Head Office: Università degli Studi di Padova

Department of Agronomy, Food, Natural resources, Animal and Environment

Ph.D. COURSE IN: ANIMAL AND FOOD SCIENCE

SERIES XXXV

Integrated agroecology approach for monitoring highlands: from GPS telemetry of grazing patterns to soil microbiology of nutrient cycle

Coordinator: Prof.ssa Angela Trocino

Supervisor: Prof. Enrico Sturaro

Co-Supervisor: Prof. Maurizio Ramanzin

Ph.D. student: Salvatore Raniolo

Contents

LIST OF ABBREVIATION

A: adenine

AIC: Akaike Information Criterion

ANOSIM: Analysis of Similarities

ANOVA: Analysis of Variance

AOA: Archaeal Ammonia-Oxidizers

AOB: Bacterial Ammonia-Oxidizers

ARPAV: Veneto Regional Agency for Environmental Protection and Prevention

BIC: Bayesian Information Criterion

C: Carbon

C: cytosine

CCI-LC: Climate Change Initiative – Land Cover

CO$_2$: Carbon dioxide

Df: degree of freedom

DNA: DeoxyriboNucleic Acid

DTM: Digital Terrain Model

E: East

EPSG: European Petroleum Survey Group

FAO: Food and Agriculture Organization of the United Nations

G: guanine

GAM: General Additive Model

GIS: Geographic information system

GNSS: Global Navigation Satellite System

GPS: Global Positioning System

INLA: Integrated Nested Laplace Approximation

IPCC: Intergovernmental Panel on Climate Change

N: Nitrogen

N: North

N_2O: Nitrous oxide

NDVI: Normalized Difference Vegetation Index

NMDS: Non-metric Multidimensional Scaling

OTU: Operational Taxonomic Unit

PCoA: Principal Coordinates Analysis

PCR: Polymerase Chain Reaction

PERMANOVA: Permutational Multivariate Analysis of Variance

qPCR: RealTime PCR

R: A (adenine) or G (guanine)

r: coefficient of correlation

R^2: coefficient of determination

rRNA: Ribosomal ribonucleic acid

RSME: Root Mean Square Error

S: C (cytosine) or G (guanine)

SD: Standard Deviation

SE: Standerd Error (Std.Error)

SOC: soil organic carbon

T: thymine

VIF: Variance Inflation Factor

Y: C (cytosine) or T (thymine)

LIST OF FIGURES

ABSTRACT

The following thesis investigates multiple methods to monitor seminatural grasslands managed by humans, and it mainly focuses on grasslands. These biomes are among the most widespread in the world, almost covering 40% of terrestrial surface and are characterized by a rich multifunctionality. Among semi-natural grasslands, pastures and meadows play important roles in agriculture by supporting livestock systems with habitat and feeds. Grassland functionality is based on soil, which hosts complex microbial communities able to drive and regulate nutrient cycles such as N and C. Local disturbance, such as animal grazing and fertilization, can influence the soil microbial communities with implications on ecosystem functions. However, the relations among local disturbances and soil microbial communities are still largely unexplored. This thesis aims to investigate the feasibility of integration between methods used to characterize grazing patterns and soil microbial communities in order to build an integrate approach for monitoring grasslands.

This thesis is articulated with three contributions based on the application of GPS telemetry combined with remote sensing and molecular analysis to monitor highlands grasslands. The first contribution tests the effects of vegetation abundance, derived from satellite imagery (NDVI index), slope and farmer conduction on grazing patterns of lactating cows in alpine pastures through GPS telemetry and remote sensing. The second contribution is also based on GPS telemetry and remote sensing, and it extends the approaches of the first one, considering the differences among multiple behaviours and parities and between local and common breeds in lactating cows at a larger alpine pasture. The third contribution tests the effect of difference in terms of soil microbial communities between highland grasslands managed as pasture or meadow in a European context through the application of real time PCR and sequencing.

Results of the first contribution revealed significant avoidance of the steepest slopes and preference for high vegetated grassland areas by the cows and a significant effect of the farmer conduction, necessary to increase the use of marginal areas. The second contribution confirmed the avoidance of the steepest slopes and the preference for grassland areas but revealed a significant difference between local and common dairy breeds as a function of parity where younger individuals of local breed moved faster and used steeper slope than common breed and older individuals. It also confirmed a diurnal activity pattern of grazing, resting a dn walking, with grazing being predominant during the day, but remarkably present also during the early night. Results of the third contribution revealed a significant effect of pH to shape both taxonomic and functional profiles of soil microbial communities, while management of pasture and meadow induced dissimilarity only in terms of OTU

lists, highlighting likely functional redundancy and uncoupling between taxonomic biodiversity and functional diversity. Thus, the results presented in this thesis confirmed the promising application of GPS telemetry-remote sensing and real time PCR-sequencing for monitoring grassland as all methods were able to detect variability at fine scales. The integration of methods can provide useful tools for monitoring the multifunctionality of grasslands and assessing impacts of human management at different temporal and spatial scales.

RIASSUNTO

La seguente tesi indaga molteplici metodi per monitorare le praterie seminaturali gestite dall'uomo, concentrandosi principalmente sui pascoli. Questi biomi sono tra i più diffusi al mondo, ricoprendo quasi il 40% della superficie terrestre, e sono caratterizzati da una ricca multifunzionalità. Tra le praterie seminaturali, i pascoli e i prati svolgono un ruolo importante in agricoltura, supportando i sistemi zootecnici con habitat e risorse alimentari. La funzionalità dei pascoli si basa sul suolo, che ospita comunità microbiche complesse in grado di guidare e regolare i cicli dei nutrienti come quelli del N e C. I disturbi locali, come il pascolo e la fertilizzazione, possono influenzare le comunità microbiche del suolo con implicazioni sulle funzioni dell'ecosistema. Tuttavia, le relazioni tra disturbi locali e comunità microbiche del suolo sono ancora in gran parte inesplorate. Questa tesi cerca di investigare la fattibilità dell'integrazione tra i metodi utilizzati per caratterizzare i modelli di pascolo e le comunità microbiche del suolo, al fine di costruire un approccio integrato per il monitoraggio delle praterie.

La tesi si articola in tre contributi basati sull'applicazione della telemetria GPS combinata con il telerilevamento e le analisi molecolari per monitorare le praterie degli altopiani. Il primo contributo verifica gli effetti dell'abbondanza della vegetazione, derivata da immagini satellitari (indice NDVI), della pendenza e della conduzione degli allevatori sui pattern di pascolo delle vacche in lattazione nei pascoli alpini attraverso la telemetria GPS e il telerilevamento. Il secondo contributo, anch'esso basato sulla telemetria GPS e sul telerilevamento, estende gli approcci del primo, considerando le differenze tra comportamenti multipli, età e tra razze locali e comuni nelle vacche in lattazione in un pascolo alpino più esteso. Il terzo contributo verifica l'effetto delle differenze in termini di comunità microbiche del suolo tra le praterie di altopiano gestite a pascolo o a prato in un contesto europeo attraverso l'applicazione della RealTimePCR e del sequenziamento.

I risultati del primo contributo hanno rivelato da parte delle vacche un significativo evitamento delle pendenze più ripide insieme una preferenza per le aree erbose con abbondante vegetazione e un significativo effetto della conduzione degli allevatori, necessaria per aumentare l'utilizzo delle aree marginali. Il secondo contributo ha confermato l'evitamento delle pendenze più ripide e la preferenza per le aree erbose, ma ha rivelato una differenza significativa tra le razze da latte locali e comuni in funzione dell'età, mostrando che gli individui più giovani della razza locale si muovevano più velocemente e utilizzavano pendenze più ripide rispetto alla razza comune e agli individui più anziani. È stato inoltre confermato un pattern di attività diurna di pascolamento, riposo e camminata, con il pascolamento predominante durante il giorno, ma notevolmente presente anche nelle prime ore della

notte. I risultati del terzo contributo hanno rivelato un effetto significativo del pH nel modellare sia i profili tassonomici che funzionali delle comunità microbiche del suolo, mentre la gestione a pascolo e a prato ha indotto una dissimilarità solo in termini di liste di OTU, evidenziando una probabile ridondanza funzionale e un disaccoppiamento tra biodiversità tassonomica e diversità funzionale. I risultati presentati in questa tesi hanno quindi confermato la promettente applicazione della combinazione tra telemetria GPS-remote sensing e di quella tra RealTime PCR-sequenziamento per il monitoraggio dei pascoli, poiché tutti i metodi sono stati in grado di rilevare la variabilità a scala fine. L'integrazione dei metodi può fornire strumenti utili per monitorare la multifunzionalità delle praterie e valutare gli impatti della gestione umana a diverse scale temporali e spaziali.

1 - GENERAL INTRODUCTION

1.1 - GRASSLANDS AND THEIR MULTIPLE FUNCTIONS

Grasslands are among the most widespread ecosystems in the world covering more than 37% of the global surface excluding Antarctica and Greenland (Zhong et al., 2015, Zhao et al. 2020, Bai and Cotrufo 2022). These ecosystems are present at all latitudes (Fig.1) and include all herbaceous vegetation types. Grasslands can be classified into three macro categories: natural, semi-natural and improved or intensive (Hejcman et al. 2013; Squires et al. 2018; Mencel et al. 2022). Natural grasslands include those that thrive without direct human intervention and are predetermined by wild herbivores and environments (Hejcman et al. 2013),as the North American prairies, the South American pampas, the African savannas, the tundra, and the Eurasian steppes (Bengtsson et al. 2019; Zhao et al. 2020). Semi-natural grasslands include those that have been formed by long-term human activity for agricultural purposes in previously woody areas and are extent managed as pastures or meadows (Veen et al. 2009; Lemaire et al. 2011; Hejcman et al. 2013; Bengtsson et al. 2019). Improved or intensive grasslands correspond to those managed intensively by sowing and high fertiliser inputs, used for productive forage grasses and legumes in modern agriculture (Hejcman et al. 2013). A further classification of grasslands is based on the type of vegetation in function of the terrestrial ecoregions identifying nine macro categories (Alpine grassland, Boreal grassland, Tropical montane grassland, Tropical freshwater grassland, Tropical lowland grassland, Mediterranean grassland, Temperate grassland, Cool semi-desert grassland and Warm semi-desert grassland - Dixon et al. 2014). Grasslands, in particular those natural and semi-natural, are also crucial for the global biodiversity as they are among the most species-rich ecosystems (Veen et al. 2009; Lemaire et al. 2011; Squires et al. 2018; Petermann and Buzhdygan 2021). Grasslands host a wide diversity of plant communities (Gibson 2009; Lemaire et al. 2011): for example, mountain grasslands can host until 89 plant species per square metre, among which many are endemic and often endangered (Petermann and Buzhdygan 2021). They also are source of habitats for various wildlife species throughout the world (Gibson 2009), as the wild herbivores (Veen et al. 2009; Fynn et al. 2016) or the multiple bird (Askins et al. 2007; Aldabe 2018) and insect species associated with meadows and pastures (Bignal and McCracken 1996; Kuussaari et al. 2007; Girardello et al. 2009; Jerrentrup et al. 2016). Thanks to the synergies between their biodiversity and functions, grasslands provide all types of ecosystem services (provisioning, cultural, supporting and regulating services): so benefits which meet the human needs (Daily 2003). In terms of provisioning services, grasslands are sources of feed and raw materials when are managed as pasture with animal grazing or meadow with mowing by humans

(Zhao et al. 2020; Petermann and Buzhdygan 2021). Possible raw materials provided by grassland, such as plant compounds used as drugs, derive directly from precious and wide genetic pools of grassland, which is part of their provisioning services (Zhao et al. 2021). In terms of cultural services, grasslands are part of cultural landscapes, as products of long-term human activities (Hejcman et al. 2013): suited to tourism (Thiene and Scarpa 2008; Zhao et al. 2020) and tied to rural heritage as summer transhumance (Sturaro et al. 2013; Zendri et al. 2016; Zhao et al. 2020). In terms of supporting services, grasslands support wildlife presence with their habitats (Askins et al. 2007) and the primary production through photosynthesis (Bengtsson et al. 2019). In terms of regulating ecosystems services, grasslands may play fundamental roles in erosion control (Bengtsson et al. 2019), water and nutrient cycling, carbon sequestration and climate cooling (Bengtsson et al. 2019; Zhao et al. 2020; Bai and Cotrufo 2022).

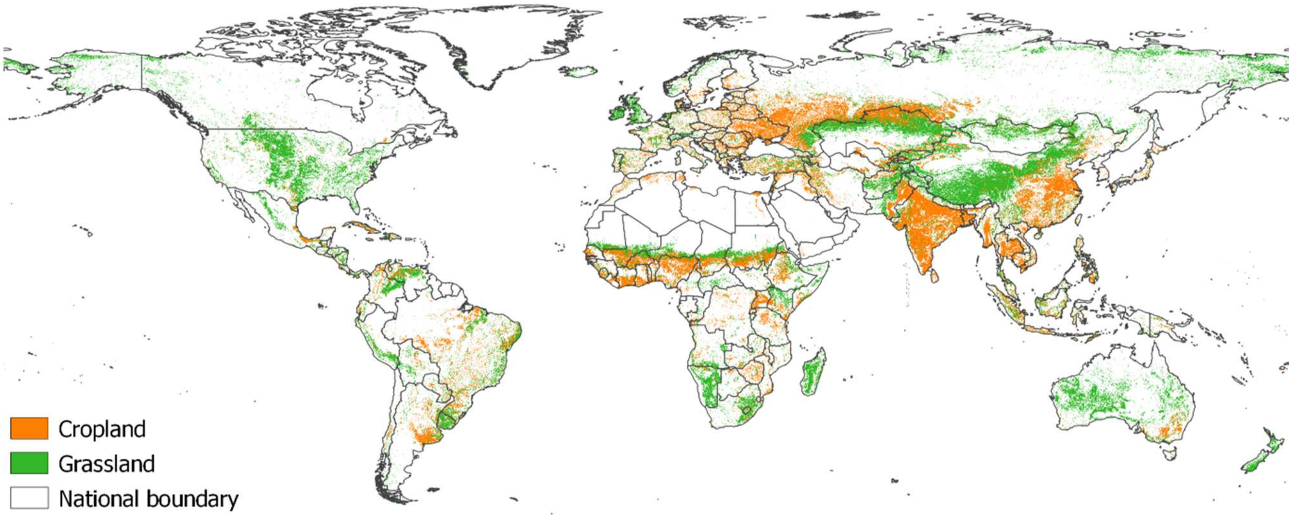


Figure 1. Global distribution of grasslands and croplands extracted from CCI-LC 2019 (Climate Change Initiative-Land Cover) products by the European Space Agency CCI projects (http://maps.elie.ucl.ac.be/CCI) with a resolution of 300 m per pixel.

Among all the regulating services provided by grasslands, one of the most relevant is carbon storage. Grasslands are among the biggest carbon sinks: their contribution to terrestrial carbon storage is about 34%, one third of total (Bai and Cotrufo 2022). Vegetation is the main driver of carbon sequestration through photosynthesis (Yang et al. 2019), which converts the atmospheric carbon into plant tissue or molecules pumped into the soil as exudates. Carbon is stored for about 90% into the soil as root biomass and soil organic carbon (SOC), which includes a broad spectrum of chemical molecules such as aliphatic compounds or root exudates (Bai and Cotrufo 2022). SOC presents two main fractions: the particulate organic matter (POM) and the mineral-associated organic matter (MAOM). The former derives from residues of plants and microbes, so it is characterised by large polymers with light molecular weight (Lavallee et al. 2020; Cotrufo and Lavallee 2022). The latter derives from small molecules, leached from plant as root exudates or residues, and associated to mineral or assimilated by microbial biomass (Lavallee et al. 2020; Cotrufo and Lavallee 2022). The nature of the two SOC factions leads to different contributions to carbon sequestration in terms of time with longer contributions for MAOMs than POMs (Bai and Cotrufo 2022). The carbon sequestration capacity of grassland soils is limited and depends on vegetation (Yang et al. 2019), climatic conditions (Cheng et al. 2011; Wu et al. 2021), and management (Byrnes et al. 2018; Eze et al. 2018). High vegetation diversity allows the introduction of carbon in various input forms so enhancing SOC storage and promoting both abundance and biodiversity of microbial communities (Yang et al. 2019; Prommer et al. 2020). Different climatic conditions, as increase of temperature and precipitation, can have direct impacts on vegetation, thus indirectly changing the SOC storage capacity, and on microbial communities, with increase or decrease of carbon respiration rates and storage (Cotrufo and Lavallee, 2022). Management practices and their intensity, as for example livestock grazing loads and fertilisation rates, can change the vegetation and the ratio between C and N with implication on the SOC storage (Byrnes et al. 2018; Eze et al. 2018). Currently, it is estimated that about 80% of European grasslands do not reach the SOC saturation, being still able to store further carbon (Bai and Cotrufo 2022).

Grasslands play a crucial role for agriculture and more generally for the global food supply (O’Mara 2012), supporting the livelihoods of about 1 billion people (Suttie et al. 2005). In fact, agricultural lands are covered by grasslands for about 70% at a global scale (Lemaire et al. 2011; Mencel et al. 2022) and the 34% at a European scale (Schils et al. 2022). Moreover, hosting various pollinators and different insect species useful for the control of phytophages, they provide ecosystem services which are essential for agricultural systems, as and pollination (Holland et al. 2017) and pest control (Honigova et al. 2012, Holland et al. 2017). Focusing on livestock systems, especially those based on ruminants, grasslands are essential for two main services: provisioning of feed requirements for ruminants and supporting grazing systems.

Ruminants can convert grass from pastures and meadows into edible food for humans without competition with them (Smith et al. 2013). Meat and milk from ruminants are fundamental into the global food supply (Smith et al. 2013). Among the livestock systems based on ruminants, those most related to grasslands in terms of surface occupied are the extensive pastoral systems, which are mainly prevalent in dry regions where agriculture is confined to marginal areas and represent from 36 to 47% of total grasslands (Kruska et al., 2003; Bouwman et al., 2005), providing globally 5% of milk and about 7% of beef and 12% of sheep meat productions (FAO, 2009). In contrast to extensive systems, the intensive systems occupy about 4% of global grasslands, but carry high densities of highly productive animals (Boval and Dixon, 2012), so contributing to 7% of milk and about 17% of beef and sheep meatproductions (FAO, 2009). Between extensive and intensive systems, the mixed crop-livestock systems, which involve interaction between livestock and crops (Sere et al. 1996), comprise about 13% of total grasslands and contribute significantly to global feedstuffs with 30% of milk and sheep meat and about 20% of beef meat (FAO, 2009; Herrero et al., 2010). Globally, forage from grasslands contributes to 35-75% of beef cattle diets and 45-95% of sheep and goat diets (Bouwman et al. 2005).

Grasslands are currently in decline, which started during the 18th century due to the conversion into arable land to meet the growing food demand (Hejcman et al. 2013; Bengtsson et al. 2019; Ridding et al. 2020) and is currently mainly caused by man through several interconnected processes (Bardgett et al. 2021), that have been summarized in the following impacts: intensification and abandonment of grasslands, desertification and climate change. The intensification of semi-natural and improved grassland management to increase the provisioning services induces the fragmentation of (semi)natural ecosystems (Andrade et al. 2015; Aune et al. 2018; Wilsey et al. 2018), while the abandonment of seminatural grassland causes the afforestation or reforestation (Aune et al. 2018; Volkò et al. 2018; Bohner et al. 2020). The change of local management of grasslands, such as overgrazing, can also induce the desertification (Archer et al. 2017; Shukla et al. 2019; Burrel et al. 2020) which is magnified by climate change through the increase of temperatures and drought (Archer et al. 2017; Shukla et al. 2019; Burrel et al. 2020). Also, the artificial afforestation and reforestation to mitigate climate change threat grasslands, reducing their surface (Posclod and Wallis DeVries 2002; Veldman et al. 2015). The decline of grasslands also involves the degradation of their vegetation biodiversity due to the nutrient enrichment by fertilisation or atmospheric deposition due to human activities (Stevens et al. 2004; Bullock et al. 2011), and by invasion of exotic species (Bardgett et al. 2021; Gaskin et al. 2021).

GRASSLANDS IN THE EUROPEAN ALPS

Grasslands, including lowland, sub-alpine and alpine grasslands occupy 17.4% of the European land (Fig. 2, adapted from Eurostat 2021). Alpine grasslands are those present above the upper limit of tree growth in mountain areas (Hejcman et al. 2013), while sub-alpine grasslands are extended below this limit (García-González 2008). These grasslands are therefore found at different elevations in the Pyrenees, Carpathians, and Scandinavian Mountains, central Apennines (García-González 2008), and European Alps, where occupy about 17% of the surface. These alpine and subalpine grasslands are characterised by species adapted to harsh environments as open stands, and animal species of high conservation value (García-González 2008; Marini et al. 2011). For example, alpine and subalpine grasslands are the main or the foraging habitat for various avian species, such as the alpine accentor *Prunella collaris*, the wheatear *Oenanthe oenanthe*, the skylark *Alauda arvensis*, the linnet *Carduelis cannabina*, the eurasian dotterel *Charadrius morinellus* and the golden eagle *Aquila chrysaetos* (Laiolo et al. 2004; García-González 2008; Pedrini and Sergio 2010). They are also essential habitats for wild herbivores such as chamois *Rupicapra rupicapra*, ibex *Capra ibex*, and marmot *Marmota marmota* (García-González 2008; Toïgo et al. 2020; Anderwald et al. 2021). Subalpine grasslands are fundamental components of mixed and extensive ruminant farming systems in the Alpine Arc, for the forage production of meadows and pastures in permanent farms which is often associated with the seasonal transhumance to summer farms (Sturaro et al., 2013a; Zendri et al. 2016; Pornaro et al., 2021), which has been practised since prehistoric times (Mack et al. 2013). Meadows are seminatural grasslands mowed to provide forage and usually allow the use of vehicles thank to their gentle slopes (Eriksson 2020). Summer farms are temporary units located at high elevations (Sturaro et al. 2013b), where domesticated herbivores, such as cattle and sheep and goat, are brought from the valley bottoms or lowland areas during the summer for grazing on alpine and sub-alpine seminatural grasslands which are managed as temporary pastures. Continuation of extensive management practices of permanent farm and summer farm meadows and pastures is fundamental for the conservation of these their grasslands, which are part of the cultural landscape (Thiene and Scarpa, 2008) allowing to maintain tradition and cultural heritage (Baudry and Thenail 2004; Kianicka et al. 2010; Erikssone 2011) and support livestock biodiversity (Sturaro et al. 2013a). Thanks to their characteristic (low agriculture input associated with high species and habitat diversity), alpine and subalpine grasslands are part of the High Nature Value farmland (HNV) and their conservation is actively supported by European Common Agricultural Policy (CAP; Paracchini et al. 2008). These grasslands have been used since the Middle Ages for producing hay as meadows and grazing cattle as pastures, but from the 19th century they have been abandoned or converted into croplands due to the intensification of agricultural practices and the depopulation of the mountain valleys (Strijker 2005; Garcìa-Martìnez et al. 2009; Bernués et al. 2011; Hejcman et al. 2013; Laiolo et al. 2004; Squires et al. 2018; Volkò et al. 2018). Other current threats to alpine and subalpine grasslands are the overgrazing, which reduces the local biodiversity (García-González 2008), construction of infrastructures and by climate change, which induces perturbations through global warming (García-González 2008; Ernakovich et al., 2014).

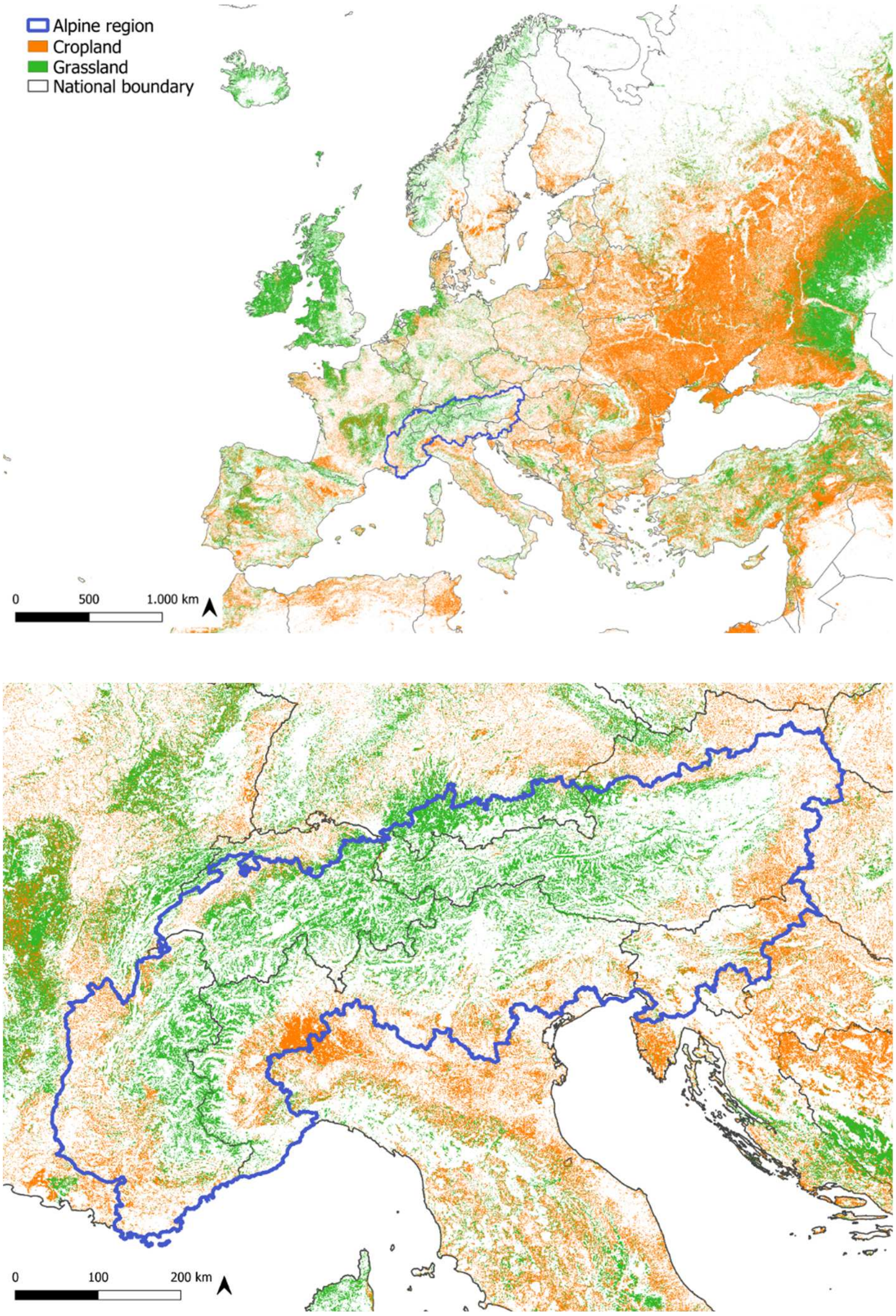


Figure 2. European distribution of grassland and cropland of Alpine region extracted from CCI-LC 2019

1.2 - POTENTIAL FUNCTIONS OF SOIL MICROBIAL COMMUNITIES

Soil is essential for all terrestrial ecosystems as a dynamic and heterogeneous environmental component, which hosts multiple communities of animals, plants, and microorganisms. The most populated part of a soil coincides with the topsoil, which is the shallow layers down to a depth of 15 cm (Paul 2014). The topsoil is characterized by a consistent presence of roots and hosts both microfauna/flora (size from 1 to 100 µm), mesofauna (size from 100 µm to 2 mm) and macrofauna (size over 2 mm). The soil microbial communities are concentrated into the microbial hotspots, spaces on the order of micrometres, where important ecosystem processes take place (Paul 2014; Kuzyakov and Blagodatskaya 2015). The microbial hotspots consist of rhizosphere, the volume occupied by living roots, drilosphere, ithe volume of biopores formed by the passage of animals through the soil, and detritosphere, which derives from the rhizosphere as result of the decomposition of roots by decomposers, such as fungi (Paul 2014; Kuzyakov and Blagodatskaya 2015).

The microbial communities are the basis for the ecosystem nutrient cycles, such as carbon and nitrogen (Zhou et al., 2012; Cavicchioli et al., 2019), which are constituted by enzyme-catalysed reactions performed by various guilds through their metabolic pathways (Rocca et al., 2015; Louca et al., 2018; Dong et al., 2020). These communities include eukaryotes, such as fungi, and prokaryotes, such as Archaea and Bacteria, and are strongly influenced by the soil physicochemical proprieties, in particular pH, moisture, soil organic carbon (SOC), and C/N ratio (Qu et al., 2016; Bahram et al., 2018; Fierer, 2017; Kuypers et al., 2018). They are also tied to the plant and animal communities both at the surface and within the soil itself through positive and negative impacts (Bardgett and Wardle 2003; Bezemer et al. 2006; Mendes et al., 2015; Delgado-Baquerizo et al., 2016; Du et al., 2019; Yin et al., 2020). Plants have a crucial role for the soil microbial communities thanks to their root systems, through which they can modify soil properties such as the amount of water, the presence of hotspots, the chemistry of the litter with their root exudates (Bardgett and Wardle 2003; Mendes et al., 2015; Delgado-Baquerizo et al., 2016; Li et al., 2016). Plants can also modify local microclimate conditions reducing the surface temperature limiting the direct income radiation (Oke 2002; D'odorico et al. 2013). Some plant species also promote certain microbial OTUs through close co-evolution processes (Qu et al., 2016). The increase/loss of plant biomass both above and below the soil can ultimately be considered as an increase/reduction in available energy for microbial growth and other biological activities (Northup et al., 1999; Song et al., 2016) with impacts on both the density and composition of the soil community (Aldezabal et al., 2015). Animals can influence microbial communities building new hotspots and modifying the vegetation by selective predation and changing soil conditions by trampling and nutrient enrichment by their excreta

(Lemaire et al. 2011; Zhao et al., 2017). The effects of animal presence on microbial communities are discussed in the paragraph below.

1.3 - GRAZING PATTERNS

Grazing is one of the most common management practices of semi-natural grasslands around the world (Tälle et al. 2016). Although various livestock species, namely cattle, sheep and goat, horses, camelids are involved, I will focus in my thesis on dairy cattle, which is the most common livestock category in grazing systems of the Italian Alps (Sturaro et al., 2013b; Zener et al, 2013). Understanding livestock grazing patterns is a crucial point to develop sustainable productive management of pastures while avoiding negative impacts on grasslands provisioning and non-provsionoing ecosystem services. Overgrazing is a threat for grasslands as it causes variation of vegetation, soil campaction, erosion and changes in nutrient conditions with negative consequences on the ecosystem (Chang et al. 2021; Bai and Cotrufo, 2022). For example, over-grazed grasslands can become sources instead of sinks of carbon (Chang et al. 2021; Bai and Cotrufo, 2022). Grazing patterns of livestock depend on multiple factors, which involve environmental conditions and animal characteristics (Rivero et al. 2021). Main environmental conditions determining grazing patterns are terrain morphology and particularly slope (Kaufmann et al. 2013; Zhong et al., 2016; Pittarello et al. 2021; Rivero et al. 2021), spatial distribution of shade, shelter, and water sources (Probo et al. 2014, Rivero et al. 2021), and climate conditions, in particular temperature (Caton and Olson 2016; Liao et al. 2017; Rivero et al. 2021). Animal characteristics significant for grazing patterns variability depend on the species, then on the breed and then on individual features such as age. Grazing species differ in mouth morphology and digestive physiology, which bring to distinct grazing patterns (Rook et al., 2004; Lemaire et al. 2011). Cattle are characterised by non-selective grazing and tend to tear up vegetation (Rook et al., 2004; Lemaire et al. 2011). Horses are selective toward grasses, avoiding dicotyledons, and practise sparse grazing because of the use of incisors, tending to leave patches of undisturbed vegetation (Rook et al., 2004; Lemaire et al. 2011). Sheep tend to be very selective toward dicotyledons if left free to graze, and they leave the turf sparse as equines (Rook et al., 2004; Lemaire et al. 2011). Finally, goats appear to be more selective toward shrub and tree species, especially young ones, than grass (Rook et al., 2004). The grazing patterns of cows are influenced by their breed. Local breeds appear more suitable to harsh environments than highly productive breeds (Isselstein et al. 2007; Bailey et al. 2010; Spiegal et al. 2019; Pauler et al. 2020; Rivero et al. 2021). Breeds tend to differ in selection of plant species (Hessle et al. 2014; Koczura et al. 2019; Spiegal et al. 2019; Pauler et al. 2020) and slope (Raniolo et al. 2022), time spent on distinct behaviours and activity budgets, and eventually in growth rate and/or milk production (Hessle et al. 2008; Spiegal et al. 2019; Pauler et al. 2020),.Another individual factor able to influence grazing patterns is the age, as younger individuals tend to be smaller and have less demanding nutritional requirements than mature individuals, while being able to move more easily across difficult terrain (Wyffels et al. 2020). Moreover, resource selection of young individuals can be less influenced by previous experiences, with consequent effects on grazing patterns (Dunn et al. 1988; Bailey et al. 2001; Walburger et al. 2009).

Grazing patterns are controlled by The grazing system adopted by farmers aim to control livestock grazing patterns. Continuous grazing systems are the most extensive ones, as the control of grazing patterns is minimised by leaving livestock free to graze without spatial andf temporal restrictions within the (usually large) pasture area. Typically, these systems have low but long-term stocking rates, and require only minimal infrastructure and labour. However, continuous grazing systems tend to increase livestock movement costs and the use of pasture can result heterogeneous with undergrazed and overgrazed patches, which can gradually reduce the forage value of the grassland and promote shrub and tree encroachment (Kothmann 2009). Rotational systems are the most intensive and controlled ones, as animals graze a sequence of small paddocks for a short period. These systems limit the animals' selectivity but tend to preserve the forage value of the pasture and enhance the livestock productivity with their high but short-term stocking rates. However, they are expensive in terms of labour and infrastructure investments and can have different impacts on grassland biodiversity and nutrient cycling (Kothmann 2009; Probo et al. 2013; 2014; Perotti et al. 2018).

1.4 - GRAZING IMPACT ON SOIL MICROORGANISMS

Livestock grazing operates both directly and indirectly on grasslands soil microbial communities through defoliation, trampling and nutrient enrichment, which impact on plants, nutrients availability, and ecological successions (Lemaire et al. 2011; Zhao et al., 2017). Through these three actions, grazing can impact on ecosystem functions, for example varying the rate of soil respiration through the nutrient enrichment from animal excreta and the change of oxygen content due to compaction from trampling (Zhao et al., 2017). The effects of grazing depend on its intensity (Aldezabal et al., 2015; Zhao et al., 2017), which can be characterised in terms of stocking rate as animals per area considering the time spent and stocking density as ratio between the number of livestock units and the area (Lemaire et al. 2011, Allen et al. 2011).

Defoliation results from the predation of plants by herbivores, which reduces the aerial biomass of plants so decreasing the input of plant organic matter for litter (Bardgett et al. 1998). Defoliation intensity varies according to the livestock species and grazing patterns, which depend on multiple factors as described in the previous paragraph. Defoliation can locally change floristic composition with cascading effects: for example, Aldezabal et al. (2015) observed that the absence of grazing increased graminoids, such as Poaceae and Cyperaceae, and reduced forbes, such as Fabaceae (). This increase in graminaceous species brought to a loss in litter quality as the C/N ratio increased. The C/N ratio is an indicator for litter decomposition: a high ratio results in a more pronounced presence of lignin, which is difficult to be degraded by microorganisms. Instead, the presence of forbs, promoted by extensive grazing, increased litter decomposition by lowering the C/N ratio thanks to a higher nitrogen supply. Defoliation can impact not only on the quality of vegetation cover and litter, but also on their quantities due to the extraction of biomass. Vegetation cover and the litter layer increase water infiltration, buffer temperature fluctuations, and reduce evaporation rates, allowing the soil to hold more moisture for longer following a rainfall (Chen et al., 2016). This improves microbial activity resulting in increased stability of soil aggregates and better support for plant development (Teague et al., 2011). In pastures, a high presence of herbaceous species with a high root density, with the consequent plant and microbial activity support an efficient assimilation of nitrogen, capable of reducing nitrogen leaching (Hackl et al., 1999). Defoliation can also induce plants to compensative development of the hypogeal part, promoting the structure of root systems and the release of exudates wich are beneficial for the development of soil microbial biomass (Hamilton III et al. 2008; Zhao et al., 2017). However, intensive grazing can bring to a substantial loss of photosynthetic tissues and subsequent reduction of carbon inputs to the roots (Aldezabal et al., 2015). This results in reduced production of root exudates with negative impacts on microbial communities (Zhao et al., 2017). Another effect of defoliation is the fluctuation of local soil temperature due to a reduction in vegetation cover and the consequent increased exposure to direct radiative flux (Aldezabal et al., 2015). This phenomenon can increase soil temperature during the day with consequences for microbial functions (Aldezabal et al., 2015). Temperature is an environmental parameter able to directly influence soil microbial community, changing the rate of enzymatic reactions (Davidson and Janssens 2006; Frey et al. 2008; Butemschoen et al. 2011). Reducing vegetation cover can also increase erosion of the soil more exposed to abiotic and biotic agents, with possible negative impacts on the primary productivity of the ecosystem and its resilience (Teague et al., 2011).

Animal trampling causes soil compaction affecting root development, pore presence and availability of oxygen and water (Qu et al., 2016). These implications have direct effects on the microbial communities by changing microbial hotspots, such as pores and the rhizosphere, but mainly by varying the concentration of molecular oxygen, which determines the oxidizing conditions of the soil. Oxidising conditions influence the nutrient bioavailability and the microbiological OTUs, favouring those able to use different electron acceptors such as nitrate (Li et al., 2016). In addition, trampling can induce changes on the local plant communities (Aldezabal et al., 2015) with consequences on the production of root exudates, which play a key role in the composition of soil microbial communities (Qu et al., 2016).

