Universitat Autònoma de Barcelona

EL PASTOREO COMO HERRAMIENTA DE PREVENCIÓN DE INCENDIOS FORESTALES EN EL BOSQUE MEDITERRÁNEO

JAVIER CIPRIAN PAREJA LOAYZA

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Tesis doctoral

Presentada por:

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Octubre de 2020

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Departament de Ciència Animal i dels Aliments

CONCYTEC 50 años

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CERTIFIQUEN:

Que **Javier Ciprian Pareja Loayza** ha realitzat sota la seva direcció el treball de recerca: **"El pastoreo como herramienta de prevención de incendios forestales en el bosque mediterráneo"**

per a obtenir el grau de Doctor per la Universitat Autònoma de Barcelona.

Que aquest treball s'ha dut a terme al Departament de Ciència Animal i dels Aliments de la Facultat de Veterinària de la Universitat Autònoma de Barcelona (UAB).

Bellaterra, octubre de 2020

Dr. Jordi Bartolomé Firella **Dra. Carme Espunya Prat**

Dra. Elena Baraza Ruíz

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Resumen

La cuenca Mediterránea, por su ubicación geográfica, presenta una vegetación adaptada a una pronunciada estacionalidad climática y a perturbaciones recurrentes como el fuego y el herbivorismo. El reciente cambio global, fenómeno que incluye el cambio climático y los cambios en los usos del suelo, ha favorecido el incremento de las superficies leñosas, con el consiguiente aumento en la intensidad de los incendios forestales.

Durante las últimas décadas se ha intentado promover el pastoreo en los sotobosques mediterráneos como estrategia para disminuir el riesgo de incendios. En esta línea, especies típicamente ramoneadoras, como la cabra doméstica (*Capra hircus* L.), han sido utilizadas en la mayor parte de iniciativas y ensayos obteniendo, en general, resultados satisfactorios. Sin embargo, la ganadería extensiva de caprino es un sector en claro declive, por lo que es necesario ensayar con otras especies ganaderas.

En el primer capítulo de esta tesis se presenta un estudio realizado con ganado bovino (*Bos taurus*), de raza *Bruna dels Pirineus*. Este estudio se realizó en pinares de pino carrasco (*Pinus halepensis*) de las inmediaciones del Parque Natural de la Montaña de Montserrat. Se evaluó el efecto de una carga ganadera elevada, sin suplementación y durante un periodo corto de tiempo. Los resultados mostraron que el ganado adaptó sus hábitos alimenticios a una dieta más leñosa que incluye taxones potencialmente inflamables, pero con algunos efectos perjudiciales sobre el estado de salud de los animales. Por ello, se concluyó que el ganado bovino requiere alimentación suplementaria para controlar la vegetación leñosa durante largos períodos de tiempo.

Otra opción para el control del sotobosque mediterráneo la constituyen los grandes herbívoros salvajes, que en las últimas décadas han incrementado notablemente sus poblaciones. Sin embargo, las dificultades de manejo para organizar un pastoreo prescrito son muy grandes y por ello, las iniciativas en este sentido son muy escasas. Una opción intermedia se podría encontrar en las poblaciones de ganado asilvestrado, las cuales también han aumentado en los últimos años, como consecuencia del abandono de la actividad ganadera tradicional. En este sentido, el segundo capítulo de la tesis aborda el papel que pueden jugar las cabras asilvestradas en el mantenimiento de franjas cortafuego. El estudio se realizó en la isla de Mallorca, donde las sierras del norte de la isla albergan una importante población de estos animales, siendo prácticamente el único gran herbívoro de la isla que puede desempeñar esta función. Los resultados mostraron que las áreas cortafuego ejercen de por sí un efecto atrayente respecto al bosque colindante y que las cabras consiguen reducir la biomasa herbácea. Este efecto se potenció con la implementación de puntos de agua y sal, consiguiendo reducir el fitovolumen de muchas especies leñosas sin afectar la biodiversidad a corto o medio plazo. Se concluyó que una gestión estratégica de los animales asilvestrados, dirigida a áreas cortafuegos, podría contribuir no sólo a reducir el riesgo de incendios sino también a distribuir estos animales hacia los bosques, evitando así su dispersión hacia lugares conflictivos como carreteras, residencias, campos agrícolas y jardines.

La gestión del ganado, como alternativa o complemento al desbroce mecánico para el control de la vegetación, requiere de un conocimiento profundo de las especies que forman parte de la dieta de los animales. Una de las técnicas que más información proporciona en este sentido es el análisis microhistológico de las heces. Sin embargo, se trata de un procedimiento que requiere mucho tiempo de capacitación y análisis. Esta técnica podría sustituirse por otras, actualmente más rápidas, como puede ser el análisis molecular. Así pues, el tercer capítulo de la tesis está dedicado a comparar ambas técnicas aplicadas a la composición de la dieta de los animales estudiados en los capítulos anteriores, es decir, vacas y cabras asilvestradas. Los resultados mostraron que ambas técnicas detectaron un número similar de componentes vegetales en las heces de ambos animales. Se concluyó que la metodología utilizada en el análisis molecular con electroforesis capilar (PCR-CE) es un método rápido para detectar los diferentes componentes vegetales en las heces de herbívoros. Sin embargo, no puede considerarse como una alternativa al análisis microhistológico, sino como un método complementario, ya que ambas técnicas pueden detectar algunos taxones que no son detectados por la otra técnica. Además, el análisis microhistológico permite detectar la presencia de los diferentes taxones, y a la vez, permite obtener datos cuantitativos de la composición de la dieta vegetal.

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Introducción

El ecosistema mediterráneo

El clima mediterráneo se caracteriza por ser muy estacional y con una elevada variabilidad interanual. Presenta dos estaciones lluviosas (primavera y otoño) y dos estaciones secas (verano e invierno) y se clasifica en el grupo de climas subtropicales secos de Köppen-Trewartha (Belda et al., 2014). En verano coinciden las altas temperaturas con la baja precipitación, fenómeno que propicia los incendios forestales. El bioma mediterráneo se sitúa entre los 30º y 45º de latitud en ambos hemisferios y en esta franja se distribuyen las cinco regiones mediterráneas del mundo (Figura 1), ocupando el 1% del total de la superficie terrestre.

Figura 1. Ubicación de las cinco regiones del bioma mediterráneo. Fuente: Terpsichores - Trabajo propio, CC BY-SA 3.0. https://commons.wikimedia.org/w/index.php?curid=22441322

En la cuenca mediterránea, la mayor parte de la Península Ibérica presenta clima mediterráneo. En la Figura 2 se muestra el diagrama ombrotérmico de dos zonas mediterráneas. La imagen A, de la isla de Mallorca (municipio de Calvià), y la imagen B a Barcelona (parque de Montserrat) (*Climate-data.org*, 2020), donde se aprecia una marcada estacionalidad, con temperatura altas en verano (julio y agosto), y mínimas en invierno (diciembre a febrero). Las precipitaciones escasas en verano y con picos en primavera y otoño (septiembre y octubre).

Los bosques mediterráneos se caracterizan por una densidad variable, altura moderada (10-15 m) y alta diversidad en el estrato arbustivo (Valette, 1992). Las principales especies arbóreas esclerófilas de estos bosques pertenecen al género *Quercus,* siendo las más abundantes en la Península Ibérica: *Quercus ilex*, *Quercus rotundifolia*, *Quercus* *suber* y *Quercus coccifera*. Estos bosques, en las zonas más húmedas y templadas, presentan un sotobosque con arbustos con hojas de tipo lauroide, enteras, perennes y coriáceas, tales como, *Arbutus, Viburnum, Myrtus, Phyllirea, Laurus* y *Prunus* (Feranec et al., 2010). También suelen presentar un rico estrato de plantas trepadoras como las de los géneros *Clematis, Lonicera, Rubia o Smilax*.

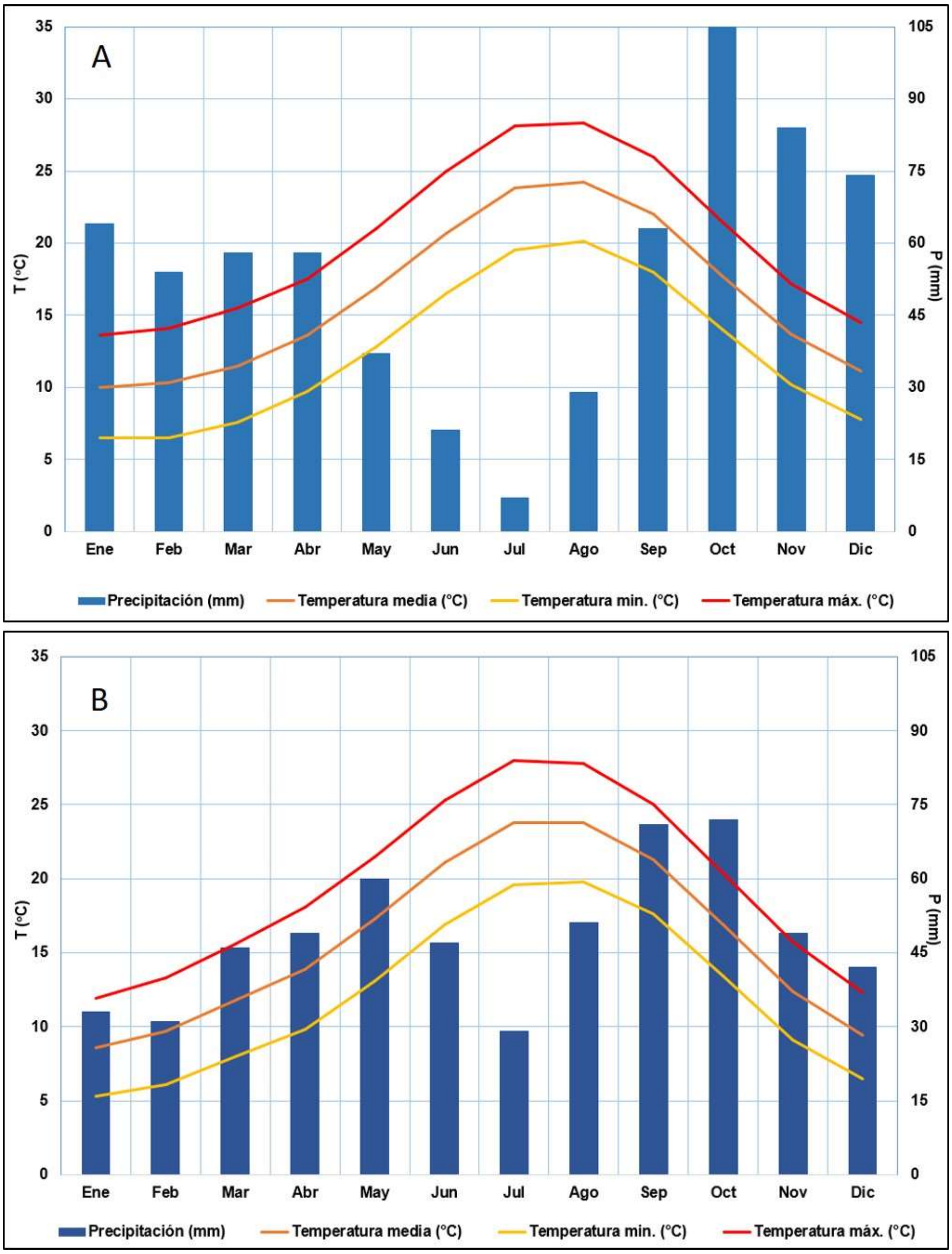

Figura 2. Diagrama ombrotérmico promedio del 2019. En la imagen (A) del Municipio de Calvià en la Isla de Mallorca y (B) del parque de Montserrat en Barcelona. *Fuente: (*Climate-data.org*, 2020)*

Los bosques mediterráneos en las zonas llanas son escasos, ya que estas tradicionalmente son aprovechadas por el hombre con fines agropecuarios (Petanidou et al., 2008), sobre todo en la Península Ibérica. Son comunes las formaciones adehesadas, bosques aclarados de árboles adultos, generalmente del género *Quercus* (Cubera & Moreno, 2007). En estas formaciones, el sotobosque ha sido eliminado mediante tala, fuegos controlados, roturaciones, ramoneo y pastoreo del ganado.

La cuenca mediterránea se caracteriza también por la formación de matorrales altos, en algunos casos, más abundantes que el propio bosque. Además de encinas como *Quercus ilex, Quercus rotundifolia,* muchas veces arbustivas, se encuentran otras fagáceas esclerófilas como *Quercus coccifera,* en la parte occidental o *Quercus calliprinos,* en la parte oriental (Cerdà, 1997). En ambientes más térmicos aparecen *Olea europaea*, *Pistacia lentiscus, Ceratonia siliqua* o *Chamaerops humilis*. Estas especies suelen formar parte del sotobosque de los pinares de *Pinus halepensis* (Nadal-Romero et al., 2018).

El paisaje mediterráneo actual es el resultado de procesos que interactúan entre sí a lo largo del tiempo, tanto de índole natural (relieve, clima, etc.), como de índole antropogénica, como son los distintos usos del suelo, ya sea con finalidad agrícola o ganadera (Ruiz-Mirazo et al., 2007).

Las perturbaciones ecológicas recurrentes: fuego y herbivorismo

Se entiende como perturbación a cualquier evento que altere la estructura de un ecosistema, comunidad o población, modificando la disponibilidad de recursos o el ambiente físico (Pickett & Cadenasso, 2009). Las perturbaciones pueden tener un origen abiótico (condiciones climáticas), biótico (biocombustible, plagas o herbivorismo) o incluir ambos componentes. Además las perturbaciones pueden ser temporales o continuas, pudiendo condicionar la evolución natural del ecosistema en algunos casos (Turner, 2010). Las perturbaciones ambientales son uno de los principales motores de cambio de poblaciones, comunidades y ecosistemas. Aunque tales cambios son inherentes al funcionamiento de los sistemas naturales, el ser humano los ha incrementado y acelerado (Chapin et al., 2008). En el contexto actual de cambio global, comprender los efectos de las perturbaciones naturales y antrópicas en la dinámica poblacional es fundamental para la conservación de la biodiversidad.

Países europeos como Francia, Italia y España vienen experimentando un incremento de su cubierta leñosa mientras que los pastizales y las tierras agrícolas han disminuido considerablemente (Debussche et al., 1999). Por ejemplo, Francia pasó en 18 años (1978-1996) de tener un 14% de cubierta de bosque a un 27% (Ciesla, 1996). En España, según el informe del sector forestal, la cubierta vegetal leñosa maderable se ha duplicado, pasando de 456,72 millones de m^3, en 1975, a 927,8 m^3 en 2010 (Montero & Serrada, 2013). La dinámica sucesional de la vegetación que se desarrolla después del abandono agrícola es bastante predecible, incluso cuando se imponen nuevas perturbaciones a lo largo del curso de la sucesión (Arroyo-Rodríguez et al., 2017). En ese aspecto, el fuego como proceso ecológico forma parte de los ecosistemas terrestres ya desde el momento en que la atmósfera pudo alcanzar una determinada concentración de oxígeno para generar una combustión eficiente (Wildman et al., 2004). En la cuenca mediterránea la relación fuego-hombre empezó a ser notoria y generalizada a partir del Neolítico, convirtiéndose en un factor relevante en la dinámica vegetal desde hace más de 5.000 años (Debussche et al., 1999). Muchos procesos ecológicos en el Mediterráneo están relacionados con los incendios, como por ejemplo la regeneración vegetal, sea mediante el rebrote de individuos sobrevivientes o bien a través de la germinación de semillas. Ambas estrategias pueden ser utilizadas con éxito por distintas especies para conseguir ventajas ecológicas después del fuego. Así pues, los incendios forestales a nivel global son considerados como la perturbación ecológica de mayor relevancia, con una distribución temporal y espacial muy variable (Turner, 2010).

Los incendios forestales y sus efectos en diferentes ecosistemas terrestres han centrado la atención de muchos científicos en las últimas décadas (Archibald et al., 2013; Pausas & Ribeiro, 2013). Las tecnologías de información geográfica, como la teledetección, se han convertido en potentes herramientas que permiten conocer los patrones temporales y espaciales de los incendios (Archibald et al., 2013; Vicente-Serrano et al., 2004).

Además, las alteraciones en el régimen de incendios pueden dar lugar a cambios en la estructura del paisaje {Formatting Citation}. Esto se debe a que los incendios determinan las especies dominantes en el territorio (Turner, 2010). Por ejemplo se ha demostrado que los pinares (*Pinus* sp.), *Erica* sp., *Quercus* sp. y *Arbutus unedo* tienen una mayor capacidad regenerativa post incendio, estructurando cambios en la dominancia de especies y en la estructura del ecosistema y moldeando la estructura vegetal (Lloret, 2004).

Otra de las perturbaciones históricas en la cuenca mediterránea es la presión ganadera, ejercida sobre todo por los rebaños domésticos desde el periodo Neolítico. Sin embargo, en la actualidad, la actividad ganadera de carácter extensivo se ha reducido notablemente en los países del norte de la cuenca (Battaglini et al., 2014). Esto ha facilitado el incremento de las poblaciones de ungulados silvestres como el ciervo (*Cervus elaphus*), el gamo (*Dama dama*), el corzo (*Capreolus capreolus*), el sarrio (*Rupicapra pyrenaica*), la cabra montesa (*Capra pyrenaica hispanica*), el muflón (*Ovis gmelini)* y la cabra doméstica asilvestrada (*Capra hircus*). Estas poblaciones continúan ejerciendo un control sobre la vegetación, ya sea modificando su capacidad de rebrote (Zamora et al., 2004) o limitando su regeneración (Piñero & Luengo, 1992).

