efectos beneficiosos sobre la salud humana.

8. El pastoreo constituyó una parte muy importante de la dieta del ganado, y pudo satisfacer completamente sus necesidades de energía en primavera y verano. El gasto de locomoción se mostró como un importante factor a tener en cuenta en el balance energético; el gasto en locomoción fue mayor en las estaciones de menor disponibilidad de alimentos.

The main conclusions that can be drawn based on the results obtained during the development of this PhD thesis are the following:

1. Goats released in the pine forest fed on a great variety of shrub species, consuming nearly all species present in the understory with different intensity and temporality. They also grazed the grassland.

2. Goats are selective "mixed-grazers". They showed a selective feeding behaviour, since they did not browse species according to their abundance. In their daily foraging, goats selected species according to preference, and consumption was not random. During grazing, goats increased their nutritional value intake (Nitrogen content) while minimizing secondary compounds (tannin and phenol).

3. Monitoring of the shrub understory has shown the positive impact of goat grazing: the resulting decrease in the quantity of total phytovolume (easily combustible vegetation) induces changes in species diversity without diminishing species richness. This calls for a reduction in plant homogeneity and the risk of forest fires.

4. Grazing changed the structure of foraged shrubs and decreased the biomass of leaves and fine twigs, thus altering their physical characteristics, and perhaps modifying the risk of wildfires (catch and spread of fire).

5. Few seeds passed through the goats guts (30%). Nevertheless, goats can act as potential seed dispersals of the plants they eat; seeds from three of the four Mediterranean species studied (*C. salvifolius*, *H. halimifolium* and *M. communis*), survived ingestion, goat gut passage and germinated afterwards.

6. Grazing increased the decomposition rate of *P. pinea* needles, promoting the incorporation of Nitrogen into the system. The higher decomposition rates recorded in the grazed area could certainly contribute to changes in vegetation composition, creating a positive feedback (plant-soil) in these areas.

7. Small but significant differences were found on goat milk yield and milk FA composition according to feeding treatment (grazing *vs.* concentrate-forage diet). Free-range grazing goats showed higher levels of n-3 FA and lower n6:n3 ratios, their beneficial effects on human health being widely recognized.

8. Grazing constituted an important part of the goats´ diet, enabling them to meet their energy requirements in spring and summer. The locomotion expenditure represented an important factor to acknowledge, increasing during the seasons of lower food availability.

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León Tolstoi