Animal excreta are labile substrates able to increase both microbial metabolic activities and microbial biomass (Chen et al., 2016). Grazing herbivores through their excreta are part of grassland nutrient cycles, reintroducing nutrients such as carbon, nitrogen, and phosphorus. However, animal excreta not only change nutrient availability of soil, but also its chemical condition. During the hydrolysis of urea to ammonia (NH_3), urine tends to increase soil pH through the release of hydroxyl anions (OH). Ammonia tends to be converted to ammonium ion (NH_4^+) through the acquisition of a proton or ion H+: this reaction increases the concentration of inorganic nitrogen and pH (Prieto et al., 2011). Animal excreta chane also the spatial availability of nutrients and depostion of faeces and urine can create local hotspots for specific microbial guilds and their processes, such as nitrifiers and denitrifiers with increases in nitrification and denitrification rates (Zhong et al., 2015). Animals through their excreta can also alter soil microbial endemisms, introducing external OTUs with possible effects on microbial communities (Nandakafle et al., 2017).

Grazing effects on microbial communities and ecosystems functioning can be exacerbated by climate change, which can increase soil erosion, alter vegetation with variation of temperature and rain precipitation and increase enzymatic reaction through warming (Bai and Cotrufo 2022). Thus, assessing possible interactions between grazing impacts and climate change on microbial communities plays an important role to develop new practices for improving ecosystem functioning and resilience.

1.5 - APPROCHES USED TO STUDY GRAZING PATTERNS: GPS TELEMETRY AND REMOTE SENSING

Global Positioning System (GPS) technology is one of the most frequently used satellite applications for real-time tracking, i.e, the monitoring of position. GPS is based on the transmission of radio signals (electromagnetic waves characterised by frequency between 0 and 300 GHz) between a network of 24 satellites and a receiver on the earth's surface. The radio signal is transmitted by each satellite and then processed by the receiver to obtain the location, which is a point value defined through longitude and latitude at a specific time. GPS systems are the most widespread of the global navigation satellite systems (GNSS), in addition to Russia's GLONASS and the more recent Europe's Galileo (Li et al., 2015). The GPS system is managed by the U.S. government, but it is freely accessible, as the other two mentioned above. The accuracy of GPS locations is affected by multiple factors, including the relative position and number of available satellites for the receiver, the presence of sky-viewing obstacles such as tree canopies or rock walls, weather conditions, and finally the quality and type of receiver (Sager-Fradkin et al., 2007; Zweifel-Schielly and Suter, 2007; Hansen and Riggs 2008). In general, receivers for civil use arrive at a resolution of a few metres, which is equal to a radius of about 5 metres from the position of the receiver (Aguado et al. 2017).

This technology in the last two decades has been widely used in the monitoring of both domestic livestock and wild animal movement (Cagnacci et al., 2010; Perotto-Baldivieso et al., 2012; Tullo, Finzi and Guarino, 2019), introducing revolutionary opportunities for the collection of large numbers of positions with high accuracy and time resolution. GPS data can also be coupled with information on individual activity and behaviour, using triaxial accelerometers integrated into geolocation devices. Thus, GPS technology has enabled a radical advancement in the study of the distribution, movement and behaviour of animals, both wild and domestic (Cagnacci et al., 2010; Lovarelli, Bacenetti and Guarino, 2020, Semenzato et al. 2021).

In the livestock sector, GPS collars with tri-axial accelerometers are currently used in precision livestock farming, which consists of the management of animal production using advanced technologies to maximise efficiency (Tullo et al. 2019). Examples of GPS applications are the remote monitoring of grazing animals to highlight in real time behavioural variations which can be linked to health status and prevent, for example, the phenomena of rustling or predation (Perotto-Baldivieso et al., 2012). They can also be used to monitor the welfare status of animals during transport, or for the implementation of virtual fences with acoustic or electrical stimuli to mark areas of use (Perotto-Baldivieso et al., 2012). GPS collars can be used to extract geometric metrics of animal movement and to define its spatial-temporal patterns. The temporal sequences of individual positions (trajectories) can be analysed to derive descriptive metrics of individual "steps" (intervals between one position and the next) such as distance, velocity, and relative angles (the directions of animal movement between the two locations; Homburger et al., 2014). These metrics, possibly in combination with acceleration values of activity sensors, can be used to classify the animal behaviour remotely through the application of Machine Learning algorithms (Valletta et al. 2017). GPS localizations can be used to identify the environment used by the animal, overlaying GPS positions with georeferenced maps of ground morphology (slope, elevation, etc.) and available habitats (Tomkins and O'Reagain, 2007; Handcock et al., 2009; Feldt and Schlecht, 2016), and to identify spatiotemporal gradients of grazing patterns (Kaufmann et al., 2013). The study of movement and behaviour can be conducted according to frequent events in the animals' routine, such as milking in the case of dairy cows, or day-night cycles, or weather conditions, or even animal states such as breed, parity, milk production, etc. Thus, GPS tracking allows a deeper understanding of the real interactions between livestock and pasture, especially when it is coupled with remote sensing. Remote sensing is the monitoring of physical characteristics of an area through measures of reflected and emitted radiation at distance using satellites (Khorram et al. 2012). Remote sensing allows to monitor habitat components, such grassland vegetation through spectral indices such as the Normalized Difference Vegetation Index (NDVI; Myneni et al. 1995) or the green-house gases emission, such as methane (DeFries et al. 2007), or surface temperature (Tomlinson et al. 2011). Combining GPS telemetry and Remote sensing is essential to assess future impacts and to develop more conscious and sustainable management practices, not only for production purposes, but also for the conservation of important agroecosystems, such as alpine grassland.

1.6 - APPROACHES USED TO STUDY MICROBIAL COMMUNITIESY

Microbial communities can be studied in terms of taxonomic but also functional groups thanks to their roles in ecosystem functions. The taxonomic groups determine the richness of microbial communities, i.e., the taxomonic diversity, while the functional groups constitute the set of possible functions, which can be interpreted as functional diversity. From the perspective of ecosystem functioning, microbial richness per se is less relevant than phenotypic traits for specific functions (Moonen and Bàrberi, 2008; Bahram et al., 2018; Louca et al., 2018). The functional diversity depends on richness as a high number of OTUs can enrich the genetic content, which ultimately defines the enzyme-catalysed reactions of communities basing on the central tenet of molecular biology where biological information passes from genes to their transcripts and then to protein synthesis. (Rocca et al., 2015; Louca et al., 2018). Thus, the presence of specific genes determines the metabolic niches of microbial communities and their potential functions (Rocca et al., 2015; Louca et al., 2018). The abundance of a gene reflects that of its carrying population, as its multiplication testifies the relevance of that microbial group within the ongoing local processes (Beule et al., 2019; De Boer and Kowalchuk, 2001; Jia and Conrad, 2009; Yergeau et al., 2007). For this reason, the relative frequency of the corresponding cells population for a specific gene can entail a correspondent high expression of the same phenotype as soon as reaction substrates of those enzymes should become available under favourable boundary conditions. From this standpoint, microbial protein-encoding genes can be used both as reporters of the leading biochemical occurrences and as predictive indicators of potential specific phenomena within the ecosystem functions (Lindsay et al., 2010; Wallenstein and Vilgalys, 2005). The analysis of microbial functions on gene indicators can be achieved by the common molecular analysis of RealTime PCR. The RealTime PCR is a molecular technique based on polymerase chain reaction (PCR) able to monitor the amplification of a target gene during the reaction thanks to the use of fluorescent dyes and reporter (Mackay et al. 2002). This technique is used to quantify the starting gene number of copies in different environmental matrices, including water (Wakelin et al., 2008) and soil (Henry et al., 2004). The gene number of copies is used as a reporting measure for the potential of ecosystem processes, such as ammonia oxidation or denitrification (Henry et al., 2004). This analysis has been used in many different agroecosystems, such as croplands (Beule et al., 2019; Colloff et al., 2008), forests (Lindsay et al., 2010), and grasslands (Wakelin et al., 2009), in order to analyse the microbial communities.

Microbial communities can be analysed from a metagenomic perspective through sequencing methods, such as Sanger sequencing and Illumina dye sequencing, which provide information on the microbial phylotypes present and their relative abundances with high sample throughput and affordable laboratory costs (Bartram et al. 2011; Berman et al. 2019). The process of sequencing determines the nucleic acid sequences or the order of DNA nucleotides, which can be used to extract taxonomic profiles. The two main sequencing methods in metagenomic analysis are the amplicon sequencing and the shotgun metagenomic sequencing (Di Bella et al. 2013). The amplicon sequencing is performed on genetic libraries made by PCR on marker genes, so a region of a genome, such as the 16S rRNA in the Bacteria and Archaea and 18S rRNA in the Eukarya (Bartram et al. 2011; Di Bella et al. 2013). Sequenced amplicons are compared to reference databases to achieve the taxonomic classification of communities. The shotgun metagenomic sequencing does not rely on the genetic library made through PCR but it reads the entire genome present in the DNA extracted. This method gives a complete inventory of genes contained in a genome, thus defining the functional genetic potential of an organism (Di Bella et al. 2013). However, the definition of functional profiles can be also obtained from amplicon sequencing thanks to modern bioinformatics tools, such as FAPROTAX (Functional Annotation of Prokaryotic Taxa; Louca et al. 2016), KEGG (Kyoto Encyclopedia of Genes and Genomes; Kanehisa and Goto 2000) and FUNGuild (Nguyen et al. 2016). These tools assign the functional profiles to a microbial community comparing its taxonomic profile of bacteria (Kanehisa and Goto 2000; Louca et al. 2016), archaea (Kanehisa and Goto 2000; Louca et al. 2016) and fungi (Nguyen et al. 2016) with a reference database.

The characterization of soil microbial community at both single gene and metagenomic levels can be essential to assess the real impact of different management practices, other disturbance factors and climate change on ecosystem functions. Thus, this can be the base for developing sustainable management practices of grasslands (and obviously of other agricultural ecosystems).

1.7 – REFERENCES

Aguado MÁP, Sturaro E, and Ramanzin M. 2017. Individual activity interacts with climate and habitat features in influencing GPS telemetry performance in an alpine herbivore. Hystrix. 28(1):1–7.

Aldezabal A, Moragues L, Odriozola I, Mijangos I. 2015. Impact of grazing abandonment on plant and soil microbial communities in an Atlantic mountain grassland. Applied Soil Ecology. 96:251-260.

Allen VG, Batello C, Berretta EJ, Hodgson J, Kothmann M, Li X, McIvor J, (…), Sanderson M. 2011. An international terminology for grazing lands and grazing animals. Grass and Forage Science. 66 (1):2-28.

Anderwald P, Ambarli H, Avramov S, Ciach M, Corlatti L, Farkas A, Jovanovic M, Papaioannou H, Peters W, Sarasa M, Šprem N, Weinberg P, Willisch C. 2021. Rupicapra rupicapra (amended version of 2020 assessment). The IUCN Red List of Threatened Species 2021: e.T39255A195863093.

Andrade BO, Koch C, Boldrini II, Vélez-Martin E, Hasenack H, Hermann JM, Kollmann J, Pillar VD, Overbeck GE. 2015. Grassland degradation and restoration: a conceptual framework of stages and thresholds illustrated by southern Brazilian grasslands. Natureza & Conservação. 13(2):95-104.

Archer SR, Andersen EM, Predick KI, Schwinning S, Steidl RJ, Woods SR. 2017. Woody plant encroachment: causes and consequences. In: Briske, D. (eds) Rangeland Systems. Springer Series on Environmental Management. Springer, Cham. pp. 25–84.

Askins RA, Chávez-Ramírez F, Dale BC, Haas CA, Herkert JR, Knopf F, Vickery PD. 2007. Conservation of grassland birds in North America: understanding ecological processes in different regions. Ornith. Monogr. 64:1–46.

Aune S, Bryn A, Hovstad KA. 2018. Loss of semi-natural grassland in a boreal landscape: Impacts of agricultural intensification and abandonment. J Land Use Sci. 13(4):375-390.

Bahram M, Hildebrand F, Forslund SK, Anderson JL, Soudzilovskaia NA, Bodegom PM, Bengtsson-Palme J, Anslan S, Coelho LP, Harend H, Huerta-Cepas J, Medema MH, Maltz MR, Mundra S, Olsson PA, Pent M, Põlme S, Sunagawa S, Ryberg M, Tedersoo L, Bork P. 2018. Structure and function of the global topsoil microbiome. Nature. 560:233–237.

Bai Y, Cotrufo MF. 2022. Grassland soil carbon sequestration: Current understanding, challenges, and solutions. Science. 377(6606):603-608.

Bailey DW, Kress DD, Anderson DC, Boss DL, Miller ET. 2001. Relationship between terrain use and performance of beef cows grazing foothill rangeland. J. Anim. Sci. 79(7):1883-1891.

Bailey DW, Thomas MG, Walker JW, Witmore BK, Tolleson D. 2010. Effect of previous experience on grazing patterns and diet selection of Brangus cows in the Chihuahuan Desert. Rangel Ecol Manag. 63(2):223-232

Bardgett RD, Bullock JM, Lavorel S, Manning P, Schaffner U, Ostle N, Chomel M, Durigan G, Fry EL, Johnson D, Lavallee JM, Le Provost G, Luo S, Png K, Sankaran M, Hou X, Zhou H, Ma L, Ren W, Li X, Ding Y, Li Y, Shi H. 2021. Combatting global grassland degradation. Nat. Rev. Phys. 2(10):720-735.

Bardgett RD, Wardle DA, Yeates GW. 1998. Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biol. Biochem. 30(14):1867-1878.

Bartram AK, Lynch MD, Stearns JC, Moreno-Hagelsieb G, Neufeld JD. 2011. Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. Appl. Environ. Microbiol. 77(11):3846-3852.

Baudry J, Thenail C. 2004. Interaction between farming systems, riparian zones, and landscape patterns: a case study in western France. Landsc Urban Plann. 67:121–129.

Bengtsson J, Bullock JM, Egoh B, Everson C, Everson T, O' Connor T, O’Farrel PJ, Smith HG, Lindborg R. 2019. Grasslands - more important for ecosystem services than you might think. Ecosphere. 10(2):e02582.

Bernués A, Ruiz R, Olaizola A, Villalba D, Casasùs I. 2011. Sustainability of pasture-based livestock farming systems in the European Mediterranean context: synergies and trade-offs. Livest Sci. 139:44–57.

Beule L, Corre MD, Schmidt M, Göbel L, Veldkamp E, Karlovsky P. 2019. Conversion of monoculture cropland and open grassland to agroforestry alters the abundance of soil bacteria, fungi and soil-N-cycling genes. PLoS One. 14:1–19.

Bezemer TM, Lawson C.S, Hedlund K, Edwards AR, Brook AJ, Igual JM, Mortimer S, Van der Putten WH. 2006. Plant species and functional group effects on abiotic and microbial soil properties and plant–soil feedback responses in two grasslands. J. Ecol. 94(5):893-904.

Bignal E, McCracken. 1996. Low-intensity farming systems in the conservation of the countryside. J Appl Ecol. 33:413–424.

Bouwman AF, Van der Hoek KW, Eickhout B, Soenario I. 2005. Exploring changes in world ruminant production systems. Agric. Syst. 84:121–153.

Boval M, Dixon RM. 2012. The importance of grasslands for animal production and other functions: a review on management and methodological progress in the tropics. Animal. 6(5):748-762.

Bullock JM, Jefferson RG, Blackstock TH, Pakeman RJ, Emett BA, Pywell RJ, Grime JP, Silvertown J. 2011. Semi-natural Grasslands In: The UK National Ecosystem Assessment Technical Report. UK National Ecosystem Assessment, UNEP-WCMC, Cambridge.

Burrell AL, Evans JP, De Kauwe MG. 2020. Anthropogenic climate change has driven over 5 million km2 of drylands towards desertification. Nat. Commun. 11(1):1-11.

Butenschoen O, Scheu S, Eisenhauer N. 2011. Interactive effects of warming, soil humidity and plant diversity on litter decomposition and microbial activity. Soil Biol. Biochem. 43(9):1902-1907.

Byrnes RC, Eastburn DJ, Tate KW, Roche LM. 2018. A global meta-analysis of grazing impacts on soil health indicators. J. Environ. Qual. 47(4):758-765.

Cagnacci F, Boitani L, Powell RA, Boyce MS. 2010. Animal ecology meets GPS-based radiotelemetry: A perfect storm of opportunities and challenges. Philos. Trans. R. Soc. B Biol. Sci. 365(1550):2157–62.

Caton JS, Olson BE. 2016. Energetics of grazing cattle: Impacts of activity and climate. J. Anim. Sci. 94:74–83.

Cavicchioli R, Ripple WJ, Timmis KN, Azam F, Bakken LR, Baylis M, Behrenfeld MJ, Boetius A, Boyd PW, Classen AT, Crowther TW, Danovaro R, Foreman CM, Huisman J, Hutchins DA, Jansson JK, Karl DM, Koskella B, Mark Welch DB, Martiny JBH, Moran MA, Orphan VJ, Reay DS, Remais JV, Rich VI, Singh BK, Stein LY, Stewart FJ, Sullivan MB, van Oppen MJH, Weaver SC, Webb EA, Webster NS. 2019. Scientists' warning to humanity: microorganisms and climate change. Nat. Rev. Microbiol. 17:569–586.

Chen J, Zhou X, Wang J, Hruska T, Shi W, Cao J, Zhang B, Xu G. Chen Y, Luo Y. 2016. Grazing exclusion reduced soil respiration but increased its temperature sensitivity in a Meadow Grassland on the Tibetan Plateau. Ecol. Evol. 6(3):675-687.

Cheng X, Luo Y, Xu X, Sherry R, Zhang Q. 2011. Soil organic matter dynamics in a North America tallgrass prairie after 9 yr of experimental warming. Biogeosciences. 8(6):1487-1498.

Colloff MJ, Wakelin SA, Gomez D, Rogers SL. 2008. Detection of nitrogen cycle genes in soils for measuring the effects of changes in land use and management. Soil Biol. Biochem. 40:1637–1645.

Cotrufo MF, Lavallee JM. 2022. Soil organic matter formation, persistence, and functioning: A synthesis of current understanding to inform its conservation and regeneration. Adv. Agron. 1-66.

Daily G. 2003. What are ecosystem services. Global environmental challenges for the twenty-first century: Resources, consumption and sustainable solutions. 227-231.

Davidson EA, Janssens IA. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature. 440(7081):165-173.

De Boer W, Kowalchuk GA. 2001. Nitrification in acid soils: Micro-organisms and mechanisms. Soil Biol. Biochem. 33:853–866.

Delgado-Baquerizo M, Oliverio AM, Brewer TE, Benavent-González A, Eldridge DJ, Bardgett RD, Maestre FT, Singh BK, Fierer N. 2018. A global atlas of the dominant bacteria found in soil. Science. 359(6373):320-325

Di Bella JM, Bao Y, Gloor GB, Burton JP, Reid G. 2013. High throughput sequencing methods and analysis for microbiome research. J. Microbiol. Methods. 95(3):401-414.

Dixon AP, Faber-Langendoen D, Josse C, Morrison J, Loucks CJ. 2014. Distribution mapping of world grassland types. J. Biogeogr. 41(11):2003-2019.

D'odorico P, He Y, Collins S, De Wekker SF, Engel V, Fuentes JD. 2013. Vegetation–microclimate feedbacks in woodland–grassland ecotones. Global Ecology and Biogeography. 22(4):364-379.

Dunn RW, Havstad KM, Ayers EL. 1988. Grazing behavior responses of rangeland beef cows to winter ambient temperatures and age. Appl. Anim. Behav. Sci. 21(3):201-207.

Eriksson C. 2011. What is traditional pastoral farming? The politics of heritage and 'real values' in Swedish summer farms. Pastoralism. 1:1–18.

Eriksson O. 2020. Origin and development of managed meadows in Sweden: A review. Rural Hist. 7(1):1-23.

Ernakovich JG, Hopping KA, Berdanier AB, Simpson RT, Kachergis EJ, Steltzer H, Wallenstein MD. 2014. Predicted responses of arctic and alpine ecosystems to altered seasonality under climate change. Glob Chang Biol. 20:3256–3269.

Eurostat. 2021. LUCAS: the EU's land use and land cover survey, 2021 edition. Publications Office of the European Union. https://data.europa.eu/doi/10.2785/608.

Eze S, Palmer SM, Chapman PJ. 2018. Soil organic carbon stock in grasslands: Effects of inorganic fertilizers, liming and grazing in different climate settings. J. Environ. Manage. 223:74-84.

Feldt T, Schlecht E. 2016. ‘Analysis of GPS trajectories to assess spatio-temporal differences in grazing patterns and land use preferences of domestic livestock in southwestern Madagascar’, Pastoralism. 6(1).

Food and Agriculture Organization (FAO). 2009. The state of food and agriculture 2009: livestock in the balance. State of Food and Agriculture, Viale delle Terme di Caracalla, Rome, Italy. pp.166.

Frey SD, Drijber R, Smith H, Melillo JJSB. 2008. Microbial biomass, functional capacity, and community structure after 12 years of soil warming. Soil Biol. Biochem. 40(11):2904-2907.

García-González R. 2008. Management of Natura 2000 habitats. Alpine and subalpine calcareous grasslands. European Commission. pp.27.

Garcìa-Martìnez A, Olaizola A, Bernués A. 2009. Trajectories of evolution and drivers of change in European mountain cattle farming systems. Animal. 3:152–165.

Gaskin JF, Espeland E, Johnson CD, Larson DL, Mangold JM, McGee RA, Milner C, Paudel S, Pearson DE, Perkins LB, Prosser CW, Runyon JB, Sing SE, Sylvain ZA, Symstad AJ, Tekiela DR. 2021. Managing invasive plants on Great Plains grasslands: A discussion of current challenges. Rangel Ecol Manag. 78:235-249.

Gibson DJ. 2009. Grasses and grassland ecology. Oxford University Press. pp.323.

Girardello M, Griggio M, Whittingham MJ, Rushton SP. 2009. Identifying important areas for butterfly conservation in Italy. Anim. Conserv. 12:20–28.

Gorelick N, Hancher M, Dixon M, Ilyushchenko S, Thau D, Moore R. 2017. Google Earth Engine: Planetary-scale geospatial analysis for everyone. Remote Sens Environ. 202:18–27.

Hackl E, Zechmeister-Boltenstern S, Kandeler E. 1999. Nitrogen dynamics in different types of pasture in the Austrian Alps. Biol. Fertil. Soils. 32:321–327.

Hamilton III EW, Frank DA, Hinchey PM, Murray TR. 2008. Defoliation induces root exudation and triggers positive rhizospheric feedbacks in a temperate grassland. Soil Biol. Biochem. 40(11):2865-2873.

Handcock RN, Swain DL, Bishop-Hurley GJ, Patison KP, Wark T, Valencia P, Corke P, O'Neill CJ. 2009. Monitoring Animal Behaviour and Environmental Interactions Using Wireless Sensor Networks, GPS Collars and Satellite Remote Sensing. Sensors. 9(5):3586-603.

Hansen MC, Riggs RA. 2008. Accuracy, precision, and observation rates of global posi- tioning system telemetry collars. J. Wildl. Manage. 72(2):518–526.

Hejcman M, Hejcmanova P, Pavlů V, Beneš J. 2013. Origin and history of grasslands in Central Europe – a review. Grass Forage Sci. 68(3):345-363.

Henry S, Baudoin E, López-Gutiérrez JC, Martin-Laurent F, Brauman A, Philippot L. 2004. Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. J. Microbiol. Methods. 59:327–335.

Herrero M, Thornton PK, Notenbaert AM, Wood S, Msangi S, Freeman HA, Bossio D, Dixon J, Peters M, van de Steeg J, Lynam J, Parthasarathy Rao P, MacMillan S, Gerard B, McDermott J, Sere C and Rosegrant M. 2010. Smart investments in sustainable food production: revisiting mixed crop-livestock production. Science. 327:822–825.

Hessle A, Rutter M, Wallin K. 2008. Effect of breed, season and pasture moisture gradient on foraging behaviour in cattle on semi-natural grasslands. Appl. Anim. Behav. Sci. 111(1–2):108–19.

Hilker T, Natsagdorj E, Waring RH, Lyapustin A, Wang Y. 2014. Satellite observed widespread decline in Mongolian grasslands largely due to overgrazing. Glob Chang Biol. 20(2):418-428.

Holland JM, Douma JC, Crowley L, James L, Kor L, Stevenson DRW, Smith BM. 2017. Semi-natural habitats support biological control, pollination and soil conservation in Europe. A review. Agron Sustain Dev. 37(4):31.

Homburger H, Schneider MK, Hilfiker S, Lüscher A. 2014. Inferring behavioral states of grazing livestock from high-frequency position data alone. PLoS One. 9(12):1–22.

Honigova I, Vackar D, Lorencova E, Melichar J, Gotzl M, Sonderegger G, Ouskova V, Hosek M, Chobot K. 2012. Survey on grassland ecosystem services. Report to the EEA—European Topic Centre on Biological Diversity. Nature Conservation Agency of the Czech Republic. Prague. pp.78.

Isselstein J, Griffith BA, Pradel P, Venerus S. 2007. Effects of livestock breed and grazing intensity on biodiversity and production in grazing systems. 1. Nutritive value of herbage and livestock performance. Grass Forage Sci. 62(2):145–58.

Jerrentrup JS, Klimek S, Marchiori E, Bittante G, Ramanzin M, Sturaro E, Marini L. 2016. Impact of dairy farming on butterfly diversity in Alpine summer pastures. Agric Ecosyst Environ. 232:38-45.

Jia Z, Conrad R, 2009. Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. Appl. Environ. Microbiol. 11:1658–1671.

Kanehisa M, Goto S. 2000. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res. 28(1):27-30.

Kaufmann J, Bork EW, Blenis P V. Alexander MJ. 2013. Cattle habitat selection and associated habitat characteristics under free-range grazing within heterogeneous Montane rangelands of Alberta. Appl. Anim. Behav. Sci. 146(1–4):1–10.

Khorram S, Koch FH, van der Wiele CF, Nelson SA. 2012. Remote sensing. SpringerBiefs in Space Development. Springer New York, NY. pp.134.

Kianicka S, Knab L, Buchecker M. 2010. Maiensäss – Swiss Alpine summer farms – an element of cultural heritage between conservation and further development: a qualitative case study. Int. J. Heritage Stud. 16:486–507.

Kozcura M, Martin B, Bouchon M, Turille G, Berard J, Farruggia A, Kreuzer M, Coppa M. 2019. Grazing behaviour of dairy cows on biodiverse mountain pastures is more influenced by slope than cow breed. Animal. 13(11):2594-2602.

Kruess A, Tscharntke T. 2002. Grazing intensity and the diversity of grasshoppers butterflies, and trap-nesting bees and wasps. Conserv. Biol. 16:1570–1580.

Kruska RL, Reid RS, Thornton PK, Henninger N, Kristjanson PM. 2003. Mapping livestock-oriented agricultural production systems for the developing world. Agric. Syst. 77:39–63.

Kuypers MM, Marchant HK, Kartal B. 2018. The microbial nitrogen-cycling network. Nat. Rev. Microbiol. 16(5):263.

Kuzyakov Y, Blagodatskaya E. 2015. Microbial hotspots and hot moments in soil: concept & review. Soil Biol. Biochem. 83:184-199.

Laiolo P, Dondero F, Ciliento E, Rolando A. 2004. Consequences of pastoral abandonment for the structure and diversity of the alpine avifauna. J Appl Ecol. 41(2):294-304.

Lavallee JM, Soong JL, Cotrufo MF. 2020. Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. Glob Chang Biol. 26(1):261-273.

Lemaire G, Hodgson J, Chabbi A. 2011. Grassland productivity and ecosystem services. CABI, Wallingford, UK. pp.287.

Li X, Chen X, Ge M, Schuh H. 2019. Improving multi-GNSS ultra-rapid orbit determination for real-time precise point positioning. J Geod. 93(1):45-64.

Li Y, Lin Q, Wang S, Li Xiangzhen Liu W, Luo C, Zhang Z, Zhu X, Jiang L, Li X. 2016. Soil bacterial community responses to warming and grazing in a Tibetan alpine meadow. Microb. Ecol. 92:1–10.

Liao C, Clark PE, Degloria SD, Barrett CB. 2017. Complexity in the spatial utilization of rangelands: pastoral mobility in the Horn of Africa. Appl Geogr. 86:208–219.

Lindsay EA, Colloff MJ, Gibb NL, Wakelin SA. 2010. The abundance of microbial functional genes in grassy woodlands is influenced more by soil nutrient enrichment than by recent weed invasion or livestock exclusion. Appl. Environ. Microbiol. 76:5547–5555.

Louca S, Parfrey LW, Doebeli M. 2016. Decoupling function and taxonomy in the global ocean microbiome. Science 353:1272-1277.

Louca S, Polz MF, Mazel F, Albright MBN, Huber JA, O'Connor MI, Ackermann M, Hahn AS, Srivastava DS, Crowe SA, Doebeli M, Parfrey LW. 2018. Function and functional redundancy in microbial systems. Nat. Ecol. Evol. 2:936–943.

Lovarelli D, Bacenetti J, Guarino M. 2020. A review on dairy cattle farming: Is precision livestock farming the compromise for an environmental, economic and social sustainable production?. J. Clean. Prod. 262:121409.

Mack G, Walterand T, Flury C. 2013. Seasonal Alpine grazing trends in Switzerland: economic importance and impact on biotic communities. Environ Sci Policy. 32:48–57.

Mackay I.M, Arden K.E, Nitsche A. 2002. Real-time PCR in virology. Nucleic Acids Res. 30(6):1292-1305.

Marini L, Klimek S, Battisti A. 2011. Mitigating the impacts of the decline of traditional farming on mountain landscapes and biodiversity: a case study in the European Alps. Environ Sci Policy. 14:258–267.

McSherry ME, Ritchie ME. 2013. Effects of grazing on grassland soil carbon: a global review. Glob Chang Biol. 19(5):1347-1357.

Mcsherry ME, Ritchie ME. 2013. Effects of grazing on grassland soil carbon: A global review. Glob Chang Biol. 19:1347–1357.

Mendes LW, Tsai SM, Navarrete AA, De Hollander M, van Veen JA, Kuramae EE. 2015. Soil-borne microbiome: linking diversity to function. Microb. Ecol. 70(1):255-265.

Moonen AC, Bàrberi P. 2008. Functional biodiversity: An agroecosystem approach. Agric Ecosyst Environ. 127:7–21.

Myneni RB, Hall FG, Sellers PJ, Marshak AL. 1995. Interpretation of spectral vegetation indexes. IEEE Trans Geosci Remote Sens. 33(2):481–86

NandaKafle G, Seale T, Flint T, Nepal M, Venter S.N, Brözel VS. 2017. Distribution of diverse Escherichia coli between cattle and pasture. Microbes Environ. 32:226–233.

Nguyen NH, Song Z, Bates ST, Branco, S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20:241-248.

Northup BK, Brown JR, Holt JA. 1999. Grazing impacts on the spatial distribution of soil microbial biomass around tussock grasses in a tropical grassland. Appl. Soil Ecol. 13(3):259-270.

Oke TR. 2002. Boundary layer climates. Routledge.

O'Mara FP. 2012. The role of grasslands in food security and climate change. Ann. Bot. 110(6):1263-1270.

Paracchini M, Petersen J, Hoogeveen Y, Bamps C, Burfield I, Van Swaay C. High Nature Value Farmland in Europe - An Estimate of the Distribution Patterns on the Basis of Land Cover and Biodiversity Data. EUR 23480 EN. Luxembourg (Luxembourg): OPOCE. 2008. JRC47063.

Paul EA. 2014. Soil microbiology, ecology and biochemistry. Academic press. pp.598.

Pauler CM, Isselstein J, Berard J, Braunbeck T, Schneider MK. 2020. Grazing Allometry: Anatomy, Movement, and Foraging Behavior of Three Cattle Breeds of Different Productivity. Front. Vet.

Sci. 7:1–17.

Pedrini P, Sergio F. 2001. Golden Eagle Aquila chrysaetos density and productivity in relation to land abandonment and forest expansion in the Alps. Bird study. 48(2):194-199.

Perotti E, Probo M, Pittarello M, Lonati M, Lombardi G. 2018. A 5-year rotational grazing changes the botanical composition of sub-alpine and alpine grasslands. Appl Veg Sci. 21(4):647–57.

Perotto-Baldivieso HL, Cooper SM, Cibils AF, Figueroa-Pagán M, Udaeta K, Black-Rubio CM. 2012. Detecting autocorrelation problems from GPS collar data in livestock studies. Appl. Anim. Behav. Sci. 136(2-4):117-125.

Petermann JS, Buzhdygan OY. 2021. Grassland biodiversity. Curr. Biol. 31(19):R1195-R1201.

Pornaro C, Spigarelli C, Pasut D, Ramanzin M, Bovolenta S, Sturaro E, Macolino S. 2021. Plant biodiversity of mountain grasslands as influenced by dairy farm management in the Eastern Alps. Agric Ecosyst Environ. 320:107583.

Poschlod P, WallisDeVries MF. 2002. The historical and socioeconomic perspective of calcareous grasslands - lessons from the distant and recent past. Biol. Conserv. 104(3):361-376.

Prieto LH, Bertiller MB, Carrera AL, Olivera NL. 2011. Soil enzyme and microbial activities in a grazing ecosystem of Patagonian Monte, Argentina. Geoderma. 162(3-4):281-287.

Probo M, Lonati M, Pittarello M, Bailey DW, Garbarino M, Gorlier A, Lombardi G. 2014. Implementation of a rotational grazing system with large paddocks changes the distribution of grazing cattle in the south-western Italian Alps. Rangel. J. 36(5):445-458.

Probo M, Massolo A, Lonati M, Bailey DW, Gorlier A, Maurino L, Lombardi G. 2013. Use of mineral mix supplements to modify the grazing patterns by cattle for the restoration of sub-alpine and alpine shrub-encroached grasslands. Rangel. J. 35(1):85–93.

Prommer J, Walker TW, Wanek W, Braun J, Zezula D, Hu Y, Hofhansl F, Richter A. 2020. Increased microbial growth, biomass, and turnover drive soil organic carbon accumulation at higher plant diversity. Glob Chang Biol. 26(2):669-681.

Qu T, Du W, Yuan X, Yang Z, Liu D, Wang D, Yu L, 2016. Impacts of Grazing Intensity and Plant Community Composition on Soil Bacterial Community Diversity in a Steppe Grassland. PLoS One. 11.

Qu TB, Du WC, Yuan X, Yang ZM, Liu DB, Wang DL, Yu LJ. 2016. Impacts of grazing intensity and plant community composition on soil bacterial community diversity in a steppe grassland. PloS one. 11(7):e015968.

Raniolo S, Sturaro E, Ramanzin M. 2022. Human choices, slope and vegetation productivity determine patterns of traditional alpine summer grazing. It. J. Anim. Sci. 21(1):1126-1139.

Ridding LE, Watson SC, Newton AC, Rowland CS, Bullock JM. 2020. Ongoing, but slowing, habitat loss in a rural landscape over 85 years. Landsc Ecol. 35(2):257-273.

Rivero MJ, Grau-Campanario P, Mullan S, Held SD, Stokes JE, Lee MR, Cardenas LM. 2021. Factors affecting site use preference of grazing cattle studied from 2000 to 2020 through GPS tracking: A review. Sensors. 21(8):2696.

Rocca JD, Hall EK, Lennon JT, Evans SE, Waldrop MP, Cotner JB, Nemergut DR, Graham EB, Wallenstein MD. 2015. Relationships between protein-encoding gene abundance and corresponding process are commonly assumed yet rarely observed. ISME J. 9:1693–1699.

Rook AJ, Dumont B, Isselstein J, Osoro K, Wallis DeVries MF, Parente G, Mills J. 2004. Matching type of livestock to desired biodiversity outcomes in pastures–a review. Biol. Conserv.119(2):137-150.

Sager-Fradkin KA, Jenkins KJ, Hoffman RA, Happe PJ, Beecham JJ, Wright RG. 2007. Fix Success and Accuracy of Global Positioning System Collars in Old-Growth Temperate Coniferous Forests. J. Wildl. Manage. 71(4):1298–1308.

Semenzato P, Cagnacci F, Ossi F, Eccel E, Morellet N, Hewison A.M, Sturaro E, Ramanzin, M. 2021. Behavioural heat-stress compensation in a cold-adapted ungulate: Forage-mediated responses to warming Alpine summers. Ecol. 24(8):1556-1568.

Sere C, Steinfeld H and Groenewold J. 1996.World livestock production systems: current status, issues and trends. FAO Animal Production and Health Papers 127. FAO, Rome, Italy.

Shukla P.R, Skeg J, Buendia E.C, Masson-Delmotte V, Pörtner H.O, Roberts D.C., Zhai P, Slade R, Connors S, van Diemen S, Ferrat M, Haughey E, Luz S, Pathak M, Petzold J, Portugal Pereira, Vyas P, Huntley E, Kissick K, Belkacemi M, Malley J. 2019. Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems.

Smith J, Sones K, Grace D, MacMillan S, Tarawali S, Herrero M. 2013. Beyond milk, meat, and eggs: Role of livestock in food and nutrition security. Anim. Front. 3(1):6-13.

Song H, Che Z, Cao W, Huang T, Wang J, Dong Z. 2016. Changing roles of ammonia-oxidizing bacteria and archaea in a continuously acidifying soil caused by over-fertilization with nitrogen. Environ. Sci. Pollut. Res. 23(12):11964-11974.

Spiegal S, Estell RE, Cibils AF, James DK, Peinetti HR, Browning DM, Romig KB, Gonzalez AL, Lyons AJ, Bestelmeyer BT. 2019. Seasonal Divergence of Landscape Use by Heritage and Conventional Cattle on Desert Rangeland. Rangel. Ecol. Manag. 72(4):590–601.

Squires V.R, Dengler J, Hua L, and Feng H. 2018. Grasslands of the World: Diversity, Management and Conservation (Boca Raton, FL: CRC Press).

Stevens CJ, Dise NB, Mountford JO, Gowing DJ. 2004. Impact of nitrogen deposition on the species richness of grasslands. Science, 303(5665):1876-1879.

Strijker D. 2005. Marginal lands in Europe – causes of decline. Basic Appl Ecol. 6:99–106.

Sturaro E, Marchiori E, Cocca G, Penasa M, Ramanzin M, Bittante G. 2013a. Dairy systems in mountainous areas: farm animal biodiversity, milk production and destination, and land use. Livest Sci. 58:157–168.

Sturaro E, Thiene M, Cocca G, Mrad M, Tempesta T, Ramanzin M. 2013b. Factors influencing summer farms management in the Alps. Ital. J. Anim. Sci. 12(2):e25.

Survey on grassland ecosystem services. Report to the EEA—European Topic Centre on Biological Diversity.