En la cuenca mediterránea, los pastos son muy leñosos, debido a las adaptaciones de la vegetación a la sequía estival, a los incendios y al herbivorismo. En estas condiciones, el ramoneo de especies leñosas y arbustivas se convierte en una práctica común en la mayoría de grandes herbívoros (Zamora et al., 2004). El ramoneo, en general, provoca un retraso en el crecimiento de las plantas, generando una adaptación en su desarrollo vegetativo (Fernández et al., 2017). Asimismo, la aplicación de perturbaciones intermedias en frecuencia e intensidad, como el pastoreo, provocan que la diversidad de especies se maximice, modelando la composición y estructura de las comunidades vegetales y aumentando la heterogeneidad de la distribución espacial de la vegetación, puesto que se disminuye la abundancia de las especies más competitivas generándose nuevos nichos para las especies menos competidoras (Zamora et al., 2004). En este sentido, un pastoreo gestionado puede convertirse en una herramienta de control de la vegetación, ya que un pastoreo prescrito permitiría disminuir el riesgo de incendios forestales (Lovreglio et al., 2014; Xanthopoulos et al., 2006).

El abandono de las actividades silvoagropecuarias tradicionales

España, al igual que otros países europeos de la cuenca mediterránea, ha registrado en los últimos tiempos cambios muy relevantes en el uso del territorio. El despoblamiento y envejecimiento de la población rural se han traducido en un abandono de muchas prácticas silvoagropecuarias (Domenech et al., 2018; Vicente-Serrano et al., 2004). La producción agraria se ha especializado e intensificado, abandonando las menos rentables para concentrarse en las zonas más fértiles y mejor conectadas (Hazell & Wood, 2008). Por otro lado, también ha aumentado el número y la extensión de espacios naturales protegidos, que actualmente representan cerca de un 6% de la superficie nacional (Ángel et al., 2005). Estos cambios han significado un aumento de la matorralización de las superficies boscosas, con el consiguiente incremento de biomasa combustible y una homogenización de los paisajes (Lasanta-Martínez et al., 2005). Además hay que añadir el aumento de los usos recreativos del bosque y el crecimiento continuo de la interfaz urbano/forestal (Fernández Leiceaga, 2000; MacDonald et al., 2000; Palang et al., 2005), que también conllevan a un incremento en el riesgo de incendios. En la Figura 3 se muestra como en menos de cuatro décadas, el área agrícola en España ha disminuido en un 15%, a la vez que el área forestal ha aumentado en un 35%. Resulta evidente que la rapidez de esta sucesión depende de factores relacionados con las características del suelo, el clima, la proximidad a otras formaciones vegetales, que actúan como fuente de propágulos, o al tipo de actividad agrícola previa, entre otros (Sluiter & De Jong, 2007).

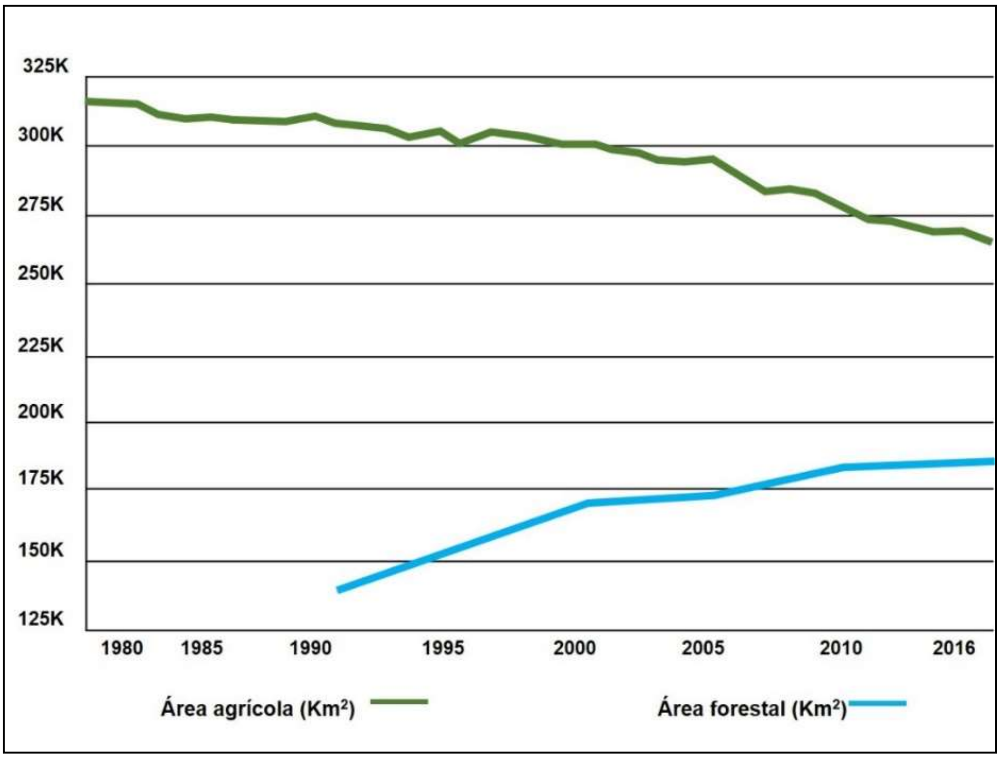

Figura 3. Evolución del área agrícola y forestal en España desde 1980 hasta 2016. Fuente: Anuario de Estadística, 2017. MAGRAMA

El incremento de los fuegos forestales

En Europa, el 90% de los incendios forestales se producen en los países del sur, Grecia, Italia, España y Portugal. El territorio español cada vez es más propicio a la recurrencia de los incendios (Lindner et al., 2010; San-Miguel-Ayanz et al., 2013). En la Figura 4, se muestra la evolución de los incendios forestales en España durante los últimos 20 años, así como la superficie afectada. Se considera que la principal causa de los incendios forestales es la actividad humana (Archibald et al., 2013; Lloret, 2004). Sin embargo, el cambio climático también contribuye a fomentar estos episodios. La temperatura media en España ha aumentado en casi 1 ºC en los últimos 30 años (Figura 5), y ésto trae como consecuencia veranos más secos y cálidos, apreciándose una relación positiva entre veranos más cálidos e incremento de incendios (Pausas & Fernández-Muñoz, 2012).

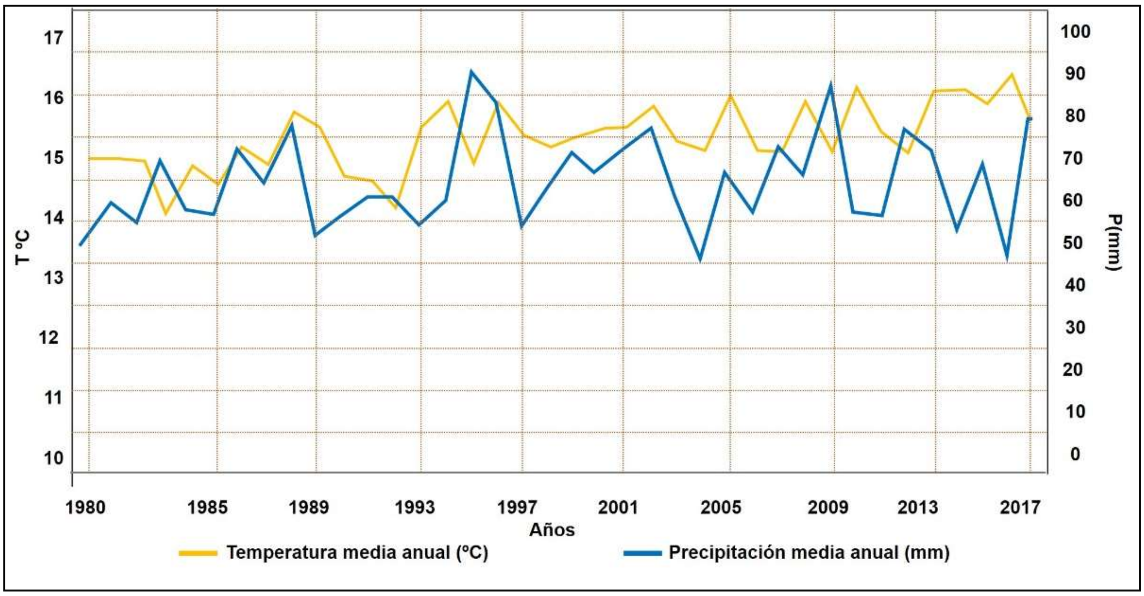

Figura 4. Evolución de la temperatura y humedad España 1980-2017. Fuente: Reporte Agencia Estatal de Meteorología - AEMET 2017

Es de vital importancia trabajar en la prevención para reducir los incendios de origen antrópico (Zamora et al., 2004), ya sea mediante la educación (persuasión, conciliación, etc.), o mediante las redes de franjas cortafuegos y el control del biocombustible (Raftoyannis et al., 2014). No obstante, existen problemas estructurales difíciles de resolver, como son las zonas rurales abandonadas, el incremento de las áreas de bosque y matorral, la poca gestión de los animales silvestres, etc. Gestionar todos estos factores supone inversiones elevadas para espacios cuya aportación monetaria es prácticamente nula a las administraciones del estado (Pausas & Fernández-Muñoz, 2012). Por ello, es urgente un cambio de percepción de las zonas forestales y en las políticas que en ellas se aplican.

Las predicciones climáticas no dibujan en la cuenca mediterránea un panorama optimista (Valladares et al., 2005). La recurrencia de sequías más severas, los mayores intervalos entre lluvias y las condiciones más cálidas y áridas, fruto del aumento de temperaturas, plantean un panorama propicio para una propagación más fácil del fuego

(Turner, 2010). Los tres factores comentados, junto a la mayor o menor eficacia en la extinción de los incendios, han contribuido a definir el escenario cambiante en que se han desarrollado los incendios forestales en las últimas décadas (Franquesa et al., 2017).

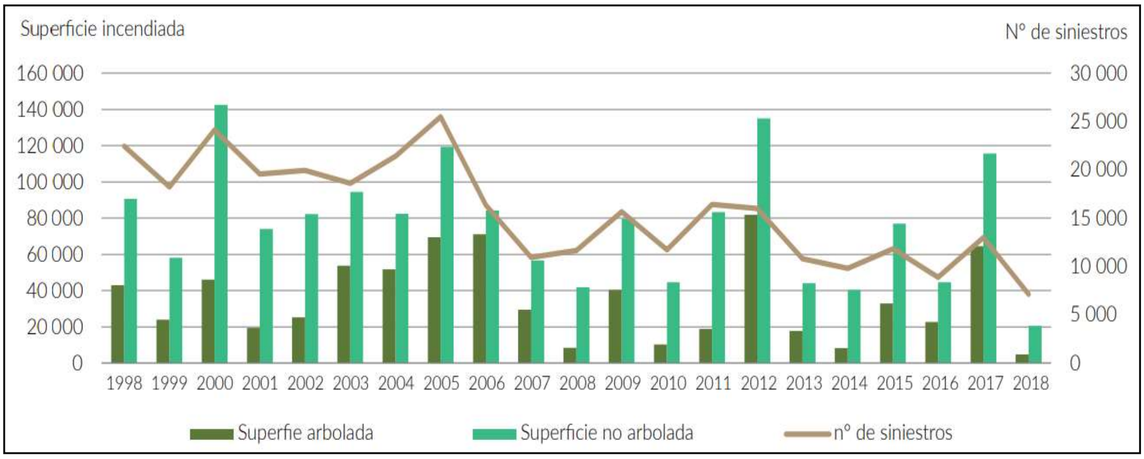

Figura 5. Evolución de los incendios forestales y superficie incendiada en España. Fuente: Informe de incendios forestales en España 2018- MAPA

La prevención de los incendios forestales

A pesar del incremento de las condiciones climáticas favorables a los incendios forestales y su propagación, en España y en los demás países del sur de Europa, la superficie quemada ha disminuido durante los últimos años. Esto se atribuye a la mejora en la eficiencia de actuaciones frente al fuego (Moreno et al., 2015). Sin embargo, las proyecciones para los próximos decenios son alarmantes respecto al cambio climático global, así como el cambio de uso de suelo (Moriondo et al., 2006; Pausas & Ribeiro, 2013). Ante este escenario, la prevención de las igniciones (básicamente antrópicas) requiere de campañas de concienciación, educación ambiental y aplicación de las normativas sobre el control del combustible (Ruiz-Mirazo, 2007; San-Miguel-Ayanz et al., 2013). En este aspecto se vienen desarrollando un conjunto de actuaciones, tales como la apertura y aclareo de franjas cortafuego (Velamazán et al., 2018), el acceso de los medios de extinción a las fuentes de agua, ya sea de forma aérea o terrestre, el uso de ganado domestico extensivo o silvestre para disminución de la biomasa forestal (Jáuregui et al., 2007; Mancilla-Leytón et al., 2013), o el uso del fuego prescrito (Piñol et al., 2005).

- *Las redes de cortafuegos*

Dentro de las acciones para la prevención de incendios se encuentra la gestión del combustible, ya que éste proporciona la energía necesaria para que un incendio se propague (Raftoyannis et al., 2014; San-Miguel-Ayanz et al., 2013). En condiciones apropiadas, todas las especies de plantas pueden ser combustible para el fuego, por ello, al disminuir la cubierta vegetal, disminuye el material combustible y se posibilita una respuesta rápida, eficaz y segura de los medios de extinción frente a los incendios (Cui et al., 2019). Las franjas cortafuegos (Figura 6) pretenden minimizar la superficie potencial de afectación en caso de un incendio forestal (Xanthopoulos et al., 2006).

Figura 6. Franja cortafuegos en la sierra de Na Burguesa, perteneciente a la red de cortafuegos de las Islas Baleares-España. Fuente: imagen propia

Estas redes de franjas cortafuego suelen estar distribuidas en los cambios de rasante u orientación dentro de los bosques, delimitando las carreteras, pistas y caminos, o debajo de los cables eléctricos. Su efectividad en la contención del incendio está relacionada con su dimensión y con la biomasa existente. En este sentido, es importante su mantenimiento, ya sea de forma mecánica o manual (Aguirre, 2001). Otra forma de mantener las franjas cortafuego, y en general el sotobosque, con poco combustible es mediante el pastoreo controlado o mediante la atracción de herbívoros silvestres (Mancilla-Leytón et al., 2014; Velamazán et al., 2018), así como el uso de las quemas controladas (Xanthopoulos et al., 2006).

- *El pastoreo como herramienta de control de la vegetación*

El uso del pastoreo en el sotobosque reduce la acumulación de biomasa, alarga el tiempo entre los desbroces para su manteniendo (Velamazán et al., 2018), revaloriza el papel del pastor (Lasanta et al., 2006; Ruiz-Mirazo et al., 2011), y promueve un paisaje en mosaico (Fernández-Olalla et al., 2006). Por ello, el aprovechamiento de las áreas cortafuegos mediante el pastoreo es una herramienta útil en la prevención de incendios, aportando sinergias medioambientales (Robles et al., 2009; Velamazán et al., 2018; Xanthopoulos et al., 2006). Estas sinergias dependen, entre otros factores, del tipo de animal a utilizar. Así se han utilizado con mayor o menor éxito: caballos (Rigueiro-Rodríguez et al., 2012), asnos (Lamoot et al., 2005), cabras (Lovreglio et al., 2014), ovejas (Ruiz-Mirazo & Robles, 2012) y vacas (Teruel-Coll et al., 2019). En otros casos, se proponen combinaciones de varios animales buscando mayor efectividad (Bartolomé et al., 2020; Bowie et al., 2016; Robles et al., 2009; Xanthopoulos et al., 2006). Sin embargo, la disminución de la ganadería extensiva es un factor limitante para este propósito (Nadal-Romero et al., 2018; Pardo Abad, 1996). Por ello, el uso de ungulados silvestres o de ganado asilvestrado ha despertado el interés en la gestión forestal (Ruiz-Mirazo et al., 2011), debido a su aumento poblacional y a la posibilidad de gestionar su comportamiento mediante el uso de atrayentes como la sal, agua, y comida (Gordon et al., 2004; Mancilla-Leytón et al., 2013; Sahlsten et al., 2010; Velamazán et al., 2018). Este manejo de ungulados silvestres a través del ramoneo y pastoreo, contribuye de manera efectiva a reducir la biomasa vegetal, ayudando así a la prevención de incendios forestales (Ruiz-Mirazo & Robles, 2012). Además, la gestión de ungulados silvestres mediante el uso de atrayentes, influye en su comportamiento dentro del territorio, llevándolos a ambientes con mayor densidad vegetal o evitando su desplazamiento hacia las áreas urbanas o agrícolas, que es un problema en torno a su gestión (Duncan et al., 2012).

- *Técnicas para determinar la dieta de los herbívoros*

La composición de la dieta en los herbívoros silvestres o en ganadería extensiva se basa en la disponibilidad y preferencia de la vegetación existente (Aldezabal & Garin, 2000). Para determinar esta composición, existen diferentes técnicas que buscan conocer hasta los taxones menores como género y especie de los forrajes (graminoides, herbáceas o arbustos). En general, las muestras que se utilizan son la vegetación existente, el contenido gástrico de animales muertos o fistulados, y las heces de los animales (Brosh et al., 2006; Shrestha & Wegge, 2006). Las técnicas más utilizadas son: el grado ramoneo o pastoreo de la vegetación, la microhistología, la espectroscopia de rayos infrarrojos, el análisis de los n-alcanos, y el análisis molecular (Niderkorn et al., 2014). Entre los métodos más utilizados se encuentre el análisis microhistológico de heces. A pesar de ser una técnica antigua (Webb, 1940), ofrece ventajas, como la facilidad para procesamiento de las muestras y el uso de equipo no especializado. Una de sus limitaciones es el tiempo que demanda para obtener los resultados y la experiencia previa en la lectura de las muestras para evitar falsos positivos (Alipayo et al., 1992; Bartolome et al., 1995; Henley et al., 2001). En los últimos años nuevas técnicas vienen siendo desarrolladas, como el análisis genético de las heces, que va abriendo nuevas perspectivas en el análisis de la dieta. Aunque la principal limitante es la calidad del DNA en las heces y su degradación en el medio ambiente, por lo cual, se viene buscando adecuar nuevos protocolos para este tipo de estudios genéticos, que seguramente en el futuro, será el camino a seguir (Espunyes et al., 2019; Valentini et al., 2009).

Objetivos

Objetivo general

En esta tesis doctoral se planteó determinar el papel de los herbívoros, vacuno doméstico y cabra domestica asilvestrada, en la prevención de incendios mediante el consumo de la biomasa presente en el bosque y el uso de atrayentes para complementar la intensificación del consumo.