Suttie J.M, Reynolds S.G, Batello C. 2005 Grasslands of the World. Food and Agriculture Organization of the United Nations, Plant Production and Protection Series (Food and Agriculture Organization, Rome): No. 34.

Tälle M, Deák B, Poschlod P, Valkó O, Westerberg L, Milberg P. 2016. Grazing vs. mowing: A meta-analysis of biodiversity benefits for grassland management. Agric Ecosyst Environ. 222:200-212.

Teague WR, Dowhower SL, Baker SA, Haile N, DeLaune PB, Conover DM. 2011. Grazing management impacts on vegetation, soil biota and soil chemical, physical and hydrological properties in tall grass prairie. Agric Ecosyst Environ. 141(3-4):310-322.

Thiene M, Scarpa R. 2008. Hiking in the Alps: exploring substitution patterns of hiking destinations. Tour. Econ. 14(2):263-282.

Toïgo C, Brambilla A, Grignolio S, Pedrotti L. 2020. Capra ibex. The IUCN Red List of Threatened Species 2020:e.T42397A161916377.

Tomkins N, O'Reagain P. 2007. 'Global positioning systems indicate landscape preferences of cattle in the subtropical savannas'. Rangel. J. 29(2):217–222.

Tomlinson C.J, Chapman L, Thornes JE, Baker C. 2011. Remote sensing land surface temperature for meteorology and climatology: A review. Meteorol. Appl. 18(3):296-306.

Tullo E, Finzi A, Guarino M. 2019. 'Review: Environmental impact of livestock farming and Precision Livestock Farming as a mitigation strategy'. Sci. Total Environ. 650:2751–2760.

Urbano F, Cagnacci F. 2014. Spatial Database for GPS Wildlife Tracking Data. A Practical Guide to Creating a Data Management System with PostgreSQL/PostGIS and R. 1st ed. Springer, Cham.

Valkó O, Venn S, Zmihorski M, Biurrun I, Labadessa R, Loos J. 2018. The challenge of abandonment for the sustainable management of Palaearctic natural and semi-natural grasslands. Hacquetia.

Valletta JJ, Torney C, Kings M, Thornton A, Madden J. 2017. Applications of machine learning in animal behaviour studies. Anim Behav, 124:203-220.

Veen P, Jefferson R, De Smidt J, Van der Straaten J. 2009. Grasslands in Europe: Of high nature value. BRILL.

Veldman JW, Overbeck GE, Negreiros D, Mahy G, Le Stradic S, Fernandes GW, Durigan G, Buisson E, Putz FE, Bond WJ. 2015. Where tree planting and forest expansion are bad for biodiversity and ecosystem services. BioScience. 65(10):1011-1018.

Wakelin S.A, Colloff M.J, Kookana R.S. 2008. Effect of wastewater treatment plant effluent on microbial function and community structure in the sediment of a freshwater stream with variable seasonal flow. Appl. Environ. Microbiol. 74(9):2659-2668.

Wakelin S.A, Gregg A.L, Simpson R.J, Li G.D, Riley I.T, McKay A.C. 2009. Pasture management clearly affects soil microbial community structure and N-cycling bacteria. Pedobiologia (Jena). 52:237–251.

Walburger K.J, Wells M, Vavra M, DelCurto T, Johnson B., Coe P. 2009. Influence of cow age on grazing distribution in a mixed-conifer forest. Rangel Ecol Manag. 62(3):290-296.

Wallenstein MD, Vilgalys RJ. 2005. Quantitative analyses of nitrogen cycling genes in soils. Pedobiologia (Jena). 49:665–672.

Wilsey B.J. 2018. The Biology of Grasslands (Oxford Univ.Press).

Wu M.H, Chen SY, Chen JW, Xue K, Chen SL, Wang XM, Chen T, Kang SC, Rui JP, Thies JE, Bardgett RD, Wang YF. 2021. Reduced microbial stability in the active layer is associated with carbon loss under alpine permafrost degradation. Proc. Natl. Acad. Sci. 118(25):e2025321118.

Wyffels SA, Boss DL, Sowell BF, DelCurto T, Bowman JG, McNew LB. 2020. Dormant season grazing on northern mixed grass prairie agroecosystems: Does protein supplement intake, cow age, weight and body condition impact beef cattle resource use and residual vegetation cover?. PloS one. 15(10):e0240629.

Yang Y, Tilman D, Furey G, Lehman C. 2019. Soil carbon sequestration accelerated by restoration of grassland biodiversity. Nat Commun 10:718.

Yergeau E, Kang S, He Z, Zhou J, Kowalchuk G.A. 2007. Functional microarray analysis of nitrogen and carbon cycling genes across an Antarctic latitudinal transect. ISME J. 1:163–179.

Zendri F, Ramanzin M, Bittante G, Sturaro E. 2016. Transhumance of dairy cows to highland summer pastures interacts with breed to influence body condition, milk yield and quality. Ital. J. Anim. Sci. 15(3):481-491.

Zhang G, Biradar CM, Xiao X, Dong J, Zhou Y, Qin Y, Zhang Y, Liu F, Ding M, Thomas RJ. 2018. Exacerbated grassland degradation and desertification in Central Asia during 2000–2014. Ecol Appl. 28(2):442-456.

Zhao F, Ren C, Shelton S, Wang Z, Pang G, Chen J, Wang J. 2017. Grazing intensity influence soil microbial communities and their implications for soil respiration. Agric Ecosyst Environ. 249:50-56.

Zhong L, Bowatte S, Newton PCD, Hoogendoorn CJ, Li FY, Wang Y, Luo D. 2015. Soil N cycling processes in a pasture after the cessation of grazing and CO2 enrichment. Geoderma 259–260:62–70.

Zhong L, Hoogendoorn C.J, Bowatte S, Li FY, Wang Y, Luo D. 2016. Slope class and grazing intensity effects on microorganisms and nitrogen transformation processes responsible for nitrous oxide emissions from hill pastures. Agric Ecosyst Environ. 217:70-78.

Zhou J, Xue K, Xie J, Deng Y, Wu L, Cheng X, Fei S, Deng S, He Z, Van Nostrand J.D, Luo Y. 2012. Microbial mediation of carbon-cycle feedbacks to climate warming. Nat. Clim. Chang. 2:106–110.

Zhu YG, Gillings M, Simonet P, Stekel D, Banwart S, Penuelas J. 2017. Microbial mass movements.

Science. 357(6356):1099-1100.

Zweifel-Schielly B, Suter W. 2007. Performance of GPS telemetry collars for red deer Cervus elaphus in rugged Alpine terrain under controlled and free-living conditions. Wildlife Biology 13(3):299–312.

2 – OBJECTIVE

The general aim of this thesis is to contribute to developing integrated and multi-scale methodological approaches to the study of the relations between grazing patterns, environmental, vegetation and soil conditions, soil microbial communities and functions in grassland ecosystems. For this purpose, GPS tracking for fine scale monitoring of livestock movement and behaviour, remote sensing for characterisation of vegetation and soil morphology, soil physical-chemical and genetic molecular analyses are tested and compared, in order to both define methodological protocols and acquire new knowledge. The aims of the specific studies conducted within this framework, and their conceptual consequential flow, are shortly described below.

The first and second contributions implement the use of GPS telemetry and remote sensing for describing grazing patterns of dairy cows in summer farms and factor influencing them. Specifically, the first contribution tests the effects of farmer conduction, slope, habitat type (grassland and larix forest) and vegetation abundance on grazing patterns and habitat selection of two local breeds of the Alps - the Alpine Grey and the Simmental - in traditional summer farm context. In this contribution, the GPS telemetry is used to monitor 9 dairy cows (4 Alpine Greys and 5 Simmentals) every minute during the day while the remote sensing to extract information about the local topology, habitat types and vegetation abundance. The second contribution expands the aims and the methods of the first one by applying machine learning model to remotely assess animal behaviours at pasture. This second contribution tests the differences between one local breed, the Alpine Grey, and one common dairy breed, the Brown Swiss, in terms of activity budget (grazing, resting, and walking) and grazing patterns, considering the effects of daily period (day and night), parity (primiparous and multiparous), habitat types (grassland, sparse forest and forest), slope and distance from barns. In this contribution, the application of GPS telemetry involves the night period and is used to monitor 18 dairy cows (9 Brown Swiss, 4 Alpine Greys primiparous and 5 Alpine Grey multiparous) while the remote sensing is used to extract information on local topology and the habitat types but not on vegetation abundance. The third and last contribution investigates the differences between soil microbial communities in term of taxonomical and functional structures of 38 European highland grasslands (18 in France, 10 in Italy and 10 in Norway) used as meadows (19) and pastures (19) testing the effect of the management type and two pedological parameters (pH and organic Carbon). In this contribution, the soil microbial communities are characterized though two molecular methods: the amplicon sequencing and the RealTime PCR. The former is applied along with FAPROTAX to define taxonomical and functional profiles while the latter to investigate the microbial potential of Nitrogen cycle functions.

The development of this thesis involved multiple partners, both academic and non-academic in different European countries, including the University of Padova and the Natural Park “Parco Naturale Paneveggio Pale di San Martino” in Italy, the VetAgro Sup and the Parc naturel régional Livradois-Forez in France, the Norwegian Institute of Bioeconomy Research (NIBIO) and Lofotlam in Norway.

3 – CONTRIBUTION

3.1 - Human choices, slope and vegetation productivity determine patterns of traditional alpine summer grazing

Raniolo S[a]., Sturaro E[a*]., Ramanzin M[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

Italian Journal of Animal Science, 2022, 21(1): 1126-1139.

Abstract

Grazing behaviour influences animal productivity and the conservation of grassland ecosystem services. We used GPS tracking and remote sensing (NDVI index) to monitor the grazing patterns of lactating cows on the 'Malga Ombretta' summer farm (1,957m asl) in the Dolomites, eastern Italian Alps, from 5th July to 5th August 2018. The pasture area (35 ha) was grazed by a mixed herd of Simmental and Alpine grey cows (stocking density = 0.6 LU/ha) under traditional management: each morning the farmer led the cows to graze in a selected sub-area of pasture, and during the afternoon he left them free to graze unrestricted until they returned to the barn for the night. GPS positions were collected every minute from 9 Simmental and 4 Alpine Grey cows with low milk production during the time they were outdoors. The farmer's choice of where to drive the herd to graze in the morning determined the distances the cows walked/day, which varied from 2.0 to 8.9 km, and favoured the use of higher and steeper areas that the cows tended otherwise to avoid. When free in the afternoon, the cows selected areas with higher NDVI values than those selected by the farmer in the morning, and Alpine Grey cows used slightly higher slopes and altitudes than Simmental cows, suggesting better adaptation to mountain pastures. The study revealed highly heterogenous grazing patterns dependent on multiple factors that can be assessed at fine temporal and spatial scales using GPS and remote sensing technologies to improve grazing management.

Highlights

- Daily distances walked and grazing patterns were influenced differently by the farmer's decisions and the animals' choices in response to environmental features.
- The NDVI index of vegetation productivity suggested that cows grazed more productive areas when free than when driven by the farmer.
- GPS tracking and remote sensing shed light on how human and animal choices regarding grazing are influenced by environmental features.

Keywords

Dairy cattle; mountain pasture; GPS tracking; NDVI

Introduction

Alpine pastures are semi-natural grasslands managed by humans for grazing herbivores, such as cattle (Bunce et al. 2004). These high-elevation agroecosystems are exploited during the summer seasonal transhumance and are still important in local livestock farming systems and for product quality, as well as for their biodiversity and delivery of non-provisioning ecosystem services (Sturaro et al. 2013; Schils et al. 2022). Livestock grazing patterns and their effects on animal welfare and productivity, and on the conservation of pasture ecosystem services depend on a complex set of interactions and trade-offs between animal characteristics (Isselstein et al. 2007; Spiegal et al. 2019; Pauler et al. 2020; Rivero et al. 2021), environmental features, such as local morphology (Kaufmann et al. 2013; Pittarello et al. 2021; Rivero et al. 2021), water location (Probo et al. 2014), the presence of shelter and shade, and climate conditions (Caton and Olson 2016; Liao et al. 2017; Rivero et al. 2021). These interactions and trade-offs are controlled by the grazing management system adopted. The most extensive continuous grazing systems minimise the control of grazing patterns by leaving livestock free to graze the whole pasture area, and have a low, long-term stocking rate. This requires only low labour and infrastructure investment, but increases livestock movement costs and, due to selective grazing patterns, results in a heterogeneous use of pasture with a mosaic of undergrazed and overgrazed patches, which gradually decreases the forage value of the pasture and allows shrub and tree encroachment (Kothmann 2009). At the opposite extreme, grazing patterns in the most intensive rotational systems are controlled by sequential grazing of cows at very high stocking rates in small paddocks for short periods, which restricts the animals' selectivity but enhances the forage value of the pasture and livestock productivity. However, these systems are labour intensive, require considerable infrastructure investment, and may impact on pasture biodiversity and nutrient cycling (Kothmann 2009; Probo et al. 2013; 2014; Perotti et al. 2018).

In order to minimise the trade-off between these two extremes, traditional grazing management in the Alps has developed around extensive rotational systems, where the whole pasture area is divided into relatively large sub-areas where livestock are brought to graze at variable stocking rates and for variable periods based on the shepherd's and/or expert's knowledge of the vegetation conditions and evolution (Probo et al. 2013; Perotti et al. 2018; Pittarello et al. 2019). In this case, a thorough understanding of grazing patterns and how they are influenced by both the farmers' and animals' choices are fundamental to tailoring grazing management to local conditions to ensure animal welfare and productivity, and conservation of soil and vegetation ecosystem functions (Ravetto Enri et al. 2017).

The study of animal movement and behaviour has improved dramatically during recent decades with the introduction of GPS tracking (Cagnacci et al. 2010; Nathan et al. 2022). This technology allows the position of individual animals to be monitored with high time-frequency resolution on a scale as small as seconds or minutes, and high spatial accuracy with location errors of <5–10m (D'Eon et al. 2002; Tomkiewicz et al. 2010; Muminov et al. 2019), and without interference with the animals' behavioural patterns by an observer (Homburger et al. 2014). GPS tracking has therefore been used extensively to study the movement ecology of wildlife (Nathan et al. 2022) and more recently, but to a lesser extent, to study livestock grazing patterns (see the reviews by Bailey et al. 2018 and Rivero et al. 2021). GPS location technology can be combined with remote sensing technologies, which are able to characterise environmental conditions at multiple spatial and temporal scales from satellite images and provide a more detailed understanding of animal-habitat interactions (Pettorelli et al. 2005, 2014). A widely-used remote sensing index is the absolute Normalised Difference Vegetation Index (NDVI; Myneni et al. 1995; Shariatinajafabadi et al. 2014). The NDVI ranges from 1 to þ1, where values below 0 are typical of habitats without vegetation, while values close to 1 are indicative of areas with very abundant vegetation (Pettorelli et al. 2005). It is therefore used to estimate vegetation biomass, but, in addition, temporal variations in the index can also be used to estimate the phenological stage and stress conditions of plants (Myneni et al. 1995; Pettorelli et al. 2005; Shariatinajafabadi et al. 2014).

In this study, we used GPS tracking on a small temporal scale to investigate the movement patterns of lactating cows in an alpine pasture managed according to a combination of rotational and continuous grazing. Specifically, we aimed to describe the animals' movement patterns and use of pasture at two spatiotemporal scales, i.e. the single movement step, defined as the segment connecting two consecutive locations, and the total daily trajectory, and assess how they were influenced by the farmer's decisions and the animals' selectivity in relation to land morphology, weather conditions, vegetation type and vegetation productivity (according to the NDVI). In addition, we took advantage of the presence of two breeds of cows, Alpine Grey and Simmental, to obtain a preliminary indication of possible differences between them.

Material and methods

Study area and summer farm management

The study was conducted in Val Ombretta, located in the Marmolada massif in the eastern Italian Alps (Dolomites; 46_2601300 N, 11_5105400 E), during the summer of 2018 (Figure 1). Val Ombretta is characterised by a typical alpine environment with subalpine grasslands and sparse forest composed mostly of *Larix decidua* with also *Pinus mugo* and *Picea abies*, surrounded by rocky cliffs that reach an altitude of over 3,000m asl. It has approximately 35 ha of grazing land at an average altitude of 1,957m asl (SD = 15) and with an average slope of 16.5° (SD = 6.1).

In summer - from mid-June until mid-September - the grasslands of the valley are grazed by a herd of dairy cattle managed by the local summer farm. During the study period, the summer farm hosted 14 Simmental and 7 Alpine Grey lactating cows at an average stocking density of 0.6 livestock units/ha. The herd was managed traditionally. After the evening milking (starting 5.30–6 pm), the cows spent the night inside the barn in fixed stalls. After the morning milking (ending around 8–8.30 am), they were conducted by the farmer to selected pasture sub-areas (Figure 1), where they grazed until lunch time (around 12 am–1 pm), after which the farmer left them free for the afternoon until they returned spontaneously to the barn for the evening milking. The cows received a very limited amount (0.5–0.8 kg/d) of a compound feed supplement (19.0% crude protein, 6.4% crude fibre, 9.2% total ash; Palumbo et al. 2021), so they had to rely mostly on grazing to fulfil their energy requirements.

The study included 9 Simmental and 4 Alpine Grey cows, which were all multiparous. The summer farm was visited once during the study period and milk yield was estimated from the volume of milk collected in a graduated bucket (0.5 l resolution) at the morning and evening milking of each cow. On the same visit, body length (BL), girth circumference (GC) and body condition scores (BCS) were measured by a single trained operator. The values obtained were used to estimate live body weight according to the equation -1099.5 + 3.37*BL + 5.47*GC + 25.3*BCS (L. Gallo, unpublished data).

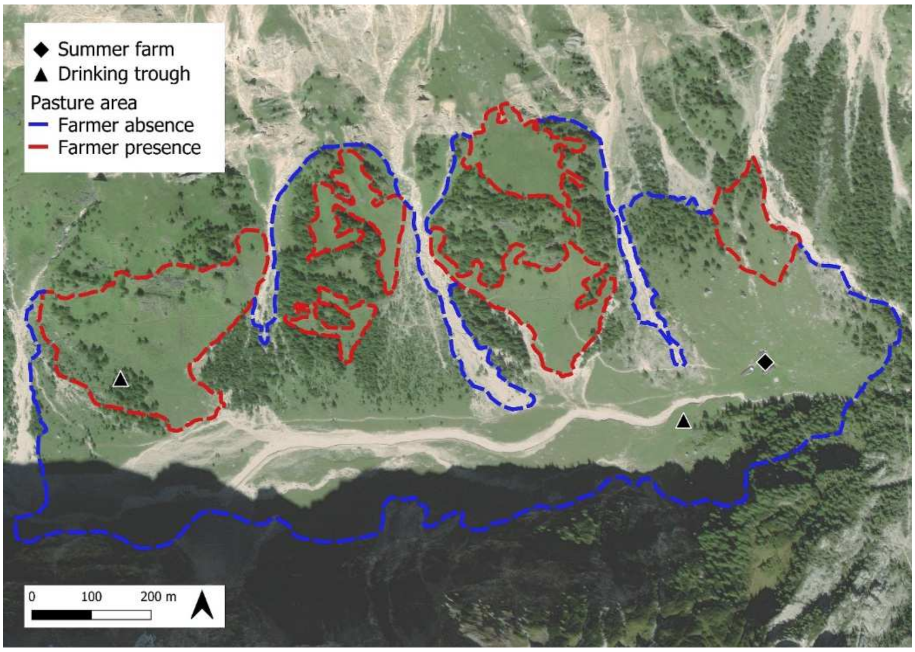


Figure 1. Val Ombretta showing the location of the summer farm, the total grazing area comprising areas used freely by the cows in the afternoon (in blue, 'Farmer absence'), the sub-areas where the farmer conducted the cows in the morning (in red, 'Farmer presence'), and the positions of drinking troughs.

Spatial covariates and time-varying

Spatial covariates

Digital maps of the study area were generated in QGIS 3.14 (http://www.qgis.osgeo.org/) using the EPSG 4326 coordinate system. We created a raster map of slope (degrees) and altitude (m) using a Digital Elevation Model with a resolution of 10m (https://www.regione.veneto.it/web/ambiente-e-territorio/ ctr). We created a categorical variable 'slope class' with four levels: <10°, 10° and <20°, 20° and <30° and 30°. We used the map produced by Scillitani et al. (2013) with a spatial resolution of 50m to categorise the land cover of the grazing area into three 'habitat types': 'Larix' (*Larix decidua* stands), 'grassland' (subalpine pastures), and 'scree' (areas covered by loose stone with little vegetation). We obtained NDVI values as an indicator of vegetation productivity of the study area from the Sentinel-2 constellation of satellites using the Google Earth Engine open-source platform (Gorelick et al. 2017). The NDVI raster maps were acquired at a 10m resolution for the dates 5th, 8th and 13th July, according to the temporal resolution of Sentinel-2 spectral acquisition. We generated daily NDVI maps by interpolating the daily variation in NDVI across the three dates. We created a categorical variable 'NDVI class'- with four levels: <0.2, 0.2 and <0.4, 0.4 and <0.6, 0.6. We also obtained average hourly temperatures and precipitation from the nearest weather station (Malga Ciapela station, 1,475m asl; Rocca Pietore BL ARPAV – Veneto Regional Agency for Environmental Protection and Prevention, https://www.arpa.veneto.it/) for every day of the study period. Because the temperatures were not recorded at the summer farm itself, but at a lower altitude, we created a categorical variable 'temperature class' with three levels: 'low' (<13.1 °C, first quartile); 'average' (13.1 and <17.4 °C, second and third quartiles); 'high' (17.4 °C, fourth quartile), which means the values of this variable are relative, rather than absolute. We also categorised precipitation as 'rain occurrence' (yes/no) as there were many days without rain.

Movement patterns of cows

The movement patterns of the cows were monitored with GPS collars (VERTEX Plus model; VECTRONIC Aerospace GmbH) scheduled to attempt a location every minute from 08:00 am to 6:00pm from 5th July to 5th August. The median location error had been previously estimated at 4.5m (Parraga Aguado et al. 2017). We obtained 198,186 GPS positions, which we pre-processed in PostgreSQL 14 (https://www.postgresql.org/docs/14/index.html) with the plugin PostGIS 3.1.5 (http://postgis.net/2022/02/01/postgis-3.1.5/). After excluding impossible locations (e.g. on steep rocky slopes or in other valleys), we associated to the second location of each 'movement step' (i.e. each consecutive pair of locations): (a) the 'step distance', calculated as the linear distance in m between the first and second locations corrected for the slope travelled as indicated by the elevation difference between them; (b) the 'speed', calculated as step distance/time interval in sec; (c) the ‘turning angle’, i.e. the cosine of the angle between the line intersecting the two locations of one step and the line intersecting the first location of that step with the second location of the preceding step (Urbano and Cagnacci 2014). We then identified the remaining outlier locations by first excluding those with an impossible speed (>15 km/h), as suggested by Liao et al. (2017) and Spiegal et al. (2019), and then those with an impossible combination of speed and turning angle (speed >2.4 km/h and cosine <–0.97), as suggested by Urbano and Cagnacci (2014). Finally, since the daily schedule of data collection by the GPS collars (8:00 am–6:00 pm) could have included periods when the cows were in the barn, we identified the indoor period for each cow on each day using a combination of the frequency of missed locations (when the GPS is inside a building most positions are lost due to obstruction of the satellite signals) and movement trajectories. Specifically, we assumed that the indoor period started in the first 10-min time interval after 4:30pm with >5 missed positions (out of 10 expected), and ended in the last interval after 8:00 am with >5 missed positions. We checked that all the 10-min intervals within the starting or ending intervals exhibited consistently high numbers of missed positions. To avoid removing any real outdoor positions from the starting and ending intervals, we deleted from these only the positions that were within a 25m buffer surrounding the barn. Finally, we visually checked the continuity of the resulting individual outdoor daily movement trajectories. The final geodatabase contained 174,171 outdoor locations with each location associated with individual features (individual cow and breed), temporal features (Julian date and hour), environmental features (slope, elevation, land cover type and NDVI class), climate conditions (hourly and daily average temperature, temperature class, rain occurrence) and step movement features (step distance, speed). We also calculated for each day and each cow the ‘outdoor time’ (in h) as the difference between the time of the first and the last outdoor locations, and divided this into two ‘day periods’ using 12.00 am as the boundary: ‘morning’ (before 12.00 am), when the cows’ movement patterns are determined by the farmer, and ‘afternoon’ (after 12.00 am), when no restrictions are placed on the cow on where to graze and when to return to the barn. For each day and cow, we also calculated: (a) the ‘distance walked in the morning’ as the sum of the step distances of the morning trajectory, and the ‘distance walked daily’ as the sum of the step distances of the morning and afternoon trajectories; and (b) the ‘daily vertical movement’, as the difference (in m) between the lowest and highest elevation/cow/day.

Statistical analysis

The statistical analyses were conducted in R 4.0.2 (R Core Team 2016). As a preliminary, we assessed possible differences in milk yield and live body weight between the two breeds with a simple one-way ANOVA.

Daily and hourly movement variables

Daily movement-related variables (distance walked and vertical movement) were analysed with linear mixed effects models using the lmer function of the lme4 package (Bates et al. 2015). The models included the continuous linear effects of Julian date, distance walked in the morning, outdoor time, and (only for daily distance walked) daily vertical movement, the categorical effects of breed, rain occurrence and temperature class, and the random effect of individual cow. As a preliminary, we checked for absence of collinearity between the explanatory variables (all VIF values were below 2.6). We assessed the models' marginal R^2, due to fixed factors only, and conditional R^2, due to fixed plus random factors (Nakagawa and Schielzeth 2013), using the Performance package (Lüdecke et al. 2021).

Hourly movement-related variables (slope, altitude and speed associated to each location/step) were analysed with generalised additive mixed models using the gam function of the mgcv package (Wood 2017), with a model that included the smoothed spline effect of hour, the categorical effects of breed, temperature class, rain occurrence and day period, the two-way interactions of day period with breed, temperature class or rain occurrence, and the random effects of Julian date and individual cow. Speed was expressed as m/h, and slope and speed were log-transformed before analysis.

Use of pasture and resource selection

We assessed the spatial evenness of the use of the pasture area during each day period by calculating Camargo's index (Payne et al. 2005; Pauler et al. 2020) on the total GPS positions acquired during the mornings and afternoons of the entire study period within each cell of a 25x25m grid overlaid onto the pasture area. The index ranges from 0, patchy or heterogeneous use of an area, to 1, homogeneous use.

To assess the selection – i.e. the preferential use of a resource limited by external conditions (Manly et al. 2002) – of spatial features (slope, NDVI, habitat type) we used a resource selection function approach (Boyce and McDonald 1999). We first extracted the areas 'available' to the cows in each day period of each date by calculating the minimum convex polygons that included the morning and afternoon locations of the monitored cows from 5th to 13th July. From within each polygon we extracted the 'non-used area' by excluding the area not forming part of the 25m buffer around each acquired location. Within the non-used area, we generated a number of random 'non-used positions' equal to the number of acquired animal locations and assigned them randomly to each cow. Thus, we obtained a database of 'used' (with animal locations) and 'non-used' (without animal locations) positions, which we categorised by NDVI class, slope class and habitat type (simplified to 'grassland' and 'forest', excluding 'scree', which was very seldom used). Finally, we used a generalised linear mixed effects model with binomial distribution and logit link function (glmer function of the lmer4 package (Bates et al. 2015)) to assess the relative probability of a position being used in response to the fixed effects of habitat type, slope class, NDVI class and day period, the two-way interactions between period and the other effects, and the random effect of the individual cow. We assessed the model's marginal R^2, due to fixed factors only, and the conditional R^2, due to fixed plus random factors (Nakagawa and Schielzeth 2013) with the Performance package (Lüdecke et al. 2021).

Results

Milk production and live body weight

Milk production was low and did not significantly differ between breeds (GLM least square means: Alpine Grey: 10.9 kg/day, SE = 2.1; Simmental: 11.2 kg/day, SE = 1.4; p = 0.63), nor did predicted live body weight (GLM least square means: Alpine Grey: 537.9 kg, SE = 39.3; Simmental: 553.0 kg, SE = 26.2; p=0.74). Therefore, we assumed that any differences in movement patterns between breeds would not be related to milk yield and live body weight.

Daily movement-related variables

The average distance walked daily was 4,585m (SD = 206, minimum = 2,019, maximum = 8,912), while the average daily vertical movement was 101m (SD = 51 m, minimum = 18 m, maximum = 225 m).

For brevity, the parametric coefficients of the models analysing the two variables are given in Supplementary Table S1. The distance walked daily was unaffected by breed, rain occurrence and temperature class (P values ranging between 0.26 and 0.73) and increased only tendentially with outdoor time (p=0.06). However, it increased significantly ($p<0.001$) with Julian date and mainly, although unsurprisingly, with distance walked in the morning (Figure 2, panels A and B). Longer distances walked daily were also associated ($p<0.001$) with higher daily vertical movement (Figure 2, panel C). Daily vertical movement did not vary significantly in response to distance walked in the morning, breed and outdoor time (p values ranging between 0.25 and 0.79) but increased markedly with Julian date ($p<0.001$; Figure 2, panel D). It was also negatively affected ($p<0.05$) by rain occurrence and temperature class (Figure 2, panels E and F).

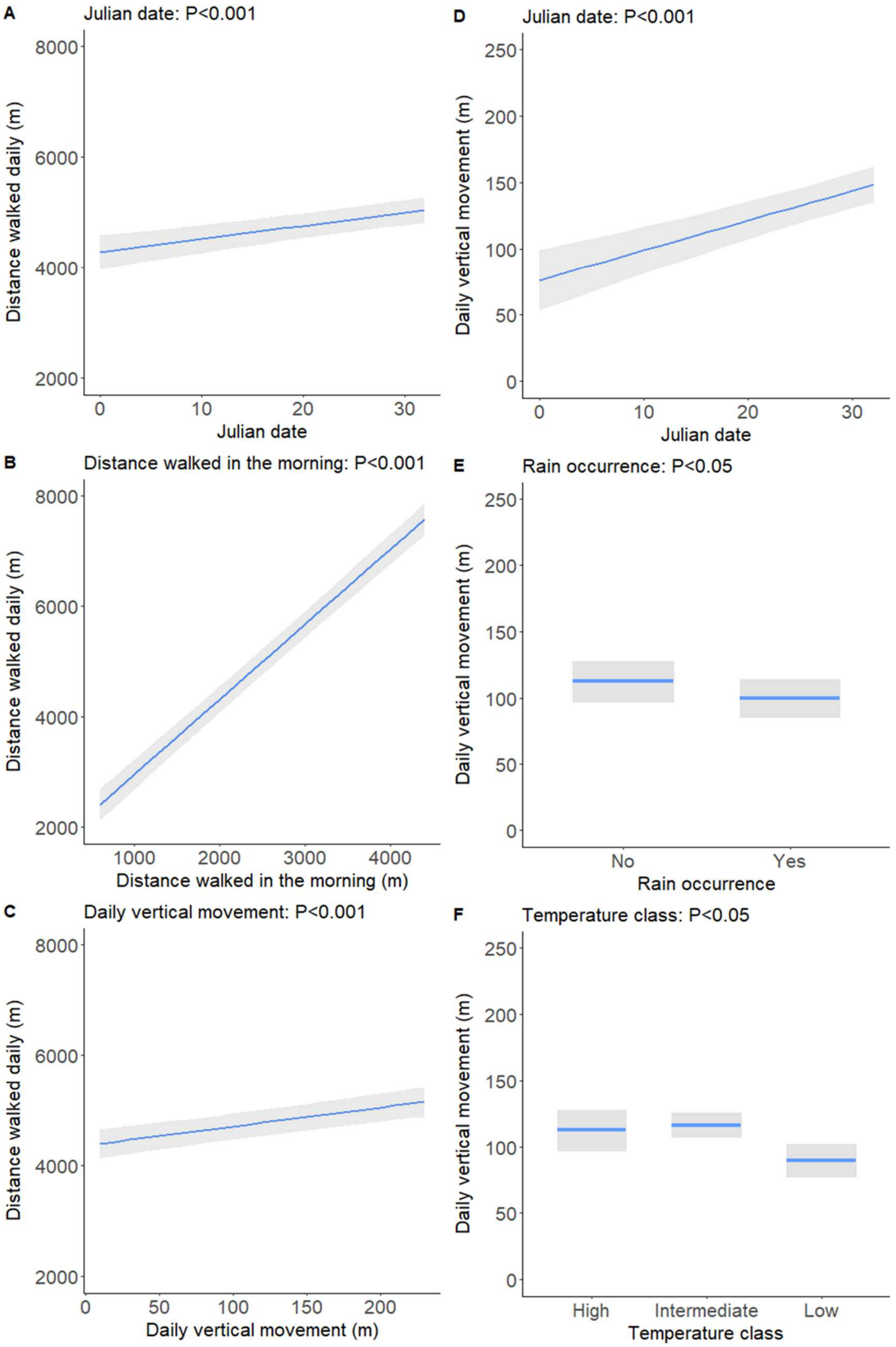


Figure 2. Effects on distance walked daily of Julian date, distance walked in the morning and daily vertical movement (panels A, B, and C, respectively), and effects on daily vertical movement of Julian date, rain occurrence, and temperature class (panels D, E, and F, respectively). Shaded areas indicate 95% confidence intervals. Only significant effects are shown; for details of the parametric coefficients of the statistical models see Supplementary Table S1.

Hourly movement-related variables

The parameters of the models analysing slope used, altitude used and speed are reported in Supplementary Table S2. Slope used followed a spiked pattern with peaks at around 11 am and 4 pm and troughs at around 9 am and 2 pm (Figure 3, panel A, smoothed spline effect of hour; p<0.001). Alpine Grey cows used steeper slopes than Simmental cows, especially in the afternoon (Figure 3, panel B, breed by day period interaction; p<0.05). The cows used a markedly gentler slope in the morning than in the afternoon in the presence of rain, and a slightly steeper slope in the absence of rain (Figure 3, panel C, rain class by day period interaction; p<0.001). At high temperatures the cows grazed on a much gentler slope in the afternoon than in the morning, but the difference was smaller at low temperatures and non-existent at intermediate temperatures (Figure 3, panel D, temperature class by day period interaction; p<0.001).

The altitude used increased rapidly during the morning, almost paralleling the increasing pattern of slope, but in the afternoon showed a constant decline (Figure 3, panel E, smoothed spline effect of hour; p<0.001) The Alpine Grey cows used a higher altitude than the Simmental cows, especially in the afternoon (Figure 3, panel F, breed by day period interaction; p<0.001). The occurrence of rain was accompanied by lower altitudes used in the morning, and by higher altitudes in the afternoon (Figure 3, panel G, rain occurrence by day period interaction; p<0.001). Higher temperatures were accompanied by higher altitudes in the morning, but by lower altitudes in the afternoon, while the opposite pattern was observed at lower temperatures (Figure 3, panel H, temperature class by day period interaction; p<0.001).

The cows left the barn at high speed, then gradually slowed until 12 am when they reached the maximum slopes and altitudes; there was then a period of slow movement until 4pm, followed by increasing speed until they returned to the barn (Figure 3, panel I, smoothed spline effect of hour; p<0.001). There were no differences between breeds (Figure 3, panel J; p=0.94), and no breed by day period interaction (p=0.33). The occurrence of rain reduced speed (Figure 3, panel K; p<0.01). Finally, speed was faster in the morning than in the afternoon, but this difference was less marked at high temperatures as morning speed was slower and afternoon speed was faster than at intermediate or low temperatures (Figure 3, panel L, temperature class by day period interaction; p<0.001).

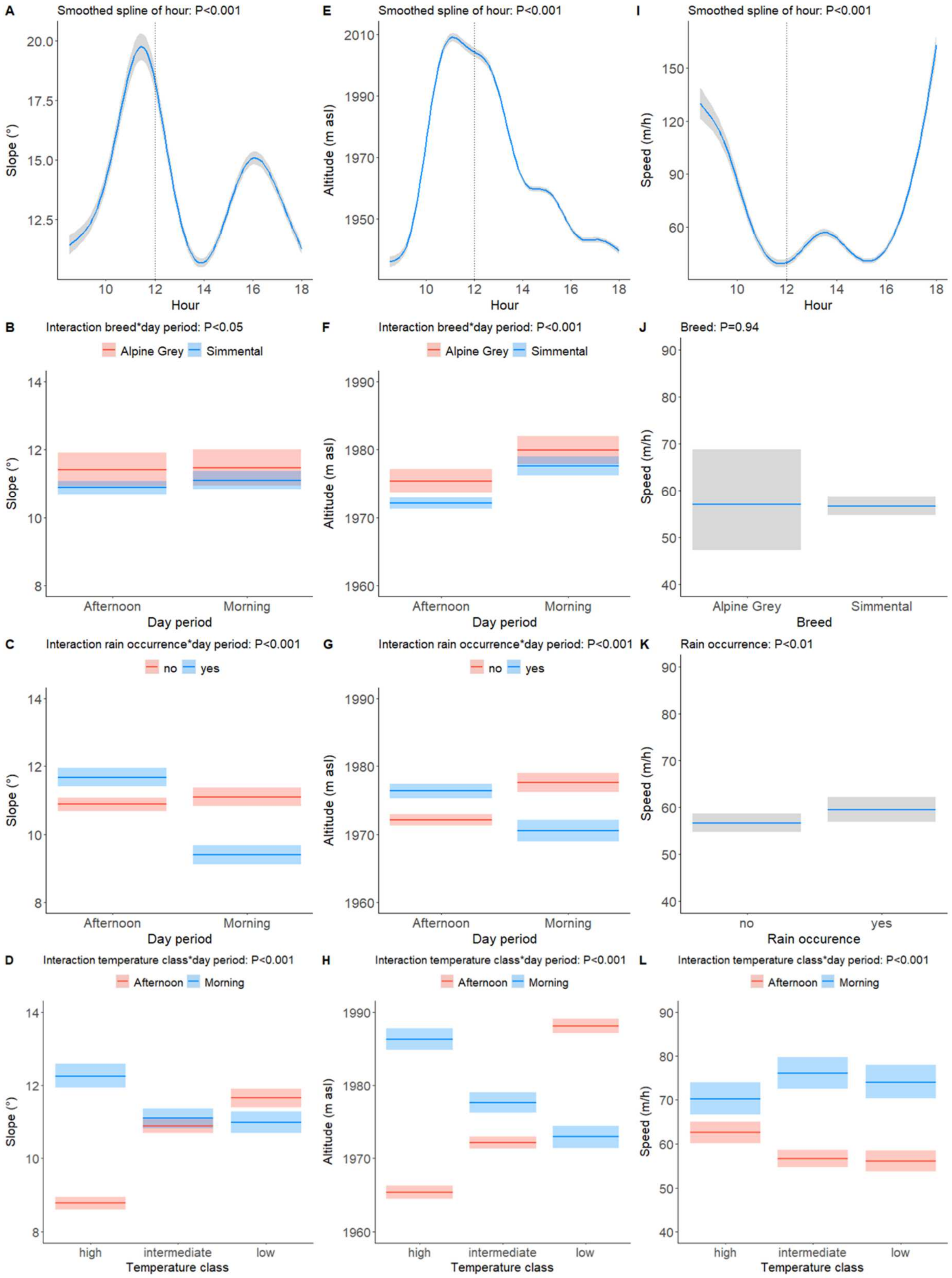


Figure 3. Effects of the smoothed spline of hour, and of the interactions breed by day period, rain occurrence by day period, and temperature class by day period on the slope (panels A, B, C and D, respectively) and on the altitude used (panels E, F, G and H, respectively), and effects of the smoothed spline of hour, breed, rain occurrence and the temperature class by period interaction on speed (panels I, J, H and K, respectively). Shaded areas indicate 95% confidence intervals. The vertical dotted line in panels A, E and I indicate the separation between the morning and afternoon day periods at 12 am. For details of the parametric coefficients of the statistical models see Supplementary Table S2.

Use of pasture and resource selection

Camargo’s index (0.11 ± 0.06 in the morning, and 0.13 ± 0.07 in the afternoon) indicated a highly uneven use of the pasture, with greater use in the steeper, peripheral areas in the morning, and in the flatter areas close to the barn in the afternoon (Figure 4).

The probability of a position being used increased markedly with increasing NDVI values (see Supplementary Table S3 for the parametric coefficients of the model), but in the afternoon the cows grazed patches with high NDVI more frequently and patches with low NDVI less frequently than in the morning (Figure 5, panel A, NDVI class by day period interaction; $p<0.01$). Slope had little influence on the probability of a position being used in the morning, but in the afternoon this probability was clearly lower for slopes steeper than 20 degrees (Figure 5, panel B, slope class by day period interaction; $p<0.001$). Finally, there was a strong preference for areas of grasslands over areas of Larix decidua stands, but less so in the afternoon than in the morning (Figure 5, panel C, habitat type by day period interaction; $p<0.001$).

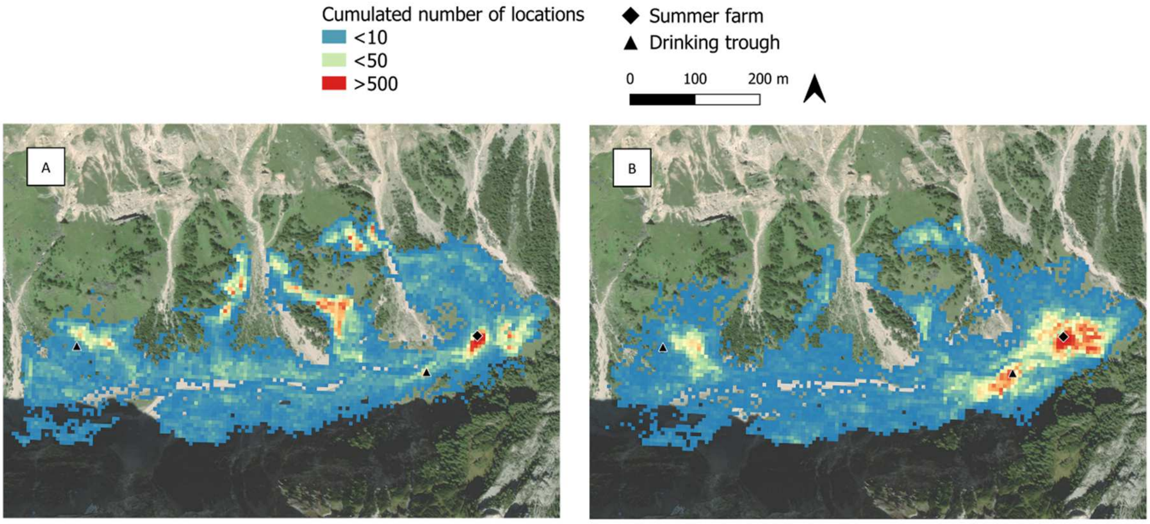


Figure 4. Intensity of use of pasture (no. of locations cumulated over the study period within a 10_10 m grid) in the morning (A) and afternoon (B).

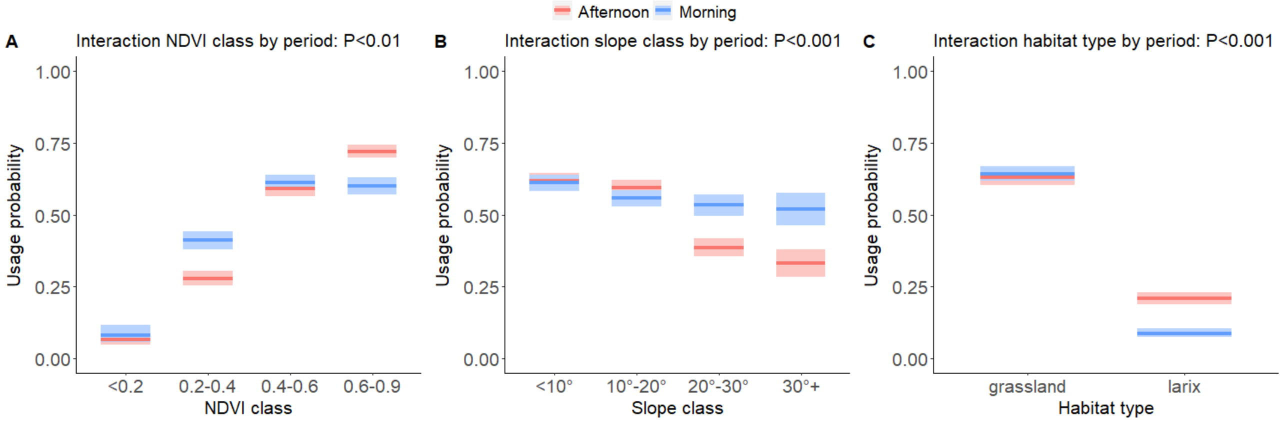


Figure 5. Predicted probability of use of pasture area according to the two-way interactions between day period and NDVI class, slope class and habitat type. Shaded areas indicate 95% confidence intervals.

Discussion

In this study, we identified the role played by the farmer's daily decisions in determining grazing patterns in a mixed rotational/continuous management system, and at the same time showed how cows seek an energy landscape characterised by low cost (gentler slope, lower elevations) and high forage biomass (according to NDVI values). However, the resulting use of the pasture areas remained highly heterogeneous. In the following sections, we will discuss these findings.

The importance of the farmer's decisions

The farmer's daily decision regarding which pasture sub-area to conduct the herd to in the morning had a major influence on the distance the cows walked daily, as indicated by the distance walked in the morning, but also on the daily vertical movement and slopes used, as evidenced by the fact that the cows reached the highest altitudes (>1,950m asl) and steepest slopes (>20 degrees) around 11 am. There was substantial variability in the distances covered daily by the cows, and in their daily vertical movement, and consequently slopes used. Since these variables are positively related, there was also considerable daily variation in movement costs, and hence maintenance requirements. There is a surprisingly small literature on the energy costs of the movement of grazing livestock. According to the Agricultural Research Council (1980), the energy costs associated with cattle walking 1 km horizontally and 0.1km vertically are 2.0 and 2.8 kJ/kg body weight, respectively. In our study, the minimum distance walked daily was 2.0 km with a vertical movement of 0.02 km, while the maximum was 8.9 km with a vertical movement of 0.16 km. For a cow with a body weight of 550 kg this would, according to ARC (1980), correspond to energy costs of 2.5 and 12.3 MJ, which would increase maintenance requirements (Nozière et al. 2017) by approximately 7 and 34%, respectively. These additional costs are within the range suggested by the Standing Committee on Agriculture (SCA; 1990) for grazing cows, but the considerable variability between days cannot be compensated for on summer farms where supplementary feeding is very limited and cows have to rely on herbage intake to fulfil their needs, as in our case study. Herd management also influenced the patterns of speed. The cows moved faster soon after leaving the barn in the morning when the farmer conducted them to the selected grazing areas. They then gradually slowed down as they increased their grazing activity until noon, when the animals, now left free by the farmer, increased their speed to reach the drinking troughs. Afterwards, they reduced speed again for 2–3 hours, before starting to move faster to reach the barn for the evening milking. In this symmetrical diurnal pattern, the cows moved faster in the morning, when the farmer was driving them, than in the afternoon.

Our results also suggest that the selection of pasture sub-areas by the farmer was influenced by weather conditions. Daily vertical movements were smaller on days with rain and low temperatures than on days without rain and with intermediate or high temperatures, which appears to be a consequence of lower altitudes (and consequently gentler slopes) being used in the morning than in the afternoon. It seems, therefore, that the farmer decided to stay closer to the bottom of the valley when the weather was less favourable. Furthermore, the positive relationship between daily horizontal and vertical movement and Julian date probably reflects an attempt by the farmer to exploit the more peripheral areas with the advancing season, possibly because the more accessible areas had already been exploited. This is consistent with Palumbo et al. (2021) findings that in the same pasture the average forage value of the plant species ingested by grazing cows gradually declined over the grazing period because of an increase in the proportion of species with low palatability and low forage value. Finally, analyses of movement variables on an hourly scale and of resource selection revealed that in various instances the farmer's decisions were in contrast to the cows' choices. This is important to consider in addressing grazing management and will be discussed in the next section.

The animal component remains important

It is well known that in terms of body size/morphology, behaviour and performance levels, traditional local breeds are more suited to grazing in harsher areas than conventional and especially highly productive breeds (Hessle et al. 2014; Zendri et al. 2016). However, comparisons of the movement patterns of different breeds or their crosses in European mountain pastures are rare and have involved genotypes that are more divergent in body size and productivity than those compared here (Hessle et al. 2008; Pauler et al. 2020). Although our results are to be treated with caution and considered preliminary, requiring verification with larger sample sizes with a wider variation in body mass and milk yield and including factors, as parity, which we could not test here, they suggest that the local Alpine Grey has a better ability to move quickly and on steeper slopes than the Simmental. Alpine Grey cows also used higher altitudes, and although the difference was very modest in absolute values (around 10 m) it indicates spatial separation between individuals of the two breeds. Overall, these findings suggest that Alpine Grey are more suited than Simmental to grazing alpine pastures. In a study involving multi-breed herds on 15 alpine summer farms, Zendri et al. (2016) found that lactating cows of local dual-purpose breeds (Alpine Grey, Rendena and crossbreds) maintained higher body condition scores during the season than Simmental cows, whose milk yields were higher at the beginning of the summer grazing periods, but similar to the other breeds at the end.

In general, land morphology and the spatial distribution of vegetation types and forage values influence animals' movement patterns in interaction with climate conditions (Rivero et al. 2021). We also found an effect of land morphology in our study. In the afternoon, when left free by the farmer, the cows showed a clear preference for gentler slopes. This was expected, since steep slopes and rocky or soft soils are avoided by cattle due to the increased energy cost of movement and risk of injuries (Kaufmann et al. 2013; Probo et al. 2014; Pittarello et al. 2021; Rivero et al. 2021). In our study, the gentler slopes and lower altitudes were also used more in the afternoon on hotter and sunny days, which might be to reduce energy expenditure and avoid the risk of heat stress. However, we could not test this hypothesis directly because we did not have the actual temperatures at the summer farm, which would probably have been lower than those we used. Furthermore, we do not have an explanation for why the cows moved faster in the afternoon and more slowly in the morning on hotter days.

The analysis of habitat selection showed a strong preference for grassland patches over Larix decidua stands, consistent with the general observation that cattle prefer open grassland to shrub or forest habitat for grazing (Meisser et al. 2014; Spedener et al. 2019; Tofastrud et al. 2019). Interestingly, however, the cows had a greater tendency to avoid the Larix decidua stands during the morning, when their grazing area was chosen by the farmer, than in the afternoon, when it was chosen by the cows. This indicates that humans and animals have different perceptions of this habitat. Larix decidua stands have a loose canopy cover that allows ground vegetation to grow, and because they offer both forage and shade or shelter they are often grazed (Da Ronch et al. 2016). Possible explanations for the higher use of Larix decidua in the afternoon could be that the cows simply passed

through the forest on their way from the high areas to the valley bottom, or alternatively that they deliberately made greater use of this habitat during the afternoon, for instance in search of shade while continuing feeding. This last hypothesis could be tested with accurate and more variable temperature values, and by investigating the types of activities the animals engage in (Homburger et al. 2014, 2015). The NDVI has been used as an indicator of vegetation productivity, especially on rangelands, often at large spatial and temporal scales (Browning et al. 2018; Spiegal et al. 2019). It has been shown that cattle have a clear preference for higher NDVI values (>0.5; Manning et al. 2017). In this study we found a similar trend, suggesting that the index can be used to shed light on grazing patterns, even at small spatial and temporal scales. Interestingly, the areas selected by the cows in the afternoon had higher NDVI values than the areas selected by the farmer in the morning. This was not related to the greater use of Larix decidua stands, which had on average a lower NDVI value than the grasslands (least squared means of a one-way ANOVA: grassland = 0.540, SE = 0.001; Larix decidua=0.507, SE = 0.002, $p<0.001$), and therefore seems to reflect the cows' ability to graze in (grassland) areas with richer vegetation than those selected by the farmer. While absolute NDVI values reflect vegetation biomass, relative variations in them over time index phenology, and hence nutritional quality, increasing rapidly during the early growth phases, more slowly towards maturation, and decreasing during senescence (Beck et al. 2008; Shariatinajafabadi et al. 2014). For this reason, various studies on wild species have found that movement and foraging patterns have a positive relationship with vegetation quality as indexed by NDVI variation (Geremia et al. 2019; Semenzato et al. 2021). To our knowledge, this has not been replicated with domestic species and we were unable to do so in this study because we had too few NDVI time intervals, although it is a promising topic for future research.

The use of pasture is highly heterogeneous

Our assessment of pasture use is partial because we did not monitor the whole grazing season, but it clearly suggests that the combination of the farmer's daily decisions and the cows' preferences when left free resulted in highly variable stock densities over the grazed area. The morning hotspots reflected the farmer's attempts to use areas at higher elevations and greater distances from the barn that the cows would otherwise probably not have used, while the afternoon hotspots were influenced in part by those of the morning, but mainly reflected the cows seeking the valley bottom, the water troughs and proximity to the barn. This pattern illustrates the difficulty faced by non-intensive grazing management systems in reducing heterogeneity in the use of pasture (Probo et al. 2013; Pittarello et al. 2019), which has various potential consequences. In pasture ecosystems, livestock presence is a local disturbance because the structure and composition of the vegetation can be shaped by selective herbivory (Isselstein et al. 2007; Rivero et al. 2021), and soil abiotic conditions and biotic functions can be modified by trampling and nutrient translocation through excreta (Teague et al. 2011; Peco et al. 2017; Koch et al. 2018). Undergrazed areas may suffer from depletion of nutrients, progressive shrub or tree encroachment, and even abandonment (Koch et al. 2018) and, in fact, visual observations of this pasture clearly reveal an ongoing expansion of Larix decidua towards the valley bottom. Overgrazed areas, on the other hand, may be impacted by erosion and depletion of grass vegetation – indeed vegetation sampling (Stefano Macolino, unpublished results) has indicated that standing grass biomass is very low in proximity to the barn during the grazing season _, and an excess of nutrients (Teague et al. 2011; Sartorello et al. 2020). In order to address the complexity of these interactions, the possibility offered by GPS tracking to accurately map the variation in animal stock density at a fine spatial scale is clearly of considerable interest. The data it yields may be used to improve grazing management, address the identification and rotation of grazing subareas or paddocks, and also the placement of points of attraction for the animals, such as water troughs and mineral supplements (Probo et al. 2013).

Conclusion

This study provides an example of the variability in grazing patterns that can be found in alpine summer pastures managed with a combination of extensive rotational and continuous grazing, an increasingly common practice in recent decades in the Alps. The farmer's driving of the herd in the morning was fundamental to using pasture areas that under continuous grazing management would have been avoided by the cows. The daily selection of these areas, which was influenced by weather conditions, was a determining factor in the variability in energy costs associated with animal movement and resulted in the cows using less productive grassland patches than those used when grazing freely in the afternoon. In addition, the intensity of use of the pasture area was highly heterogeneous. Given the wide diversity in the environmental and management conditions of alpine pastures, and pastures managed extensively in general, in other contexts an even greater variability in grazing patterns than found in this study may be expected. Describing this variability is important for addressing the multifunctional management of pastures, which needs to combine animal welfare and productivity with the conservation of ecosystem services, both provisioning (i.e. forage production) and non-provisioning (e.g. biodiversity, carbon and nutrient balance, aesthetic and cultural values). In this regard, our study shows that the rapidly expanding GPS and remote sensing technologies may be used not only to understand spontaneous animal grazing patterns and to index land morphology and grassland productivity, but also to verify farmers' choices and their consequences. In geographic contexts other than Europe, this knowledge and technology is being increasingly expanded and used to develop precision grazing management tools. We suggest that further studies in different alpine contexts, with larger sample sizes and a wider range of environmental conditions (e.g. the actual temperature animals are exposed to) would provide useful information for improving grazing management of alpine pastures.

Acknowledgments

We thank the farmers of Malga Ombretta for their support and Dr. Luca Carraro for taking measurements and calculating body condition scores.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Ethical approval

The study was approved by the ethical committee of the University of Padova with prot. number 389576 09/11/2017.

ORCID

Salvatore Raniolo http://orcid.org/0000-0003-1989-0376

Data availability statement

The data of this study are freely available from the corresponding author upon request. The data are not publicly available due to the involvement of private partners (farmers).

Funding

The study was funded by the University of Padova, Project No. BIRD 171044/17.

References

Agricultural Research Council. 1980. The Nutrient Requirements of Ruminant Livestock. CAB, UK: Slough.

Bailey DW, Trotter, MG, Knight, CW, Thomas, MG. 2018. Use of GPS tracking collars and accelerometers for rangeland livestock production research. Transl. Anim. Sci. 2(1):81-88.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Softw. 67(1):1–48.

Beck PSA, Wang TJ, Skidmore AK, Liu XH. 2008. Displaying remotely sensed vegetation dynamics along natural gradients for ecological studies. Int. J. Remote Sens. 29(14):4277–83.

Boyce MS, McDonald LL. 1999. Relating populations to habitats using resource selection functions. Trends Ecol. Evol. 14(7):268–72.

Browning DM, Spiegal S, Estell RE, Cibils AF, Peinetti RH. 2018. Integrating space and time: A case for phenological context in grazing studies and management. Front. Agric. Sci. Eng. 5(1):44–56.

Bunce RGH, Pérez-Soba M, Jongman RH, Gómez Sal A, Herzog F, Austad I. 2004. Transhumance and biodiversity in european mountains. IALE publication series nr 1. Wageningen UR.

Cagnacci F, Boitani L, Powell RA, Boyce MS. 2010. Animal ecology meets GPS-based radiotelemetry: A perfect storm of opportunities and challenges. Philos. Trans. R. Soc. B Biol. Sci. 365(1550):2157–62.

Caton JS, Olson BE. 2016. Energetics of grazing cattle: Impacts of activity and climate. J. Anim. Sci. 94:74–83.

Da Ronch F, Caudullo G, Tinner W, de Rigo D. 2016. Larix decidua and other larches in Europe: distribution, habitat, usage and threats. Eur. Atlas For. Tree Species. 108–10.

D'Eon RG, Serrouya R, Smith G, Kochanny C. O. 2002. GPS radiotelemetry error and bias in mountainous terrain. Wildl. Soc. Bull. 30(2):430–39.

Geremia C, Merkle JA, Eacker DR, Wallen RL, White PJ, Hebblewhite M, Kauffman MJ. 2019. Migrating bison engineer the green wave. Proc. Natl. Acad. Sci. U.S.A. 116(51):25707-25713.

Gorelick N, Hancher M, Dixon M, Ilyushchenko S, Thau D, Moore R. 2017. Google Earth Engine:

Planetary-scale geospatial analysis for everyone. Remote Sens. Environ. 202:18–27.

Hessle A, Rutter M, Wallin K. 2008. Effect of breed, season and pasture moisture gradient on foraging behaviour in cattle on semi-natural grasslands. Appl. Anim. Behav. Sci. 111(1–2):108–19.

Hessle A, Dahlström F, Bele B, Norderhaug A, Söderström M. 2014. Effects of breed on foraging sites and diets in dairy cows on mountain pasture. Int J Biodivers Sci Ecosyst Serv Manag. 10(4):334-342.

Homburger H, Schneider MK, Hilfiker S, Lüscher A. 2014. Inferring behavioral states of grazing livestock from high-frequency position data alone. PLoS One. 9(12):1–22.

Homburger H, Lüscher A, Scherer-Lorenzen M, Schneider MK. 2015. Patterns of livestock activity on heterogeneous subalpine pastures reveal distinct responses to spatial autocorrelation, environment and management. Mov. Ecol. 3(1):1-15.

Isselstein J, Griffith BA, Pradel P, Venerus S. 2007. Effects of livestock breed and grazing intensity on biodiversity and production in grazing systems. 1. Nutritive value of herbage and livestock performance. Grass Forage Sci. 62(2):145–58.

Kaufmann J, Bork EW, Blenis P V. Alexander MJ. 2013. Cattle habitat selection and associated habitat characteristics under free-range grazing within heterogeneous Montane rangelands of Alberta. Appl. Anim. Behav. Sci. 146(1–4):1–10.

Koch B, Homburger H, Edwards PJ, Schneider MK. 2018. Phosphorus redistribution by dairy cattle on a heterogeneous subalpine pasture, quantified using GPS tracking. Agric. Ecosyst. Environ. 257:183-192.

Kothmann M. 2009. Grazing methods: A viewpoint. Rangel. 31(5):5–10.

Liao C, Clark PE, Degloria SD, Barrett CB. 2017. Complexity in the spatial utilization of rangelands : Pastoral mobility in the Horn of Africa. Appl. Geogr. 86:208–19.

Lüdecke D, Ben-Shachar MS, Patil I, Waggoner P, Makowski D. 2021. performance: An R package for assessment, comparison and testing of statistical models. J. Open Source Softw. 6(60).

Manning J, Cronin G, González L, Hall E, Merchant A, Ingram L. 2017. The behavioural responses of beef cattle (Bos taurus) to declining pasture availability and the use of gnss technology to determine grazing preference. Agric. 7(5):1–12.

Manly BFL, McDonald L, Thomas DL, McDonald TL, Erickson WP. 2002. Resource selection by animals: statistical design and analysis for field studies. 2nd ed. Springer, Dordrecht.

Meisser M, Deléglise C, Freléchoux F, Chassot A, Jeangros B, Mosimann E. 2014. Foraging behaviour and occupation pattern of beef cows on a heterogeneous pasture in the Swiss alps. Czech J. Anim. Sci. 59(2):84–95.

Muminov A, Sattarov O, Lee CW, Kang HK, Ko MC, Oh R, Ahn J, Oh HJ, Jeon HS. 2019. Reducing GPS error for smart collars based on animal's behavior. Appl. Sci. 9(16):3408.

Myneni RB, Hall FG, Sellers PJ, Marshak AL. 1995. Interpretation of spectral vegetation indexes. IEEE Trans. Geosci. Remote Sens. 33(2):481–86.

Nakagawa S, Schielzeth H. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods Ecol. Evol. 4(2):133–42.

Nathan R, Monk CT, Arlinghaus R, Adam T, Alós J, Assaf M, Baktoft H, Beardsworth CE, Bertram MG, Bijleveld AI, Brodin T, Brooks JL, Campos-Candela A, Cooke SJ, Gjelland K Ø, Gupte PR, Harel R, Hellström G, Jeltsch F, Killen SS, Klefoth T, Langrock R, Lennox RJ, Lourie E, Madden JR, Orchan Y, Pauwels IS, Říha M, Roeleke M, Schlägel UE, Shohami D, Signer J, Toledo S, Vilk O, Westrelin S, Whiteside MA, Jarić I. 2022. Big-data approaches lead to an increased understanding of the ecology of animal movement. Science. 375(6582): eabg1780.

Nozière P,Sauvant D, Delaby L. 2017. INRA feeding system for ruminants. Wageningen Academic Publishers.

Palumbo F, Squartini A, Barcaccia G, Macolino S, Pornaro C, Pindo M, Sturaro E, Ramanzin M. 2021. A multi-kingdom metabarcoding study on cattle grazing Alpine pastures discloses intra-seasonal shifts in plant selection and faecal microbiota. Sci. Rep. 11(1):1–14.

Parraga Aguado MÁ, Sturaro E, Ramanzin M. 2017. Individual activity interacts with climate and habitat features in influencing GPS telemetry performance in an alpine herbivore. Hystrix. 28(1):1–7.

Pauler CM, Isselstein J, Berard J, Braunbeck T, Schneider MK. 2020. Grazing Allometry: Anatomy, Movement, and Foraging Behavior of Three Cattle Breeds of Different Productivity. Front. Vet. Sci. 7:1–17.

Payne LX, Schindler DE, Parrish JK, Temple SA. 2005. Quantifying spatial pattern with evenness indices. Ecol. Appl. 15(2):507–20.

Peco B, Navarro E, Carmona CP, Medina NG, Marques MJ. 2017. Effects of grazing abandonment on soil multifunctionality: The role of plant functional traits. Agric. Ecosyst. Environ. 249: 215-225.

Perotti E, Probo M, Pittarello M, Lonati M, Lombardi G. 2018. A 5-year rotational grazing changes the botanical composition of sub-alpine and alpine grasslands. Appl. Veg. Sci. 21(4):647–57.

Pettorelli N, Laurance WF, O'Brien TG, Wegmann M, Nagendra H, Turner W. 2014. Satellite remote sensing for applied ecologists: Opportunities and challenges. J. Appl. Ecol. 51(4):839–48.

Pettorelli N, Vik JO, Mysterud A, Gaillard JM, Tucker CJ, Stenseth NC. 2005. Using the satellite-derived NDVI to assess ecological responses to environmental change. Trends Ecol. Evol. 20(9):503–10.

Pittarello M, Probo M, Perotti E, Lonati M, Lombardi G, Ravetto Enri S. 2019. Grazing Management Plans improve pasture selection by cattle and forage quality in sub-alpine and alpine grasslands. J. Mt. Sci. 16(9):2126–35.

Pittarello M, Ravetto Enri S, Lonati M, Lombardi G. 2021. Slope and distance from buildings are easy-to-retrieve proxies for estimating livestock site-use intensity in alpine summer pastures. PLoS ONE. 16(11): e0259120.

Probo M, Massolo A, Lonati M, Bailey DW, Gorlier A, Maurino L, Lombardi G. 2013. Use of mineral mix supplements to modify the grazing patterns by cattle for the restoration of sub-alpine and alpine shrub-encroached grasslands. Rangel. J. 35(1):85–93.

Probo M, Lonati M, Pittarello M, Bailey DW, Garbarino M, Gorlier A, Lombardi G. 2014. Implementation of a rotational grazing system with large paddocks changes the distribution of grazing cattle in the south-western Italian Alps. Rangel. J. 36(5):445-458.

R Core Team. 2016. R: A language and environment for statistical computing. Vienna, Austria: The R Foundation for Statistical Computing.

Ravetto Enri S, Probo M, Farruggia A, Lanore L, Blanchetete A, Dumont B. 2017. A biodiversity-friendly rotational grazing system enhancing flower-visiting insect assemblages while maintaining animal and grassland productivity. Agric. Ecosyst. Environ. 241:1–10.

Rivero MJ, Grau-Campanario P, Mullan S, Held SD, Stokes JE, Lee MR, Cardenas LM. 2021. Factors affecting site use preference of grazing cattle studied from 2000 to 2020 through GPS tracking: A review. Sensors. 21(8):2696.

Sartorello Y, Pastorino A, Bogliani G, Ghidotti S, Viterbi R, Cerrato C. 2020. The impact of pastoral activities on animal biodiversity in Europe: A systematic review and meta-analysis. J. Nat. Conserv. 56:125863.

Schils RLM, Bufe C, Rhymer CM, Francksen RM, Klaus VH, Abdalla M, Milazzo F, Lellei-Kovács E, ten Berge H, Bertora C, Chodkiewicz A, Dămătîrcă C, Feigenwinter I, Fernández-Rebollo P, Ghiasi S, Hejduk S, Hiron M, Janicka M, Pellaton R, Smith KE, Thorman R, Vanwalleghem T, Williams J, Zavattaro L, Kampen J, Derkx R, Smith P, Whittingham MJ, Buchmann N, Newell Price JW. 2022. Permanent grasslands in Europe: Land use change and intensification decrease their multifunctionality. Agric. Ecosyst. Environ. 330(November 2021):107891.

Scillitani L, Darmon G, Monaco A, Cocca G, Sturaro E, Rossi L, Ramanzin M. 2013. Habitat selection in translocated gregarious ungulate species: an interplay between sociality and ecological requirements. J. Wildl. Manag. 77(4): 761-769.

Semenzato P, Cagnacci F, Ossi F, Eccel E, Morellet N, Hewison AJM, Sturaro E, Ramanzin M. 2021. Behavioural heat-stress compensation in a cold-adapted ungulate: Forage-mediated responses to warming Alpine summers. Ecol. Lett. 24(8):1556–68.

Shariatinajafabadi M, Wang T, Skidmore AK, Toxopeus AG, Kölzsch A, Nolet BA, Exo KM, Griffin L, Stahl J, Cabot D. 2014. Migratory herbivorous waterfowl track satellite-derived green wave index. PLoS One. 9(9):1–11.

Spedener M, Tofastrud M, Devineau O, Zimmermann B. 2019. Microhabitat selection of free-ranging beef cattle in south-boreal forest. Appl. Anim. Behav. Sci. 213(February):33–39.

Spiegal S, Estell RE, Cibils AF, James DK, Peinetti HR, Browning DM, Romig KB, Gonzalez AL, Lyons AJ, Bestelmeyer BT. 2019. Seasonal Divergence of Landscape Use by Heritage and Conventional Cattle on Desert Rangeland. Rangel. Ecol. Manag. 72(4):590–601.

Standing Committee on Agriculture (SCA). 1990. Feeding standards for Australian livestock — Ruminants. CSIRO, Australia.

Sturaro E, Marchiori E, Cocca G, Penasa M, Ramanzin M, Bittante G. 2013. Dairy systems in mountainous areas: Farm animal biodiversity, milk production and destination, and land use. Livest. Sci. 158(1–3):157–68.

Teague WR, Dowhower SL, Baker SA, Haile N, DeLaune PB, Conover DM. 2011. Grazing management impacts on vegetation, soil biota and soil chemical, physical and hydrological properties in tall grass prairie. Agric. Ecosyst. Environ. 141(3–4):310–22.

Tofastrud M, Devineau O, Zimmermann B. 2019. Habitat selection of free-ranging cattle in productive coniferous forests of south-eastern Norway. For. Ecol. Manage. 437:1–9.

Tomkiewicz SM, Fuller MR, Kie JG, Bates KK. 2010. Global positioning system and associated technologies in animal behaviour and ecological research. Philos. Trans. R. Soc. B Biol. Sci. 365(1550):2163–76.

Urbano F, Cagnacci F. 2014. Spatial Database for GPS Wildlife Tracking Data. A Practical Guide to Creating a Data Management System with PostgreSQL/PostGIS and R. 1st ed. Springer, Cham.

Wood S. 2017. Generalized Additive Models: An Introduction with R. 2nd ed. Chapman and Hall/CRC.

Zendri F, Ramanzin M, Bittante G, Sturaro E. 2016. Transhumance of dairy cows to highland summer pastures interacts with breed to influence body condition, milk yield and quality. Ital. J. Anim. Sci. 15(3):481–91.

Supplementary

Table S1: parameters of the Linear Mixed Effects Models analysing distance walked daily and daily altitudinal gradient

	Distance walked daily (m)					Daily altitudinal gradient (m)				
	Estimate	Std. Error	DF	t value	P	Estimate	Std. Error	DF	t value	P
(Intercept)	1840.23	470.70	307.61	3.91	<0.001	70.47	36.76	382	1.92	0.056
Julian Date	24.00	4.24	377.49	5.66	<0.001	2.25	0.35	382	6.52	<0.001
Distance walked in the morning	1.36	0.05	380.96	28.73	<0.001	0.00	0.00	382	-0.51	0.61
Outdoor time	-103.51	55.70	336.58	-1.86	0.064	1.17	4.34	382	0.27	0.79
Altitudinal gradient	3.44	0.59	370.40	5.86	<0.001					
Breed:Simmental	-65.51	107.75	10.03	-0.61	0.56	-5.53	4.74	382	-1.17	0.25
Rain:yes	-30.14	60.82	369.53	-0.49	0.62	-12.65	5.27	382	-2.40	<0.05
Temperature class:Intermediate	98.45	87.92	371.87	1.12	0.26	3.88	7.64	382	0.51	0.61
Temperature class:low	-35.88	102.05	369.86	-0.35	0.73	-22.75	8.82	382	-2.58	<0.05
RMSE	480.98					42.51				
Model marginal R squared[1]	0.75					0.29				
Model conditional R squared[1]	0.77					0.29				

[1] We assessed the model marginal R^2, due to fixed factors only, and the conditional R^2, due to fixed plus random factors (Nakagawa and Schielzeth 2013), using the package “performance”(Lüdecke 2020). In the model analysing daily altitudinal gradient the random effect was negligible, and hence the conditional R^2 was equal to the marginal one.

Table S2: parameters of the Generalized Additive Mixed Models analysing the hourly average slope used, altitude used and speed of movement.

	Slope used (degrees)				Altitude used (m)				Speed of movement (m/h)			
Parametric terms	Estimate	Std. Error	t value	P value	Estimate	Std. Error	t value	P value	Estimate	Std. Error	t value	P value
Intercept	2.20	0.037	59.75	<0.001	1919.16	4.17	460.35	<0.001	4.41	0.083	53.29	<0.001
Breed (B):Simmental	-0.047	0.020	-2.28	<0.05	-3.25	0.77	-4.23	<0.001	-0.007	0.094	-0.078	0.94
Day period (DP):morning	0.32	0.012	26.55	<0.001	19.91	0.64	30.90	<0.001	0.10	0.023	4.49	<0.001
Rain:yes	0.070	0.008	9.24	<0.001	4.21	0.35	12.13	<0.001	0.048	0.015	3.29	<0.01
Temperature class (TC):intermediate	0.22	0.006	36.18	<0.001	6.80	0.27	24.92	<0.001	-0.10	0.011	-8.60	<0.001
Temperature class:low	0.28	0.008	33.71	<0.001	22.70	0.38	58.98	<0.001	-0.11	0.016	-6.75	<0.001
B Simmental:P morning	0.014	0.006	2.41	<0.05	0.93	0.27	3.47	<0.001	0.011	0.011	0.97	0.33
P morning:R yes	-0.24	0.010	-23.44	<0.001	-11.29	0.46	-24.58	<0.001	0.008	0.019	0.39	0.69
P morning:TC intermediate	-0.31	0.007	-42.96	<0.001	-15.40	0.33	-45.98	<0.001	0.18	0.014	12.48	<0.001
P morning:TC low	-0.39	0.008	-46.93	<0.001	-35.97	0.38	-94.01	<0.001	0.16	0.016	9.97	<0.001
Smooth terms	edf	Ref.df	F	p-value	edf	Ref.df	F	p-value	edf	Ref.df	F	p-value
Spline (hour)	6.00	6	1740.4	<0.001	9.00	9	7586	<0.001	6.00	6	2975.2	<0.001
Spline (individual)	10.77	11	82.2	<0.001	10.66	11	182	<0.001	29.64	30	119.6	<0.001
Spline (Julian date)	29.94	30	613.5	<0.001	29.99	30	2393	<0.001	10.96	11	312.2	<0.001
Adjusted R squared	0.21				0.62				0.14			
RSME	0.55				25.19				1.068			

Table S3: parametric coefficients of the generalized linear mixed model with binomial distribution and logit link function analysing the probability of a position to be used in response to NDVI class, day period, slope class, and habitat type

	Estimate	Std. Error	z value	P(>|z|)
(Intercept)	-2.022	0.21	-9.88	<0.001
NDVI class 0.2-0.4	2.11	0.20	10.54	<0.001
NDVI class 0.4-0.6	2.93	0.19	14.75	<0.001
NDVI class 0.6-0.9	2.88	0.20	14.40	<0.001
Day period (DP) afternoon	-0.33	0.25	-1.28	0.200
Slope class 10-20	-0.22	0.032	-6.87	<0.001
Slope class 20-30	-0.32	0.059	-5.44	<0.001
Slope class >30	-0.39	0.11	-3.71	<0.001
Habitat: *Larix decidua*	-2.95	0.082	-36.13	<0.001
NDVI class 0.2-0.4:Day period afternoon	-0.39	0.26	-1.51	0.131
NDVI class 0.4-0.6:Day period afternoon	0.12	0.26	0.47	0.640
NDVI class 0.6-0.9:Day period afternoon	0.74	0.26	2.89	<0.01
Slope class 10-20:Day period afternoon	0.12	0.039	3.04	<0.01
Slope class 20-30:Day period afternoon	-0.63	0.075	-8.38	<0.01
Slope class >30:Day period afternoon	-0.80	0.14	-5.56	<0.001
Habitat Larix decidua:Day period afternoon	1.082	0.090	12.03	<0.001
Model marginal R squared[1]		0.22		
Model conditional R squared[1]		0.23		
RSME		0.45		

[1] We assessed the model marginal R squared, due to fixed factors only, and the conditional R squared, due to fixed plus random factors

(Nakagawa and Schielzeth 2013), using the package “performance”(Lüdecke 2020).

References

Lüdecke D, Ben-Shachar MS, Patil I, Waggoner P, Makowski D. 2021. performance: An R package for assessment, comparison and testing of statistical models. J. Open Source Softw. 6(60).

Nakagawa S, Schielzeth H. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol. Evol. 4(2):133–42.