Objetivos específicos:

- Evaluar la utilidad del uso de ganado vacuno doméstico bajo altas densidades y corto periodo sin suplementación.
- Evaluar el efecto del pastoreo del sotobosque en el ganado vacuno.
- Analizar el efecto de las cabras asilvestradas sobre la vegetación de las áreas cortafuegos en un medio insular mediterráneo.
- Analizar el uso de atrayentes sobre el comportamiento de las cabras asilvestradas y su efecto en la vegetación mediterránea.
- Determinar la complementariedad entre la técnica microhistológica (CMA) y molecular (PCR-CE) para determinar la dieta de ganado vacuno doméstico con alimentación extensiva y cabra domestica asilvestrada en pastoreo libre.

Capítulo 1

Effects of boom and bust grazing management on vegetation and health of beef cattle used for wildfire prevention in a Mediterranean forest

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Effects of boom and bust grazing management on vegetation and health of beef cattle used for wildfire prevention in a Mediterranean forest

Abstract

Humans and wildfires have historically driven landscape structure in the Mediterranean basin. The Iberian Peninsula is not an exception to that rule, and therefore, farmers, researchers, and governments seek alternative tools to minimize the loss of biodiversity and wildfire risks. Extensive livestock including beef cattle is currently promoted as a suitable management tool by European agro-environmental policies yet pieces of evidence exist regarding the reciprocal effects between cows and Mediterranean woody vegetation. In this work, we performed a field manipulation to evaluate whether free-ranging beef cattle without supplementary feeding, at high density (2 livestock units (LU)/ ha) for a short period of time i.e. "boom and bust grazing" management, are able to adapt their grazing preferences to the Mediterranean woody vegetation without health impairment, and prevent from bush encroachment and wildfires. For our purposes, a native herd of 14 adult cows was kept captive without supplementary feeding in a 14 ha enclosure covered by Mediterranean vegetation for two months (April–June 2016). Plant and cattle fecal and blood samples were collected to assess diet composition (plant cuticle microhistological analysis), fecal nitrogen and protein contents of consumed plants, and the nutritional status (non-esterified fatty acids) of cattle. Our results showed that cattle adapted their feeding habits toward a more woody diet including potentially flammable taxa but with some detrimental effects on health status. Hence, cattle cannot control woody vegetation for long periods of time without supplementary feeding. Further research should be oriented to explore other alternative approaches to minimize the health impairment of cattle used for control flammable vegetation in Mediterranean regions.

Keywords: Agro-environmental policies, Diet, Encroachment, Microhistology, Silvopastoral

Introduction

In the late 1990s, there has been a growing interest for the use of free-ranging livestock for the management of vegetation structure to prevent bush encroachment and halt the loss of biodiversity and wildfire risk in the Mediterranean basin (Etienne et al., 1996). Although cows are considered grass feeders (Hofmann R R, 1989), some research has indicated its ability to feed on Mediterranean woody plants, in particular at moderate to high densities (Henkin et al., 2005; Schoenbaum et al., 2017). The European agro-environmental policies have promoted the use of beef cattle to reconcile agricultural land use and wildfire prevention (Bernués et al., 2011). However, the toxicity and poor quality of the edible phytomass of many Mediterranean woody plants, along with the low productivity of some Mediterranean grasslands, encourage farmers to use supplementary feeding and recurrent technological intervention (Bartolomé et al., 2011; Brosh et al., 2006). Previous studies appointed that these management practices minimize the ability of cattle to control woody vegetation in Mediterranean landscapes (Provenza et al., 2003; Schoenbaum et al., 2017).

Some authors like Provenza et al.(2003) have suggested the use of short-duration grazing at high stock densities, called "boom and bust grazing", with limited or no use of supplementary feeding to force cattle to feed on a broad array of plants including more woody plants. However, we currently lack empirical comprehensive data on the impact of browsing in Mediterranean scrublands on diet quality and health status of these large ruminants (Casasús et al., 2005), and hence support for the current European agro-environmental policies. We, therefore, empirically study by using a multidisciplinary approach, some specific short-term effects of the "boom and bust grazing" management on cattle health and consumption of Mediterranean woody vegetation. We aim to provide relevant insights to evaluate the environmental utility of this management and eventually help to design more efficient monitoring protocols and agro-environmental policies focused on halt bush encroachment and wildfire risk in Mediterranean forested areas.

In this work, we took advantage of a field manipulative experiment to study the short-terme ffects of the "boom and bust grazing" management in a beef cattle herd fenced in a Mediterranean woody environment. We specifically evaluated: (I) the ability of beef cattle to adapt their typical grasser behaviour to a more woody vegetation in a Mediterranean forest at a high livestock rate and without supplementary feeding for two months; (II) the impact of the new feeding habits on diet quality; and (III) the impact on the nutritional status of beef cattle.

Material and methods

The study was conducted within the Montserrat range, in NE of Spain (central point 41°40′09″N, 01°46′38″E) in 2016. The study area was fenced and consisted of 14 ha at 370–440ma.s.l.with a mild topography (slopes b15%) integrated by small hills and valleys with multiple aspects except East. Soils are poorly developed (haplic regosols), derived from calcareous conglomerates, sandstone, and marls(*Icgc.cat*, 2018; IUSS, 2015). The climate is typically Mediterranean with a long-term (1955–1999) average annual mean temperature of 13.3 °C, and an annual rainfall of 610 mm distributed from autumn to spring, with scattered summer storms (Ninyerola et al., 2000). The vegetation comprises 10 ha of a dense planted forest of *Pinus halepensis* Mill., with the understory saturated with Mediterranean woody plants (e.g., *Erica multiflora* L., *Pistacia lentiscus* L., *Rosmarinus officinalis* L.) and 4 ha of shrubby patches dominated by the same woody species growing in the pinewoods. Among herbaceous plants, Poaceae species predominate (*Brachypodium retusum* (Pers.) Beauv., and B. *phoenicoides* (L.) Roem. et Schultes), plus underlying bryophytes carpets (*Pleurochaete squarrosa* (Brid.) Lindb., *Homalothecium* sp.) on northern or shady slopes.

A herd of 14 adult (> 4 years old) cows of the native breed *Bruna dels Pirineus* (130cm tall to the withers and the top of the hips, and weighing around 600 kg.) was introduced in the fenced area at the beginning of April. Then, the herd was monitored from the 19th of April (mid-spring) to the 21st of June of 2016 (early summer). Animals were kept with water supply but without supplementary feeding, and under high density: 2 livestock units (LU)/ha. Diet composition and quality including bromatological analyses of plants as well as nutritional and energy status of cattle were studied by collecting plant, fecal and blood samples at the onset (19th of April) and at the end of the experiment (21st of June).

The fecal samples from ten (out of 14) different individuals were taken and kept in sealed plastic bags and then frozen until analysis. The diet composition was analyzed by microscopic identification of 200–240 vegetal epidermic fragments per fecal sample, using the cuticle microhistological analysis (Stewart, 1967). The total 4800 fragments were classified into four major groups: 1. Graminoids (Poaceae, Cyperaceae, Juncaceae, etc.), 2. Woody plants, 3. Forbs and 4. Bryophytes.

Diet quality and nutritional status of different cows (n = 10) were assessed by fecal nitrogen (FN) (Villamuelas et al., 2017). The percentage of FN was estimated using Near-Infrared Reflection Spectroscopy (Lyons & Stuth, 1992) implemented in *NIRSystem 5000® por Foss Electric A/S*. Fecal samples were air-dried at 65 °C, milled to 0.5 mm, and scanned from 1100 to 2500 nm. Reflectance was recorded at 2-nm intervals and the fecal nitrogen percentage was predicted from the resultant reflectance pattern. The energy status (i.e., energy status=energy intake−energy requirement) of the same ten individuals was assessed by non-esterified fatty acids (NEFA) (Rudik et al., 2004) that has largely been used for monitoring the energetic status of cows (Adewuyi et al., 2005). Blood samples were collected into Vacutainer tubes with $C_2K_2O_4$ and NaF as a glycolytic inhibitor. At the lab, samples were centrifuged (915 × g) for 5 min immediately after collection; plasma was decanted and stored at −20 °C until analysis. NEFA assessment was performed by a WAKO NEFA C Kit (Wako Chemicals GmbH, Germany).

Bromatological analyses were performed to determine the quality of seven browsed and grazed taxa: four shrubs (*Erica multiflora*, *Rhamnus alaternus* L., *Rosmarinus officinalis*, *Rubus ulmifolius* Schott) and three graminoids (*Brachypodium retusum*, B. *phoenicoides* y *Carex* sp.). All these taxa were selected on the basis of previous studies (e.g., Bartolomé et al., 2011) and direct observations. Ten samples (200 g in total) from different sites and individuals within the fenced area were collected and mixed for each plant taxa and date (April and June).Then, three subsamples of each plant taxa and date were used to obtain the percentage of the protein content following the procedures of Helrick (1991) and Van Soest et al. (1991).

To explore whether cattle modified their feeding habits during the study period, we used Generalized Linear Mixed Models (GLMM) including the proportion of plants consumed (assessed by fecal cuticle microhistological analysis), as response variable and plant type (i.e., graminoids, woods and forbs), sampling period (onset vs end) and their two-way interaction as fixed explanatory variables. To control for individual effects, cow identification was included as a random intercept in our GLMM (Zuur et al., 2009). Model selection was based on the Akaike information criterion (lowest AIC) and the Akaike differences (Δi) with respect to the best model. When the Δi was <2 units, we applied the principle of parsimony to select the simplest model (Burnham & Anderson, 2002). We then estimated the Akaike weight (Wi), that is, the relative likelihood of the model given the data available. Previous to any model interpretation, we checked for evidence of overdispersion of residuals. Our response variable (plant fragments in %) were log transformed to achieve the GLMM assumptions (Zuur & Ieno, 2016). Pseudo R^2 for our set of GLMM was estimated following Nakagawa y Schielzeth (2013). Finally, mean FN and NEFA concentrations, as well as percentages of protein content from comparisons between the onset and the end of our experiment, were performed using Student's *t*-tests. However, due to the reduced sample size for protein content, the *t*-test was based on 1000 bootstrapped replications.

GLMM were performed using the “lme4” package 1.1–17 version (Douglas Bates et al., 2015), pseudo R2 using “MuMIn” package 1.40.4 version (Bartoń, 2018) and bootstrapping using the “boot” package 1.3–20 version (Canty & Ripley, 2017) of the R software 3.5.1 version (R Development Core Tea, 2018).

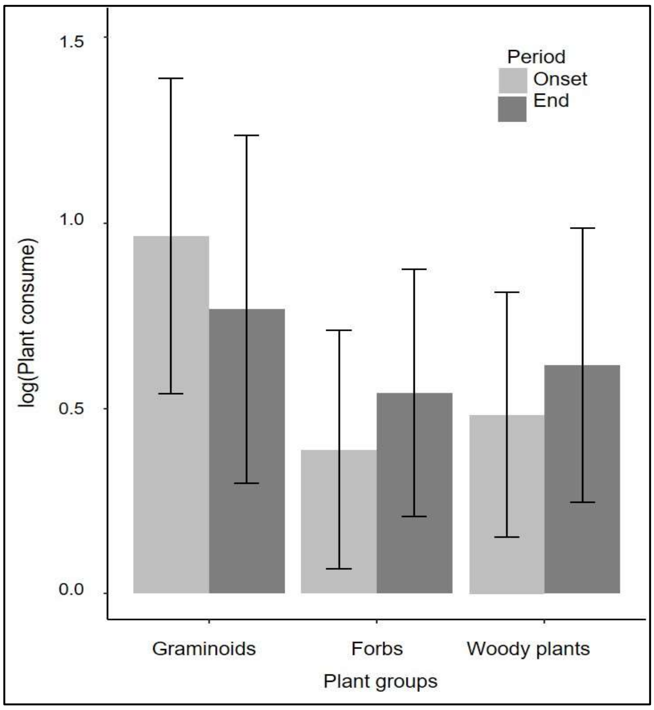

Figure 1. Feeding habits of a herd of ten adult cows. Percentage of fragments summarised into three informal groups. Bars and whiskers represent mean and standard deviation values, respectively

Results

The model including the interaction between plant type and the period best explained the feeding habits of our herd of cows (Wi Period ∗ Plant type = 1, R2 = 25.8, Table 1 and Figure 1). Plant type explained most (13.1%) of the observed variability in the feeding habits whereas changes between sampling periods explained 3.5%. That indicates that changes in the plant consume between periods significantly varied among all the plant types ($p < 0.05$). For example, cattle increased the consumption of woody plants and forbs (by 15.5 and 4.4% in the use respectively) whereas the use of graminoid plants decreased (by 19%) over time.

Table 1. Model selection for exploring changes in the feeding habits of ten different adult cows by analysing vegetal fragments grouped into three plant types (Woody plants, Forbs and Graminoids) from their respective 20 fecal samples collected in two periods: 10 in April and 10 in June. The Null model only included the intercepts as explanatory fixed variable. K=degrees of freedom of the models, AIC=Akaike Information Criterion, Δi = difference of AIC respect to the best model, Wi=Akaike weight. The percentage of the observed feeding patterns variability explained by both the fixed and random terms (Conditional), by the fix (Marginal) and the random term are also provided. The best model is shown in bold.

Biological model	K	AIC	Δi	Wi	Conditional R^2	Marginal R2	Random Factor R^2
Period∗plant type	**8**	**262.01**	**0**	**1**	**25.8**	**16.7**	**9.1**
Plant type	5	268.15	6.14	0.05	20.3	13.2	7.1
Period + plant type	6	269.13	7.12	0.03	20.7	13.4	7.3
Null model	3	304.08	42.07	< 0.01	-	-	-
Period	4	305.71	43.71	< 0.01	0.2	0.1	0.1

Overall, cattle consumed a wide array of species common to the understory layers including bryophytes, the first target plants in spring were three graminoids, *Brachypodium phoenicoides*, *B. retusum* y *Carex* sp. Secondarily, two woody plants, *Rhamnus alaternus* L. and *Rubus ulmifolius*, which doubled the percentage of protein of the graminoids (Table 2). Several sclerophyllous shrubs (e.g. *Erica multiflora*, *Olea europaea* L., *Phillyrea angustifolia* L., *Pistacia lentiscus* and *Viburnum tinus* L.) were also consumed yet mainly at the end of the study period.

Table 2. Percentage of protein (mean ± standard deviation) obtained at the bromatological analysis of three samples per taxa and date. Significant differences ($p < 0.05$, bootstrapped *t*-student test) in protein content between dates were observed in all plant taxa.

Taxa	Woody /Herbaceous	Protein (%)	
		19th April	21st June
Brachypodium phoenicoides	Herbaceous	7.6 ± 1.01	5.0 ± 0.84
Brachypodium retusum	Herbaceous	7.6 ± 0.46	5.1± 0.47
Carex sp.	Herbaceous	10.3 ± 0.64	8.2 ± 47
Erica multiflora	Woody	5.2 ± 0.56	5.1 ± 0.49
Rhamnus alaternus	Woody	12.8 ± 0.63	11.5 ± 0.55
Rosmarinus officinalis	Woody	9.7 ± 0.50	6.0 ± 53
Rubus ulmifolius	Woody	15.7 ± 0.27	11.4 ± 0.35

The bromatological analysis by bootstrapped *t*-test showed a significant ($p < 0.01$) decreasing tendency in the percentage of protein in every analyzed planta taxa but *Erica multiflora* (Table 2). Likewise, at the end of the field experiment, the FN of cows decreased by 1.6 times ($t = -3.5$, p-value = 0.006) confirming the consumption of less nutritious plants (Table 3). Along the same lines, NEFA concentrations in the herd increased two-fold ($t = -3.5$, p-value = 0.006) after two months without supplementary feeding (Table 3).

Table 3. Mean ± standard deviation of ten fecal nitrogen (FN) and non-esterified fatty acid (NEFA) concentrations recorded in ten different adult cows at the onset (19th of April) and at the end of the experiment (21st of June).

Period	**FN (%)**	**NEFA (mmol/L)**
Onset	2.76 ± 0.320	0.14 ± 0.03
End	1.67 ± 0.31	0.28 ± 0.13

Discussion

We confirm the prediction regarding the implementation of the “boom-bust grazing” management (Provenza et al., 2003). Cattle, without supplementary feeding and under high density, increased the consumption of sclerophyllous woody plants over two months while fenced in a Mediterranean forest. As expected in grazing animals (Hofmann R R, 1989), cattle initially focused on herbaceous plants and some shrubs with high protein content (e.g. *Rhamnus alaternus* y *Rubus ulmifolius*). Moreover, cattle also browsed a large series of woody plants including taxa avoided in similar Mediterranean environments under similar or even higher livestock rates but with supplementary feeding (Bartolomé et al., 2011). In fact, after two months, cattle increased the consumption of several woody taxa (e.g. *Erica multiflora*, *Olea europaea*, *Pistacia lentiscus*) Cattle only seemed to avoid the dominant tree species (*Pinus halepensis*) probably due to the scarcity of saplings (i.e. young *Pinus* individuals with accessible branches), which otherwise are consumed in other Mediterranean areas (Pfister et al., 2008). Cattle also consumed bryophytes, not by chance, given that mosses grow close to the ground (<3 cm height) and possess deterrent chemical components that exert detrimental effects on nitrogen absorption (Ihl & Barboza, 2007 and references therein). This result might reveal the lack of optimal resources or overgrazing (Ihl & Barboza, 2007). Thus, without supplementary feeding, cattle might effectively modify forest structure by changing, in the short-term, the density of different understory vegetation strata including woody and mossy vegetation.

In the spring (April), at the beginning of the study, all the studied plants, except Erica multiflora, showed higher protein values than those needed for cattle maintenance, considering that the protein requirement for cattle is around 14% for production and 7% for basal maintenance (NRC, 1996). Thus, Mediterranean forests can meet cattle requirements in the spring (Casasús et al., 2005) even though nutritive values of grasses can be reduced by shrubs abundance (Zarovali et al., 2007). In contrast, in early summer (June), Mediterranean plants showed much lower protein contents and some highly consumed taxa, *Brachypodium*, *Erica multiflora* y *Rosmarinus officinalis*, yielded values under the maintenance threshold. This poor-quality diet might be the cause of the nitrogen content decrease in cattle feces. Although the registered nitrogen values resemble those recorded for extensive grazing cattle (Aldezabal et al., 2011), NEFA concentrations were slightly above the reference values for a cow with adequate nutrition (<0.2 mmol/L, (Drackley, 2000). In fact, concentrations in some individuals got close to 0.6 mmol/L, the limit beyond which a severe negative energy balance would be seen (Adewuyi et al., 2005). Thus, if the grazing period was prolonged, the boom-bust grazing management (sensu Provenza et al., 2003) would reduce the woody cover but severely compromise livestock health and herd productivity.

Conclusion

Cattle, without supplementary feeding and under high density, actively graze herbaceous taxa including bryophytes but also browse woody taxa. The consumption of woody taxa responsible for bush encroachment increases over time which in turn might cause a reduction of the shrub understory layer of the forests. Besides, cattle feed on key taxa, like *Brachypodium* spp. and *E. multiflora*, that possess a relatively high percentage of dry matter, and are potentially flammable (Elvira Martín, L. M., & Hernando Lara, 1989).

Therefore, these preliminary results might support extensive cattle as a suitable target for incoming studies focused on the environmental problems linked to land abandonment: bush encroachment and the concomitant increase in wildfire risks. However, further studies will have to address whether the “boom and bust grazing” management significantly modifies the vegetation structure (woody and herbaceous biomass spatial patterns) and the species composition to make Mediterranean forests less prone to wildfires.

Nonetheless, the studied Mediterranean forest failed to truly provide the energetic requirements of beef cattle. The use of the boom-bust grazing management would be constrained to no more than two months to conciliate bush encroachment control, livestock welfare, and herd productivity. Still, further studies are needed on different and complementary options like the use of native breeds (Bartolomé et al., 2011), improving the adaptability of cattle to the Mediterranean woody vegetation (Estell et al., 2012;

Gutman et al., 2000) or use nonprofitable livestock as an environmental management tool (Bernués et al., 2005). Optimally, future studies should incorporate long-termex periments comprising analysis of cattle body conditions to meet the ambitious goal of sustainable (profitable) extensive grazing systems in Mediterranean regions(Nadal-Romero et al., 2018).

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Capítulo 2

The role of feral goats in maintaining firebreaks by using attractants

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The role of feral goats in maintaining firebreaks by using attractants

Abstract:

The threat of large forest fires is increasing, and the main causes are the depopulation of rural areas, along with the effects of climate change. To counter this threat in recent decades, there have been numerous proposals and actions aimed at promoting grazing in the forest as a tool for controlling biomass fuel. However, the continued disappearance of traditional herds makes this activity difficult. Rural depopulation has also meant that domestic species become feral, being habitual in the case of goats. Currently, little is known about the role that feral goats can play in the fight against forest fires. In this work, an analysis is made on the effect of feral goats on the control of the vegetation in firebreak areas. Furthermore, the effect of attractants, such as water, salt, or food, on goat behavior is also studied. The study was carried out on the island of Mallorca, where a population of feral goats occupies the mountain areas, and where it is common for them to graze on the network of firebreaks. The results showed that these areas in themselves exert an attractive effect with respect to the neighboring forest, and that the herbaceous biomass is reduced. This effect was enhanced with the implementation of water and salt points, although only in certain periods of the year. In general, it was possible to reduce the phytovolume of many species without affecting biodiversity in the short or medium term. Therefore, strategic management of feral animals, aimed at firebreak areas, could contribute not only to reducing the risk of fires and, consequently, to the mitigation of climate change, but also to attracting these animals to the forests, thus avoiding their dispersion to conflictive places such as roads, residences, agricultural fields, and gardens.

Keywords: browsing; silvopastoralism; fecal groups; forest fires; fire prevention

Introduction

In recent decades, Europe, North America and parts of Asia have experienced an increase in their forest area (Keenan et al., 2015). This increase corresponds to the abandonment of agricultural land in remote and poorly productive areas, coinciding with the increase in urban area (García-Ruiz & Lana-Renault, 2011). The decrease in agricultural, livestock, and forestry activity leads to the loss of the mosaic structure of the landscape, reducing its heterogeneity (Navarro & Pereira, 2015). One of the effects of this change in land use is colonization by woody species of abandoned land (Loepfe et al., 2010). In Mediterranean forests, this transformation leads to increasingly dense undergrowth, with a greater accumulation of biomass that, in turn, increases the risk of forest fires (Lasanta-Martínez et al., 2005). The increase in fuel continuity at landscape scale has led to the generation of large fires in the last decade, as has occurred in the Mediterranean area (San-Miguel-Ayanz et al., 2013), including the Balearic Islands (Domenech, 2015). Furthermore, the existence of an understory composed of highly flammable sclerophyllous species, or large tufts of grasses, aids the spread of fire from the understory to the canopy, thus increasing its devastating effect (Pausas & Paula, 2012).

On the other hand, global change also includes the effect of climate change, for which forecasts in the Mediterranean basin indicate an increase in periods of drought, torrential rain, and lightning storms (Lindner et al., 2010), which are great hazards in the generation of forest fires. Higher risk is expected in areas where the forest cover is already very high, for example, in the Alps, the Pyrenees, or the Balkans (Fernández-Olalla et al., 2006; Moreira et al., 2011). The increase in man-made fires, either intentionally or accidentally (Radeloff et al., 2018), must also be accounted for.

One action in the prevention of forest fires is the opening of firebreak areas, which, in practice, involves clearing or cutting forest strips to contain the spread of fire (Loepfe et al., 2010). The maintenance of these strips can be carried out by grazing, thus reducing the costs of mechanical clearing (Lovreglio et al., 2014; Velamazán et al., 2018). This practice constitutes a good example of the multifunctionality of the livestock sector and allows the local population to be involved in fire prevention (Ruiz-Mirazo et al., 2007). Despite this, the availability of domestic livestock for the maintenance of these areas is limited due to the increasing abandonment of extensive livestock farming (Benjamin et al., 2008; Lasanta et al., 2015).

Parallel to the decrease in the livestock herd, an increase has been observed in the populations of feral herbivores and unmanaged feral livestock, with the highest densities for many decades being recorded (Velamazán et al., 2018). These populations have also started to be perceived as possible tools in the prevention of forest fires, while guaranteeing the conservation of biodiversity (Ruiz-Mirazo et al., 2011). Along these same lines, some authors have shown that it is possible to influence the behavior of wild herbivores through the use of attractants, such as water, food, or salt (Bailey, 2005; Sahlsten et al., 2010; Velamazán et al., 2018), thus causing changes in the structure and composition of the vegetation. However, the use of attractants for wild animals, even for a management purpose, could have an impact on their behavior and population dynamics (Sahlsten et al., 2010), and if this were the case, it could be perceived by conservation sectors as an interference in ecological processes.

Feral livestock could be a tool for clime mitigation by the prevention of forest fires since, as they are not wild populations, their management is more justified. Furthermore, these animals, especially feral goats (*Capra hircus* L.), often cause damage to crops and gardens, forcing administrations to devote a lot of effort to their control or eradication (Roque, 2015; Vives & Baraza, 2010). So far, there are no known works that analyze the role that feral goats can play in the maintenance of firebreaks. The island of Mallorca, in the western Mediterranean, enables us to address this issue, since it has a network of firebreak areas and a population of feral goats, with serious management problems (Mayol et al., 2017; Vives & Baraza, 2010).

For this reason, the objective of this work is to analyze the effect of feral goats on the vegetation of the firebreak areas in pine forests. An analysis will also be performed on the use of attractants (water, food, and salt) to increase their presence in these areas and thus increase their impact on vegetation. The initial hypothesis is that the firebreak areas exert an attractive effect on feral goats, and this effect can be accentuated with the use of other attractants, thus helping to control plant biomass, and therefore the functionality of the firebreaks, for a longer time.

Material and methods

Study Area

The work was carried out in the Sierra de Na Burguesa (39°35'11" N 2°33'55" E) located in the municipality of Calvià in the southwestern part of the island of Mallorca (Balearic Islands, Spain). The altitude of the study area ranges between 400 and 503 m above sea level (asl). The climate is dry supra-Mediterranean, with a mean temperature of 17 °C, with January being the coldest month and August being the warmest. Annual rainfall is irregular, distributed mainly in spring and autumn, with annual lows of 313 mm and highs of 863 mm. The mean annual temperature was 18 °C for the two years of study, and the annual rainfall was 528 and 326 mm for the first and second year, respectively (Agencia Estatal de Meteorología-AEMET, 2019).

The vegetation is characterized by the dominance of Aleppo pine (*Pinus halepensis* M.) forests with an understory of Mediterranean scrubs, where species such as *Erica multiflora* L., *Pistacia lentiscus* L., *Phillyrea angustifolia* L., *Cistus albidus* L., *Cistus monspeliensis* L., and *Anthyllis cytisoides* L. are common. Perennial grasses predominate in the herbaceous layer, including *Ampelodesmos mauritanica* (Poiret) and *Brachypodium retusum* (Pers.), as well as other annual species.

The study area is part of the network of fire prevention firebreak areas on the island of Mallorca. The study area was located in a firebreak strip of variable width ranging between 30 and 50 m and a length of 2 km. Maintenance is the responsibility of the Council of Agriculture, Environment, and Territory of the Balearic Islands. It is carried out by means of selective mechanical or manual brush clearing, in addition to thinning and the occasional pruning of trees, with a frequency of 5–6 years, stipulated in the IV General Plan of defense against forest fires of the Balearic Islands (Gobierno de las Islas Baleares, 2015), with the last clearing being carried out in early spring 2017. According to reports from the Ministry of Agriculture, Fisheries and Food of Spain, the last forest fire in the study area was in 1993, with a total surface of 492 hectares affected.

The area is frequented by feral goats (*Capra hircus*), weighing between 30–50 kg, generally forming small groups that do not exceed 50 animals, with one or more males. Their behavior is territorial, with foraging areas of between 45 and 170 hectares (Ibáñez et al., 2019). These goat populations probably come from the gradual abandoning of livestock from old traditional extensive farms, and as they currently lack management, they graze all over the Na Burguesa mountains.

Data Collection

To determine the effect of goats on plant species in the firebreak areas, 12 plots of 5 × 5 m were marked out. Plots were distributed in 6 pairs (one fenced plot and its respective unfenced plot). An attempt was made for each pair of plots to have the same ecological conditions (same inclination, orientation, and height), and they were less than 5 m apart. Fencing of the plots was performed immediately after the clearing maintenance of the firebreak areas, using a galvanized metal mesh 25 mm thick and 1.80 m high, thus guaranteeing the complete exclusion of goats. Each pair of plots was randomly distributed in the firebreak areas. The phytovolume of the bushes (including *A. mauritanica*) and the biodiversity of the vegetation in these plots were estimated before the clearing and at 2 years after clearing. In order to calculate the phytovolume (V) of each individual, the height (h), largest diameter (D), and smallest diameter (d) were measured, and then the inverted cone formula was used:

$$V = \pi \cdot D \cdot d \cdot h/3$$

To determine the biodiversity, a variant of the point interception method was used (Elzinga et al., 1998), which consisted of tracing 2 linear transects, following the two diagonals of the plot. Every 10 cm, a 1 m rod was driven into the ground and all species that came into contact with it were noted.

The biomass of the herbaceous group was determined after two years of the clearing. The herbaceous biomass was obtained by collecting samples from the herbaceous stratum in 5 quadrants of 50 × 50 cm distributed randomly in each plot at the end of the study period. The samples were dried to constant weight at 103 °C.

The effect of attractants on the habitat used by feral goats was studied from fecal group counts, assuming that the greater the use of a territory, the greater the amount of deposited feces (Fattorini et al., 2011; Härkönen et al., 2018). A total of 32 strip transects of 20 × 1 m were established in two areas, 16 in each, separated by a distance of 490 m. In each zone, eight transects were distributed in parallel in the firebreak area and another eight in the adjacent pine forest. These transects were cleared of excrement at the start of the study, and the accumulated excrement was counted and removed periodically. Six fecal pellets together were established as a minimum fecal group (Acevedo et al., 2010), with a similar appearance in color, consistency, and humidity (Sutherland, 2006). The sampling period was two consecutive years (from July 2017 to July 2019). During the first year, no type of attractant was used, and for the second year, two water points were installed in aluminum drinking troughs, 16 × 14.5 × 6 cm with outlet regulators, connected to a 210-L water container and attached to a stake 60 cm above the ground. The containers were filled every 15 days on average, guaranteeing the availability of water. Also, from the second year, a 10 kg block of salt was placed about 3 m from each trough, at a height of approximately 80 cm. In each water replacement, 1 kg of corn grain was spread around each water container. The 16 transects closest to the attractants were placed at an average distance of 70 m, and the 16 farthest were placed 490 m away.

Statistical Data Processing

To evaluate habitat use, the accumulation of fecal groups in transects was analyzed as an indirect measurement of the number of animals present. Two study periods were established to analyze the effect of the attractants: “before”, referring to the first year prior to the use of attractants, and “after”, referring to the second year after placement. The total fecal group count was divided by the total days of accumulation and multiplied by 30 to obtain a mean value of fecal groups per month. The mean monthly accumulation of fecal groups was analyzed by means of a generalized linear mixed model (GLMM), using the transect as a random factor and using the following as fixed factors: the period, referring to before and after the placement of attractants; the season, referring to the four seasons of the year; the area, split between near and far with respect to the distance towards the attractants; and the habitat, differentiating between the firebreaks area and the forest.

The effect of the exclusion of goats on the accumulation of dry biomass of grasses was analyzed by means of a mixed general model with the treatment (inside referring to the fenced plot, and outside referring to the unfenced plot), and the area (near and far with respect to the distance from the attractant) as fixed factors and plot as a random factor.

As expected, the height, cover, and phytovolume of the vegetation were highly correlated (Spearman $\rho > 0.77$ $p < 0.0001$), so only the phytovolume was analyzed. This was normalized by a logarithmic transformation. To analyze the effect of the exclusion of goats on the phytovolume of woody species (including *A. mauritanica*), a general mixed model was performed, with plot and species as random factors and treatment, time (before clearing and two years after clearing), and zone as fixed factors, including interactions when they were significant. For the analysis, data from species that did not have a minimum of 10 individuals were excluded.

Similar analyses were carried out separately for the species groups according to the flammability index, since this index evaluates the ease of igniting and producing a flame when subjected to constant heat energy (Valette, 1992). Table 1 shows the flammability index of the main species in the study area. Statistical analyses were adjusted with mixed models, with the plot as a random factor and treatment, period, and area as fixed factors. In some cases, it was only possible to test some of the interactions since there were not enough replicates at the different levels of each factor. For the most abundant species, this same model was re-adjusted considering only the individuals of the species.

Table 1. Flammability index of the main species in the Sierra de Na Burguesa, Mallorca, Spain.

Species	Stratum	Flammability Index[1]
Ampelodesmos mauritanica	Herbaceous	3
Anthyllis cytisoides	Shrubby	2
Arbutus unedo	Arboreous	2
Asparagus acutifolius	Shrubby	3
Ceratonia siliqua	Arboreous	3
Cistus albidus	Shrubby	2
Cistus monspeliensis	Shrubby	2
Erica multiflora	Shrubby	2
Euphorbia characias	Shrubby	3
Globularia alypum	Shrubby	3
Lonicera sp.	Arboreous	1
Olea europaea	Arboreous	2
Phagnalon saxatile	Shrubby	3
Phillyrea angustifolia	Shrubby	4
Pinus halepensis	Arboreous	4
Pistacia lentiscus	Shrubby	1
Quercus coccifera	Arboreous	3
Quercus ilex	Arboreous	4
Rhamnus oleoides	Shrubby	2

[1]Flammability Index: 1 = Non-flammable, 2 = Slightly flammable, 3 = Flammable, 4 = Highly flammable, according to El-Amine Henaoui et al. (Henaoui et al., 2013) and Mancilla-Leytón et al. (Mancilla-Leytón et al., 2013).

All the aforementioned mixed models were adjusted using the lmer function of the lme4 package (D Bates et al., 2015) by R, R Core Team (R Development Core Tea, 2018). The best model was selected following the Akaike information criterion (AIC) (models with lower AIC value were selected), and the residual distribution graphs were reviewed to ensure the adequacy of the models (Zuur et al., 2009).

For the analysis of the effect of exclusion on diversity, the Shannon diversity index was calculated for each of the plots. The effect of the treatment and area as fixed factors was analyzed using a two-way ANOVA with the avo function by R, R Core Team (R Development Core Tea, 2018).

Results

Effect of Habitat and Attractants on the Accumulation of Fecal Groups

Statistically significant differences were found in the accumulation of fecal groups according to the period (p = 0.0005 GLMM), the season (p = 0.0002 GLMM), and the habitat (p < 0.0001 GLMM). The interactions between the period and habitat (p = 0.002 GLMM) and period and zone (p < 0.0001 GLMM) were also significant. Figure 1 illustrates the habitat effect, showing the number of fecal groups in the firebreaks area being 70% higher in relation to the forest (note the difference in scale between Figure 1 a,b). Before the attractants were placed, the presence of fecal groups in the area furthest from attractants versus those closest was the same in most seasons of the year, except in autumn in the forest, and autumn and winter in the firebreaks, where it was greater in the furthest (Figure 1). After the attractants were placed, there was a general decrease in the accumulation of fecal groups in the forest habitat, except in autumn and winter in the nearby area, which increased. On the other hand, within the firebreak areas, after the placement of the attractants, fecal groups always decreased in the distant zone, whereas in the near zone they only decreased in spring, remaining stable in summer and winter and increasing in autumn.