3.2 - Activity budget and movement patterns of Brown Swiss and Alpine Grey lactating cows during summer grazing in alpine pastures

The paper will be submitted in a scientific journal (probably Italian Animal Science) as soon as we have collected all the revision from all the authors involved

Activity budget and movement patterns of Brown Swiss and Alpine Grey lactating cows during summer grazing in alpine pastures

Raniolo S[a]., Sturaro E[a*]., Bovolenta S[b]., Gianelle D[c]. Ramanzin M[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

[b] Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Udine 33100, Italy

[c] C.R.I. Unità di Ecologia Forestale, Fondazione Edmund Mach, San Michele All'Adige, 38098, Italy

[*] Corresponding author: E. Sturaro, Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy. Phone: +39 0498 272641; Email: enrico.sturaro@phd.unipd.it
https://orcid.org/0000-0001-9508-5622

Abstract

Monitoring activity budgets and grazing patterns is necessary to manage semi-natural grasslands for both productivity and conservation of grassland ecosystem services. We used GPS tracking to monitor the grazing patterns and activity budget of lactating cows on the 'Malga Vallazza' summer farm (1,937m asl) in the Dolomites, eastern Italian Alps, from 5th July to 5th September 2020. The pasture was grazed by a mixed herd of Brown Swiss and Alpine grey cows (stocking density = 0.52 LU/ha) under continuous grazing system characterized by morning and evening milking and free movement during the night. GPS positions were collected every two minute all day from 9 Brown Swiss multiparous and 9 Alpine Grey (4 primiparous and 5 multiparous) cows. Activity budgets strongly differed between night and day with some dissimilarities among breeds and parity. Resting was the prevalent behavior during the night while the day was dominated by grazing, which was also present during the late evening. Cows mainly used grasslands close to the barn although forest and sparse forest were also used. Despite a general avoidance of steep slopes, Alpine Grey primiparous cows were less influenced by slope and more selective in term of habitat and areas used than multiparous cows, suggesting different mountain pasture use as function of parity and breed. The study outlined highly heterogenous grazing patterns for both breeds influenced by multiple factors which can be assessed at fine temporal and spatial scales through the integration between GPS and remote sensing technologies to improve grazing management.

Highlights

- The main behaviors were resting during the night and grazing during the day and the late evening.
- Despite grasslands were the most used habitat used, even forest and sparse forest were used.
- GPS tracking and remote sensing shed light on how grazing is influenced by breed-parity and environmental features.

Keywords

Dairy cattle; mountain pasture; GPS tracking; NDVI; animal behaviour

Introduction

Alpine pastures are semi-natural ecosystems that play a crucial role for mountain livestock farming by providing forage for grazing herbivores, and may also deliver multiple non-provisioning ecosystem services, e.g., carbon stocking, protection from soil erosion, water flows regulation, natural biodiversity (Bunce et al. 2004; Sturaro et al. 2013; Zendri et al. 2016; Schils et al. 2022). The conservation of these pastures and their multiple functions depends directly on the local livestock systems that manage them through a variety of grazing methods with variable degrees of spatio-temporal animals' control and grazing pressure (Probo et al. 2013; 2014; Perotti et al. 2018, Pittarello et al. 2019; Raniolo et al. 2022), which influence livestock patterns of pasture use with relevant consequences on the animal welfare and productivity and on the ecosystem services associated with the grasslands (Sturaro et al. 2013; Schils et al. 2022; Bai and Cotrufo 2022). For example, high stocking rates may lead to over-grazing, which can transform grassland areas from sinks to sources of carbon and modify soil conditions, microbial communities, and consequently vegetation through animal trampling and excreta deposition (Chang et al. 2021; Bai and Cotrufo, 2022).

Among the multiple factors that interact with grazing methods in determining the livestock grazing patterns, main elements external to animals are slope (Kaufmann et al. 2013; Pittarello et al. 2021;

Rivero et al. 2021), climate conditions (Caton and Olson 2016; Liao et al. 2017; Rivero et al. 2021), and the availability and spatial distribution of shade, shelter, and water sources (Probo et al. 2014, Rivero et al. 2021), while main animals' internal features are productivity, nutritional requirements, body weight and conformation, ability to move on difficult terrain and attitude to grazing, which depend first on the species, then on breed (Isselstein et al. 2007; Bailey et al. 2010; Spiegal et al. 2019; Pauler et al. 2020; Rivero et al. 2021), and, within breed, on the age class (Bailey et al. 2001; Walburger et al. 2009; Lopes et al. 2013). Cattle breeds may differ in selection of slopes and altitudes (Raniolo et al. 2022), activity budgets and time spent on distinct behaviours, with likely consequences on the areas explored by individuals (Hessle et al. 2008; Spiegal et al. 2019; Pauler et al. 2020), and foraging behaviour with selection of diverse plant species (Hessle et al. 2014; Koczura et al. 2019; Spiegal et al. 2019; Pauler et al. 2020), which may ultimately influence animals' growth rate and milk production and quality. In general, local breeds show a better adaptation to harsher areas than highly productive breeds, such as Holstein or Brown Swiss (Hessle et al. 2014; Zendri et al. 2016). The age class can be important because younger individuals are smaller than mature individuals, which favours them in moving over difficult terrain, and in the case of dairy breeds have lower productivity levels and hence nutritional requirements that may be more easily fulfilled by grazing (Wyffels et al. 2020). Moreover, younger individuals might not benefit by previous experience of summer grazing, which may affect resource selection (Dunn et al. 1988; Bailey et al. 2001; Walburger et al. 2009).

Recently, the study of grazing patterns has greatly benefitted of the rapid expansion of the GPS tracking technology, which allows to monitor the individual animals' position with high spatial accuracy, location error being normally below 10 m, and with high time-frequency resolution, as low as minutes or even seconds (D'Eon et al. 2002; Tomkiewicz et al. 2010; Muminov et al. 2019). Additionally, sensors associated with GPS tracking devices enable researchers to monitor animal behaviours in continuum and without observers' interferences (Homburger et al. 2014; Semenzato et al., 2021), which has improved dramatically the understanding of grazing patterns of domestic livestock (Bailey et al. 2018; Rivero et al. 2021) and in general of movement ecology of free-roaming animals (Nathan et al. 2022).

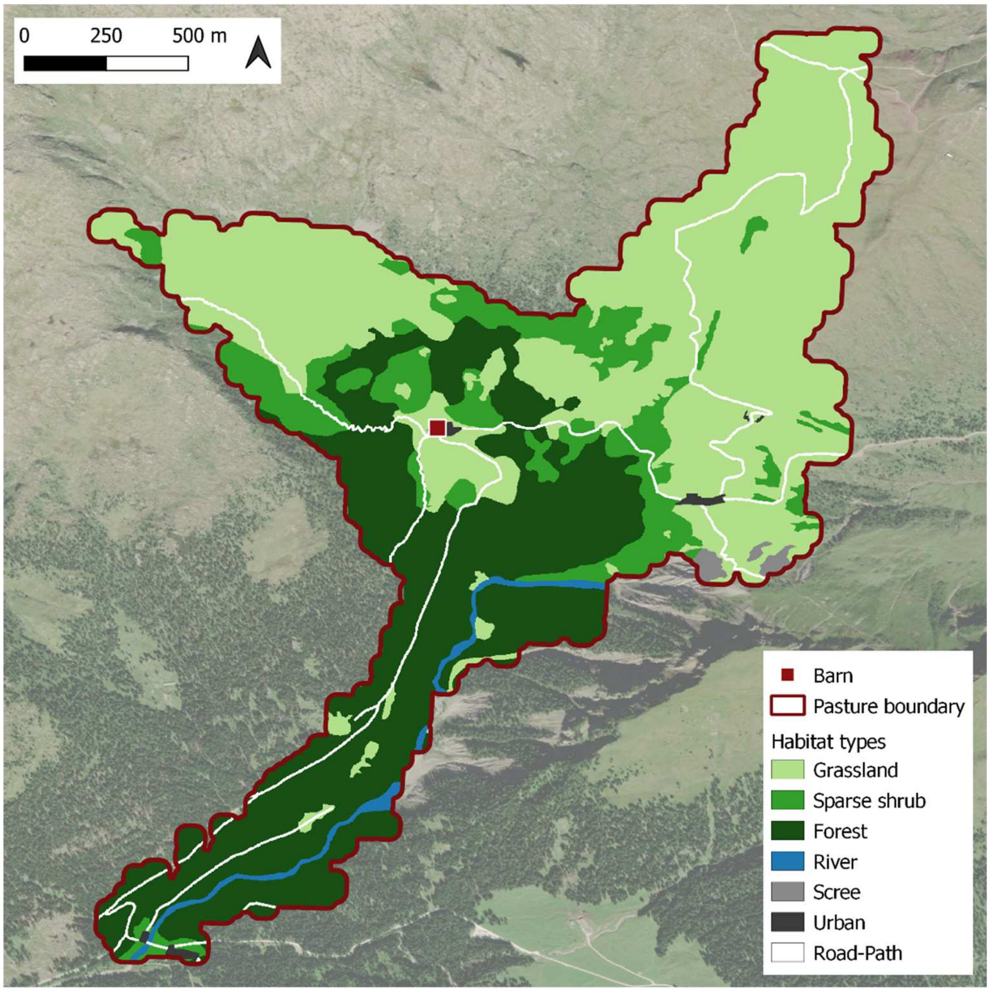
The literature comparing breeds in alpine grazing conditions has so far mainly focused on morphology, behaviour, and performance (Zendri et al. 2016; Toledo-Alvarado et al. 2017), while grazing patterns have been compared between genotypes highly divergent for productivity and body size (Hessle et al. 2008; Pauler et al. 2020). In this study, we aimed to compare the activity budgets, movement patterns and spatial use of pastures of lactating Alpine Grey and Brown Swiss lactating cows during summer grazing in a high-elevation alpine pasture managed with a combination of loose rotational and continuous grazing. Alpine Grey is a local dual-purpose breed of the eastern Alps (https://www.grigioalpina.it/), while Brown Swiss is a typical cosmopolitan dairy breed (http://www.anarb.it/en/home/). A previous study (Raniolo et al. 2022) had suggested that Alpine Grey might be more suitable than Simmental, a larger and more productive dual-purpose breed (https://www.anapri.eu/it/), to grazing in mountain pastures, and we aimed to expand this finding by including a more specialized dairy breed as Brown Swiss. Additionally, we took advantage of the availability of different parity categories within the Alpine Grey breed to compare primiparous and pluriparous cows.

Material and methods

Study area

The study was conducted during the summer of 2020 in the “Vallazza” summer farm, located in the Natural Park “Parco Naturale Paneveggio Pale di San Martino” in the Trento province, eastern Italian Alps (46°18′28″N, 11°44′38″E; Fig.1). Summer farms are temporary units traditionally used in the Alps for seasonal transhumance of livestock that is moved from lowland permanent farms to graze alpine pastures (Zendri et al., 2016; Sturaro et al., 2013). The Vallazza summer farm is located at 2038 m a.s.l. (SD=146) and is characterised by an alpine climate (Tattoni et al., 2010) with long and cold winters (mean October-April 2000 to 2021: precipitation = 132.8mm ± 295.3 mm; temperature = -1.1° ± 5.5°), and fresh and rainy summers (mean June-September 2000-2021: precipitation = 147.5mm ± 48.1mm; temperature = 10.9° ± 3.9°).

We generated digital maps of the study area in QGIS 3.22.7 (http://www.qgis.osgeo.org/) using the EPSG 4326 and 32632 coordinate systems. The “grazed area” (171 ha, Figure 1) was delimited as the surface enclosing all the retained GPS positions (see below for details on GPS positioning) with a buffer of 50 m. A raster map of slope of the grazed area (mean=15.3°; SD = 7.9°) was created with a resolution of 25 m from the Digital Terrain Model (DTM) provided by the Natural Park “Parco Naturale Paneveggio Pale di San Martino” (https://siat.provincia.tn.it/stem/). A vector map of the habitats of the grazed area was digitized on a fixed scale of 1:2500 from the satellite images of the ArcGIS server (https://server.arcgisonline.com/ArcGIS/rest/services/World_Imagery/MapServer), with 7 “habitat types”: “grassland” (48%), “forest” (*Picea abies* and/or *Larix decidua* stands, 34%), “sparse shrubs” (grassland mixed with shrub – mostly *Rhododendron* spp., 12.6), “road-path” (paved roads or forest paths, 3.3%); “stream” (stream beds, 1.3%),‘scree” (areas covered by loose stone with little vegetation, 0.3%), “farm” and “Urban” (0.5% all together).



Figur 1. Area grazed with the main habitat types. The external red line delimits a raster map (25 m resolution) encompassing all the GPS locations plus an external buffer of 50 m. The habitats are mapped as vectors at a fixed scale of 1:2500 (see main text for details).

Summer farm management and animals sampled

This summer farm has a long history of continuous grazing (Zanella et al., 2010) and during the study period hosted a herd of dairy cattle (89 livestock units – LU) of mixed breeds (Brown Swiss, Alpine Grey), with a low stocking rate (0.52 Livestock LU/ha). Lactating cows were milked twice daily in the barn, starting at approximately 6 a.m. and 5 p.m. Each day, after the morning milking, they were conducted by the shepherds to graze in a different section of the pasture area, where they were then left free until they returned to the barn for the evening milking, after which they were again released free to spend the night outdoor. During the milking, all cows received a concentrate supplement (Alpine Grey: 4 Kg/d; Brown Swiss: 6 Kg/d. Crude protein: 14.7%; crude fibre: 6.1%; crude fat: 3.4%; total ash: 5.2%), which is a common practice in these grazing systems (Zendri et al., 2016).

We monitored 9 Alpine Grey (4 primiparous and 5 multiparous) and 9 multiparous Brown Swiss cows from 5 July to 5 September 2020. Due to the herd composition, we could not match parity distribution of the two breeds. Individual milk yield was obtained from the monthly milk recordings (excluding August) collected by the Breeders Association of Trento and averaged for each cow. Individual live body weight was recorded at the beginning and at the end of the grazing season and averaged for each cow. We classified cows for “breed-parity” as “Brown Swiss multiparous”, “Alpine Grey primiparous” and "Alpine Grey multiparous” and compared milk yields and live body weight of the three categories with a simple one-way ANOVA.

Movement data collection

Movement of the cows was monitored with GPS collars (Vertex Plus model, Vectronic Aerospace GmbH) scheduled to record a position every 2 minutes. Since we had only 9 collars available, cows were divided into two groups of 9 individuals, distributed across breed-parity categories, which were sequentially monitored for one month each. One GPS collar failed to acquire positions after August 1. We obtained 362,157 positions out of the 372,803 scheduled (position acquisition rate: 97,1%). With this high acquisition rate, median position error was assumed to be within 6 m (Párraga Aguado et al., 2017). We pre-processed acquired positions data in PostgreSQL 14 (https://www.postgresql.org/docs/14/index.html) with the plugin PostGIS 3.1.5 (http://postgis.net/2022/02/01/postgis-3.1.5/). We first eliminated all impossible positions (e.g., peaks of mountains, etc.), and then excluded the remaining outlier positions with the procedure used by Raniolo et al. (2022). Briefly, this procedure identifies outlier positions as those that are associated with unreliable movement speed and/or abnormal deviations from the movement trajectory (e.g., the spatial-temporal sequence of locations). Since our time schedule of position collecting covered the whole day, we needed to exclude the periods spent in the barn by each cow during milking. For this purpose, we used a procedure like that used by Raniolo et al (2022). We first identified all the individual locations collected inside a 50 m buffer surrounding the barn during the periods 5:00-8:00 a.m. and 4:00-7:00 p.m., to delimit the potential individual milking periods. Within these periods, we then identified the actual individual milking periods as the sequences of 10 minutes time intervals having more than 3 missed locations (when GPS are inside buildings, the probability of acquiring locations drops because of the physical obstruction to the satellites’ signal) and average acceleration values of the x and y activity sensors (see below) lower than 35 (which indicates inactivity). The positions recorded during the actual individual milking periods were excluded, and we checked visually for consistency of the resulting outdoor movement daily trajectories of individual cows.

The GPS collars were equipped with a tri-axial activity sensor set by manufacturer to store acceleration values (0-255) as averages over five-minute intervals. We used accelerometer data combined with metrics of movement steps (e.g., the segments linking consecutive pairs of locations) to classify positions according to three behaviour categories: “grazing” (i.e., bouts of biting, chewing, and swallowing, also if interrupted by relocation movements between clusters of plants; Owen-Smith et al. 2010); “resting” (standing without leg movements or lying); “walking” (with a clear directionality, without interruptions for grazing). The classification and validation procedures are described in detail in Supplementary Appendix S.1. Briefly, we trained a random forest classifier (Liaw and Wiener 2002; Homburger et al. 2015) by matching the known behaviour associated with 2,237 positions to the corresponding accelerometer values and movement metrics. The final geodatabase contained 269,963 outdoor locations, which we classified into two “day-periods”: “day” (between the morning and evening milking) and “night” (between the evening and morning milking) and associated with the corresponding individual cows’ features (“individual”, breed-parity), temporal variables (“Julian date”, “hour”), linear distance from the barn (“distance”), movement features (“slope” and “speed”, calculated as the distance in m between each consecutive pair of locations divided by the time in seconds separating their acquisition), “habitat” (the habitat type corresponding to the position), and behaviour.

Daily activity budget of cows

For each day of monitoring, we computed the hours spent in each behaviour category (“grazing time”, “resting time”, “walking time”) by each cow during each day-period as $NB_i/30$, where NB_i is the number of locations (collected every 2 minutes) assigned to each behaviour. We analysed grazing time, resting time and walking time separately for each day-period, because their values across the whole day had a bimodal distribution. We used linear mixed models with the “lmer4” function of the “lme4” library (Bates et al. 2015) in R 4.2.0 (R Core Team 2016) and tested the effects of breed-parity as a fixed factor and of the individual cow nested into breed-parity as a random factor (Table S.2.1). We assessed the model’s marginal R^2, due to fixed factors only, and the conditional R^2, due to fixed plus random factors (Nakagawa and Schielzeth, 2013), with the Performance package (Lüdecke et al. 2021).

Movement and use of pasture

We evaluated the cows’ movement and use of pasture with three approaches. First, we examined the slope and movement speed associated with each cow’s position; second, we assessed the effects of environmental covariates on the fine-scale spatial use of pasture; third, we compared the size of the areas that individual cows used daily.

Slope used and speed of movement

We analysed slope and speed with generalized additive mixed models using the function “gam” of the package “mgcv” (Wood 2017) in R 4.2.0 (R Core Team 2016). For the analysis of slope and speed, since we wanted to include the effects of habitat type, we excluded 12,290 positions (4.5%) intersecting habitat types with a very small area (“road-path”, “scree”, “stream”), and retained only positions in grassland, sparse shrub, and forest. Additionally, for the analysis of speed we excluded the positions collected during the “night” day-period (N = 116,672), because it was dominated by resting, and all the resting positions (N = 32,565) during the “day” day-period. The final sub-dataset of speed had 83,242 positions. Slope was analysed with a model (Table S.2.2) including the fixed effects of the smoothed splines of hour and distance and of the categorical variables breed-parity, behaviour, day-period, and habitat, the two-way interactions between them, the three-way interactions between breed-parity, day-period and behaviour and between breed-parity, day-period and habitat, and the random effect of individual cow nested within breed-parity. Speed was expressed as m/h and log-transformed, and then analysed with a model (Table S.2.3) including the fixed effects of the smoothed splines of hour and distance and of the categorical variables breed-parity, habitat, behaviour, and of the two-way and three-way interactions between them, plus the random effect of the individual cow nested within breed-parity.

Fine-scale intensity of use of pasture

We examined how spatially structured effects affected the fine-scale spatial use of the grazed area based on the frequency distribution of cows’ positions discretized within a grid of 25x25 m cells aligned with the DTM. We classified each cell for the summed number of positions of each breed-parity category during each day-period of the whole study duration. Spatially structured factors assigned to each cell were the “prevalent habitat”, which we defined as “grassland”, “sparse forest”, and “forest” when their percent cover was higher than 50% of the cell, the slope, and the linear distance (m) from the centre of each pixel to the barn. We analysed the positions’ frequency with the INLA (Integrated Nested Laplace Approximation) approach, which works on a Bayesian framework using the SPDE (Stochastic Partial Differential Equations) methodology and allows to account for and manage error covariance, as spatial autocorrelation (Rue et all. 2009, Homburger et all. 2015). We used the “INLA” function of the INLA library (Rue et all. 2009) in R 4.2.0 (RCore Team 2016) to build six models (Table S.2.4), one for each combination of breed-parity and day-period, using six corresponding sub-datasets. The models were based on a zero-inflated negative binomial distribution, due to the high frequency of 0 values, and included the random spatial effect of the single cell, which accounted for the spatial autocorrelation and included the spatial effects of neighbouring cells, and the fixed effects of habitat prevalence (“grassland”, “sparse forest”, “forest”, each one expressed as a separate binomial variable yes/no), slope, and the log-transformed distance from the barn. For hyperparameter specification, we set the parameters and the diffuse prior distribution as in

Homburger et al. 2015. The approximations of model posterior marginals were computed with the empirical Bayes approach and the Gaussian method to balance between accuracy and computational cost. The models were compared with a forest plot to highlight possible differences in terms of statistical relevance.

Daily areas used by cows

We used a utilization distribution (UD) method to calculate the surface of the areas used daily by individual cows during day and night with the function “kernelUD” of the adehabitatHR library (Calenge 2021) in R 4.2.0 (RCore Team 2016), using 90% and 50% of the locations with the smoothing parameter set at 25 m. This is a common approach to estimate the total area used (90%, ”total area”, excluding the 10% most peripheric and occasional locations) and, within that, the portion used most intensively (50%, “core area”) by free-roaming animals (Viana et al. 2018, Floyd et al. 2022). We analysed total areas and core areas with a generalized linear mixed model using the “glmer” function of the “lme4” library (Bates et al. 2015) based on a Gamma distribution and a log link function. The model (Table S.2.5) included the fixed effects of the 2-way interaction between breed-parity and day-period and of Julian date as a linear covariate, plus the random effect of individual cow nested into breed-parity.

Results

Milk production and live body weight

Live body weight differed markedly between breed-parities ($p<0.001$) with the expected ranking order, being lowest for Alpine Grey primiparous, intermediate for Alpine Grey multiparous, and highest for Brown Swiss multiparous (GLM least square means: 565.6 kg, SE = 18.7; 603.8 kg, SE = 20.8; 689 kg, SE = 13.9; respectively). Milk yield varied also significantly ($p <0.05$) between breed-parities, but with a different ranking order: it was again lowest for Alpine Grey primiparous, slightly higher for Brown Swiss multiparous, and clearly highest for Alpine Grey multiparous (GLM least square means: 15.1 kg/day, SE = 1.7; 17.1 kg/day; SE = 1.2, 22.2 kg/day, SE = 1.8; respectively). These differences in milk yield and especially in body weight between breeds and parities will be considered when discussing the movement and behavioural patterns.

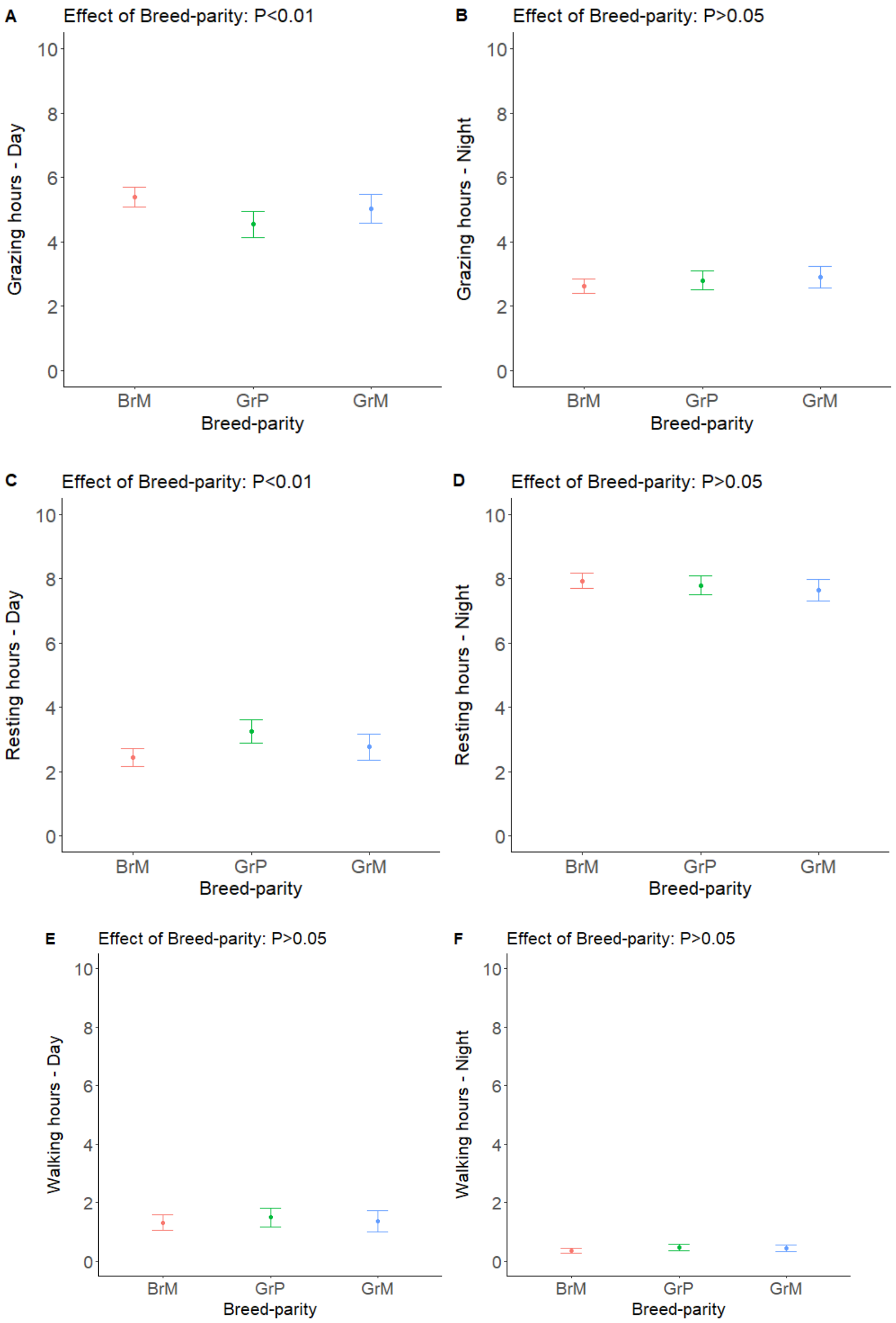


Figure 2. Effects of breed-parity on the hours spent grazing (A and B panels), resting (B and C panels) and walking (D and F panels) during day and night. “BrM”: Brown Swiss multiparous; GrM: Alpine Grey multiparous; GrP: Alpine Grey primiparous. Whiskers indicate 95% confidence intervals. For details of the parametric coefficients of the statistical models see Supplementary table S.2.1.

Activity budgets during day and night

The hours spent grazing were influenced by breed-parity (p <0.01) during the day, but not during the night (Table S.2.1). During the day (Figure 2A), Alpine Grey primiparous cows grazed on average for 4:30 hours (SE = 0:12), Alpine Grey multiparous for 5:00 hours (SE: 0:13 hours), and Brown Swiss multiparous for 5:18 hours (SE: 0:09 hours). During the night (Figure 2B), cows of all breed-parity categories grazed for 2:36-2:54 hours (SE = 0:06-0:09). The hours spent resting were also influenced by breed-parity during the day, but, again, not during the night (Table S.2.1). During the day (Figure 2C), Alpine Grey primiparous cows rested longer (3:12 hours, SE = 0:10) than Brown Swiss and Alpine Grey multiparous cows (2:24-2:42 hours; SE = 0:08-0:12); during the night (Figure 2D), resting time varied between 7.36 and 7:54 hours (SE = 0:06-0:10) among breed-parity categories. Finally, time spent walking was unaffected by breed-parity during both day and night (Table S.2.1; Figure 2.E and 2.F, respectively). During the day cows walked for 1:18-1:24 hours (SE= 0:07-0:11 hours), and during the night for 0:18-0:24 hours (SE = 0:02-0:03 hours).

Use of pasture

Slope used and movement speed

After accounting for the spline effects of hour and distance from barn (Table S.2.2 and figure S.2.1), slope used was significantly influenced (P<0.001, Table S.2.2) by the three-way interactions breed-parity by day-period by behaviour and breed-parity by day-period by habitat. During the day (Figure 3A) and when grazing and resting, Brown Swiss multiparous cows used the gentlest slopes (grazing: 13.9°, SE = 0.03; resting: 13.2°, SE= 0.04), Alpine Grey multiparous cows used intermediate slopes (grazing: 13.9°, SE = 0.04; resting: 13.6°, SE = 0.06), and Alpine Grey primiparous used the steepest slopes (grazing: 14.4°, SE = 0.04; resting: 13.5°, SE = 0.05), while when walking all breed-parity categories used similar slopes (Brown Swiss multiparous: 13.4°, SE = 0.05; Alpine Grey multiparous: 13.35°, SE = 0.07; Alpine Grey primiparous: 13.5°, SE = 0.06). During the night (Figure 3B) and when grazing and walking, Alpine Grey primiparous used steeper slopes (grazing: 14.24°, SE = 0.09; walking: 13.5° ±0.1) than Alpine Grey multiparous and Brown Swiss pluriparous (Alpine Grey multiparous, grazing: 13.3°, SE = 0.09; Alpine Grey multiparous, walking: 12.8°, SE = 0.1; Brown Swiss multiparous, grazing: 13.3°, SE =0.09; Brown Swiss multiparous, walking: 12.8°, SE = 0.10), while when resting Brown Swiss pluriparous used the steepest slopes (12.9°, SE = 0.08), Alpine Grey primiparous the intermediate ones (12.2°, SE = 0.09), and Alpine Grey pluriparous the gentlest ones (11.1°, SE = 0.09). In general, slope used when grazing (13.1 – 14.5°) was steeper than that used when resting (12.0 – 13.6°) and walking (12.5 – 13.7°; Figure 3A and 3B).

During the day and when cows were in forest (Figure 3C), Brown Swiss multiparous used slightly steeper slopes than Alpine Grey primiparous and Alpine grey multiparous (Brown Swiss multiparous: 16.8°, SE = 0.04; Alpine Grey primiparous: 16.6°, SE = 0.06; Alpine grey multiparous: 16.2°, SE = 0.06;), but when cows were in grassland Brown Swiss multiparous used clearly gentler slopesthan the other breed-parity categories (Brown Swiss multiparous: 10.5°, SE = 0.04; Alpine Grey primiparous: 11.5°, SE = 0.05; Alpine grey multiparous: 11.3°, SE = 0.05). There were no differences between breed-parity categories when cows were in sparse shrub (Brown Swiss multiparous: 13.2°, SE = 0.05; Alpine Grey primiparous and Alpine Grey multiparousboth categories: 13.3°, SE = 0.06). During the night (Figure 3D) and when cows were in forest and grassland, Alpine Grey multiparous used gentler slopes (forest: 15.2°, SE = 0.1; grassland: 10.2°, SE = 0.09) than the other two categories (Brown Swiss multiparous, forest: 16.9°, SE = 0.08; Brown Swiss multiparous, grassland: 11.1°, SE = 0.08; Alpine Grey primiparous, forest: 16.9°, SE = 0.1; Alpine Grey primiparous, grassland: 10.8°, SE =0.09), while when cows were in sparse shrub the gentlest slopes were used by Brown Swiss multiparous (Brown Swiss multiparous: 10.9°, SE = 0.08; Alpine Grey multiparous: 11.8°, SE = 0.09; Alpine Grey primiparous: 12.02°, SE = 0.09). In general, slope used by cows of all breed-parity categories was steeper when they were in forest (16.3 – 16.6°) than when they were in sparse shrub (12.4 – 12.6°) and especially in grassland (10.8 – 11.0°), which reflects the morphology of these habitats.

After accounting for the spline effects of hour and distance (Table S.2.3 and figure S.2.2), speed was significantly influenced ($P<0.01$, Table S.2.3) by the three-way interactions breed-parity by day-period by behaviour and breed-parity by day-period by habitat. When grazing in forest and in sparse shrub (Figure 3D), Brown Swiss multiparous cows moved at a slower speed (forest: 256 m/h, SE = 0.011; sparse shrub: 229 m/h, SE = 0.012) than the cows of the other two categories (Alpine Grey primiparous, forest: 276 m/h, SE = 0.016; Alpine Grey primiparous, sparse shrub: 242 m/h, SE = 0.015; Alpine Grey multiparous, forest: 276 m/h, SE = 0.015; Alpine Grey multiparous, sparse shrub: 248 m/h, SE = 0.016) when grazing in grassland, cows of all breed-parity categories moved at a similar speed, remarkably lower speed than that observed in the other habitats (Brown Swiss multiparous: 190 m/h, SE = 0.01; Alpine Grey primiparous: 189 m/h, SE = 0.011; Alpine Grey multiparous 185 m/h, SE = 0.012).

When walking (Figure 3E), cows moved at a much faster speed than when grazing, without differences between breed-parity categories (Brown Swiss multiparous 1149 m/h, SE = 0.012; Alpine Grey primiparous 1156 m/h, SE = 0.015; Alpine Grey multiparous 1105 m/h, SE = 0.014)

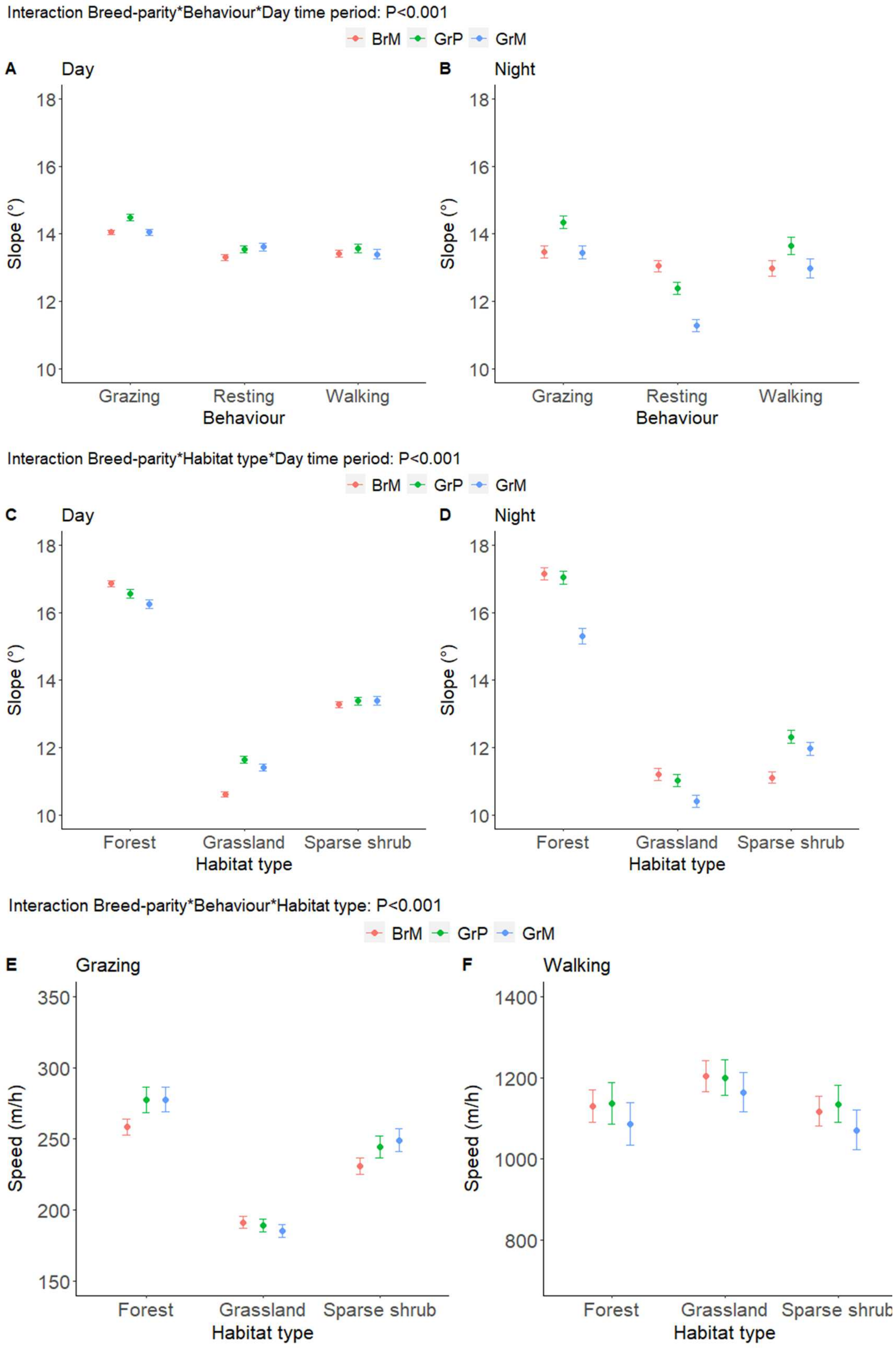


Figure 3. Effect of the three-way interaction breed-parity by day-period by behaviour (panels A and B) and breed-parity by day-period by habitat (panels C and D) on the slope used by the cows, and effect of the interaction breed-parity by habitat by behaviour (panels E and F, note that the scale of the y axis differs between these two panels) on the speed of movement. BrM: Brown Swiss multiparous; GrP: Alpine Grey primiparous; GrM: Alpine Grey multiparous.

Fine scale intensity of spatial use

The posterior estimates of the INLA models analysing the fine-scale spatial use of the cows are shown in table S.2.4. During the day (Figure 4A) there was in general a tendency for intensity of use to be higher than expected in grassland (positive effect), intermediate in sparse shrub (no effect), and lowest in forest (negative effect). The positive effect of grassland on intensity of use was significant for Brown Swiss multiparous and Alpine Grey primiparous and close to significance for Alpine Grey pluriparous, while the effect of forest was negative and significant for Alpine Grey primiparous, non-significant for Alpine Grey multiparous, and positive and significant for Brown Swiss multiparous. Distance from barn and slope had a significant negative effect on intensity of use for all breed-parity categories, less marked for Alpine Grey primiparous than for the other bred-parity categories. During the night (Figure 4B), grassland had a similar, significant, and positive effect on intensity of use for all breed-parity categories, while sparse shrub and forest had no significant effect. Distance from barn and slope confirmed the negative effect on intensity of use observed during the day.