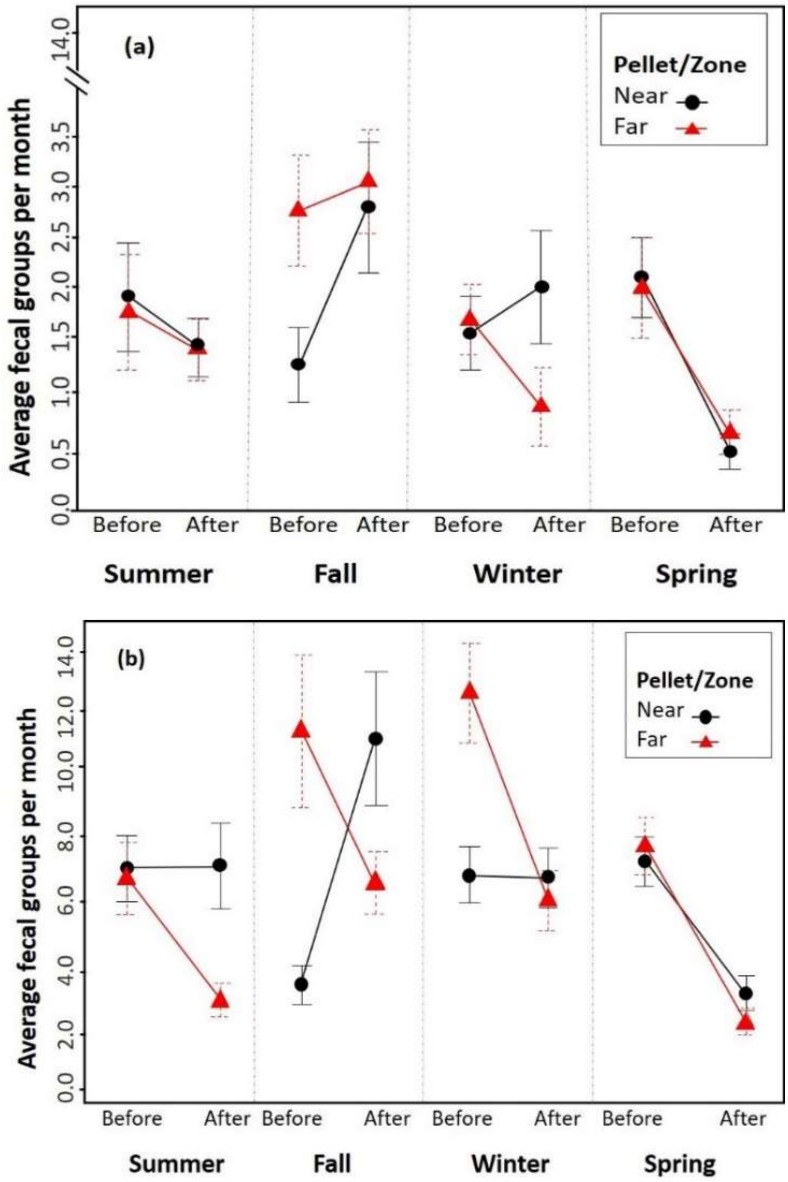

Figure 1. Number of fecal groups in the forest (**a**) and firebreaks (**b**) throughout the seasons, depending on the period of installation of attractants (Before or After) and the distance to them. Circles and triangles represent the mean, and vertical lines represent the standard error.

Effect of the Exclusion of Goats on the Evolution of the Vegetation in the Firebreaks

Significant differences were observed in the biomass of the herbaceous stratum, both between treatments (p = 0.02 GLMM) and between areas (p = 0.01 GLMM). The biomass of grasses in the fenced plot is 53% higher compared with the unfenced plot (Figure 2). This difference is more noticeable in plots near the attractant where it reaches 76%, while in the fenced plots far from the attractant, the biomass difference was only 37%.

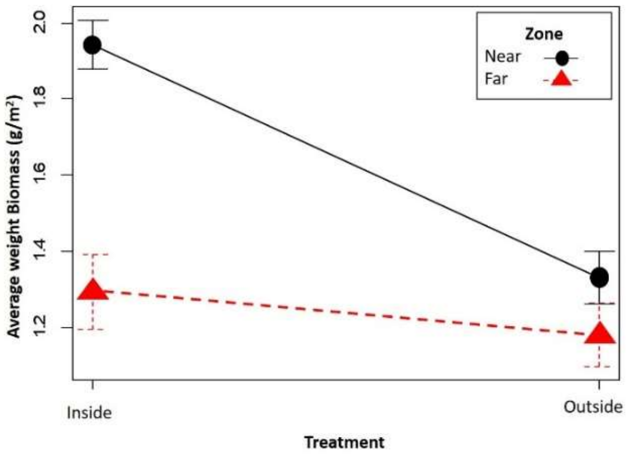

Figure 2. Variation of the weight of the herbaceous biomass in the firebreaks, depending on the fenced and unfenced plots (inside and outside) and the distance to the area of attractants (near and far). Circles and triangles represent the mean, and vertical lines represent their standard error.

The results of the analysis of the variation of the phytovolume of the bushes and scrubs, including *A. mauritanica* and excluding species with less than 10 individuals in the study plots, are shown in Figure 3. There are significant differences regarding the treatment (p = 0.01 GLMM), with the phytovolume being greater within the fenced plots. There were also differences regarding time (p < 0.0001 GLMM), with greater phytovolume before the clearing. In contrast, no differences were found with respect to the area (p > 0.05 GLMM). A significant effect (p = 0.0002 GLMM) was observed in the interaction between the time and zone, showing that, after two years, the phytovolume within the plots far from the attractant was similar to the phytovolume before the clearing, whereas this effect was not noticeable in fenced plots near the attractant.

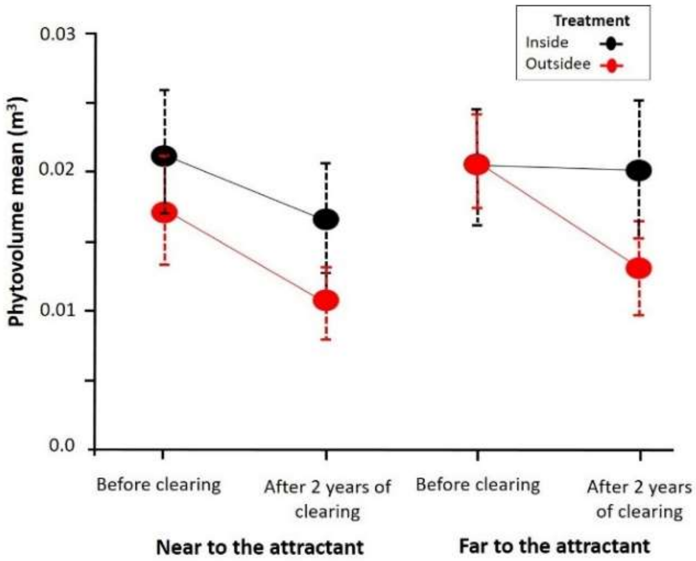

Figure 3. Variation of the phytovolume of the firebreaks bushes according to the treatment, location inside and outside of the plots, zone, location near and far with respect to the distance far from the attractants, and time before the clearing and after two years. The circle represents the mean, and vertical lines represent its standard error.

Figure 4 shows variations of the plant volume grouped according to the flammability index in the different bushes and shrubs, including *A. mauritanica*. For the species with flammability index 1, no significant differences ($p > 0.05$ GLMM) were detected for the treatment, area, and time factors. The species with flammability index 2 did not show significant differences with respect to treatment and area ($p > 0.05$ GLMM), while the time and treatment*time interaction showed significant differences ($p = 0.001$ GLMM) and ($p = 0.002$ GLMM), respectively, indicating that variation of the phytovolume is less within fenced plots after clearing. The species with flammability index 3 showed significant differences for the three factors: treatment ($p = 0.005$ GLMM), zone ($p = 0.003$ GLMM), and time ($p = 0.02$ GLMM), indicating differences in phytovolume inside and outside the fenced plots, as well as the effect of the distance to the attractant and differences after two years of clearing. In this case, the interaction between factors could not be calculated due to lack of replicates. For species with flammability index 4, no significant differences were found with any of the three factors ($p > 0.05$ GLMM). However, significant differences were observed for the treatment and time interaction ($p < 0.05$ GLMM). In this case, a significant increase in the phytovolume of these species was observed after two years, although only outside the fenced plots of the nearby area.

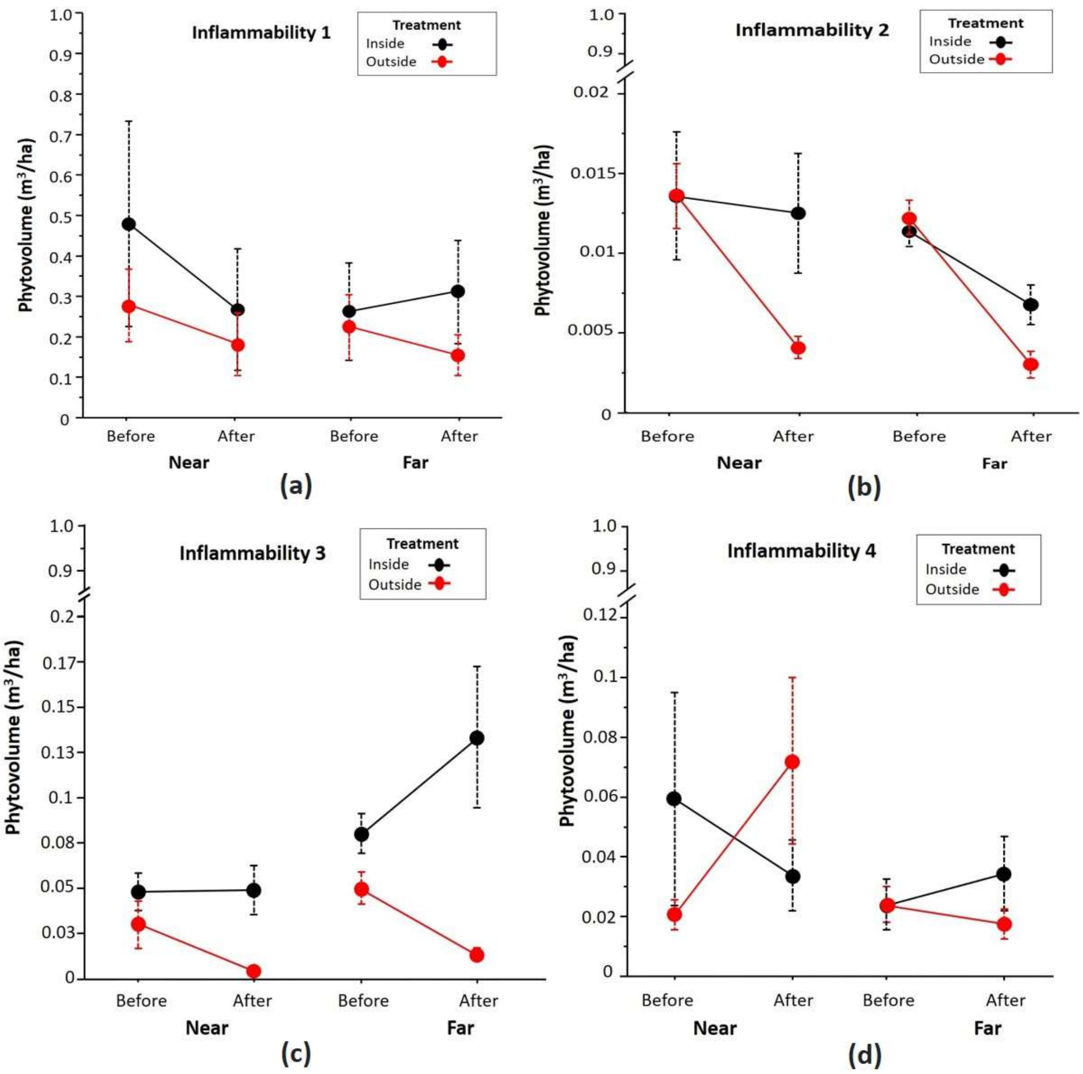

Figure 4. Variation of the phytovolume of the bushes grouped by the flammability index on (**a**) scale 1, (**b**) scale 2, (**c**) scale 3, and (**d**) scale 4 with respect to the treatment factors (inside and outside the fenced plots), time (before and two years after clearance), and area (near and far from the attractant). The circles represent the means and the vertical lines their standard error.

Figure 5 shows the plant volume of some of the most abundant species in the study area. In general, in the nearby plots a lower recovery of the phytovolume is observed in the unfenced plots (outside) compared with the fenced ones (inside). The phytovolume in some fenced plots, whether near or far from the attractant, showed a phytovolume higher than the initial record (two previous years), such as *A. unedo* (near), *A. mauritanica* (far), and *P. angustifolia* (far). Observing the phytovolume from outside the fenced plots, differences were observed both near and far from the attractant, except in *P. angustifolia*, with phytovolume around zero.

The Shannon biodiversity index (H) did not show significant differences between inside and outside the exclusions, nor between study areas (p > 0.05 ANOVA). H in the area near the attractants within the exclusions was 1.98 ± 0.04, while outside it was 1.85 ± 0.38. In the case of the exclusions of the area far from the attractants, within exclusion H was 1.99 ± 0.16 and outside was 1.91 ± 0.05.

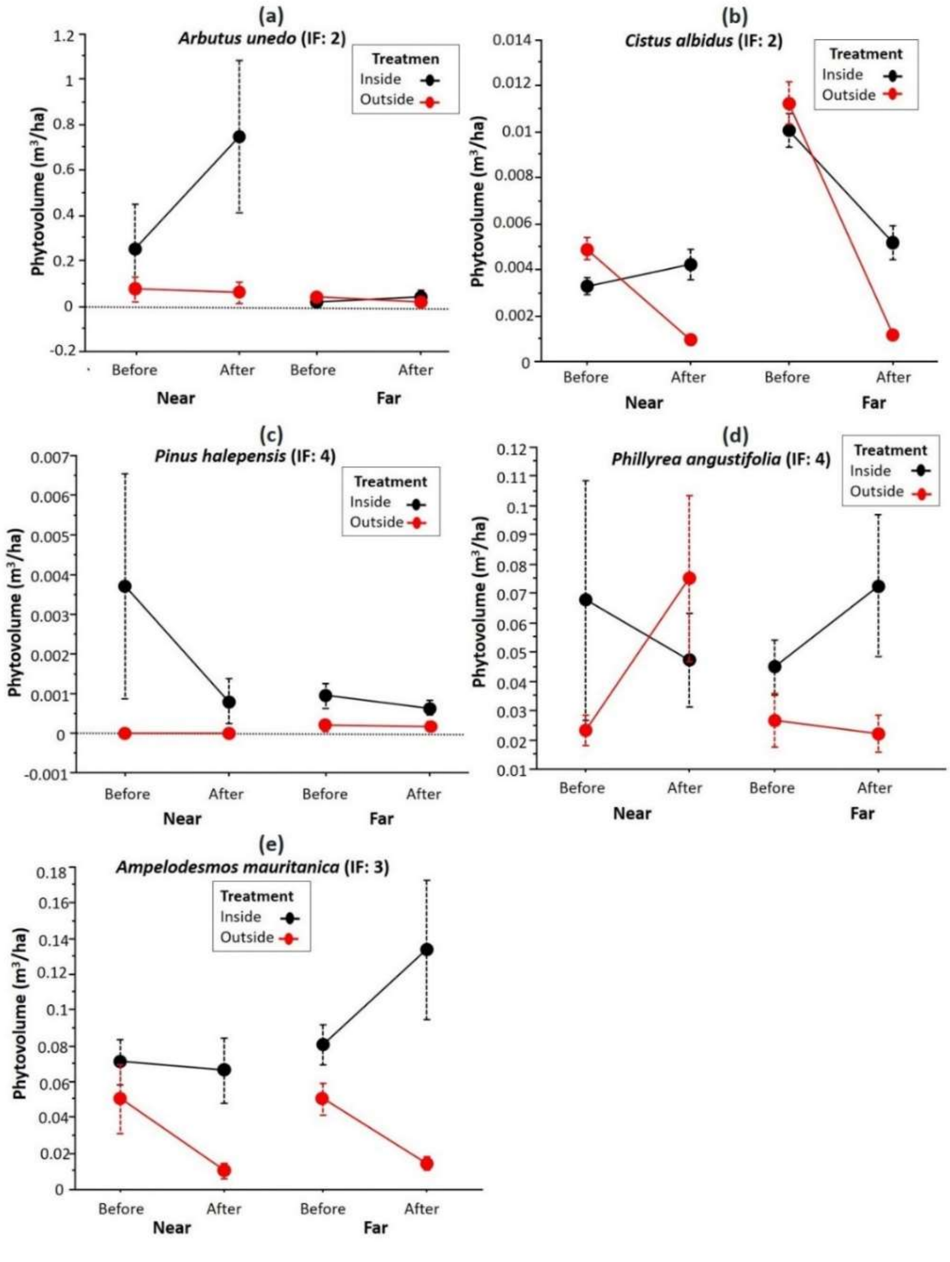

Figure 5. Variation of the phytovolume of the most abundant shrub species in the firebreaks (**a**) *Arbutus unedu*, (**b**) *Cistus albidus*, (**c**) *Pinus halepensis*, (**d**) *Phyllirea angustifolia*, and (**e**) *Ampelodesmos mauritanica* depending on the treatment factors (fenced plots: inside, and unfenced plots: outside), time (before clearing and two years after clearing), and zone (near and far regarding the attractant). The circle represents the mean, the vertical lines represent its standard error, and the IF represents the flammability index.

Discussion

The present study shows the important role that feral goats play in the maintenance of firebreak areas and how the use of attractants increases these effects. The results show that feral goats use the firebreak area more than the adjacent forest, putting significant pressure on its vegetation. This is in agreement with other results in which it was observed that firebreak areas have herbaceous covers of higher quality and abundance for feral ungulates and extensive livestock, compared with woody formations (Mancilla-Leytón et al., 2013; Ruiz-Mirazo et al., 2011). For many ungulates, modified linear areas such as firebreaks and roads act as attractants, either because of their current biodiversity, the quantity or quality of existing food, or because they are corridors for movement (Ruiz-Mirazo et al., 2011; Suárez-Esteban et al., 2013; Velamazán et al., 2018). In the case of goats, it should be added that, being prey animals, they usually prefer open spaces where they have a wide visual field to monitor possible predators (Wittmer, 2008).