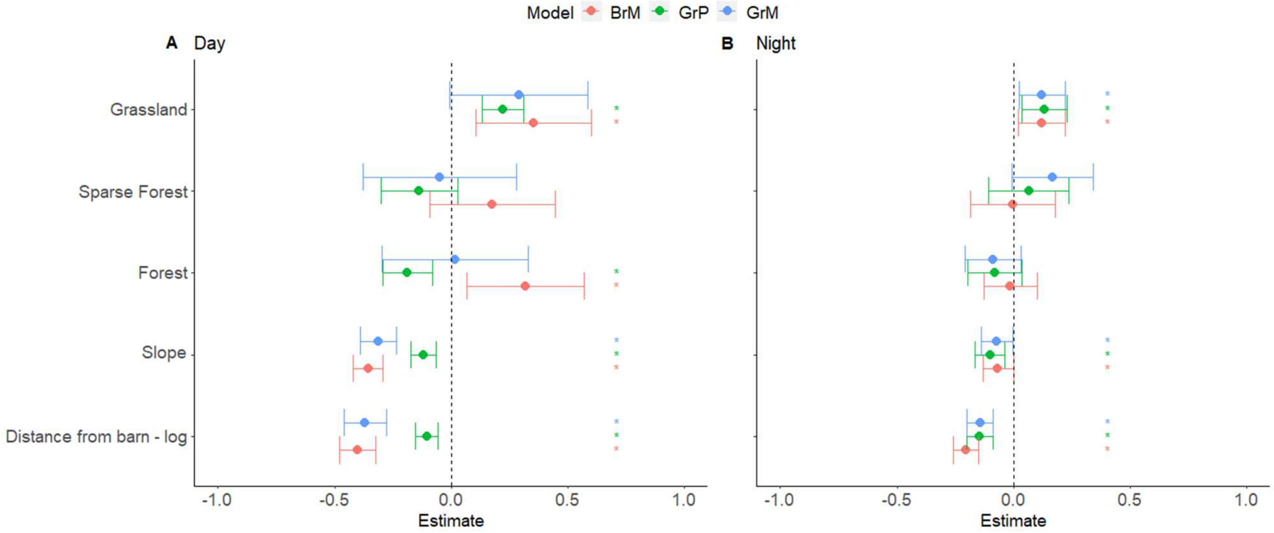


Figure 4. Estimated effects of prevalent habitat type (grassland, sparse shrub and forest), distance from barn, slope, and breed-parity ("BrM": Brown Swiss multiparous; GrM: Alpine Grey multiparous; GrP: Alpine Grey primiparous) on intensity of pasture use during day (panel A) and night (panel B). Horizontal whiskers indicate 95% credibility intervals of posterior estimates, which differ significantly when whiskers do not overlap with the dotted line at estimate = 0. The asterisks (*) indicate statistical relevance. For details of the parametric coefficients of the statistical models see Supplementary table S.2.4.

Total areas and core areas

The size of both the total areas and core areas was influenced by Julian date ($p<0.001$; Table S.2.5) with a decreasing trend (Figure S.2.2). It was also, and predictably, strongly influenced by day-period ($p<0.001$), which interacted with breed-parity ($p<0.05$ for total areas, and $p<0.01$ for core areas (Table

S.2.5). Total areas' size (Figure 5A) was much larger during the day (12.60 ha, SE =0.02), when it did not differ between breed-parity categories, than during the night, when it was smaller for Brown Swiss multiparous (4.38 ha, SE = 0.02) than for Alpine Grey multiparous and Alpine Grey primiparous (4.99 ha, SE = 0.03, and 5.06 ha, SE = 0.03, respectively). Core areas' sizes (Figure 5B) were much were larger for Brown Swiss multiparous than for Alpine Grey multiparous and Alpine Grey primiparous cows (2.14 ha, SE = 0.03, 1.99 ha, SE = 0.04, and 1.85 ha, SE = 0.03, respectively) during the day, and did not differ between breed-parity categories during the night (0.88 ha, SE = 0.02, 0.90 ha, SE = 0.03, 0.92 ha, SE = 0.03, respectively).

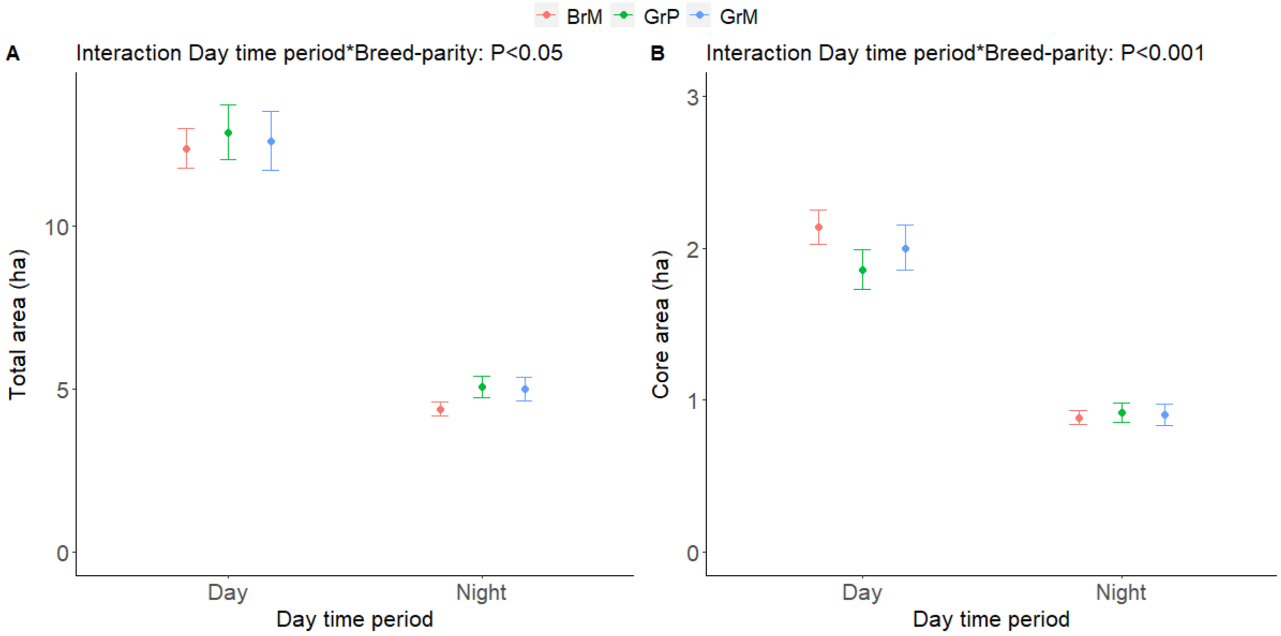


Figure 5. Effects of the 2-way interaction between breed-parity and day-period on the surface (ha) of total areas (90%of locations) and core areas (50% of locations; note that the scale of the y axis differs from that of panel A). Whiskers indicate 95% confidence intervals. "BrM": Brown Swiss multiparous; GrM: Alpine Grey multiparous; GrP: Alpine Grey primiparous. For details of the parametric coefficients of the statistical models see Supplementary tab S.2.5.

Discussion

In this study, we found that breed and parity influenced the activity budget of the cows and their use of pasture in a complex interaction with day period, behaviour displayed, and habitat type used. In the following discussion, we will first examine the general patterns of the activity budget during day and night, then discuss the influence of day-period and environmental features on pasture use, and finally address how breed-parity categories differed within the general framework previously outlined.

In general, during the whole day (24 hours) the cows spent approximately 8 hours grazing, 10 -11 hours resting, and little longer than 1.5 hours walking. The total grazing time was in the lower limits of the values observed in the studies reviewed by Kilgour (2011), which can probably be justified by the substantial concentrate supplementation that the cows received, which has a well-known negative effect on both grazing time and herbage intake (Krysl and Hess, 1993, Gekara et al. 2001, Bovolenta et al. 2002; Soca et al. 2014). Of the total daily grazing time, approximately 30% occurred in the "night" period between the evening and the morning milking. Therefore, grazing during the night period is important for the daily forage intake of the cows, and this might conflict with certain management practices. Traditionally, cows were kept inside the barn after the evening milking and released only after the next morning's milking, a practice that is now rare but still practiced (Raniolo et al. 2022), and likely reduces the time cows have available for grazing. Maintaining high forage intakes might be difficult also if night fencing or keeping in the barn were adopted as measures to protect livestock from the rapidly expanding alpine wolf population (Marucco et al. 20202), although this practice should be relevant especially for more vulnerable cattle categories than adult cows (Faccioni et al. 2005). Kilgour (2011) found, in the studies he examined, a very wide variability in the proportion of time spent grazing between day and night by cattle at pasture but did not attempt to provide an explanation. One possible factor influencing the daily grazing rhythms might be climate, and especially temperature since cattle respond to heat stress by reducing activity and feed intake (Silanikove 2000). When free to roam at pasture, cattle show two daily major bouts of grazing generally associated with sunrise and sunset (Kilgour et al. 2011). To avoid heat stress in warm days, which is not uncommon even in temperate climates during summer (Veissier et al 2018) and will become more frequent because of global warming, they might show behavioural plasticity by anticipating the morning grazing bout and delaying and prolonging the evening bout, as observed in other heat-sensitive herbivores (Semenzato et al. 2021) and as claimed by shepherds (Ramanzin M. personal communication). This should be considered in the management practices. For example, when the day and night periods of our study are decomposed into hourly sub-periods (Figure S.2.3), it becomes clear how milking times and grazing management may interfere with the diurnal grazing patterns: the sunrise grazing bout did not start until after the morning milking, the afternoon grazing decreased rapidly when cows had to walk back to the barn from the areas where they had been conducted in the morning for the evening milking, and only after it the sunset grazing bout could take place. Therefore, a reduction of grazing during the day should be compensated by an increase during the deep hours of the night that now appear dedicated most exclusively to resting.

The use of pasture was highly heterogenous, (Probo et al. 2014; Homburger et al. 2015; Raniolo et al. 2022), as indicated by the fact that half of the locations collected daily for each cow were inside core areas which were 5 times smaller than the total areas used. During the day, when the cows were conducted by the shepherd to graze at longer distances from the barn (see figure S.2.4), they stayed into total areas and core areas that were 2-2.5 times larger than during the night, when they grazed but mostly rested close to the barn (see figure S.2.4). Therefore, during the night the animals' load per unit of surface was higher and spatially concentrated in areas that were likely subject to intense trampling and deposition of excreta (White et al, 2001), which might strongly impact on soil physical properties and nutrient balance and vegetation (Pietola et al. 2005; Jewell et al. 2007). Since in most alpine grazing systems as the one we examined here the cows are left free during the night, reducing the animals' load, balancing the release of excreta, and maintaining good sward conditions in such areas might be challenging without devising a rotational system with the shepherd conduction also during the night.

In addition to the herd management practised, the heterogeneity of pasture use was also clearly related with environmental variables, as indicated by the analysis of fine-scale spatial intensity of use. The cows showed a clear tendency to select grassland patches, did not show any preference nor avoidance for sparse shrub patches, and tended to avoid only tendentially but mostly in non-significant way forest patches. While the positive selection for grassland patches was expected, given that these are typically forage-rich habitats (Homburger et al.2015), the lack of a negative selection for sparse shrub and forest was unexpected (Raniolo et al. 2022). During the day, this might be partially explained by the grazing management, since to reach most of the grassland areas when conducted by the shepherd the cows had to pass through forest and sparse shrub (see Fig. 1). Additionally, while cattle avoid thick stands of high shrubs, as *Alnus* spp., they could move easily and find forage in sparse shrub, which was composed by patches of dwarf shrub (*Rhododendron* and *Vaccinium* spp.) that did not impede movement interspersed with grassland. Finally, forest might be used by cattle for a variety of reasons, including finding shade and forage in sunny and warm days (de Weerd et al. 2014). In fact, the cows spent more time grazing in grassland than in forest and sparse shrub, but the use of these two latter habitats for grazing was not negligible (see table S.2.5). Different environmental factors may respond to/impact on different needs of the animals, which will consequently make different choices according to the activity in which they are involved (Kohler et al. 2006; Homburger et al. 2015). Slope, and distance from barn, had a negative effect on the intensity of use, which was expected and confirmed the findings of other studies (Kaufmann et al. 2013; Pittarello et al. 2021; Rivero et al. 2021 Raniolo et al. 2022).

When considering differences between breeds and parity categories, we should discuss the breed effect by comparing Alpine Grey multiparous with Brown Swiss multiparous cows, and the parity effect by comparing primiparous and multiparous cows within the Alpine Grey breed. Since during the night differences between breed-parity categories were small and non-significant (activity budget) or followed patterns consistent with those of the day (fine scale analysis of spatial intensity of use and analysis of areas used daily), and only the analysis of slope at the movement step scale showed somewhat different patterns (most probably because during night cows spent 70% of their time resting), we will address these differences considering the day, when relevant. Overall, the results suggest that, as respect to Alpine Grey multiparous, Brown Swiss multiparous did not differ in activity budget and might be less selective in their use of habitat (only for them forest had a positive effect on intensity of spatial use; they moved at a lower speed in forest and sparse shrub, and they used less heterogeneously the total daily areas used daily, as indicated by larger core areas). Whether in moving they are more limited by slope can only be suggested but needs further confirmation. We have also to consider that in this study Brown Swiss multiparous were heavier than Alpine Grey multiparous, which should reduce grazing time (Aharaoni et al. 2013) and modify their movement patterns (Rivero et al. 2021), but produced less milk than Alpine Grey multiparous and received more concentrate supplement, which should have a negative effect on grazing time and reduce movement (Heublein et al. 2016), and it is impossible to disentangle these possibly contrasting effects. In general, differences between Alpine Grey primiparous and Alpine Grey multiparous, when present, were more marked than those between the multiparous cows of the two breeds. As respect to the older cows of the same breed, primiparous Alpine Grey spent less time grazing and more resting, used steeper slopes when grazing and in grassland, showed a higher tendency to avoid sparse shrub and especially forest, were influenced less negatively by slope and distance from barn in their spatial intensity of use. The reduced grazing time might be explained by the fact that they had lower feed requirements, being lighter and producing less milk, but received the same amount of concentrate, while their ability to move on steeper ground is in accord with their smaller body size (Rivero et al. 20121). That they were more selective in their use of habitats and the pasture area in general might possibly be related to a lack of knowledge and previous experience. Experience can be as important as breed in foraging behaviour (Orr et al. 2013) and in use of pasture (Bailey et al. 2010; Wyffels et al. 2020), but we cannot speculate on this because we don’t know whether primiparous cows had been grazing in the summer farm when heifers.

Conclusion

In this study we monitored both movement and activity budget of lactating cows in an extensive grazing system, outlining the main differences between day and night, the influence of environmental covariates, and some dissimilarities between breeds and parity. Grazing was predominant during the day but extended remarkably during late evening, while night was dominated by resting. We suggest that further research should elucidate the role of concentrate supplementation, climate conditions and herd management on the daily activity budget of domestic livestock at a detailed time scale, because they might be influenced by herd and grazing management practices and by climate, especially considering the risks of heat stress associated with global warming. Use of the pasture area was highly heterogeneous, which was partly explained by the avoidance of steeper slopes and by cows concentrating their presence close to the barn, and by a selection for grassland patches. However, also habitats with a lower forage abundance, as sparce shrub and forest, were used. We did not address specifically the spatial intensity of use and choice of habitats by the cows when grazing, resting, or walking, but we recommend that they should be further investigated in relation with other environmental covariates and climate conditions. The comparisons between breeds and parities did not yield conclusive indications, partly because of the limitation of our sample, but the local Alpine Grey breed appeared to be more selective in the choice of habitats and in the internal use of the total daily areas explored than the specialized Brown Swiss breed, while primiparous Alpine Grey cows showed clearly to be less limited by slope in their movement and use of pasture, while being more selective in habitats' use. These results indicate that different breeds, even if not genetically and phenotypically as divergent as those so far studied, and parity levels influence grazing patterns and use of pasture, which might be exploited in grazing management. For this purpose, understanding the different external and internal drivers of activity budgets and use of pasture by grazing livestock is crucial to animals' combine productivity and welfare with conservation of grasslands with their associated multiple ecosystem services. In this regard, GPS telemetry proved to be a powerful instrument to monitor both movement and behaviour of free-ranging animals, and should be implemented in further studies with increased sample sizes and environmental variability, and possibly complemented with insights into their possibly different sociality and physiological adaptations y.

Acknowledgments

We thank the farmers of Malga Vallazza for their support, the Breeders Association of Trento for providing milk recording data and Dr. Luca Carraro for his precious field support.

Disclosure Statement

No potential conflict of interest was reported by the author(s).

Ethical Approval

The study was approved by the ethical committee of the University of Padova with prot. number 389576 09/11/2017.

Data availability statement

The data of this study are freely available from the corresponding author upon request. The data are not publicly available due to the involvement of private partners (farmers).

Funding

The study was funded by the “SmartAlp” and Cariparo Foundation

References

Aharoni Y; Dolev A; Henkin Z; Yehuda Y; Ezra A; Ungar ED; Shabtay A; Brosh A. 2013. Foraging behavior of two cattle breeds, a whole-year study: I. Heat production, activity, and energy costs. J. Anim. Sci. 91:1381–1390.

Bailey D.W, Kress D.D, Anderson D.C, Boss D.L., Miller E.T. 2001. Relationship between terrain use and performance of beef cows grazing foothill rangeland. J. Anim. Sci. 79(7): 1883-1891.

Bailey D.W, Thomas M.G, Walker J.W, Witmore B.K, Tolleson D. 2010. Effect of previous experience on grazing patterns and diet selection of Brangus cows in the Chihuahuan Desert. Rangel Ecol Manag, 63(2): 223-232.

Bailey DW, Trotter, MG, Knight, CW, Thomas, MG. 2018. Use of GPS tracking collars and accelerometers for rangeland livestock production research. Transl. Anim. Sci. 2(1):81-88.

Bates D, Machler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67(1):1–48.

Bai Y, Cotrufo M. F. 2022. Grassland soil carbon sequestration: Current understanding, challenges, and solutions. Science. 377(6606): 603-608.

Bestelmeyer BT. 2019. Seasonal divergence of landscape use by heritage and conventional cattle on desert rangeland. Rangel. Ecol Manag. 72(4):590–601.

Bovolenta S, Ventura W, Malossini F. 2002. Dairy cows grazing an alpine pasture: effect of pattern of supplement allocation on herbage intake, body condition, milk yield and coagulation properties. Anim. Res. 51 (2002) 15-23.

Bunce RGH, Pérez-Soba M, Jongman RH, Gómez Sal A, Herzog F, Austad I. 2004. Transhumance and biodiversity in european mountains. IALE publication series nr 1. Wageningen UR.

Cagnacci F, Boitani L, Powell RA, Boyce MS. 2010. Animal ecology meets GPS-based radiotelemetry: A perfect storm of opportunities and challenges. Philos. Trans. R. Soc. B Biol. Sci. 365(1550):2157–62.

Caton JS, Olson BE. 2016. Energetics of grazing cattle: Impacts of activity and climate. J. Anim. Sci. 94:74–83.

Chang J, Ciais P, Gasser T, Smith P, Herrero M, Havlìk P, Obersteiner M, Guenet B, Goll D.S, Li

W, Naipal V, Peng S, Qiu C, Tian H, Viovy N, Yue C, Zhu D. 2021. Climate warming from managed grasslands cancels the cooling effect of carbon sinks in sparsely grazed and natural grasslands. Nat. Commun. 12:118.

D'Eon RG, Serrouya R, Smith G, Kochanny C. O. 2002. GPS radiotelemetry error and bias in mountainous terrain. Wildl. Soc. Bull. 30(2):430–39.

de Weerd N, van Langevelde F, van Oeveren H, Nolet B.A, Kölzsch A, Prins H.H, de Boer W.F. 201. Deriving animal behaviour from high-frequency GPS: tracking cows in open and forested habitat. Plos one. 10(6):e0129030

Dunn R.W, Havstad K.M., Ayers E.L. 1988. Grazing behavior responses of rangeland beef cows to winter ambient temperatures and age. Appl. Anim. Behav. Sci. 21(3): 201-207.

Faccioni G, Sturaro E, Calderola S, Ramanzin M. 2015. Wolf (Canis lupus) predation on dairy cattle in eastern Italian Alps. Poljoprivreda 21(1) Supplement: 138-141

Floyd, J.R., Kwoba, E., Mwangi, T., Okotto-Okotto J., Wanza P., Wardrop N., Yu W., Wright J. A. 2022. A spatiotemporal analysis of cattle herd movement in relation to drinking-water sources: implications for Cryptosporidium control in rural Kenya. Environ Sci Pollut Res. 29:34314–34324.

Gekara OJ, Prigge EC, Bryan WB, Schettini M, Nestor EL, Townsend EC. 2001. Influence of pasture sward height and concentrate supplementation on intake, digestibility, and grazing time of lactating beef cows. J. Anim. Sci. 79(3): 745–752.

Hessle A, Rutter M, Wallin K. 2008. Effect of breed, season and pasture moisture gradient on foraging behaviour in cattle on semi-natural grasslands. Appl. Anim. Behav. Sci. 111(1–2):108–19.

Hessle A, Dahlström F, Bele B, Norderhaug A, Söderström M. 2014. Effects of breed on foraging sites and diets in dairy cows on mountain pasture. Int J Biodivers Sci Ecosyst Serv Manag. 10(4):334-342.

Heublein C, Dohme-Meier F, Südekum K-H, Bruckmaier R. M, Thanner S, Schori F. 2016. Impact of cow strain and concentrate supplementation on grazing behaviour, milk yield and metabolic state of dairy cows in an organic pasture-based feeding system. Animal. 11: 1163-1173.

Homburger H, Schneider MK, Hilfiker S, Lüscher A. 2014. Inferring behavioral states of grazing livestock from high-frequency position data alone. PLoS One. 9(12):1–22.

Homburger H, Lüscher A, Scherer-Lorenzen M, Schneider MK. 2015. Patterns of livestock activity on heterogeneous subalpine pastures reveal distinct responses to spatial autocorrelation, environment and management. Mov Ecol. 3(1):1–15.

Isselstein J, Griffith BA, Pradel P, Venerus S. 2007. Effects of livestock breed and grazing intensity on biodiversity and production in grazing systems. 1. Nutritive value of herbage and livestock performance. Grass Forage Sci. 62(2):145–58.

Jewell PL, Käuferle D, Güsewell S, Berry NR, Kreuzer M, Edwards PJ. 2007. Redistribution of phosphorus by cattle on a traditional mountain pasture in the Alps. Agric Ecosyst Environ. 122: 377-386.

Kaufmann J, Bork EW, Blenis P V. Alexander MJ. 2013. Cattle habitat selection and associated habitat characteristics under free-range grazing within heterogeneous Montane rangelands of Alberta. Appl. Anim. Behav. Sci. 146(1–4):1–10.

Kilgour R.J. 2012. In pursuit of “normal”: A review of the behaviour of cattle at pasture. Applied Animal Behaviour Science, 138(1-2):1-11.

Koczura M, Martin B, Bouchon M, Turille G, Berard J, Farruggia A, Kreuzer M,Coppa M. 2019. Grazing behaviour of dairy cows on biodiverse mountain pastures is more influenced by slope than cow breed. Animal. 13(11):2594-2602.

Kohler F, Gillet F, Reust S, Wagner HH4, Gadallah F, Gobat J-M, Buttler A. 2006. Spatial and seasonal patterns of cattle habitat use in a mountain wooded pasture. Landsc. Ecol. 21:281–295.

Krysl LJ, Hess BW. 1993.Influence of supplementation on behavior of grazing cattle. J. Anim. Sci. 71(9):2546-2555.

Liao C, Clark PE, Degloria SD, Barrett CB. 2017. Complexity in the spatial utilization of rangelands: pastoral mobility in the Horn of. Africa Appl Geogr. 86:208–219.

Liaw A., Wiener M. 2002. Classification and regression by Random Forest. R News. 2:18–22.

Lopes F, Coblentz W, Hoffman P.C, Combs D.K. 2013. Assessment of heifer grazing experience on short-term adaptation to pasture and performance as lactating cows. J. Dairy Sci. 96(5): 3138-3152.

Lüdecke D, Ben-Shachar MS, Patil I, Waggoner P, Makowski D. 2021. Performance: a R package for assessment, comparison and testing of statistical models. J Open Source Softw. 6(60):139.

Marucco F, Avanzinelli M, Boiani V, Menzano A, Perrone S, Dupont P, Bischof R, Milleret C, von

Hardenberg A, pilgrim K, Friard O, Bisi F, Bombieri G, Calderola S, Carolfi S, Chioso C, Fattori U, Ferrari P, Pedrotti L, Righetti D, Tomasella M, Truc F, Aragno P, La Morgia V, Genovesi P. 2022. La popolazione di lupo nelle regioni alpine italiane 2020-2021. Relazione tecnica dell'attività di monitoraggio nazionale nell'ambito del Piano di Azione del lupo ai sensi della Convenzione ISPRA-MITE e nell'ambito del Progetto LIFE 18 NAT/IT/000972 WOLFALPS EU. https://www.lifewolfalps.eu/wpcontent/uploads/2022/05/REPORT_REGIONI_ALPINE_16_05_2022_FINALE.pdf

Muminov A, Sattarov O, Lee CW, Kang HK, Ko MC, Oh R, Ahn J, Oh HJ, Jeon HS. 2019. Reducing GPS error for smart collars based on animal's behavior. Appl. Sci. 9(16):3408.

Nakagawa S, Schielzeth H. 2013. A general and simple method for obtaining R2 from generalized linear mixedeffects models. Methods Ecol Evol. 4(2):133–142.

Nathan R, Monk CT, Arlinghaus R, Adam T, Alós J, Assaf M, Baktoft H, Beardsworth CE, Bertram MG, Bijleveld AI, Brodin T, Brooks JL, Campos-Candela A, Cooke SJ, Gjelland K Ø, Gupte PR, Harel R, Hellström G, Jeltsch F, Killen SS, Klefoth T, Langrock R, Lennox RJ, Lourie E, Madden JR, Orchan Y, Pauwels IS, Říha M, Roeleke M, Schlägel UE, Shohami D, Signer J, Toledo S, Vilk O, Westrelin S, Whiteside MA, Jarić I. 2022. Big-data approaches lead to an increased understanding of the ecology of animal movement. Science. 375(6582):eabg1780.

Orr RJ, Tallowin JRBB, Griffith BA, Rutter SM. 2013. Effects of livestock breed and rearing experience on foraging behaviour of yearling beef cattle grazing unimproved grasslands. Grass Forage Sci. 69:90-103.

Párraga Aguado MA, Sturaro E., Ramanzin M. 2017. Individual activity interacts with climate and habitat features in influencing GPS telemetry performance in an alpine herbivore. Hystrix. 28(1):1–7.

Pauler CM, Isselstein J, Berard J, Braunbeck T, Schneider MK. 2020. Grazing Allometry: Anatomy, Movement, and Foraging Behavior of Three Cattle Breeds of Different Productivity. Front. Vet. Sci. 7:1–17.

Perotti E, Probo M, Pittarello M, Lonati M, Lombardi G. 2018. A 5-year rotational grazing changes the botanical composition of sub-alpine and alpine grasslands. Appl. Veg. Sci. 21(4):647–57.

Pietola L, Horn R, Yli-Halla M. 2005. Effects of trampling by cattle on the hydraulic and mechanical properties of soil. Soil Tillage Res. 82: 99-108.

Pittarello M, Probo M, Perotti E, Lonati M, Lombardi G, Ravetto E. S. 2019. Grazing Management Plans improve pasture selection by cattle and forage quality in sub-alpine and alpine grasslands. J. Mt. Sci. 16(9):2126–35.

Pittarello M, Ravetto Enri S, Lonati M, Lombardi G. 2021. Slope and distance from buildings are easy-to-retrieve proxies for estimating livestock site-use intensity in alpine summer pastures. PLoS ONE. 16(11): e0259120.

Probo M, Massolo A, Lonati M, Bailey DW, Gorlier A, Maurino L, Lombardi G. 2013. Use of mineral mix supplements to modify the grazing patterns by cattle for the restoration of sub-alpine and alpine shrub-encroached grasslands. Rangel. J. 35(1):85–93.

Probo M, Lonati M, Pittarello M, Bailey DW, Garbarino M, Gorlier A, Lombardi G. 2014. Implementation of a rotational grazing system with large paddocks changes the distribution of grazing cattle in the south-western Italian Alps. Rangel. J. 36(5):445-458.

R Core Team. 2016. R: A language and environment for statistical computing. Vienna, Austria: The R Foundation for Statistical Computing.

Raniolo S., Sturaro E., Ramanzin M. 2022. Human choices, slope and vegetation productivity determine patterns of traditional alpine summer grazing. It. J. Anim. Sci. 21(1):1126-1139.

Rivero MJ, Grau-Campanario P, Mullan S, Held SD, Stokes JE, Lee MR, Cardenas LM. 2021. Factors affecting site use preference of grazing cattle studied from 2000 to 2020 through GPS tracking: A review. Sensors. 21(8):2696.

Rue H., Martino S., Chopin N. 2009. Approximate Bayesian inference for latent Gaussian models using integrated nested Laplace approximations (with discussion). J R Stat Soc Series B Stat Methodol. 71(2):319-392.

Schiavon S., Sturaro E., Tagliapietra F., Ramanzin M. Bittante G. 2019. Nitrogen and phosphorus excretion on mountain farms of different dairy systems, Agricultural Systems, 168:36-47.

Schils RLM, Bufe C, Rhymer CM, Francksen RM, Klaus VH, Abdalla M, Milazzo F, Lellei-Kovács E, ten Berge H, Bertora C, Chodkiewicz A, Dămătîrcă C, Feigenwinter I, Fernández-Rebollo P, Ghiasi S, Hejduk S, Hiron M, Janicka M, Pellaton R, Smith KE, Thorman R, Vanwalleghem T, Williams J, Zavattaro L, Kampen J, Derkx R, Smith P, Whittingham MJ, Buchmann N, Newell Price JW. 2022. Permanent grasslands in Europe: Land use change and intensification decrease their multifunctionality. Agric. Ecosyst. Environ. 330(November 2021):107891.

Semenzato P. Cagnacci F., Ossi F., Eccel E., Morellet N., Hewison A. J. M., Sturaro E., Ramanzin M. 2021. Behavioural heat-stress compensation in a cold-adapted ungulate: Forage-mediated responses to warming Alpine summers. Ecology Letters. 24:1556 –1568.

Spiegal S., Estell R. E., Cibils A. F., James D. K., Peinetti H. R., Browning D. M., Romig K. B., Gonzalez A. L., Lyons A. J., Bestelmeyer B.T. 2019. Seasonal divergence of landscape use by heritage and conventional cattle on desert rangeland. Rangel Ecol Manag. 72(4):590–601.

Silanikove N. 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. Livest Prod Sci 67:1–18.

Soca P, González H, Manterola H, Bruni M, Mattiauda D, Chilibroste P, Gregorini P. 2014. Effect of restricting time at pasture and concentrate supplementation on herbage intake, grazing behaviour and performance of lactating dairy cows. Livest. Sci. 170: 35-42.

Sturaro E, Marchiori E, Cocca G, Penasa M, Ramanzin M, Bittante G. 2013. Dairy systems in mountainous areas: Farm animal biodiversity, milk production and destination, and land use. Livest. Sci. 158(1–3):157–68.

Tattoni C, Ciolli M, Ferretti F, Cantiani M.G. 2010. Monitoring spatial and temporal pattern of Paneveggio forest (Northern Italy) from 1859 to 2006. IForest. 3:72–80.

Toledo-Alvarado H, Cecchinato A, Bittante G. 2017. Fertility traits of Holstein, Brown Swiss, Simmental, and Alpine Grey cows are differently affected by herd productivity and milk yield of individual cows. J. Dairy Sci. 100(10):8220-8231.

Tomkiewicz SM, Fuller MR, Kie JG, Bates KK. 2010. Global positioning system and associated technologies in animal behaviour and ecological research. Philos. Trans. R. Soc. B Biol. Sci. 365(1550):2163–76.

Urbano F, Cagnacci F. 2014. Spatial Database for GPS Wildlife Tracking Data. A Practical Guide to Creating a Data Management System with PostgreSQL/PostGIS and R. 1st ed. Springer, Cham.

Veissier I, Van laer E, Palme R, Moons CPH, Ampe B, Sonck B, Andanson S, Tuyttens FAM. 2018. Heat stress in cows at pasture and benefit of shade in a temperate climate region. Int. J. Biometeorol. 62:585–595.

Viana D. S., Granados J. E., Fandos P., Pérez J. M., Cano-Manuel F. J., Burón D., Fandos G., Párraga Aguado M. Á., Figuerola J., Soriguer R. C. 2018. Linking seasonal total areae size with habitat selection and movement in a mountain ungulate. Mov Ecol 6:1.

White SL, Sheffield RE, Washburn SP, King LD, Green JT. 2001. Spatial and time distribution of dairy cattle excreta in an intensive pasture system. J. Environ. Qual. 30: 2180–2187.

Wood S. 2017. Generalized Additive Models: An Introduction with R. 2nd ed. Chapman and Hall/CRC.

Wyffels SA; Boss, DL; Sowell BF; DelCurto T; Bowman JGP; McNew LB. 2020. Dormant season grazing on northern mixed grass prairie agroecosystems: Does protein supplement intake, cow age, weight and body condition impact beef cattle resource use and residual vegetation cover? PLoS ONE. 15:e0240629.

Zanella A., Tattoni C., Ciolli M. 2010. Studio della variazione temporale della quantità e qualità del bestiame nel Parco di Paneveggio Pale di San Martino e influenza sui cambiamenti del paesaggio forestale. Dendronatura. 1:24–33.

Zendri F, Ramanzin M, Bittante G, Sturaro E. 2016. Transhumance of dairy cows to highland summer pastures interacts with breed to influence body condition, milk yield and quality. Ital. J. Anim. Sci. 15(3):481–91.

Wyffels S.A, Boss D.L, Sowell B.F, DelCurto T, Bowman J.G, McNew L.B. 2020. Dormant season grazing on northern mixed grass prairie agroecosystems: Does protein supplement intake, cow age, weight and body condition impact beef cattle resource use and residual vegetation cover?. PloS one. 15(10) e0240629.

Walburger K.J, Wells M, Vavra M, DelCurto T, Johnson B., Coe P. 2009. Influence of cow age on grazing distribution in a mixed-conifer forest. Rangeland ecology & management, 62(3):290-296.

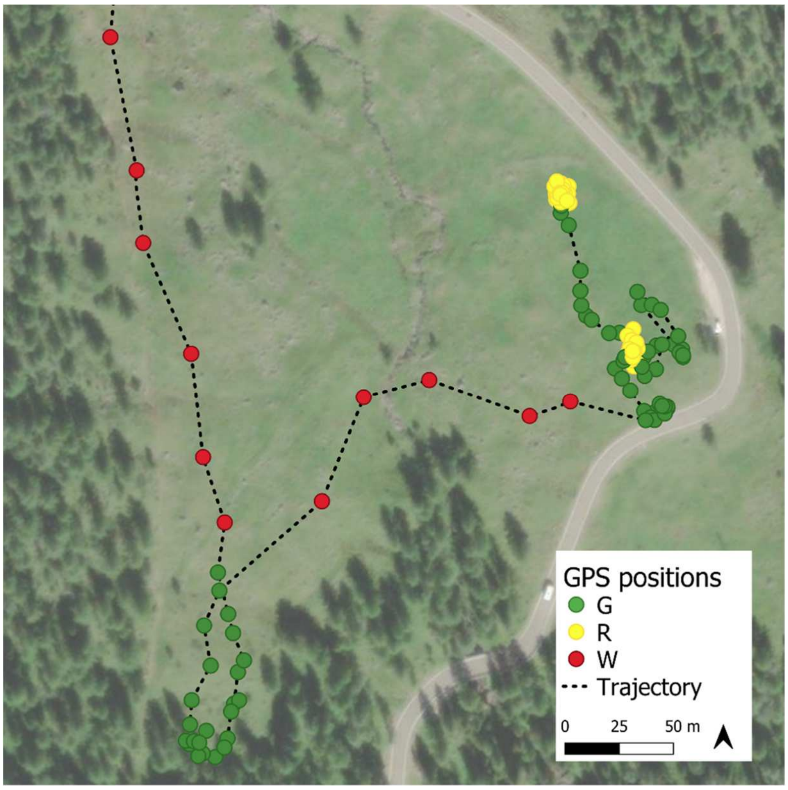
Skuce P.J, Morgan E.R, Van Dijk J, Mitchell M. 2013. Animal health aspects of adaptation to climate change: beating the heat and parasites in a warming Europe. Animal. 7(s2):333-345.

Supplementary

Appendix S.1. Classification of behaviours from acceleration data and movement features

The GPS collars used in this study (VERTEX Plus, Vectronic Aerospace GmbH) were equipped with 3-axes accelerometer (x, y, z: range 0-255) sensors. The sensors were set by the manufacturer to store acceleration values as averages over five-minute intervals. Since acceleration values are correlated with animal's motion, they have been used, alone or in combination with metrics describing movement features, to discriminate between "active" (e.g., walking, grazing) and "inactive" (e.g., lying, standing without motion) behaviours of free-ranging herbivores (Löttker et al. 2009; Moreau et al 2009; Homburger et al. 2014; Semenzato et al, 2021. For this study we developed a random forest classifier (Valletta et al. 2017) using a database of cows' positions which had been assigned directly (from visual observation) or indirectly (from unequivocal features of positions' sequences, see below) to three classes of individual behaviours ("grazing", "walking", and "resting").

Figure S.1.1. Example of movement phases that can be unequivocally assigned to three behavioural states. Green points: grazing, indicated by a sequence of locations separated by short distances with no clear directionality; yellow points: resting, indicated by a sequence of locations separated by very short distances with random directions around a centre, determined by the error of GPS sensors; red points: walking, indicated by a sequence of locations separated by long distances following a clear directionality)



To directly classify positions, we conducted visual observations on the cows equipped with GPS collars between 7:00 am and 4:00 pm from 6 to 10 July 2020, for a total of 32 hours. With the aid of a digital clock with 1 second resolution, synchronized on the activity sensors' time, we recorded the time spent into behavioral bouts of at least 10 seconds according to the following states: "grazing" (i.e., biting, chewing and swallowing, also if interrupted by relocation movements between clusters of plants; Owen-Smith et al. 2010); "walking" (with a clear directionality, without interruptions for grazing); "standing" (standing without leg movements) and "lying". We then classified the GPS positions (N = 920) collected during the observation time from the observed individuals as "grazing", "walking", or "resting" when these observed behaviors accounted for 60% or more (≥ 72 seconds) of the 120 seconds separating each position from the previous one. To integrate the database of positions classified with direct observations, we used a selected dataset of 1317 positions which, based on the movement features (see Figure S.1.1 for a visual example), could be unequivocally assigned to "grazing" (the position is part of a sequence with slow movement at short distances along irregular directions); "walking" (the position is part of a sequence with faster movement at longer distances with clear directionality); "resting" (the position is part of a sequence with short distances at random directions around a centre, due to the random error of location).

Table S.1.1. Metrics used to categorize each position for developing a random forest classifier (Calenge et al. 2009, Urbano and Cagnacci 2014, Wang et al. 2015, Edelhoff et al. 2016)

Metrics	Unit	Description
step 1	m	Step length: distance (m) between two consecutive positions, which correspond to a step
step 3	m	Distance (m) between the first and the last position of a sequence of 4 consecutive positions, corresponding to 3 steps
step 5	m	Distance (m) between the first and the last position of a sequence of 6 consecutive positions, corresponding to 5 steps
speed 1	m/h	Speed: step length/ time interval (in sec)
speed 3	m/h	Speed calculated on the distance and time interval between the first and last position of a sequence of 4 consecutive positions, corresponding to 3 steps
speed 5	m/h	Speed calculated on the distance and time interval between the first and last position of a sequence of 6 consecutive positions, corresponding to 5 steps
rel angle	rad	Cosine of turning angle: the cosine of the angle between the line intersecting the two locations of one step and the line intersecting the first location of that step with the second location of the preceding step
abs angle	rad	Cosine of absolute angle between the line intersecting the two locations of one step and the line of the X axes (east-west, "horizontal" direction)
x		Forward/backward acceleration values (4 measures/sec<) averaged over 5 minutes periods
y		Left/right acceleration values (4 measures/sec<) averaged over 5 minutes periods
z		Vertical acceleration values (4 measures/sec<) averaged over 5 minutes periods
xy		Sum of forward/backward (x) and left/right (y) acceleration values; xy= x + y
m		Square root of sum of squared single axes acceleration values; $m=(x^2+y^2+z^2)^{0.5}$

Each classified position of this integrated dataset was then categorized for the average x, y, and z acceleration values of the 5 minutes interval within which it was collected and for a series of movement metrics (Calenge et al. 2009, Urbano and Cagnacci 2014, Wang et al. 2015, Edelhoff et al. 2016), as described in table S1.1. The dataset was then randomly split into a "training" (80% of positions) and a "testing" (20% of positions) dataset ("test dataset"). In addition, we used the sub-database of only directly observed behaviours ("direct observation dataset") for further validation (see below). The random forest model was built in R environment using the function "random forest" of the library "random forest" (Liaw and Wiener, 2002). Random forest is a non-parametric model based on the result aggregation of an ensemble of decision trees created on a subsample of a dataset (Liaw and Wiener, 2002). A single decision tree is characterized by a 2-step process: firstly, it divides the predictor space into separate regions, which are split to minimize the residual sum of squares for regression trees and the Gini index of entropy (Nicodemus, 2011) for the classification; secondly, it calculates the mean of outcome values for each region, which is then used to predict new data. We built the model on 10,000 trees with the variables described in table S.1 and validated it with both the test dataset and the direct observations dataset.

The performance of the model was very good with few mislabelled predictions (Table S.1.2)

Table S.1.2. Confusion matrix of the Test dataset and of the direct observation dataset. The rows indicate the true behaviours, and the columns indicate predicted behaviours. Values on the diagonal represent the number of times where the predicted behaviour matches the true one. Values in the other cells represent instances where the classifier mislabelled an observation.

	Test - Dataset			Direct observation - Dataset		
	Grazing	Resting	Walking	Grazing	Resting	Walking
Grazing	200	1	7	616	1	7
Resting	7	345	0	6	119	0
Walking	9	1	92	8	0	154

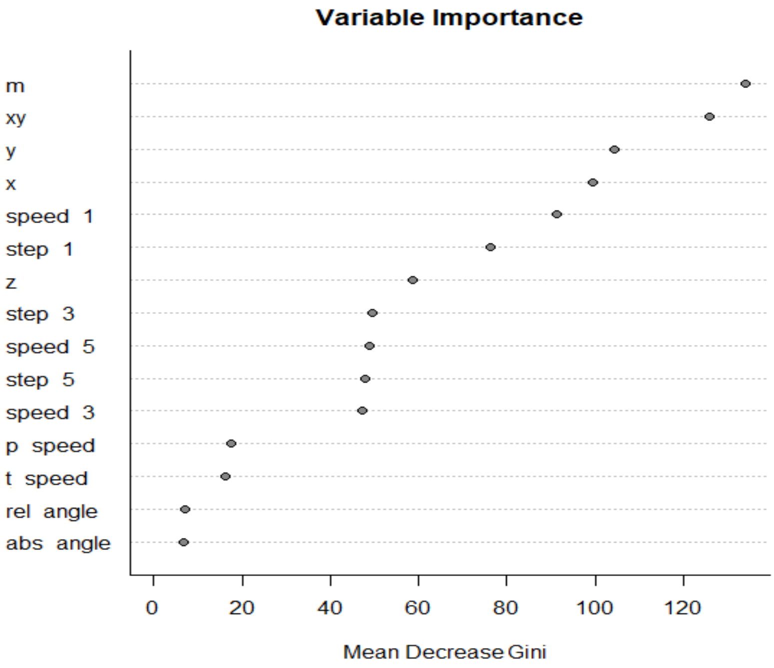
The accuracy and the Kappa statistics of the test dataset were 0.96 and 0.93 while those of the direct observation dataset were 0.97 and 0.95, respectively (Table S.1.3). The no information rate was highly significantly ($p < 0.001$) lower than the observed accuracy. The variables most contributing to the classification were "m" followed by "yx","y","x", "speed 1" and "step 1" (fig. S.1.2., see Table S.1.1 for the meaning of abbreviated variables).

After testing, the random forest model was used to classify all the locations of the geodatabase, to introduce the factor "behaviour".

Table S.1.3. Summary statistics of the random forest model. Accuracy is the proportion of observations correctly classified. The Kappa statistic is a normalized metric that compares the observed accuracy with an expected accuracy (random chance). No information rate is the largest proportion of the observed behaviours, which should be significantly lower than overall accuracy, as indicated here by P Value.

	Test	Direct observation
Accuracy	0.962	0.976
Kappa statistic	0.937	0.949
No Information Rate (NIR)	0.524	0.692
P value (Accuracy > NIR)	<0.001	<0.001

Figure S.1.2. Variable Importance of the random forest model (the mean decrease of Gini score is a measure of the contribution of a variable to the homogeneity of nodes and leaves in a random forest (Nicodemus, 2011)). The variables with the highest decrease in the Gini index of entropy are those that most contribute to the classification



References

Calenge C., Dray S., and Royer-Carenzi M. 2009. The concept of animals' trajectories from a data analysis perspective. Ecol Inform. 4(1):34-41.

Edelhoff H., Signer J., and Balkenhol N. 2016. Path segmentation for beginners: an overview of current methods for detecting changes in animal movement patterns. Mov.Ecol. 4(1):1-21.

Homburger H, Schneider MK, Hilfiker S, Lüscher A. 2014. Inferring behavioral states of grazing livestock from high-frequency position data alone. PLoS One. 9(12):1–22.

Liaw A., Wiener M. 2002. Classification and regression by Random Forest. R News. 2: 18–22.

Löttker P., Rummel, A., Traube, M., Stache, A., Šustr, P., Müller, J., et al. 2009. New Possibilities of Observing Individual Behaviour from a Distance Using Activity Sensors in Gps-Collars: An Attempt to Calibrate Remotely Collected Activity Data with Direct Behavioural Observations in Red Deer Cervus elaphus. Wildl. Biol. 15:425–434.

Moreau, M., Siebert, S., Buerkert, A., & Schlecht, E. (2009). Use of a tri-axial accelerometer for automated recording and classification of goats' grazing behaviour. Appl. Anim. Behave. Sci. 119(3-4):158-170.

Nicodemus K. K. 2011. On the stability and ranking of predictors from random forest variable importance measures. Brief. Bioinformatics. 12(4):369-373.

Owen-Smith N., Fryxell J.M. and Merrill E.H. (2010). Foraging theory upscaled: the behavioural ecology of herbivore movement. Philos. Trans. R. Soc. B Biol. Sci. 365:2267–2278.

Semenzato P. Cagnacci F., Ossi F., Eccel E., Morellet N., Hewison A. J. M., Sturaro E., Ramanzin M. 2021. Behavioural heat-stress compensation in a cold-adapted ungulate: Forage-mediated responses to warming Alpine summers. Ecol. Lett. 24:1556 –1568.

Wang Y., Nickel, B., Rutishauser, M., Bryce, C. M., Williams, T. M., Elkaim, G., & Wilmers, C. C. 2015. Movement, resting, and attack behaviors of wild pumas are revealed by tri-axial accelerometer measurements. Mov. Ecol. 3(1):1-12.

Urbano F, Cagnacci F. 2014. Spatial database for GPS Wildlife tracking data. A practical guide to creating a data management system with PostgreSQL/PostGIS and R. 1st ed. Cham: Springer.

Valletta J.J, Torney C, Kings M, Thornton A, Madden J. 2017. Applications of machine learning in animal behaviour studies. Anim. Behav. 124:203-220.

Appendix S.2: Statistical analyses and results not reported in the main text

Table S.2.1. Parametric coefficients of the generalized additive mixed models (lmer(hours~breed-parity + (1|breed-parity:individual)) analysing grazing time, resting time and walking time (hours). “Breed-parity” = 3 levels categorical factor: “Brown Swiss multiparous - BrM”; “Alpine Grey-primiparous – GrP”; “Alpine Grey multiparous – GrM”). “Individual” = 18 levels factor: individual cow. “Day- period”: 2 levels categorical factor (day/night). Marginal R^2 and conditional R^2 indicate the variance explained by fixed factors and fixed+random factors, respectively (Nakagawa and Schielzeth, 2013), which were estimated with the Performance package (Lüdecke et al. 2021) in R 4.2.0 (RCore Team 2016).

			Day-period: day						Day period: night					
Grazing time	Random effects				Variance	SD					Variance	SD		
	Breed-parity: Individual	(Intercept)			0.177	0.421					0.087	0.295		
	Residual				0.884	0.940					0.809	0.899		
	Fixed effects		Estimate	SE	df	t value	Pr(>|t|)		Estimate	SE	df	t value	Pr(>|t|)	
	(Intercept)		5.393	0.159	13.539	3.39E+01	<0.001	***	2.618	0.118	13.502	2.23E+01	<0.001	***
	Breed-parity GrP		-0.852	0.258	13.868	-3.296	<0.01	**	0.184	0.191	14.019	0.959	0.354	
	Breed-parity GrM		-0.368	0.277	13.720	-1.328	0.2058		0.291	0.205	13.814	1.422	0.177	
	Marginal R^2			0.108						0.016				
	Conditional R^2			0.257						0.112				
Resting time	Random effects				Variance	SD					Variance	SD		
	Breed-parity: Individual	(Intercept)			0.142	0.3769					0.04909	0.2216		
	Residual				0.8495	0.9217					2.15043	1.4664		
	Fixed effects		Estimate	SE	df	t value	Pr(>|t|)		Estimate	SE	df	t value	Pr(>|t|)	
	(Intercept)		2.447	0.145	13.829	1.69E+01	<0.001	***	7.937	0.118	12.757	6.74E+01	<0.001	***
	Breed-parity GrP		0.804	0.235	14.224	3.428	<0.01	**	-0.139	0.195	14.316	-0.715	0.486	
	Breed-parity GrM		0.323	0.251	14.050	1.286	0.219		-0.281	0.207	13.901	-1.355	0.197	
	Marginal R^2			0.103						0.006				
	Conditional R^2			0.231						0.028				

Table S.2.1. (Continued)

		Day						Night					
Walking time	Random effects			Variance	SD					Variance	SD		
	Breed-parity: Individual	(Intercept)	0.1266	0.3558				0.01291	0.1136				
	Residual			0.2677	0.5174					0.04628	0.2151		
	Fixed effects	Estimate	SE	df	t value	Pr(>|t|)			SE	df	t value	Pr(>|t|)	
	(Intercept)	1.320	0.130	13.923	1.02E+01	<0.001	***	0.363	0.042	13.819	8.60E+00	<0.001	***
	Breed-parity GrP	0.179	0.210	14.075	0.853	0.408		0.111	0.068	14.045	1.624	0.127	
	Breed-parity Gr M	0.040	0.225	14.002	0.178	0.861		0.069	0.073	13.945	0.945	0.361	
	Marginal R^2		0.014						0.038				
	Conditional R^2		0.331						0.248				

Table S.2.2. Parametric coefficients of the generalized additive mixed model gam(slope~s(individual, bs = “re”)+ s(individual, breed-parity, bs = “re”)+s(hour, bs = “cc”, k = 12) + s(distance, k = 6) + breed-parity*day period* behaviour+ breed-parity*habitat*day-period) analysing slope used. “Individual” = 18 levels factor: individual cow; “hour”: continuous covariate, 24 levels; “distance”: distance from the barn, continuous covariate (log-transformed); “breed-parity” = 3 levels categorical factor: “Brown Swiss multiparous - BrM”; “Alpine Grey-primiparous – GrP”; “Alpine Grey multiparous – GrM”); “habitat”: 3 levels categorical factor: “grassland”, “forest”; sparse shrub”; “day period”: 2 levels categorical factor (day/night); “behaviour”: 3 levels factor: “grazing”, “resting”, “walking”.

	Estimate	Std. Error	t	value Pr(>|t|)	
(Intercept)	17.504	0.252	69.432	< 2e-16	***
Breed-parity BrP	0.687	0.404	1.700	0.089	.
Breed-parity BrM	-1.091	0.437	-2.498	0.012	*
Day-period day	-0.392	0.089	-4.420	0.000	***
Behaviour resting	-0.400	0.042	-9.436	< 2e-16	***
Behaviour walking	-0.539	0.094	-5.725	0.000	***
Habitat grassland	-5.916	0.042	-142.159	< 2e-16	***
Habitat sparse shrub	-6.030	0.044	-138.160	< 2e-16	***
Breed-parity BrP:day-period day	-0.751	0.098	-7.646	0.000	***
Breed-parity BrM:day-period day	0.364	0.114	3.191	0.001	**
Breed-parity BrP:behaviour resting	-1.738	0.062	-27.830	< 2e-16	***
Breed-parity BrM:behaviour resting	-1.913	0.066	-29.025	< 2e-16	***
Breed-parity BrP:behaviour walking	-0.251	0.146	-1.717	0.086	.
Breed-parity BrM:behaviour walking	-0.028	0.160	-0.176	0.860	
Day-period day:behaviour resting	-0.381	0.058	-6.593	0.000	***
Day-period day:behaviour walking	-0.031	0.107	-0.285	0.776	
Breed-parity BrP:habitat grassland	-0.173	0.072	-2.410	0.016	*
Breed-parity BrM:habitat grassland	0.958	0.095	10.134	< 2e-16	***
Breed-parity BrP:habitat sparse shrub	1.298	0.080	16.261	< 2e-16	***
Breed-parity BrM:habitat sparse shrub	2.682	0.101	26.520	< 2e-16	***
Day-period day:habitat grassland	-0.496	0.059	-8.395	< 2e-16	***
Day-period day:habitat sparse shrub	2.348	0.066	35.813	< 2e-16	***
Breed-parity BrP:day-period day:behaviour resting	1.592	0.091	17.584	< 2e-16	***
Breed-parity BrM:day-period day:behaviour resting	2.288	0.096	23.718	< 2e-16	***
Breed-parity BrP:day-period day:behaviour walking	-0.089	0.169	-0.527	0.598	

Breed-parity BrM day-period day:behaviour walking	-0.011	0.184	-0.059	0.953	
Breed-parity BrP:day-period day:habitat grassland	1.468	0.104	14.121	< 2e-16	***
Breed-parity BrM:day-period day:habitat grassland	0.480	0.122	3.925	0.000	***
Breed-parity BrP:day-period day:habitat sparse shrub	-0.936	0.119	-7.846	0.000	***
Breed-parity BrM:day-period day:habitat sparse shrub	-1.946	0.137	-14.241	< 2e-16	***

	edf	Ref.df	F	p-value	
s(individual)	0.3146	14	8.05	0.985	
s(individual:breed-parity	13.652	14	2365.87	< 2e-16	***
s(hour)	6.970	7	133.33	< 2e-16	***
s(distance)	4.996	5	3765.87	< 2e-16	***
Adjusted R^2			0.290		
Deviance explained (%)			29.0		

Figure S.2.1. Effects of hour (smoothed cubic cyclic spline; panel A) and distance from barn (smoothed spline; panel B) on the slope used by cows. The statistical model and the parametric coefficients are given in table S.2.2.

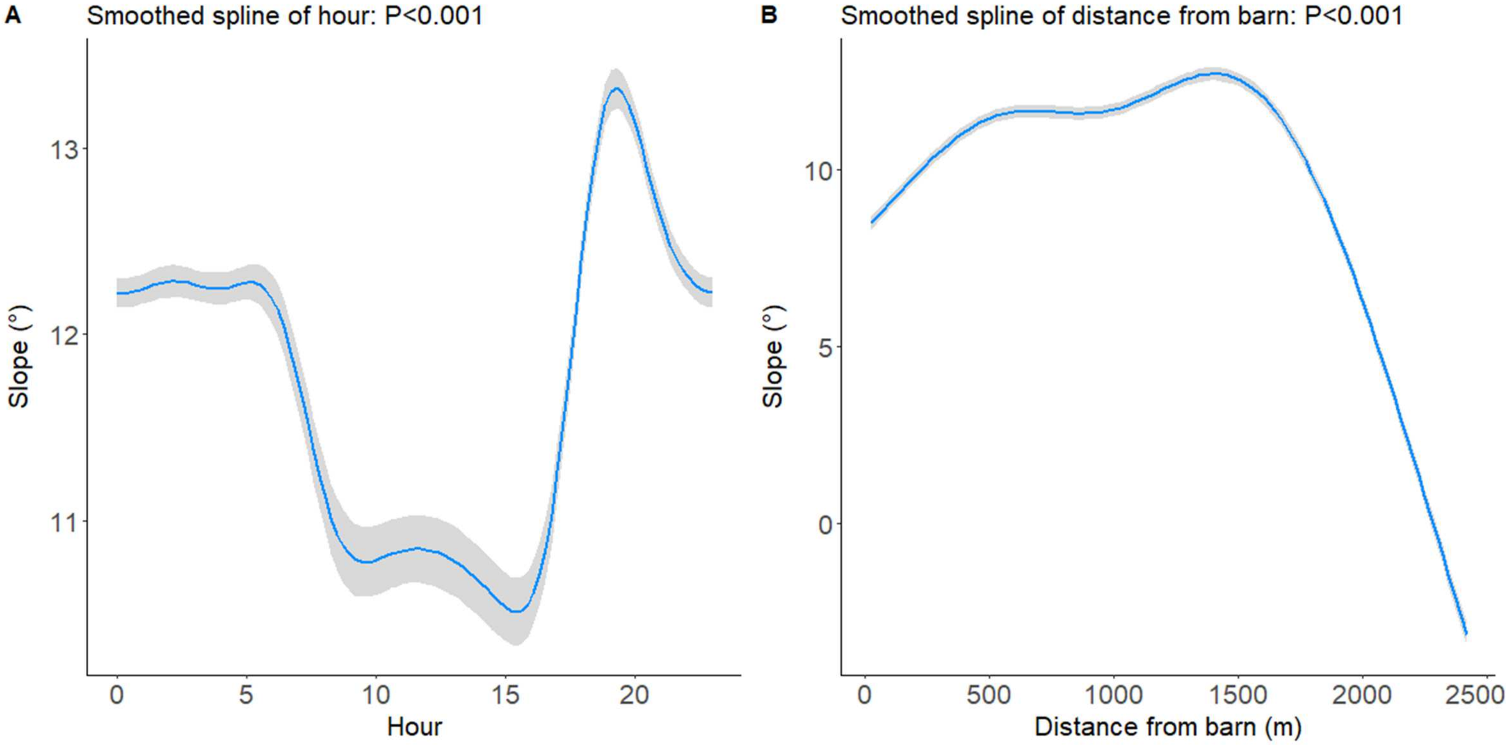


Table S.2.3. Parametric coefficients of the generalized additive mixed model gam (speed~ s(individual, bs = “re”)+ s(individual, breed-parity, bs = “re”)+s(hour, bs = “cc”, k = 12) + s(distance, k = 6) + breed-parity*habitat + breed-parity*day period* behaviour) analysing speed (log (m/hour)). “Individual” = 18 levels factor: individual cow; “hour”: continuous covariate, 24 levels; “distance”: distance from the barn, continuous covariate (log-transformed); “breed-parity” = 3 levels categorical factor: “Brown Swiss multiparous - BrM”; “Alpine Grey-primiparous – GrP”; “Alpine Grey multiparous – GrM”); “habitat”: 3 levels categorical factor: “grassland”, “forest”; sparse shrub”; “behaviour”: 2 levels factor: “grazing”, “walking” (resting was excluded because animals were static).

	Estimate	Std.Error	t value	Pr(>|t|)	
(Intercept)	5.669	0.053	106.858	< 2e-16	***
Breed-parity BrP	0.073	0.086	0.846	0.397	
Breed-parity BrM	0.074	0.092	0.802	0.422	
Behaviour: walking	1.486	0.017	88.244	< 2e-16	***
Habitat grassland	-0.297	0.010	-29.359	< 2e-16	***
Habitat sparse_forest	-0.112	0.011	-10.296	< 2e-16	***
Breed-parity BrP: behaviour walking	-0.071	0.029	-2.435	0.015	*
Breed-parity BrM: behaviour walking	-0.113	0.030	-3.752	0.000	***
Breed-parity BrP: habitat grassland	-0.081	0.018	-4.517	0.000	***
Breed-parity BrM: habitat grassland	-0.105	0.018	-5.991	0.000	***
Breed-parity BrP: habitat sparse shrub	-0.017	0.021	-0.815	0.415	
Breed-parity BrM: habitat sparse shrub	0.002	0.021	0.110	0.913	
Behaviour walking: habitat grassland	0.354	0.022	16.203	< 2e-16	***
Behaviour walking: habitat sparse shrub	0.093	0.023	4.061	0.000	***
Breed-parity BrP: behaviour walking: habitat grassland	0.078	0.037	2.118	0.034	*
Breed-parity BrM: behaviour walking: habitat grassland	0.113	0.039	2.912	0.004	**
Breed-parity BrP: behaviour walking: habitat sparse shrub	0.030	0.040	0.755	0.450	
Breed-parity BrM: behaviour walking: habitat sparse shrub	-0.005	0.042	-0.127	0.899	
Spline effectst	edf	Ref.df	F	p-value	
s(individual)	0.012	14.000	0.01	0.813	
s(individual:breed-parity	13.915	14.000	242.32	< 2e-16	***
s(hour)	9.911	10.000	321.31	< 2e-16	***
s(distance)	4.912	4.996	73.87	< 2e-16	***
Adjusted R^2			0.473		
Deviance explained (%)			47.3		

Figure S.2.2. Effects of hour (smoothed cubic cyclic spline; panel A) and distance from barn (smoothed spline; panel B) on speed. The statistical model and the parametric coefficients are given in table S.2.3.

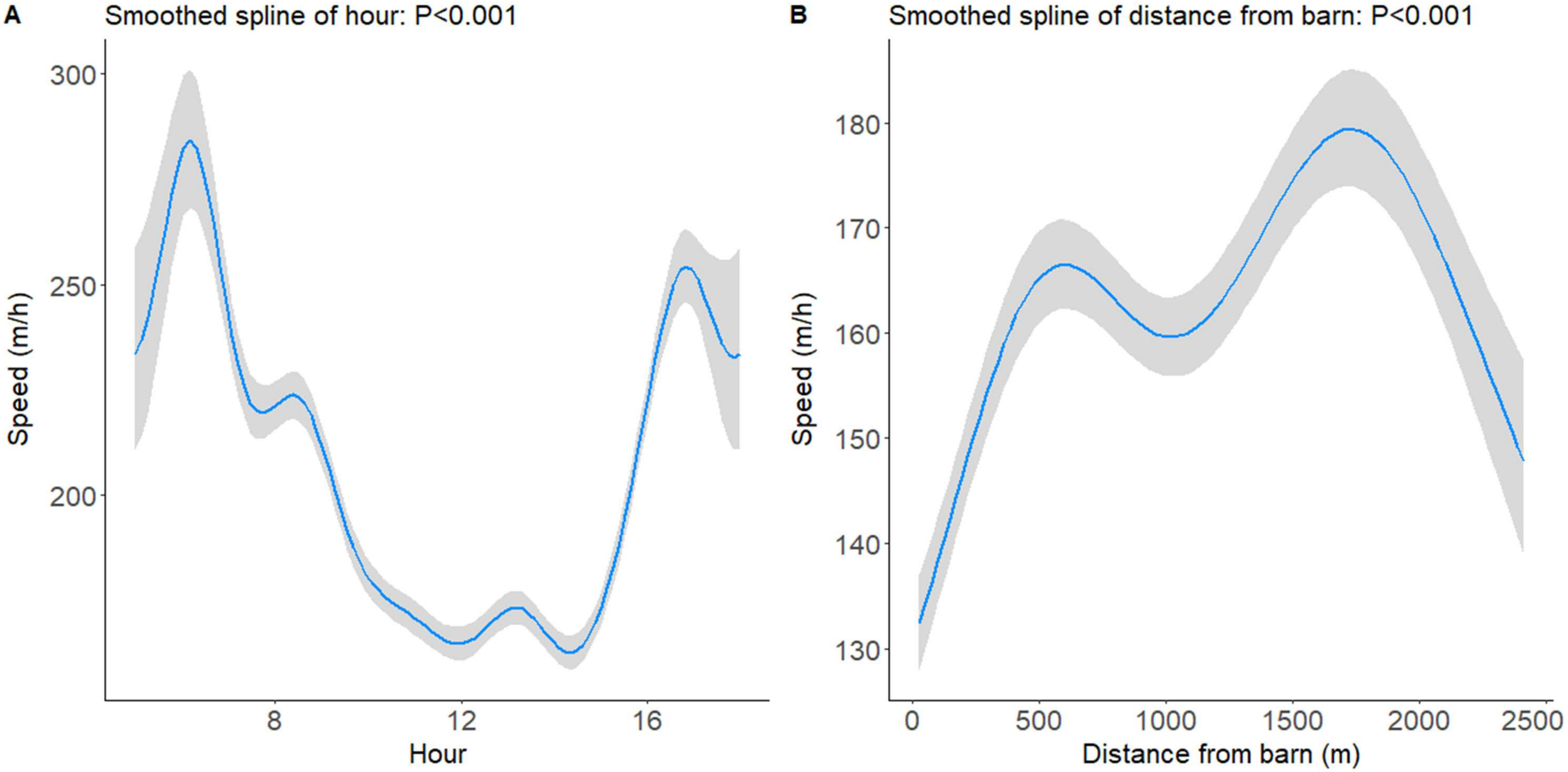


Table S.2.4. Posterior estimates with credibility intervals of the INLA models (y~ 1 + grassland + sparse_forest + forest + log_distance + slope + f(cell, model = ”bym”, graph = adjacency_matrix_neighbour_list, scale.model=T, hyper=(hyper_unstructured_effect, hyper_ precision_spatial_structured_effect), family= zeroinflatednbinomial0, control.inla = (strategy = "gaussian", int.strategy = "eb")) analysing positions’ spatial distribution for each combination of breed-parity and day-period. “Y”: position counts/cell. “Grassland”: binomial variable (1: cell area ≥ 50% of grassland; 0: cell area< 50% of grassland). “Sparse shrub”: binomial variable (1: cell area ≥ 50% of sparse shrub; 0: cell area < 50% of sparse shrub). “Forest”: binomial variable (1: cell area ≥ 50% of forest; 0: cell area< 50% of forest). “Barn distance”: distance from the barn in $\log_{10}$ scale. “Slope”: mean slope. “f(cell): random spatial effect considering the neighbouring of the grid areas and specifying the hyperparameters ((hyper_unstructured_effect, hyper_ precision_spatial_structured_effect), based on Homburger et al., 2015. Statistical relevance is marked with *.

Brown Swiss multiparous-day

Fixed effects	Mean	SD	0.025 Quant	0.5 Quant	0.975 Quant	Mode	Kld	
Intercept)	1.952	0.118	1.72	1.952	2.183	1.952	0	*
Grassland	0.354	0.126	0.107	0.354	0.601	0.354	0	*
Sparse shrub	0.176	0.137	-0.092	0.176	0.445	0.176	0	
Forest	0.319	0.129	0.066	0.319	0.571	0.319	0	*
Barn distance (log)	-0.355	0.032	-0.419	-0.355	-0.292	-0.355	0	*
Slope	-0.401	0.04	-0.479	-0.401	-0.323	-0.401	0	*

Alpine Grey multiparous-day

Fixed effects	Mean	SD	0.025 Quant	0.5 Quant	0.975 Quant	Mode	Kld	
Intercept)	1.466	0.143	1.186	1.466	1.745	1.466	0	*
Grassland	0.289	0.151	-0.007	0.289	0.586	0.289	0	
Sparse shrub	-0.048	0.168	-0.377	-0.048	0.281	-0.048	0	
Forest	0.015	0.159	-0.297	0.015	0.328	0.015	0	
Barn distance (log)	-0.312	0.039	-0.388	-0.312	-0.236	-0.312	0	*
Slope	-0.37	0.047	-0.461	-0.37	-0.279	-0.37	0	*

Alpine Grey primiparous - day

Fixed effects	Mean	SD	0.025 Quant	0.5 Quant	0.975 Quant	Mode	Kld	
Intercept)	0.038	0.037	-0.033	0.038	0.11	0.038	0	
Grassland	0.221	0.046	0.131	0.221	0.31	0.221	0	*
Sparse shrub	-0.137	0.084	-0.301	-0.137	0.027	-0.137	0	
Forest	-0.188	0.055	-0.295	-0.188	-0.08	-0.188	0	*
Barn distance (log)	-0.12	0.028	-0.174	-0.12	-0.065	-0.12	0	*
Slope	-0.105	0.025	-0.155	-0.105	-0.056	-0.105	0	*

Brown Swiss multiparous - night

Fixed effects	Mean	SD	0.025 Quant	0.5 Quant	0.975 Quant	Mode	Kld	
Intercept)	0.067	0.039	-0.009	0.067	0.143	0.067	0	
Grassland	0.12	0.051	0.021	0.12	0.22	0.12	0	*
Sparse shrub	-0.002	0.092	-0.183	-0.002	0.18	-0.002	0	
Forest	-0.014	0.058	-0.128	-0.014	0.101	-0.014	0	
Barn distance (log)	-0.067	0.034	-0.132	-0.067	-0.001	-0.067	0	*
Slope	-0.205	0.028	-0.26	-0.205	-0.15	-0.205	0	*

Alpine Grey multiparous - night

Fixed effects	Mean	SD	0.025 Quant	0.5 Quant	0.975 Quant	Mode	Kld	
Intercept)	0.049	0.039	-0.028	0.049	0.125	0.049	0	
Grassland	0.122	0.051	0.023	0.122	0.221	0.122	0	*
Sparse shrub	0.168	0.089	-0.007	0.168	0.342	0.168	0	
Forest	-0.089	0.061	-0.209	-0.089	0.031	-0.089	0	
Barn distance (log)	-0.071	0.034	-0.137	-0.071	-0.004	-0.071	0	*
Slope	-0.143	0.029	-0.199	-0.143	-0.087	-0.143	0	*

Alpine Grey primiparous – night

Fixed effects	Mean	SD	0.025 Quant	0.5 Quant	0.975 Quant	Mode	Kld	
Intercept)	0.049	0.039	-0.026	0.049	0.125	0.049	0	
Grassland	0.132	0.05	0.034	0.132	0.23	0.132	0	*
Sparse shrub	0.066	0.088	-0.106	0.066	0.238	0.066	0	
Forest	-0.081	0.059	-0.198	-0.081	0.035	-0.081	0	
Barn distance (log)	-0.101	0.033	-0.166	-0.101	-0.036	-0.101	0	*
Slope	-0.145	0.028	-0.2	-0.145	-0.09	-0.145	0	*

Table S.2.5. Parametric coefficients of the generalized linear mixed models glmer(area ~(1|breed-parity:individual)+Julian date+breed-parity*day period, family=Gamma(link=log), nAGQ = 0) analysing total areas and core areas (ha). "Breed-parity" = three-levels categorical factor: "Brown Swiss multiparous - BrM"; "Alpine Grey-primiparous – GrP"; "Alpine Grey primiparous – AgP"). "Individual" = 18 levels factor: individual cow. "Julian date": linear covariate, calculated from 05/07/2020 to 05/09/2020. "Day period": two-levels categorical factor (day/night). Marginal R^2 and conditional R^2 indicate the variance explained by fixed factors and fixed+random factors, respectively (Nakagawa and Schielzeth, 2013)), which were estimated with the Performance package (Lüdecke et al. 2021) in R 4.2.0 (RCore Team 2016).

Total areae (90% positions)						Core areas (50% positions)				
Random factors		Variance	SD				Variance	SD		
Breed_parity:individual	(Intercept)	0.001724	0.04152			(Intercept)	0.003382	0.05816		
Residual		0.114443	0.33829				0.080832	0.28431		
Fixed Factors	Estimate	SE	t-value	Pr(>|z|)		Estimate	SE	t-value	Pr(>|z|)	
(Intercept)	4.213	0.151	27.969	<0.001	***	1.254	0.145	8.642	<0.001	***
Julian date	-0.008	0.001	-11.523	<0.001	***	-0.002	0.001	-3.494	<0.001	***
Breed-parity GrM	0.019	0.044	0.417	0.676		-0.067	0.047	-1.432	0.152	
Breed-parity GrP	0.039	0.042	0.920	0.357		-0.142	0.044	-3.211	<0.01	**
Day period Night	-1.038	0.028	-36.680	<0.001	***	-0.883	0.024	-37.118	<0.001	***
Breed-parity GrM: period night	0.113	0.051	2.217	0.026	*	0.087	0.043	2.034	<0.05	*
Breed-parity GrP: period night	0.106	0.048	2.205	0.027	*	0.178	0.040	4.393	<0.001	***
Marginal R^2		0.696			RMSE			0.668		RMSE
Conditional R^2		0.701			3.378			0.682		0.483

Figure S.2.2. effect of Julian date on total areas (90% - panel A) and core areas (50% - panel B). The model and its parametric coefficient are given in table S.2.3

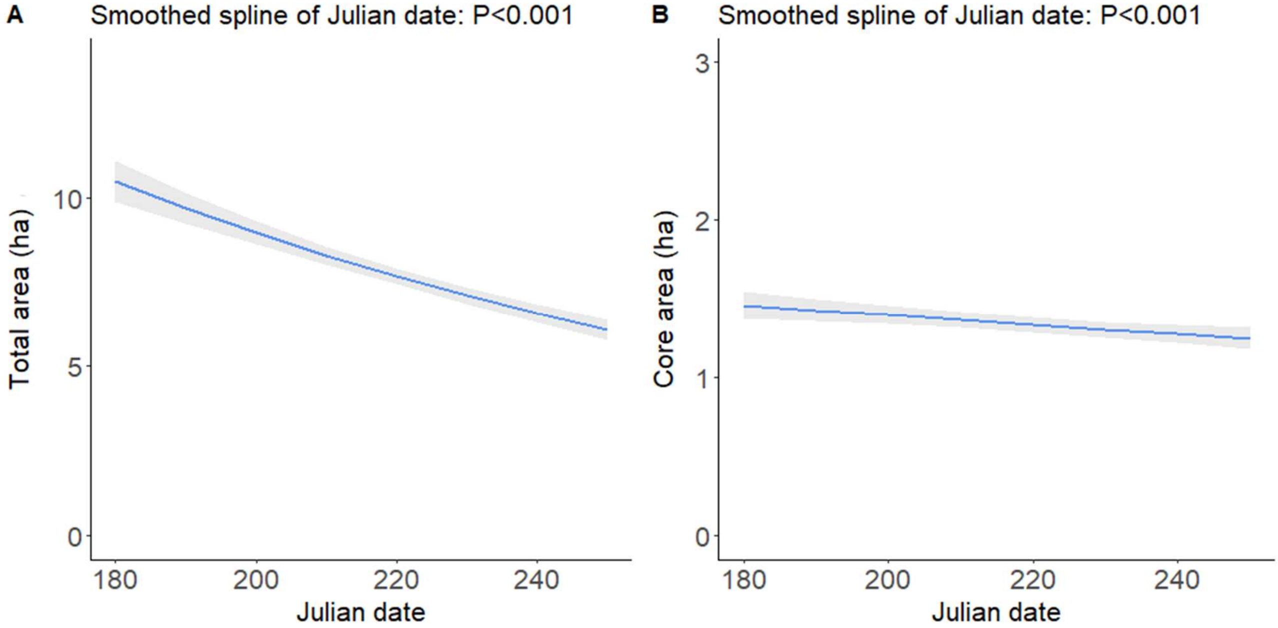


Figure S.2.3. Hourly proportions of time spent in grazing (G, green bars), resting (R, yellow bars), and walking (W, red bars) by Brown Swiss multiparous (BrM), Alpine Grey multiparous (GrM), and Alpine Grey Primiparous (GrP) cows. Dashed lines indicate median milking times.