Other authors also conclude that it is possible to reduce phytovolume and biomass by different species of domestic livestock, such as cows, sheep, and goats, and highlight the potentiating effect of the attractants (Mancilla-Leytón & Vicente, 2014; Ruiz-Mirazo, J., Robles, A. B., & González-Rebollar, 2010). The results obtained also show the effect on the distribution of feral goats in the autumn and winter seasons, with a greater effect on vegetation near the attractants.

It is worth noting the scarce difference detected in the number of fecal groups in the summer before the use of attractants and after their placement, when water shortage could have intensified its effect. This could be due to hunting carried out 2 km from the firebreak between the months of April and June, when 257 feral goats were killed, as well as the hunting in September, when another 67 goats were killed. This would explain the general decrease in excrement accumulation in the second year of study compared with the first. Across the island, feral goat population management systems are highly heterogeneous, from administration controlled areas to completely abandoned areas, including hunting areas (Ruiz-Mirazo et al., 2011). This makes population management very difficult since there are significant fluctuations in populations at a local level. Public administration will have to make a coordinated effort to harness the potential of these herds in maintaining firewall areas (Roque, 2015).

The behavior of feral goats, in regard to the range areas, was modified after placing attractants during the summer, winter, and autumn seasons, as the use of the area increased compared with before their placement. This phenomenon has also been observed in elk management, where lumps of salt were distributed at strategic points in their territories to avoid collisions with vehicles on the roads (Grosman et al., 2011; Laurian et al., 2008). These results are of special relevance for the management of the territory, since extensive livestock farming is in decline, and the populations of feral animals and the forest biomass are increasing (Ruiz-Mirazo et al., 2011).

In this work, the differences in the biomass of herbaceous plants within the fenced plots compared with the biomass of those outside them were particularly notable, indicating that grazing decreases their biomass, especially in areas near water and salt supply points. Similar results have been obtained with equine cattle in Galicia (Spain), where it was evident that the continuous and rotational management of the grazing system modifies the productivity of herbaceous plants and the evolution of understory biodiversity (Rigueiro-Rodríguez et al., 2012). This has also been observed with sheep in Andalusia (Spain), where a decrease in biomass of between 53% and 72% was observed (Robles et al., 2009). In the case of domestic goats in mountain areas where grazed and ungrazed areas were compared, a decrease in understory biomass was also observed (Marques et al., 2017).

It should be noted that the herbaceous layer of the study area is dominated by *Brachypodium retusum*, which is a common grass in the Mediterranean basin with flammable characteristics and early post-fire regeneration (Santana et al., 2013). On the other hand, this grass constitutes the fine fuel for the initiation of many forest fires (Vilà-Cabrera et al., 2008), so its reduction must be considered a success in the management of feral goats. From the results obtained, it also follows that the use of supplementary attractants, such as water and salt, increase the clearing effect of animals within a discrete radius of the supply point, in the order of tens of meters in this case, a distance similar to that obtained in other studies (Månsson et al., 2015; Velamazán et al., 2018).

Key data in firebreaks management refer to the time it takes for shrub vegetation to recover after mechanical cutting. These data will determine the frequency of clearing and, therefore, the efficiency of prevention tasks. In this work, it has been observed that part of the parcels in exclusion recover the phytovolume that existed before clearing in two years, while others require more time. It is also observed that the non-fenced plots (with free grazing), adjacent to those that recover the phytovolume in two years, have not recovered it in this period. This indicates that feral goats could delay the frequency of clearing, thereby saving management resources for fuel treatment. This has already been observed using domestic livestock, especially goats (Bowie et al., 2016; Jáuregui et al., 2007; Torrano & Valderrábano, 2005). However, in the case of feral goats, this effect does not occur in all the plots, possibly due to lack of management that forces them to remain in the area. In the case of this study, reduction in plant volume depends on the area and the shrub species.

The results of the analysis of phytovolume variations as a function of flammability showed that, in general, outside the exclusion plots there is a reduction in phytovolume for all classes of flammability, although in the group of non-flammable species (1) this reduction is not significant. However, in the case of the highly flammable group of species (4), it was observed that in plots near the supplementary attractants the phenomenon was reversed, with a significant increase in the phytovolume of this group of species outside the fences. Aleppo pine (*P. halepensis*), a dominant tree in the area, is found in this group and is not very appealing to animals due to its high content of secondary compounds (Burney & Jacobs, 2013). In this particular case, the browsing effect is likely to reduce competition with other species and favor the growth of highly flammable and poorly palatable species, such as pine. Something similar could happen with *P. angustifolia*, which is also highly flammable but much more palatable.

When the variability of the phytovolume by species in the grazed plots is analyzed after the two years of treatment, a lower recovery is observed in most of them. The effectiveness of feral goats in reducing fuel in firebreaks is thus verified. In addition, the use of feral goats also offers the potential to reduce fuel used in mechanical brush clearing, with a likely reduction in greenhouse gas emission. However, as previously discussed for pine, in some species the effect seems to be the opposite. This would be the case for *P. angustifolia*, which increased the phytovolume in the ungrazed plots near the attractant supply points. When browsing, animals are likely to reduce the competition of other species, thus favoring their growth.

Regarding biodiversity, grazing treatment and supply of attractants had no effect in the short–medium term. After two years of clearing and one year with attractants, no differences were observed in the Shannon indices. The values were low, below 2, probably due to low floristic richness and the dominance of some species. Two years is likely to be too short a period for changes in vegetation biodiversity to occur, especially in perennial species (Velamazán et al., 2018).

Conclusions

From the results obtained, it can be concluded that firebreak areas are places of attraction for feral goats, and their browsing activity contributes to slowing down the recovery of vegetation after mechanical clearing. This effect can be enhanced by supplying attractants, such as water and salt, at certain times of year and is especially evident in the reduction of the biomass of fine fuel, mainly *Brachypodium retusum*. As for the control of woody species, the effect depends on each area and each species, although, in general, it is possible to reduce the phytovolume of many species without affecting biodiversity in the short or medium term. The effect is probably less forceful than that achieved by directed grazing with domesticated herds. In this sense, support and recovery policies for rural pastoral communities would be necessary. However, this livestock has been decreasing since giving territory to populations of feral animals is a management strategy to attract feral goat populations to firebreak areas. This would contribute not only to reducing the risk of fires and, consequently, to the mitigation of climate change but also to the distribution of these animals to less conflictive places that do not cause damage in particularly fragile areas.

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Capítulo 3

Complementarity between microhistological analysis and polymerase chain reaction - capillary electrophoresis in diet analysis of ungulates in Mediterranean forest

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ABSTRACT

An evaluation is made of the complementarity between two non-invasive techniques, cuticle microhistological analysis (CMA) and polymerase chain reaction-capillary electrophoresis (PCR-CE) DNA-based analysis, for the determination of herbivore diet composition from faecal samples. CMA is based on the different microanatomical characteristics of the epidermal fragments remaining in the faeces. The PCR-CE technique combines PCR amplification of a *trnL*(UAA) genomic DNA region with amplicon length determination by capillary electrophoresis, with this length being characteristic for each species or taxon. A total of 37 fresh stool samples were analysed, including 16 from feral goats (*Capra hircus*) from the Tramuntana mountain range (Mallorca, Baleares), and 11 from *Bruna dels Pirineus* cattle breed (*Bos taurus*) from the surrounding Montserrat mountain range (Barcelona, Cataluña. All the animals were in a free grazing Mediterranean pine habitat, dominated by Aleppo pine (*Pinus halepensis*). The results showed that both techniques detected a similar number of plant components in the faeces of goats and cows. In the case of goats, a positive correlation was obtained between the percentage of samples in which a particular taxon is detected by CMA and the percentage of samples in which that taxon is detected by PCR-CE. This correlation was not observed in the case of cows. It is concluded that PCR-CE is a fast and precise method to detect the different plant components in the faeces of herbivores. However, it cannot be considered as an alternative to CMA, but as a complementary method, since both techniques can detect some taxons that are not detected by the other technique. In addition, CMA detected the presence of the different taxa in a greater number of samples, and at the same time it enables quantitative data to be obtained of plant diet composition. The species of herbivore also seems to influence the results obtained by PCR-CE, so more studies are required to address this aspect.

Keywords: *Capra hircus*, *Bos taurus*, faecal analysis, plant epidermis, free grazing.

Introduction

Many analysis techniques have been used in the past years to quantitatively or qualitatively evaluate the composition of the herbivore diet, the most relevant being: the direct observation of the animal(Shrestha & Wegge, 2006), including the bite or time counting methods (Pinto-Ruiz et al., 2014); near infrared reflectance spectroscopy (NIRS) (Coates & Dixon, 2008), quantification of n-alkanes in faeces (Ferreira et al., 2007), isotope stability analysis (Codron & Brink, 2007; Sponheimer et al., 2003), esophageal fistula sampling (Bautista et al., 1996), rumen content sampling.(Bertolino et al., 2009; Norbury & Sanson, 1992), microhistological analysis of faeces (Bartolomé et al., 1995; Stewart, 1967), and more recently, molecular analysis based on DNA (Pegard et al., 2009; Soininen et al., 2009). Each method has a number of advantages and limitations when interpreting eating habits (Henley et al., 2001). Among these techniques, cuticle microhistological analysis (CMA) is the most used method to study the diet in herbivores, although it requires a considerable training effort (Holechek & Gross, 1982), and there could be errors due to epidermal degradation and the microhistological similarity of some plant epidermis (Henley et al., 2001). On the other hand, molecular analyses based on the presence of genomic DNA remnants of ingested plants in faecal samples have been shown to be a useful and rapid tool to determine the components of the herbivore diet (Taberlet et al., 2007; Valentini et al., 2009). Within the molecular techniques, the PCR-CE technique (PCR-Capillary Electrophoresis), based on the PCR amplification of a gene fragment *trnL*(UAA), followed by the determination of the length of the amplicon by capillary electrophoresis, has been used to qualitatively estimate the presence of a small number of species in a controlled diet for chamois (Espunyes et al., 2019). However, this technique has not yet been used to determine the nature of the plants consumed by herbivores in free ranging situations.

The objectives of this work are to determine if the technique of the PCR-CE is able to identify the taxa of the diet in field grazing animals and to compare the results obtained by PCR-CE with those obtained by CMA, by evaluating the complementarity between both techniques. For this evaluation, faecal samples of animals that graze in Mediterranean environments were used, such as cows (*Bos taurus*) of the *Bruna dels Pirineus* breed in the surroundings of the Montserrat mountain, Barcelona, and feral goats (*Capra hircus*) on the island of Mallorca. Our results indicated that, in terms of species identification both techniques are similar, both for cows and for goats.
Regarding the percentage of individuals in which the presence of a certain species is detected, the microhistological analysis provided better results in both animal species.

Material and methods

Study area

This study was conducted with faecal samples of goats (*Capra hircus*) and cows (*Bos taurus*) that were fed in the undergrowth of Mediterranean pine forests. Samples of goats were collected in the Tramuntana mountain range, on the island of Mallorca (39°48′28″N2°47′37 ″E), where there is a population of feral animals (Vives & Baraza, 2010). The mean annual temperature and precipitation in the mountains is 18ºC and 650mm, respectively. The cows were located around the Montserrat Mountain Natural Park in the province of Barcelona (41°40'28"N1°46'33"E), where the mean annual temperature is of 13ºC, and rainfall of 610mm. In this case, the animals spend the winter months and part of the spring in the area, then they transhumance to the Pyrenees. The vegetation of both areas is dominated by trees of Aleppo pine (*Pinus halepensis* M.) with a Mediterranean scrub undergrowth with abundance of *Erica multiflora* L., *Pistacia lentiscus* L. and *Rosmarinus officinalis* L. The herbaceous stratum is dominated by the poaceae of the genus *Brachypodium* and, in the case of Mallorca, also by *Ampelodesmos mauritanica* (Poiret) T. Durand et Schinz.

Sample collection

Faecal samples were collected between the winter of 2016 and spring of 2017. Goat faeces were obtained directly from the rectum, from the animals killed during the culls made by the local administration. Cow faeces were collected right after their defecation. In order to prepare a reference collection for microhistological and molecular analysis, 100 g of leaves of the most abundant plant species in each study area were collected. A total of 27 faecal samples were collected, 11 from cows and 16 from feral goats. All samples were stored at -20ºC until analysis.

Cuticle microhistological analysis (CMA)

To determine the botanical composition of the diet in faeces by (CMA), the procedure used by Stewart (1967) was followed. The faecal samples were crushed in a mortar, and then 0.5 g of sample were placed in a test tube, where the content was digested with 3 ml of 65% concentrated nitric acid in a thermal bath at 80°C for 2 minutes. This content was diluted in 250 ml of distilled water and successively filtered through two sieves of 1 mm and 0.125 mm pore size. The material obtained in the second sieve was homogeneously distributed onto three slides with 50% glycerine and were sealed with DPX resin (Herter Instruments, Spain). To obtain the reference epidermis collection, the leaf samples were subjected to a scalpel scrape until the epidermis of both faces (abaxial and adaxial) was obtained. This epidermis was mounted onto slides with glycerine. The most recalcitrant species, in which epidermal tissue could not be obtained by this method, were subjected to acid digestion, following the same protocol as in the case of faecal samples. Preparations were observed under an optical microscope (Motic BA210, MoticEurope SLU, Spain) at 100 and 400 magnification, equipped with a Moticam 2300 camera. By using the Motic Images Plus 2.0 software, images of cellular forms, trichomes, and stomata were obtained from each plant sample, as a reference shown in Supplementary Figure S1. Subsequently, the faecal samples were then observed under the same microscope, making linear transects until a total of 200 epidermal fragments per sample were identified. The microhistological analysis was performed at the animal production laboratory of the Veterinary Faculty of the Universitat Autònoma de Barcelona (UAB). This laboratory is part of the ASFAC-LAB quality program consisting of an exercise of intercomparison of laboratories performing analysis of feed and raw materials for animal feed (http://www.asfac-lab.com/en/).

Molecular Analysis (PCR-CE)

Genomic DNA (gDNA) from the most abundant plant species in each area studied and from faeces samples was extracted with the DNeasy Plant Mini Kit and the QIAamp DNA faeces Kit (QIAGEN, Germany), respectively, following manufacturer's instructions. Genomic DNA of plants and faeces were diluted in 100 µl of milli-Q water and stored at -20°C. DNA concentration was measured with a NanoDrop 1000 Spectrophotometer (ThermoScientific, USA), and the quality of the gDNA was analysed on a 0.8% agarose gel electrophoresis in 1 x TAE buffer (40 mM Tris pH 7.6, 20 mM acetic acid, 1 mM EDTA). The sequences corresponding to the chloroplast gene *trnL*(UAA) were amplified by PCR from the oligonucleotides *trnL*-G (5'gggcaatcctgagccaaatc3 ') and *trnL*-D (5'ggggatagagggacttgaac3') (Taberlet et al., 2007), using plant and faeces gDNA as a template. The PCR reactions contained 1 x MyTaq reaction buffer (Bioline Reagents Ltd., United Kingdom), oligonucleotides at a concentration of 0.4 µM each (Stab Vida, Portugal), 1 U MyTaq DNA polymerase and 20 ng of gDNA, in a final reaction volume of 20 µl. The *trnL*-D oligonucleotide was labeled with 6-FAM (carboxyfluorescein) fluorochrome to allow fluorescent detection by capillary electrophoresis. The amplification reaction comprised different steps: initial denaturation of the gDNA at 95ºC for 1 min, followed by 35 cycles of denaturation at 95ºC for 15 s, annealing at 56ºC for 15 s, and elongation at 72ºC for 45 s, ending with a final elongation step at 72ºC for 3 min. The amplifications were performed on a MJ Research PTC-100 Thermal Cycler (MJ Research Inc., Canada). The amplification results were visualized on a 1.5% agarose gel electrophoresis in 1x TAE.

The length of the amplicons was determined by capillary electrophoresis using a Genetic Analyzer 3130xl system (Applied Biosystems, USA), using the LizS600 marker as standard length. The results were obtained as electropherograms where the amplified DNA fragment appeared as a fluorescence peak in the corresponding length. The electropherograms were analysed using the Peak Scanner version 2.0 software (Applied Biosystems, USA).The components of the diet were identified by comparing the lengths of the different amplicons obtained from the analysis of the faeces of cows and goats, with the reference collection established from the taxa considered in Table 1.

The molecular analysis was performed at the Genomics and Bioinformatics service of the UAB, which has a quality management system based on the ISO9001: 2015 standard and complies with the UAB code of good practice in research (https://sct.uab.cat/genomica-bioinformatica/en).

Statistical data processing

The analyses were performed separately for each animal species. For CMA analyses, the abundance of each taxon in the diet was determined as a percentage of epidermal fragments identified in the faeces. A generalized linear mixed model of binomial distribution was performed in order to determine the relationship between % of epidermal fragments identify in each sample and the probability of being detected through PCR-CE analysis. The percentage of fragments quantified was considered as a fixed predictive variable, the probability of being detected by PCR-CE as a response variable, and each species as a random factor ($B(nt/x) = \alpha + \beta 1$ percentage of epidermal + γ specie + ε). The glmer function of the lme4 package of R (Douglas Bates et al., 2015) was used.

A no parametric Spearman correlation was used to analyse the relationship between the percentage of samples on was detected each taxon by PCR-CE and the percentage of sample on was detected by CMA. In addition, the overall composition of the analysed diet was compared with each of the techniques using non-metric multidimensional scaling (NMDS). In this analysis, each sample was considered separately, and the distance matrix was calculated based on the binomial distance of Jaccard. The monoMDS function of the vegan package of R (Oksanen et al., 2019) was used.

Results

By means of the molecular technique of PCR-CE applied to the 39 selected taxa, it was possible to amplify a fragment corresponding to the gene *trnL*(UAA) in all of them, with lengths varying between 647 and 260 base pairs (bp) (Table 1). These data were used as a reference, shown in Supplementary Figure S2, for comparison with the size of amplicons detected in faeces.

Table 1. Length of the amplicons, in base pairs (bp), of the plant species considered in this study.

Species[1]	**Amplicon length (bp)**
Aphyllanthes monspeliensis	647
Carex sp.	598
Smilax aspera	540
Lolium perenne	524
Dorycnium sp.	520
Brachypodium sp.	515
Osyris alba	504
Ampelodesmos mauritanica	496
Cneorum tricoccon	495
Rhamnus alaternus	494
Arbutus unedo	492
Cistus sp.	489
Rubia peregrina	485
Chamaerops humilis	484
Psoralea bituminosa	483
Buxus balearica/Genista scorpius	479
Erica sp.	477
Bupleurum rigidum	472
Rosmarinus officinalis	461
Rubus ulmifolius	460
Genista lucida/Quercus sp./*Sanguisorba minor*	458
Pinus halepensis	456
Globularia alypum	445
Olea europaea/Phillyrea angustifolia	444
Pistacia lentiscus	425
Hieracium pilosella	418
Ononis sp.	395
Juniperus oxycedrus	265
Ephedra fragilis	260

[1] Plant species in the study areas; bp = Base pairs.

Some species that belong to the same genus had the same amplicon length, and therefore they were grouped into genus: *Dorycnium pentaphyllum* and *Dorycnium hirsutum* (*Dorycnium* sp.); *Brachypodium retusum* and *Brachypodium phoenicoides* (*Brachypodium* sp.); *Cistus albidus*, *Cistus monspeliensis* and *Cistus salviifolius* (*Cistus* sp.); *Erica multiflora* and *Erica arborea* (*Erica* sp.); *Quercus coccifera* and *Quercus ilex* (*Quercus* sp). Taxa in which the species could not be determined at the time of collection were also considered at the genus level, as was the case of *Carex* sp. and *Ononis* sp. Finally, species of a different genus or family but with identical amplicons were considered in the same group (*Buxus balearica* and *Genista scorpius*; *Genista lucida*, *Quercus* sp. and *Sanguisorba minor*; *Olea europaea* and *Phillyrea angustifolia*). The mean number of taxa identified per sample was higher when CMA was employed in both species, cows and goats (Table 2).

Table 2. Number of taxa identified per sample (maximum, minimum, and mean) by each technique (CMA and PCR-CE). On average, the CMA technique could identify 30% and 50% more taxa in cows and goats, respectively.

Taxon/sample	Cows		Feral Goats	
	CMA	**PCR-CE**	**CMA**	**PCR-CE**
Maximum	16	13	14	8
Minimum	11	3	10	2
Mean	13	9	12	6
SD	2	4	1	2

CMA = Cuticle microhistological analysis; PCR-CE = Polymerase chain reaction-Capillary electrophoresis

Table 3 shows the percentages of epidermal fragments identified in the faeces of cows together with the percentages of individuals in which a specific taxon analysed by CMA and/or PCR-CE appears. CMA analysis succeeded in identifying 19 taxa, while 20 were identified by PCR-CE. Some taxa were only detected with only one of the techniques. These were *Aphyllanthes monspeliensis*, *Carex* sp. and *Rosmarinus officinalis* by CMA and *Ephedra fragilis*, *Genista scorpius*, *Hieracium pilosella* and *Osyris alba* by PCR-CE., Eight taxa appeared in a greater proportion using CMA technique, and six using PCR-CE technique. The dominant taxon in the diet of cows was *Brachypodium* sp., which was more than a quarter of the diet. The PCR-CE technique, forced to group different species that has the same number of amplicon base pairs, as is the case of *Genista lucida*, *Quercus* sp. and *Sanguisorba minor*, and in the case of *Olea europaea* and *Phillyrea angustifolia*. In both cases, the CMA technique only detected fragments of one of the taxa, specifically *Quercus* sp. and *Olea europaea* respectively. In addition, considering that *Genista lucida* is not in the Montserrat study area, this species would no longer be part of the first group, in the case of cows.

Table 3. Percentage (%) of epidermal fragments in cow faeces determined by CMA and percentages of individuals in which a particular taxon appears by CMA and PCR-CE techniques.

<table>
<tr><th>Taxon</th><th>Faecal epidermal fragments (CMA)</th><th>Individuals with the presence of the taxon (CMA)</th><th>Individuals with the presence of the taxon (PCR-CE)</th></tr>
<tr><td>Aphyllanthes monspeliensis[1]</td><td>5.5</td><td>100.0</td><td>0.0</td></tr>
<tr><td>Arbutus unedo</td><td>1.7</td><td>81.8</td><td>54.5</td></tr>
<tr><td>Brachypodium sp.</td><td>27.6</td><td>100.0</td><td>63.6</td></tr>
<tr><td>Bupleurum rigidum</td><td>0.6</td><td>9.1</td><td>36.4</td></tr>
<tr><td>Carex sp. [1]</td><td>6.1</td><td>90.9</td><td>0.0</td></tr>
<tr><td>Cistus sp.</td><td>0.7</td><td>63.6</td><td>54.5</td></tr>
<tr><td>Dorycnium sp.</td><td>4.1</td><td>100.0</td><td>27.3</td></tr>
<tr><td>Ephedra fragilis[2]</td><td>0.0</td><td>0.0</td><td>18.2</td></tr>
<tr><td>Erica sp.</td><td>4.8</td><td>90.9</td><td>72.7</td></tr>
<tr><td>Sanguisorba minor</td><td>0.0</td><td>0.0</td><td rowspan="2">81.8</td></tr>
<tr><td>Quercus sp.</td><td>3.5</td><td>72.7</td></tr>
<tr><td>Genista scorpius[2]</td><td>0.0</td><td>0.0</td><td>9.1</td></tr>
<tr><td>Globularia alypum</td><td>0.3</td><td>18.2</td><td>18.2</td></tr>
<tr><td>Hieracium pilosella[2]</td><td>0.0</td><td>0.0</td><td>9.1</td></tr>
<tr><td>Juniperus oxycedrus</td><td>1.3</td><td>81.8</td><td>72.7</td></tr>
<tr><td>Olea europaea</td><td>6.5</td><td>100.0</td><td rowspan="2">90.9</td></tr>
<tr><td>Phillyrea angustifolia</td><td>0.0</td><td>0.0</td></tr>
<tr><td>Ononis sp.</td><td>0.05</td><td>9.1</td><td>27.3</td></tr>
<tr><td>Osyris alba[2]</td><td>0.0</td><td>0.0</td><td>9.1</td></tr>
<tr><td>Pinus halepensis</td><td>6.0</td><td>100.0</td><td>54.5</td></tr>
<tr><td>Pistacia lentiscus</td><td>4.1</td><td>90.9</td><td>100.0</td></tr>
<tr><td>Rhamnus alaternus</td><td>1.5</td><td>54.5</td><td>54.5</td></tr>
<tr><td>Rosmarinus officinalis[1]</td><td>9.0</td><td>100.0</td><td>0.0</td></tr>
<tr><td>Rubus ulmifolius</td><td>3.7</td><td>90.9</td><td>27.3</td></tr>
<tr><td>Smilax aspera</td><td>1.3</td><td>54.5</td><td>72.7</td></tr>
<tr><td>Other taxa</td><td>11.65</td><td>100.0</td><td>-</td></tr>
</table>

CMA = Cuticle microhistological analysis; PCR-CE = Polymerase chain reaction-Capillary electrophoresis.
[1] Taxa detected only by CME; [2] Taxa detected only by PCR-CE.

Table 4 shows the percentages of epidermal fragments identified in the faeces of goats, together with the percentages of individuals in which a specific taxon analysed by CMA and/or PCR-CE appears. In goat faeces, a total of 17 taxa were identified by CMA technique, and 15 by PCR-CE technique. *Smilax aspera* was only detected by CMA, and *Genista lucida* only by PCR-CE technique. Most taxa appeared in a greater proportion of individuals using CMA technique, and only *Arbutus unedo* using PCR-CE technique.

Table 4. Percentage (%) of epidermal fragments in goat faeces determined by CMA and percentages of individuals in which a certain taxa determined by CMA and by PCR-CE appears. Bold highlights those taxa only detected by one of the techniques.

Taxon	Faecal epidermal fragments (CMA)	Individuals with the presence of the taxon (CMA)	Individuals with the presence of the taxon (PCR-CE)
Ampelodesmos mauritanica	12.2	100.0	25.0
Cneorum tricoccon	2.1	62.5	6.3
Arbutus unedo	0.5	25.0	68.8
Brachypodium sp.	9.3	100.0	31.3
Chamaerops humilis	6.1	87.5	31.3
Cistus sp.	19.2	93.8	93.8
Ephedra fragilis	1.0	50.0	6.3
Erica sp.	4.6	93.8	56.3
Genista lucida	0.0	0.0	
Quercus sp.	2.1	62.5	43.8
Sanguisorba minor	0.0	0.0	
Globularia alypum	1.2	62.5	12.5
Juniperus oxycedrus	1.0	56.3	6.3
Olea europaea	8.7	100.0	68.8
Phillyrea angustifolia	0.9	43.8	
Pinus halepensis	3.7	93.8	6.3
Pistacia lentiscus	5.7	93.8	93.8
Rosmarinus officinalis	5.1	100.0	18.8
Smilax aspera[1]	1.8	56.3	0.0
Others	14.8	100.0	-

CMA = Cuticle microhistological analysis; PCR-CE = Polymerase chain reaction-Capillary electrophoresis.

[1] Taxa detected only by CME.

As in the case of cows, from the group formed by *Genista lucida*, *Quercus* sp. and *Sanguisorba minor* by PCR-CE, only *Quercus* sp., was identified by CMA. On the contrary, from the group of *Olea europaea* and *Phillyrea angustifolia*, the CMA technique distinguished fragments of both.

As regards the statistical analysis on cows results, no significant correlation was found between the percentage of samples in which a particular taxon is detected by CMA with the percentage of samples that the taxon is detected by PCR-CE ($\rho = 0.17$; $P = 0.44$ Spearman correlation test). However, in the case of goats, this correlation is positive and significant ($\rho = 0.56$; $P = 0.02$ Spearman correlation test). Similarly, the percentage of fragments of a taxon detected by CMA does not appear to have a significant effect on the probability that that taxon is detected by PCR-CE in cow faeces ($P = 0.4$ GLMM-binomial, Figure 1). However, in the case of goats, this effect is significant ($p = 0.006$ GLMM-binomial, Figure 1).

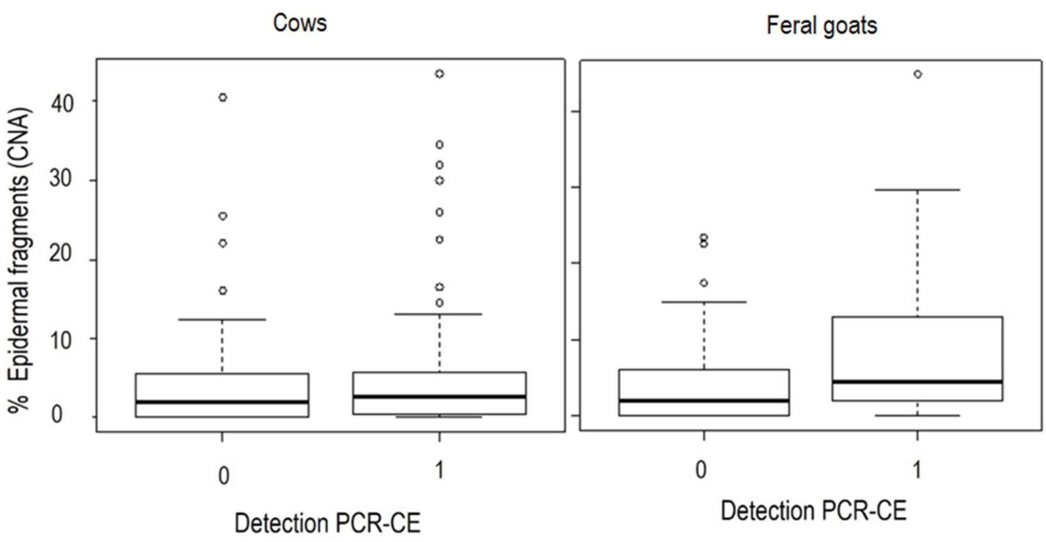

Figure 1. Box diagram showing the median (thick line) and distribution of the percentage of fragments found by CMA for taxa not detected (0) and detected (1) by PCR-CE.

Considering the overall diet composition of each sample in terms of the presence/absence of each taxon, the diets of both cows and goats are more similar to each other using the CMA method than using the PCR-CE, as shown by the results of the non-metric multidimensional scaling analysis (Figure 2). In both cases, the stress function was minimal, 0.05 in the case of cows, and 0.1 in the case of goats, ensuring that the two-dimensional representation adequately collects the multidimensional information of all taxa.

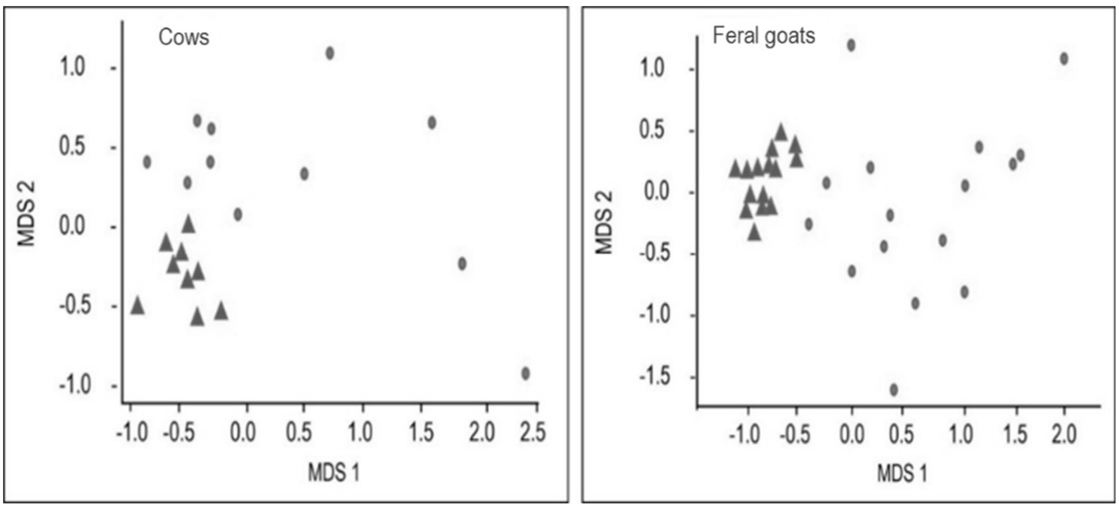

Figure 2. Non-metric multidimensional scaling (NMDS) representing the distance between samples as a function of the species composition of each sample analysed by CMA (triangle) and PCR-CE (circle) using the Jaccard distance matrix.

Discussion

The results obtained in this work show that the two analysis techniques, CMA and PCR-CE, are able to detect a similar number of plant components in the faeces of cows and goats. Similar results have been obtained recently in the study of the chamois diet (*Rupicapra rupicapra*) (Espunyes et al., 2019), although in this case, with controlled diets and using two amplicons in molecular analysis. The use of a single amplicon in the PCR-CE technique simplifies the method. Thus, the PCR-CE analysis would be a good alternative to CMA, since the latter requires a long training process (Holechek & Gross, 1982), and can be affected by the subjectivity of the observer and the differential digestibility of each plant species (Bartolomé et al., 1995; Leslie et al., 1983). For this reason, molecular analysis to determine the composition of the diet is becoming widespread and fast. Although they are not exempt from limitations, such as their extreme dependence on the degradation of the gDNA caused by the animal's own digestion or by environmental factors (Deagle et al., 2006; Espunyes et al., 2019). However, the results obtained here suggest that both techniques can be complemented rather than substituted. This is based on the fact that some species are only detected by one of the two methods.

In this work, differences between individuals have also been detected when both techniques are used. Thus, in the case of cows no correlation was observed between techniques, indicating that if a taxon appears in many individuals applying one technique, the same does not have to happen when applying the other. In contrast, a positive correlation was observed in goats, which suggested that both techniques will detect a greater or lesser percentage of individuals with a given taxon. In addition, the fact that taxa with higher percentages in the average diet of goats appear in more individuals, and that this does not occur in the case of cows, reinforces the idea that the results of both techniques vary depending on the animal species. This difference is difficult to explain, and may be based on the particularities of each digestive tract (Garnick et al., 2018; Sugimoto et al., 2018), or the amplification bias due to DNA degradation (Valentini et al., 2009). It is also worth noting the fact that, as regards the presence or absence of a certain taxon, the samples of both animals are more similar to each other when the CMA technique is applied. In this sense, the CMA technique would provide more information than the PCR-CE in the analysis of the components of the diet. These results agree with those obtained by Murphree (2012), who, when working with controlled diets obtained a mean of 89% of correct identifications using CMA, and only 50% using molecular analysis. In this sense, Valentini et al. (2009) indicate that by using the molecular method, 50% of the ingested taxa could be identified.

Finally, it should be noted that the main difference between the two methods is the possibility of quantifying the components of the diet. In this case, the CMA technique can obtain the proportions of the different fragments detected in the faeces. In general, it is assumed that these proportions correspond to the animal's intake, although if it is required to calculate the amount ingested, it may be necessary to apply correction factors (Sierra et al., 2005). The PCR-CE technique has inaccuracies when quantifying the components of the diet since the DNA extracted in the faeces is not related to the amount of food ingested, probably due to the variability in the degradation of the DNA during digestion (Deagle et al., 2009, 2010; Valentini et al., 2009). Another limitation is the similarity of the size of the amplicon that can occur between some species. Valentini et al. (2009) recommend, for these “problematic” taxa, to complement with another additional system, such as the CMA, or simply work with larger groups such as genders or families.

Conclusions

It is concluded that the molecular analysis by PCR-CE, based on the length of a single amplicon is a fast and precise method to detect the different plant components in the faeces of herbivores. The type of herbivore seems to influence the results obtained with this technique, so more studies are required to address this aspect. There is no doubt that the PCR-CE can complement the cuticle microhistological analysis (CMA) by being able to detect some species that leave few epidermal fragments and consequently are not detected by CMA. However, it cannot be considered as an alternative, since CMA detects the presence of the different taxa in a greater number of samples, and at the same time is able to obtain percentage data of the diet content.

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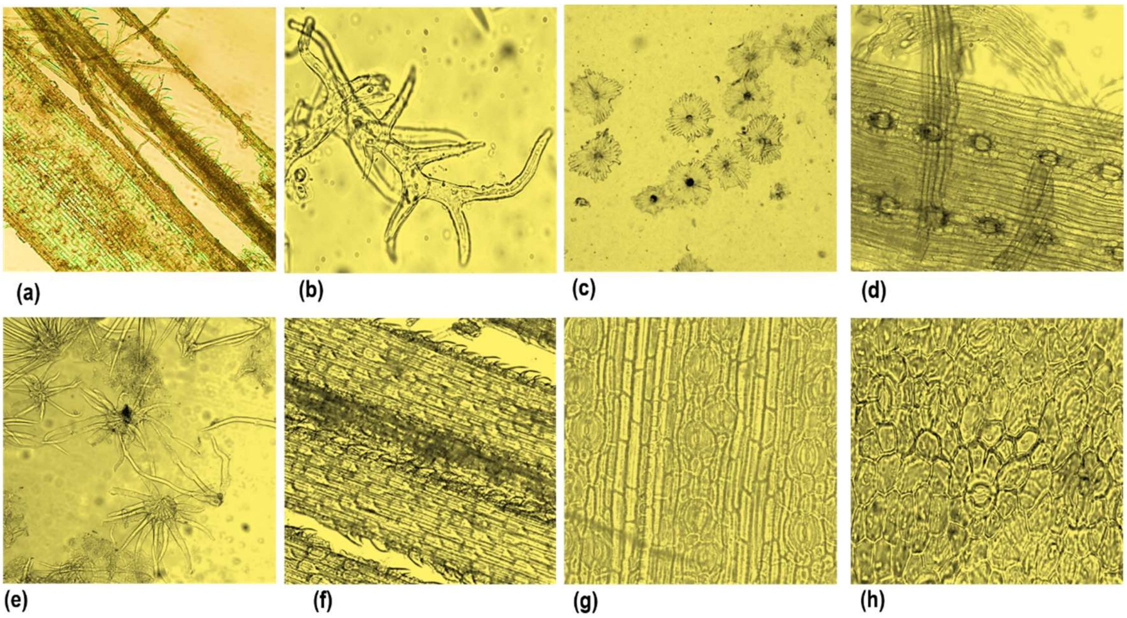
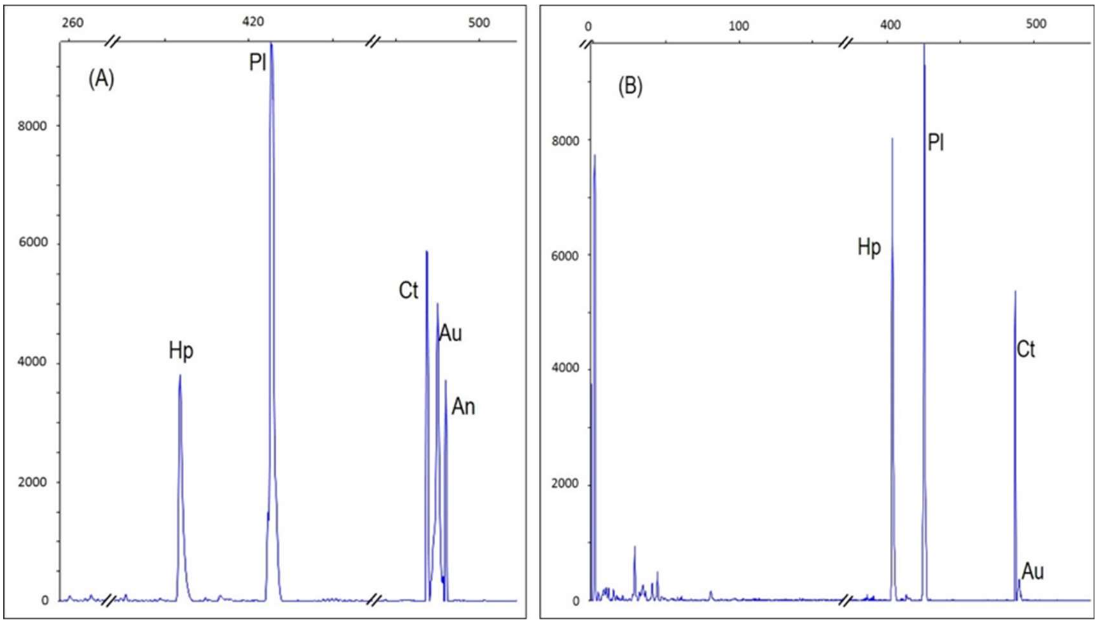
Territories of the Balearic Islands that, through the staff of the Consortium for the Recovery of the Fauna of the Balearic Islands (COFIB), provided us with the excrements of the goats wild. The capillary electrophoresis analysis was performed at the Genomics and Bioinformatics Services of the UAB.

Supplementary material



Supplementary Figure S1. Microhistological images obtained with the Moticam 2300 camera of taxa with the greatest presence in feces of cows and feral goats; (a) epidermal fragment of *Brachypodium retusum* (X 100 magnification), (b) trichomes of *Rosmarinus officinalis* (X 400 magnification), (c) trichomes of *Olea europaea* (X 100 magnification), (d) stomata and cellular forms of *Pinus halepensis* (X 100 magnification), (e) trichomes of *Cistus albidus* (X 100 magnification), (f) trichomes of *Ampelodesmos mauritanica* (X 100 magnification), (g) stomata and cellular forms of *Chamaerops humilis* (X 100 magnification), (h) stomata and cellular forms of *Pistacia lentiscus* (X 400 magnification).



Supplementary Figure S2. Representative electropherograms obtained by the PCR-CE technique, corresponding to the *trnL (UAA)* gene of the taxa present in the feces samples in cows (A) and goats (B). (Hp) *Hieracium pilosella*, 418pb; (Pl) *Pistacia lentiscus*, 425 bp; (Ct) *Cistus* sp., 489 bp; (Au) *Arbutus unedo*, 492 bp; (An) *Ampelodesmos mauritanica*, 496 bp.

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Discusión general

Discusión general

El uso del ganado doméstico en la gestión forestal ha despertado interés en las últimas décadas como consecuencia del incremento de la biomasa forestal y del riesgo de incendios (Ruiz-Mirazo, 2007; Bernués et al., 2011). Las razones de este incremento se detallaron en la introducción de la tesis, y, en resumen, son el despoblamiento rural, el abandono de la actividad agropecuaria extensiva o el incremento de zonas protegidas. A este proceso se suma el cambio global, que en la cuenca mediterránea conlleva mayores temperaturas y menores precipitaciones, aumentando también el riesgo de incendio.

Una de las estrategias para reducir la biomasa vegetal combustible del sotobosque se centra en promover el pastoreo del ganado doméstico en los bosques (Velamazán et al., 2018). Desde hace algunas décadas, se está trabajado con especies ganaderas de carácter ramoneador, como las cabras, para controlar principalmente la vegetación arbustiva (Lovreglio et al., 2014). También se han ensayado con bastante éxito otras especies como las ovejas, con un comportamiento más intermedio, entre ramoneador y pastador (Ruiz-Mirazo & Robles, 2012; Taylor, 1994). Algunos trabajos también destacan el papel que pueden jugar los equinos en la reducción de la biomasa forestal (Bartolomé et al., 2020; Gulías et al., 2016). Sin embargo, el ganado bovino, catalogado como muy pastador (Lamoot et al., 2005), apenas se ha considerado para desarrollar esta función, aunque algunas razas, como la *Vaca de les Alberes*, presentan un gran potencial para ello (Bartolomé et al., 2011). Tampoco se están teniendo en cuenta las crecientes poblaciones de ganado asilvestrado, como consecuencia del abandono de la ganadería tradicional (Carpio et al., 2014; Velamazán et al., 2018), que se refugia en bosques y matorrales. En algunas regiones, como el caso de las cabras asilvestradas en Mallorca, estos animales se dispersan por las sierras y buscan su alimento en los brotes tiernos de zonas desbrozadas, como pueden ser las franjas cortafuegos. En esta tesis, se valora por primera vez el papel de ganado poco convencional en la lucha contra los incendios forestales, como son una raza bovina típicamente pastadora, la *Bruna dels Pirineus*, y cabras sin apenas manejo, como las asilvestradas de Mallorca. Ambas presentan aún un número de efectivos considerable en el territorio, lo que aumenta su interés para la gestión forestal, en comparación con otros animales, bien adaptados a condiciones de sotobosque pero en clara regresión, como el ganado asnal o como la misma *Vaca de les Alberes.* Por otro lado, resulta evidente que el papel que juega el ganado en el control del sotobosque depende de sus preferencias alimentarias. En este sentido, el conocimiento de la composición de la dieta se convierte en uno de los principales factores a considerar en este tipo de gestión. Sin embargo, los métodos tradicionales para el estudio de dietas, como el análisis microhistológico de las heces, son laboriosos y difíciles de automatizar. Por esta razón, en esta tesis se compara por primera vez el análisis microhistológico de las heces con un análisis molecular de protocolo sencillo. Se trata de un intento de buscar alternativas ágiles para conocer la composición de la dieta del ganado y poder valorar así su potencial como herramienta de prevención de incendios.

El primer capítulo de esta tesis mostró como el ganado bovino, procedente de los pastos herbáceos del Pirineo, sin alimentación suplementaria y con alta densidad, aumentó el consumo de plantas leñosas esclerófilas durante dos meses mientras estaba cercado en un pinar mediterráneo. El ganado se centró inicialmente en plantas herbáceas y algunos arbustos con alto contenido de proteínas. Después de dos meses, el ganado aumentó el consumo de varias especies leñosas, como *Erica multiflora*, *Olea europaea* y *Pistacia lentiscus*. El ganado evitó la especie arbórea dominante (*Pinus halepensis*), probablemente debido a la escasez de árboles jóvenes y a su baja palatabilidad. El ganado también consumió briófitas, las cuales poseen componentes químicos disuasivos (Ihl & Barboza, 2007 y referencias en el mismo). Este resultado podría revelar la falta de recursos óptimos o el pastoreo excesivo (Ihl & Barboza, 2007). Por lo tanto, sin alimentación suplementaria, el ganado podría modificar efectivamente la estructura del bosque al cambiar, a corto plazo, la densidad de los diferentes estratos de vegetación del sotobosque, pero con implicaciones en su salud. En este sentido, aunque los bosques mediterráneos pueden satisfacer las necesidades del ganado bovino en la primavera (Casasús et al., 2005), hacia el verano las principales plantas del sotobosque, como *Brachypodium spp.*, *Erica multiflora* y *Rosmarinus officinalis,* muestran contenidos de proteínas por debajo del umbral de mantenimiento, situado alrededor del 7% (NRC, 1996). Esta dieta de baja calidad podría ser la causa de la disminución del contenido de nitrógeno en las heces de ganado. Además, las concentraciones de NEFA en algunos individuos se acercaron a 0.6 mmol/L, el límite más allá del cual se produciría un severo balance energético negativo (Adewuyi et al., 2005). Por lo tanto, si se prolongara el período de este tipo de pastoreo, basado en cargas ganaderas elevadas en pequeñas parcelas, se reduciría la cubierta leñosa, pero se comprometería la salud del ganado. Se demuestra pues que la eficacia de las razas de vacas típicamente pastadoras, como la *Bruna dels Pirineus*, en el control del sotobosque queda limitada por cuestión de calidad del alimento.

La disminución de los rebaños domésticos en extensivo conduce a interesarse por las poblaciones de animales asilvestrados dentro de la estrategia de uso del pastoreo como herramienta de gestión forestal. En el segundo capítulo de esta tesis, se muestra el papel que ejercen las cabras asilvestradas en el mantenimiento de las áreas cortafuegos y como el uso de atrayentes permite incrementar sus efectos. Los resultados muestran que las cabras asilvestradas usan más el área cortafuegos que el bosque adyacente, ejerciendo una importante presión sobre su vegetación. Esto concuerda con otros resultados en los que se observa como las áreas cortafuegos presentan cubiertas herbáceas de mayor calidad y abundancia para los ungulados silvestres y ganado extensivo respecto a las formaciones leñosas (Mancilla-Leytón et al., 2013; Ruiz-Mirazo et al., 2011). Las diferencias obtenidas en la biomasa de las herbáceas dentro de las parcelas cercadas respecto a fuera de éstas fueron particularmente notables, indicando que el pastoreo disminuye esta biomasa, especialmente en las zonas cercanas a los puntos de suministro de agua y sal. Otros autores concluyen también que es posible la reducción del fitovolumen y la biomasa por distintas especies de ganado doméstico, como vacas, ovejas y cabras y destacan el efecto potenciador de los atrayentes (Ruiz-Mirazo et al., 2010; Mancilla-Leytón & Vicente, 2014). Cabe destacar que el estrato herbáceo de la zona de estudio está dominado por *Brachypodium retusum*, que es una gramínea común en la cuenca mediterránea con características inflamables y de regeneración precoz post incendios (Santana et al., 2013). Por otro lado, esta gramínea constituye el combustible fino para el inicio de muchos incendios de superficie (Vilà-Cabrera et al., 2008), por lo que su reducción debe considerarse un éxito en la gestión de cabras asilvestradas. Los resultados también indican que las cabras asilvestradas podrían retrasar la frecuencia del desbroce del cortafuegos, con el consiguiente ahorro de recursos de gestión para el tratamiento del combustible. Esto ya ha sido observado utilizando ganado doméstico, especialmente cabras (Bowie et al., 2016; Jáuregui et al., 2007; Torrano & Valderrábano, 2005).

En los resultados obtenidos también se muestra el efecto sobre la distribución de las cabras asilvestradas en las estaciones de otoño e invierno, con un mayor efecto sobre la vegetación cerca de los atrayentes. La conducta de las cabras asilvestradas, en cuanto a las zonas de campeo, resultó modificada después de colocar los atrayentes, en las estaciones de verano, invierno y otoño, al incrementarse el uso de la zona respecto a antes de su colocación.

Estos resultados son de especial relevancia para la gestión del territorio, ya que la ganadería extensiva se encuentra en declive y las poblaciones de animales asilvestrados y la masa forestal van incrementándose (Ruiz-Mirazo et al., 2011).

La composición de la dieta del ganado constituye una información muy relevante en la gestión del ganado en zonas boscosas. En el tercer capítulo de esta tesis se comparó la técnica más tradicional de análisis microhistológico de las heces (CMA) con el análisis molecular (PCR-CE). Los resultados obtenidos mostraron que las dos técnicas de análisis permiten detectar un número similar de componentes vegetales en las heces de vacas y cabras. Resultados parecidos se han obtenido recientemente en el estudio de la dieta del rebeco (*Rupicapra rupicapra*) por Espunyes et al. (2019), aunque en este caso, con dietas controladas y utilizando dos amplicones en el análisis molecular. El uso de un solo amplicón en la técnica PCR-CE permite simplificar el método. Los resultados aquí obtenidos, con un solo amplicón, sugieren que ambas técnicas pueden complementarse en lugar de sustituirse. Esto se fundamenta en el hecho de que algunas especies solo se detectan con uno de ambos métodos. Además, el hecho de que los taxones con mayores porcentajes en la dieta promedio de las cabras aparezcan en más individuos y que esto no ocurra en el caso de las vacas, refuerza la idea de que los resultados de ambas técnicas varían en función de la especie animal. Esta diferencia resulta difícil de explicar y quizás se fundamente en un posible efecto diferencial entre las particularidades de cada tracto digestivo (Garnick et al., 2018; Sugimoto et al., 2018), o al sesgo en la amplificación debido a la degradación del ADN (Valentini et al., 2009). Cabe también destacar el hecho de que, en cuanto a la presencia o ausencia de un determinado taxon, las muestras de ambos animales son más parecidas entre sí cuando se aplica la técnica CMA. En este sentido, la técnica CMA aportaría más información que la PCR-CE en el análisis de los componentes de la dieta. Finalmente, hay que destacar que la principal diferencia entre ambos métodos se encuentra en la posibilidad de cuantificar los componentes de la dieta. En este caso, la técnica de CMA permite obtener las proporciones de los distintos fragmentos detectados en las heces.

En resumen, en esta tesis se demuestra que especies de ganado poco convencionales en la gestión forestal, pero abundantes en el territorio, pueden desempeñar un importante papel en la reducción de biomasa combustible. Para ello se requiere de suplemento alimentario, en el caso del ganado bovino, y actuaciones de manejo, como uso de atrayentes, en el caso del ganado caprino asilvestrado. Para determinar las dietas de estos animales se continúa utilizando técnicas laboriosas, pero que pueden complementarse para mejorar su precisión con técnicas más novedosas.

Conclusiones

Conclusiones

- El ganado bovino puede utilizarse en el control del sotobosque mediterráneo aplicando cargas ganaderas elevadas en un corto periodo de tiempo.
- Para periodos de pastoreo más largos sería necesaria alimentación suplementaria para evitar efectos perjudiciales sobre la salud del ganado bovino.
- Las franjas cortafuegos podrían ejercer un papel atrayente para las cabras asilvestradas, siendo una herramienta de gestión en el uso del territorio.
- Las cabras asilvestradas pueden contribuir en el mantenimiento de franjas cortafuegos puesto que ralentizan la recuperación de la vegetación después de desbroces mecánicos.
- El control de la vegetación de las franjas cortafuego mediante el pastoreo con cabras asilvestradas puede potenciarse mediante el uso de atrayentes, como agua y sal, en ciertas épocas del año.
- La técnica molecular del PCR-CE puede complementar a la técnica microhistológica (CMA) para determinar la composición de la dieta de vacas y cabras en pastoreo.
- La técnica microhistológica (CMA), a diferencia de la técnica molecular del PCR-CE, permite obtener datos porcentuales del contenido de la dieta.

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