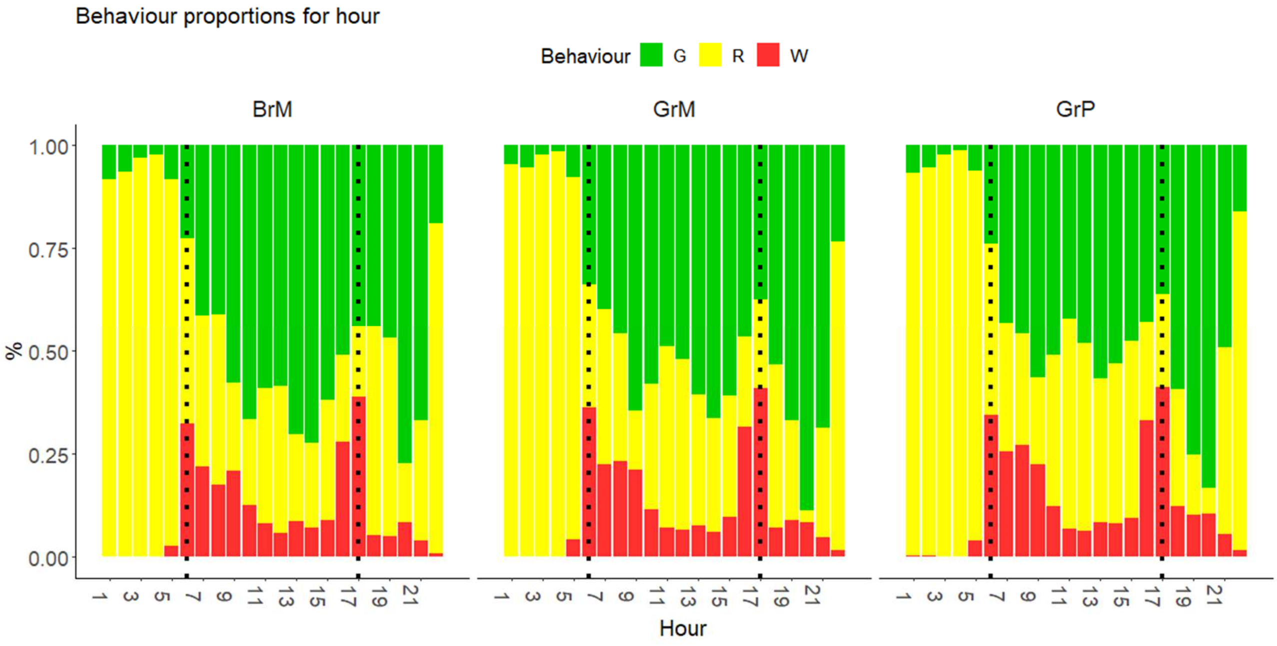


Figure S.2.4. Smoothed spline of the hourly distance from the barn (m) of the cows

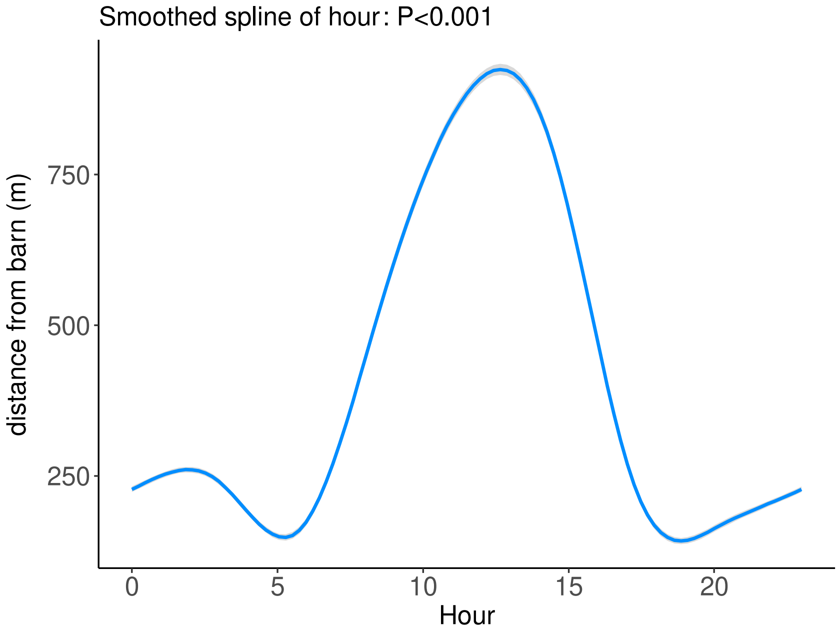
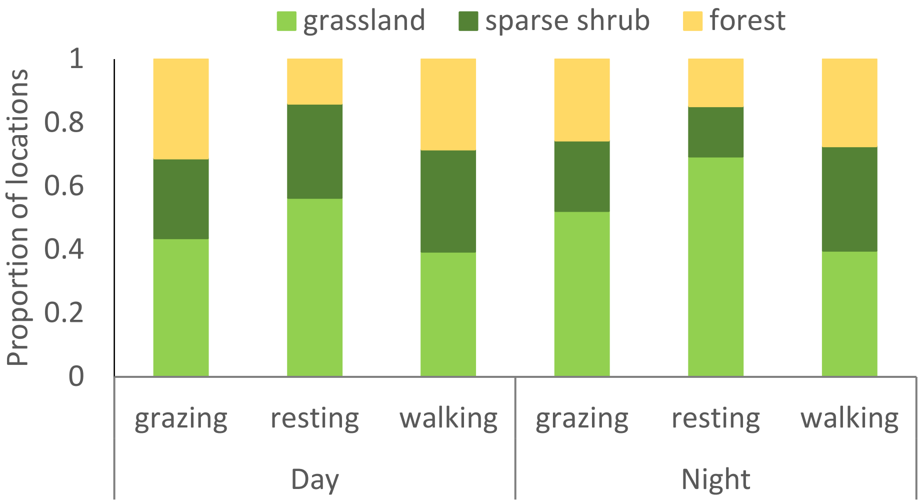


Figure S.2.5. Proportion of grazing, resting, and walking locations collected in grassland, sparse shrub and forest during day and night



References

Lüdecke D, Ben-Shachar MS, Patil I, Waggoner P, Makowski D. 2021. Performance: a R package for assessment, comparison and testing of statistical models. J Open Source Softw. 6(60):139.

Homburger H, Lüscher A, Scherer-Lorenzen M, Schneider MK. 2015. Patterns of livestock activity on heterogeneous subalpine pastures reveal distinct responses to spatial autocorrelation, environment and management. Mov Ecol. 3(1):1–15.

Liaw A., Wiener M. 2002. Classification and regression by Random Forest. R News 2, 18–22.

Nakagawa S, Schielzeth H. 2013. A general and simple method for obtaining R2 from generalized linear mixedeffects models. Methods Ecol Evol. 4(2):133–142.

R Core Team. 2016. R: A language and environment for statistical computing. Vienna, Austria: The R Foundation for Statistical Computing.

3.3 - Soil pH dominance over livestock management in determining bacterial assemblages through a latitudinal gradient of European meadows and pastures

The paper will be submitted in a scientific journal (probably Science of the Total Environment) as soon as we have collected all the revision from all the authors involved

Soil pH dominance over livestock management in determining bacterial assemblages through a latitudinal gradient of European meadows and pastures

Raniolo S[a], Squartini A[a]*, Cournut S[b], Michaud A[b], Cremilleux M[b], Nowak B[b], Lind V[c], Maretto L[a], Benedetti del Rio E[a], Ramanzin M[a], Sturaro E[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

[b] UMR Herbivores, VetAgro Sup, Av. de l'Europe 89, 63370 Lempdes, France

[c] Division of Food Production and Society Grassland and Livestock, NIBIO, 8861 Tjøtta, Norway

* Corresponding author: E. Sturaro, Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy. Phone: +39 0498 272641; Email: enrico.sturaro@phd.unipd.it

https://orcid.org/0000-0001-9508-5622

Abstract

Grasslands represent key functional ecosystems due to their global contribution to macronutrients cycling, and as reservoirs of microbial diversity. The strategic importance of these habitats rests on their involvement in carbon and nitrogen fluxes from atmosphere to soil, while at the same time offering extensive sites for livestock rearing. The management type, distinguishing the pasture or meadow land use, was investigated as a variable for its possible effects on overall bacterial diversity and on specific genes linked to functional guilds. Its contribution was compared to that of other variables as region, soil pH and soil organic carbon in order to rank their respective hierarchies in shaping microbial community structure. A latitudinal gradient across the European continent was studied with three sampling clusters located in Norway, France, and Northern Italy. Methods involved 16S DNA metabarcoding and quantitative PCR for bacterial and archaeal nitrification, intermediate or terminal denitrification, and nitrogen fixation genetic determinants. Results outlined that soil pH exerted the dominant role, affecting high taxonomy ranks and functions, along with organic carbon and region, with whom it partly co-varied. On the contrary, the management type showed its effects mostly at deeper taxonomical resolution as the OTU level and had no significant influence on the quantitative counts of functional genes. This suggests an ecological equivalence between the impacts of pasture and meadow practices, which are both perturbations sharing the aspect of vegetation withdrawal by browsing or cutting, respectively.

Introduction

Grasslands cover over 37% of the planet's emerged areas (Zhong et al., 2015, Bai et al. 2022), 70% of global agricultural land (Mencel et al. 2022) and 34% of European agricultural area (Schils et al. 2022), contributing ~34% of the terrestrial carbon store (Bai et al. 2022). They are ecosystems with a fundamental role for the conservation of biodiversity, landscape diversity, and multiple ecosystem services provided, including carbon storage, erosion control, and water management (Burczyk et al. 2018; Schils et al. 2022). Moreover, grasslands are fundamental in livestock systems providing feed for ruminants and herbivores, when used as meadows for hay/silage production or as pastures for animal grazing (Bunce et al. 2004, Mencel et al. 2022). Thanks to their importance as ecosystems and to their role in agriculture, a part of grasslands, the ones characterized by low intensity farming, are considered as High Nature Value (HNV) farmland (Lomba et al. 2014). Despite their relevance as ecosystems, grasslands are still poorly understood with respect to the microbial communities in their soils and the possible effects of different human use, such as pasture and meadow, on these communities. Different grassland uses can transform these ecosystems into sinks or sources of green-house gasses, such as CH4 or N2O, with implications on climate change in relation to processes mediated directly by the microbial community (Chadwick et al. 2018; Chang et al. 2021). In fact, microbial soil communities are the basis of the terrestrial ecosystems functioning as they mediate multiple processes of biogeochemical cycles, as those of nitrogen and carbon, through enzyme-catalysed reactions within metabolic pathways (Zhou et al. 2012; Rocca et al. 2015; Louca et al. 2018; Cavicchioli et al. 2019; Dong et al. 2020; Mencel et al. 2022). Diversity and functional redundancy within microbial communities are fundamental for sustaining ecological functions and resilience in biogeochemical processes (Louca et al. 2018; Maron et al. 2018). Thus, microbial communities can be considered as sets of functional groups in which species richness per se is less relevant than phenotypic traits for specific reactions, which define the functional richness of the ecosystem processes (Bahram et al., 2018; Louca et al., 2018; Moonen and Bàrberi, 2008). In ecosystems as grasslands, microbial community composition is shaped by both pedological conditions, such as pH and organic C content, and local disturbances, such as animal grazing or mowing or fertilization, which alter soil conditions (Mencel et al. 2022). Among pedological conditions, pH, moisture, soil organic carbon (SOC) and nitrogen contents are considered the most important drivers for microbial community structure (Bahram et al., 2018; Fierer, 2017; Kuypers et al., 2018). Instead, in terms of local disturbance, animal grazing can directly and indirectly affect soil conditions through trampling-induced asphyxia, selective removal of vegetation and deposition of urine and faeces (Du et al. 2019; Yin et al. 2020; Mencel et al. 2022; Wang et al. 2022). Animal trampling induces soil compaction changing the oxygen concentrations and soil water potential with direct effects on its microbial composition (Chroňáková et al. 2009; Yin et al., 2020; Mencel et al. 2022; Wang et al. 2022). Browsing of vegetation through animal grazing can shift grassland plant composition with changes in primary production, litter, and root exudates, which are part of the C inputs into the soil. These changes have direct effects on microbial communities (Mueller et al. 2017; Qu et al. 2016; Mencel et al. 2022; Wang et al. 2022), as those due to animal excreta deposition which directly increase the content and availability of nutrients such as C and N (Kohler et al. 2005, Wang et al. 2022). Also, mowing and fertilization practices can induce shifts on soil microbial communities in grasslands used as meadows. Mowing can partly simulate the plant consumption that would occur during grazing, inducing stimulation of root exudation and shifts on N and C cycles (Mencel et al. 2022). Instead, fertilization through animal dung or chemical compounds can change nutrients content and availability contextually to changing soil conditions such as pH (Schroder et al. 2011; Liu et al. 2014). Thus, grasslands used by agriculture as pastures or meadows can present similar microbial communities due to some similarities among the local disturbances exerted through grazing by animals or directly by humans. However, fractions of the grassland microbial communities can be resistant to local disturbance with respect to their taxonomic profiles and ensuing functional ones. Comparing the microbial communities of different grasslands used as pastures or meadows is crucial to define their differences in terms of biodiversity, functions, and possible effects of different human practices, providing insight into possible management improvements. In this study we compared the microbial communities of multiple grasslands used as either pastures, or meadows, in both cases fertilized with animal dung, in three European countries (France, Italy, and Norway) in terms of taxonomic and functional profiles, and pools of specific genes, combining 16S rDNA metabarcoding and qPCR analysis. We tested two main hypotheses: 1) grasslands managed as pastures or meadows but fertilised with animal dung differed or not in terms of both taxonomic and functional profiles; 2) the relative importance of pedo-climatic conditions and human management as drivers in determining microbial community structures and functions of grasslands.

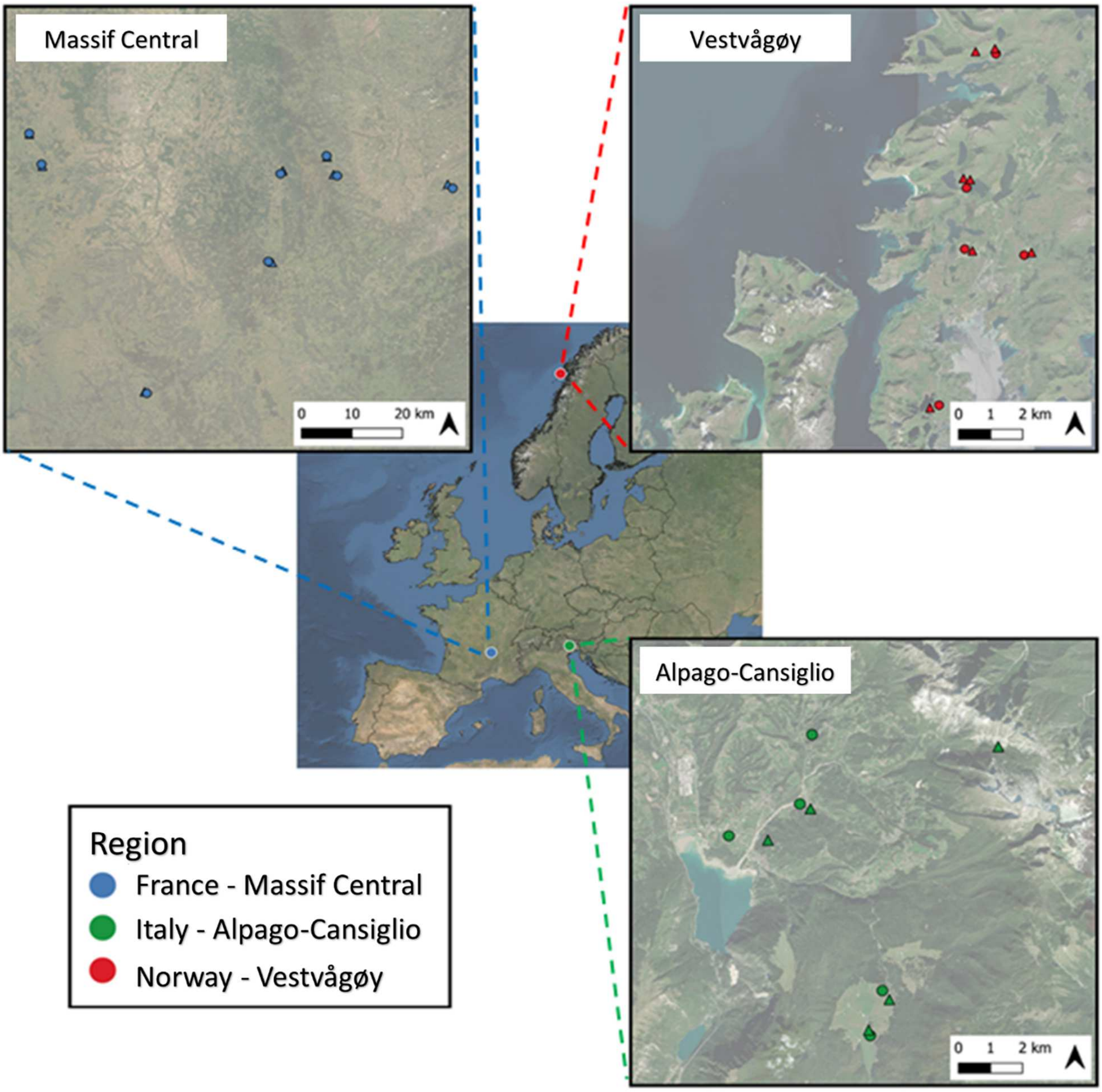
Material and methods

Site location

The study was part of the European Project "Highlands.3" and involved 18 farms in three European mountain areas with a historical presence of agricultural systems: the Vestvågøy area in Norway (NR - 5 farms), the Massif Central in France (FR - 8 farms), and the Alpago-Cansiglio in Italy (IT - 5 farms). For each farm, representative pastures and meadows were sampled for a total of 38 areas (FR: 8 meadows and 8 pastures; IT: 5 meadows and 5 pastures; NR: 5 meadows and 7 pastures). Pastures coincided with areas used for livestock grazing while meadows with areas used to produce hay and/or silage without animal presence. All meadows selected were cut twice during the summer and fertilized with animal dung and without inorganic fertilizers, except for three areas in Norway where farmers used both fertilizer types. For each area, two replicates of 12 topsoil samples (the top 15 cm of soil) were taken randomly and then used to form two representative bulks per area, one for the chemical analyses and the other for the molecular ones. Soil samples were air-dried before making the bulks and the subsequent analyses.

The three highland regions presented different extensions and pedo-climatic conditions in accord with the FAO soil map (http://www.fao.org/soils-portal/soil-survey/soil-maps-and-databases/faounesco-soil-map-of-the-world/en/) and the Köppen climate classification (Rubel and Kottek, 2010). The largest area, with the consequent highest number of farms, was the Massif Central, located in the south of France. This region is characterized by a temperate climate and features dystric cambisols. The Italian region is located in the Eastern Alps and presents a subartic or alpine climate with eutric cambisols soil types. Instead, the Norwegian region is located in the Lofoten archipelago, above the Arctic Circle, and is characterized by a subartic climate, mitigated by the presence of the ocean (Uleberg et al. 2014). Sampling took place during July 2021, at which time the three regions (countries) were characterized by different temperatures and precipitation (Norway: mean temperature = 11.3±0.2°C, mean rainfall = 1.7±0.07mm); France: mean temperature = 16.0 ± 1.1°C, mean rainfall = 2.2 ± 0.5mm; Italy: mean temperature = 17.8 ± 0.7°C, mean rainfall = 2.5 ± 0.1mm; (Muñoz Sabater, 2019).

Fig. 1 Area locations: Sampling points in France (Auvergne) are marked in blue, in Italy (Alpago-Cansiglio) in green, and in Norway (Vestvågøy) in red. Triangles indicate pastures (areas grazed, hence with animal presence), and circles indicate meadows (areas without animal presence, managed by two cuts per year, and fertilized with manure).



Chemical and molecular analyses

Soil chemistry analyses included quantification of pH by suspension of soil in water (ISO 10390) and organic C by high temperature dry combustion (ISO 10694).

Total DNA was extracted from an amount of 0.25 g of dried soil using the Qiagen DNeasy PowerSoil kit as described by the manufacturer's protocol. The extracted DNA was quantified with a Qubit 3.0

fluorimeter (Thermo Fisher Scientific, Carlsbad, CA) using the Qubit™ DNA HS Assay Kit (Thermo Fisher Scientific) and stored at -20°C.

The purified DNA was sequenced upon 16S rDNA metabarcoding on an Illumina MiSeq sequencer in the Paired End 2x300 bp format at the BMR Genomics s.r.l., Padova, Italy facilities, targeting the V4 region using the following universal primers: Modified 515f : GTGYCAGCMGCCGCGGTAA, (Parada et al. 2015), and Modified 806r:GGACTACNVGGGTWTCTAAT (Apprill et al. 2015).

Tab. 1 List of primers used for qPCR with functions associated and references.

Primer	Function	Sequence	Amplicon length	References
16S F	-	ATGGYTGTCGTCAGCTCGTG	1550 bp	Leigh *et al.* (2007)
16S R	-	GGGTTGCGCTCGTTGC		
Archaeal amoA - AOA F	Ammonia oxidation	STAATGGTCTGGCTTAGACG	635 bp	Francis *et al.*(2005)
Archaeal amoA - AOA R	Ammonia oxidation	GCGGCCATCCATCTGTATGT		
Bacterial amoA - AOB F	Ammonia oxidation	GGGGTTTCTACTGGTGGT	500 bp	Rotthauwe *et al.* (1997)
Bacterial amoA - AOB R	Ammonia oxidation	CCCCTCKGSAAAGCCTTCTTC		
nifH F	Nitrogen-fixation	AAAGGYGGWATCGGYAARTCCACCAC	432 bp	Rosch *et al.* (2002)
nifH R	Nitrogen-fixation	TTGTTSGCSGCRTACATSGCCATCAT		
nosZ F	Nitrous Oxide reduction	CGYTGTTCMTCGACAGCCAG	706 bp	Rosch *et al.* (2002)
nosZ R	Nitrous Oxide reduction	CATGTGCAGNGCRTGGCAGAA		
nirK F	Nitrite reduction	ATYGGCGGVCAYGGCGA	160 bp	Henry *et al.* (2004)
nirK R	Nitrite reduction	RGCCTCGATCAGRTTRTGGTT		

RealTime qPCR was performed by a QuantStudio 5 system (Life Technologies, Carlsbad, CA, USA. The qPCR reaction volume was equal to 5 μL, 1 μL of purified DNA solution and 4 μL of reaction mix, composed by 1.2 μL PCR-grade water, 0.15 μL each of F and R primers (Table 1) and 2.5 μL Power SYBR Green PCR Master Mix with Taq polymerase (Applied Biosystems, Foster City, CA, USA). The qPCR thermal conditions were set to a pre-denaturing stage at 95 °C for 10 minutes, followed by 40 cycles with a denaturation step at 95 °C for 15 sec, an annealing step at 57 °C for 60 sec and an extension at 72 °C for 60 sec. For each amplification, a negative control of sterile MilliQ water was run with three replicates. The Ct threshold cycles were transformed into gene copies using the equation by Dong et al. (2020). The undetermined Ct were set 40 to equate to 0 genes copies by the transformation.

The bioinformatics processing of the sequencing data was based on QIIME (Caporaso et al. 2010) and the OTU profiles were analysed by FAPROTAX 1.2.4 (Louca et al. 2016) to extract the functional profiles. The DNA sequences have been deposited in the GenBank repository SRA Archive, under the project code https://www.ebi.ac.uk/ena/browser/view/PRJEB56444.

Statistical analysis

The statistical analyses were conducted in R 4.2.0 (RCore Team 2016).

pH and organic carbon content (C org) were preliminarily analysed as function of the 2-way interaction between region (Norway - NR, France - FR, Italy - IT) and management type (meadow – M - or pasture - P) through a permutation ANOVA using the "lmp" function of the "lmperm" library (Bates et al. 2015). An analysis of pH and C org variance was performed to detect possible nested features of the data, indicating significant effects of region on pH (Supplementary Table S.1). In addition, pH and C org effects were analysed through separate models categorizing the pH and C org on their quartiles and considering as factors with 4 levels, where level 1 corresponds to the lowest values and level 4 to the highest (pH class: 4.5<"1"≤5.4; 5.4<"2" ≤5.8; 5.8<"3" ≤6.4; 6.4<"4" ≤7.6 – C org class: 1.9<"1"≤4.7; 4.7<"2" ≤6.9; 6.9<"3" ≤9.8; 9.8<"4" ≤35.9).

Diversity indices of microbial communities

The Shannon and Simpson alpha-diversity indices and the Pielou evenness index were calculated for the microbial communities of each sample at the OTU and phylum level using the "vegan" library (Dixon 2003). The indices were first analysed in terms of correlations between both taxonomic levels on the Kendall's coefficient and then as a function of the 2-way interaction between region (NR, FR, IT) and management type (M or P) through an ANOVA with permutation test using the "lmp" function of the "lmperm" library (Bates et al. 2015). The analysis of differences between indices also included two ANOVAs with permutation tests as function of pH class and C org class separately. All ANOVAs were based on 5000 permutations. To assess which factor between organic C and pH class best described the gene variability, the ANOVA models with either C org or pH class were compared in terms of AIC indices and R2 (Johnson and Omland 2004).

Taxonomic and functional profiles of microbial communities

The analysis of taxonomic and functional profiles was performed on dissimilarity matrices based on the Bray-Curtis distance of abundances applying multiple non-parametric tests (Warton et al. 2012) in function of 4 factors: region, management type, pH and C org classes. Firstly, the profile cores with respect to the four factors were extracted and represented through Venn Diagrams to identify the suite of units shared. Then, an ANOSIM (Analysis of Similarity – Clarke 1993) was performed for each single factor to test whether the similarity between factor levels was greater than within factor levels. The ANOSIM provides an R statistic constrained between -1 and +1, where the negative limit indicates strong similarity between factor levels while the positive limit indicates strong similarity within factor levels. The ANOSIM was performed at the OTU and phylum level with the "anosim" function of the "vegan" library (Dixon 2003) setting 9999 permutations. Subsequently, a NMDS (Non-metric multidimensional scaling) on 2 dimensions was performed to represent the similarities among sample profiles in function of the most significant factors of ANOSIM, considering all taxonomic rank levels. The goodness of each NMDS was verified by extracting and comparing the stress value to the threshold of 0.2 (Dexter et al. 2018). To identify the lists of phyla, OTUs and functions associated to each factor level, an indicator species analysis was performed for each factor through the "multipatt" function of the "indicspecies" library (Caceres et al. 2016). This analysis allows to measure the association between a factor level and a single phylum, OTU and function through an Indicator Value (stat), constrained from 0 to 1 where higher value corresponds to stronger associations (Dufrêne and Legendre 1997). The differences among taxonomic and functional profiles expressed as spatial distances were also analysed through PERMANOVA (Permutational Multivariate Analysis of Variance – Anderson 2001) considering firstly the 2-way interactions between management type and region, then the C org and pH classes separately. The analysis of variance was combined with the analysis of the dispersion effects between levels of single factors, which was performed to assess the possible differences in terms of beta diversity (Anderson et al. 2006). When the beta dispersion was significantly affected by a factor, an ANOVA of the distances between communities/samples and their centroids was performed to assess the effect on beta diversity. The PERMANOVA was computed with the function "adonis2" of the "vegan" library setting 9999 permutations while the analysis of dispersion with the function "betadisper" function of the same library. Then, a Mantel test (Mantel 1967) was performed with the function "mantel" of the "vegan" library to assess a possible linear correlation between community dissimilarities and single soil parameters (pH and C org) of which dissimilarities were calculated on Euclidean distances. Finally, Kendall's correlations were computed between selected functions (chemoheterotrophy and aerobic chemoheterotrophy, methanogenesis, nitrification, denitrification, nitrogen fixation, ureolysis and the number of OTUs and the percentage of FAPROTAX assignment of microbial communities.

Genes functional profiles

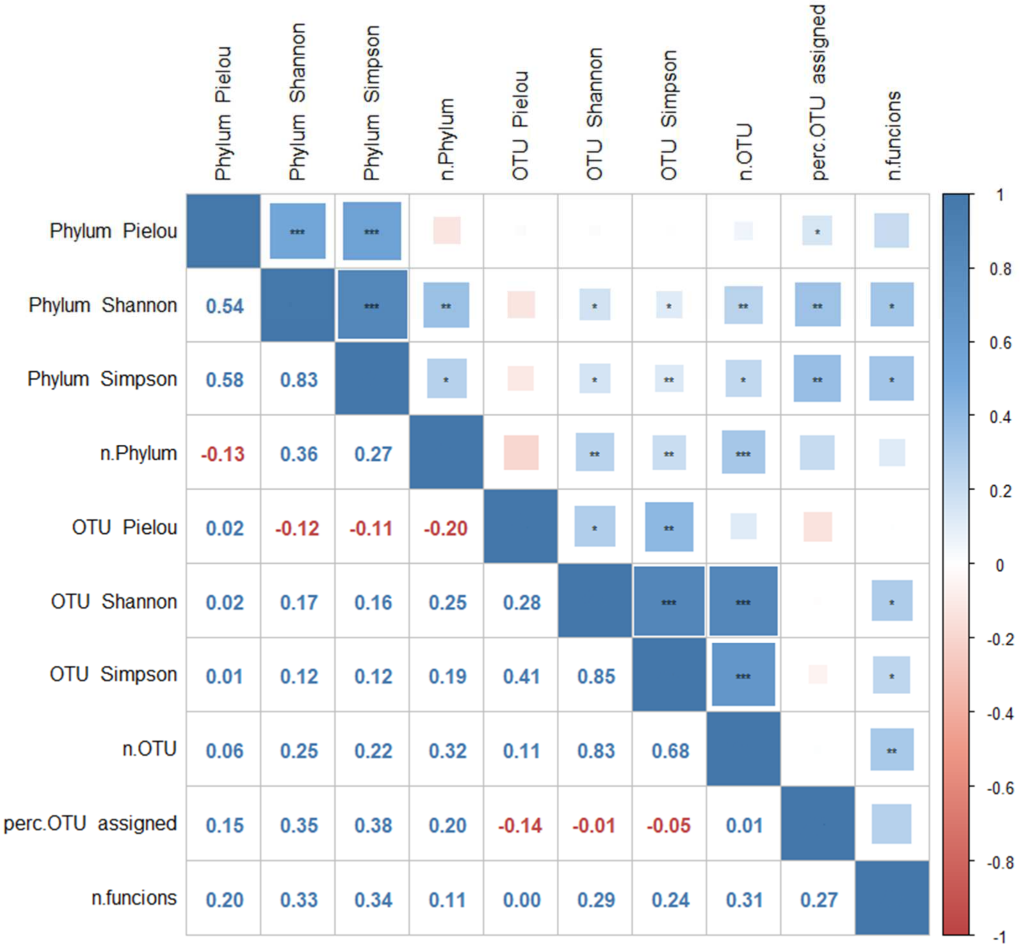
Gene copies' abundances from amplifications were log-transformed before analysis. The gene analysis included Kendall's correlations among genes and ANOVA based on permutation tests using the "lmp" function from the "lmPerm" library (Wheeler and Torchiano 2010) as function of the 2-way interactions between management type and region, the C org and pH classes separately. To assess which factor between organic C and pH classes best described the gene abundance variability, the ANOVA models were compared in terms of AIC indices and R2. Moreover, to assess possible niche differences between archaeal and bacterial amoA genes, we calculated the ratio between AOB and the total ammonia-oxidation guild (AOA+AOB) and analysed it as function of with respect to factors used in microbial community analyses the 2-way interactions between management type and region, the C org and pH classes separately by means of a general additive model based on beta distribution and log link function, using the function "gam" of the "mgcv" package (Wood, 2017).

Results

A total of 3,323 different bacterial OTUs were detected from 695,182 quality-filtered reads. The average number of different OTUs per sample was 134 ±31 with a percentage of unassigned cases of 21 ± 5 % at genus level and 1 ± 1% at phylum level, while the function prediction tool FAPROTAX had an assignment rate of 25 ± 5%. The most abundant phyla were Firmicutes (31 ± 12%), Proteobacteria (20.2 ± 5.7%) and Actinobacteriota (14.8 ± 6.8%) (Fig. 2 A). Instead, the most abundant functions resulted Chemoheterotrophy (30.2 ± 7.4%), Aerobic chemoheterotrophy (25.5 ± 6.3%), Aerobic ammonia oxidation (9.4 ± 8.1%) and Nitrification (9.4 ± 8.1%) (Fig.4 B).

Alpha-diversity and evenness indices

Fig.2 Plot of Kendall rank correlations among index of alfa-diversity and evenness at phylum and OTU rank level, number of phyla and OTUs, percentage of OTUs assigned by FAPTORAX and number of functions identified.



The analysis of correlations revealed significant and positive relations between the number of OTU and alpha-diversity indices and number of functions (Simpson: r=0.83, Shannon: r=0.68, n_fuctions:

r=0.33 - Fig. 2). The alpha diversity indices for phyla resulted positively and significantly related to the percentage of the functional phenotypic FAPROTAX predictive assignment and consequently to the number of functions (Percentage of OTUs assigned: Shannon of phylum: r=0.35, p-value<0.01; Simpson of phylum: r=0.38, p-value<0.01; n of functions: Shannon of phylum: r=0.33, p-value<0.05; Simpson of phylum: r=0.34, p-value<0.05 - Fig. 2). Instead, the alpha diversity indices for OTU resulted both negatively and positively related to the percentage and the number of the functions assigned by FAPROTAX (Percentage of OTUs assigned: Shannon of OTU: r=-0.01; Simpson of OTU: r=-0.05; n of functions: Shannon of OTU: r=0.29, p-value<0.05; Simpson of OTU: r=0.24, p-value<0.05 - Fig. 2).

The ANOVA on permutation test of alpha-diversity indices revealed no significant differences at both OTU and phylum levels as a function of region and management type, but there were significant differences with respect to pH and C org classes at phylum level. (Supplementary Material Table S2). In particular, the alpha diversity indices of phyla were significantly and positively correlated to the pH class (Shannon of phylum: p-value<0.05; Simpson of phylum: p-value<0.05) while they were significantly and negatively correlated to the C org class (Shannon of phylum: p-value<0.01; Simpson of phylum: p-value<0.01) (Supplementary Material Table S2). The index of Pielou, which gives information about the evenness, was significantly affected by region at OTU level (p<0.05) (Supplementary Material Figure S2) and pH class at both phylum and OTU levels (phylum: p-value<0.05; OTU: p-value<0.05) (Supplementary Material Table S2). For all indices, the pH class models presented the lowest AIC at phylum level while the C org class models presented the lowest AIC at OTU level.

Fig. 3 Venn diagrams of phylum, OTUs and function distinguished by region (row A – “FR” France, “IT” Italy and “NR” Norway), pH class (row B), C org class (row C) and management type (row D – (“M” meadow and “P” pasture). The pH class and the C org class present 4 levels on quartiles (pH class: 4.5<“1”≤5.4; 5.4<”2” ≤5.8; 5.8<”3” ≤6.4; 6.4<”4” ≤7.6 – C org class: 1.9<“1”≤4.7; 4.7<”2” ≤6.9; 6.9<”3” ≤9.8; 9.8<”4” ≤35.9).

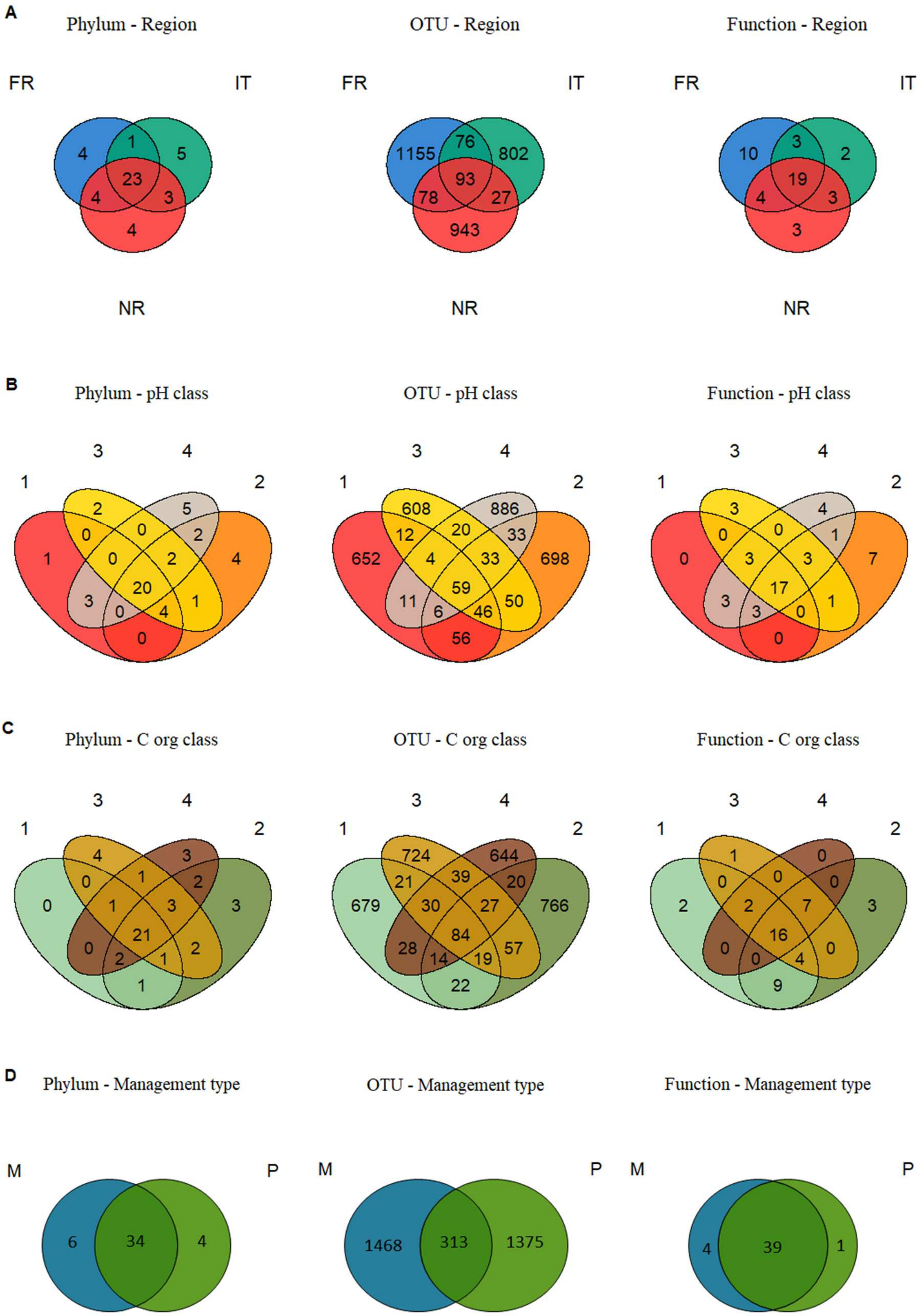
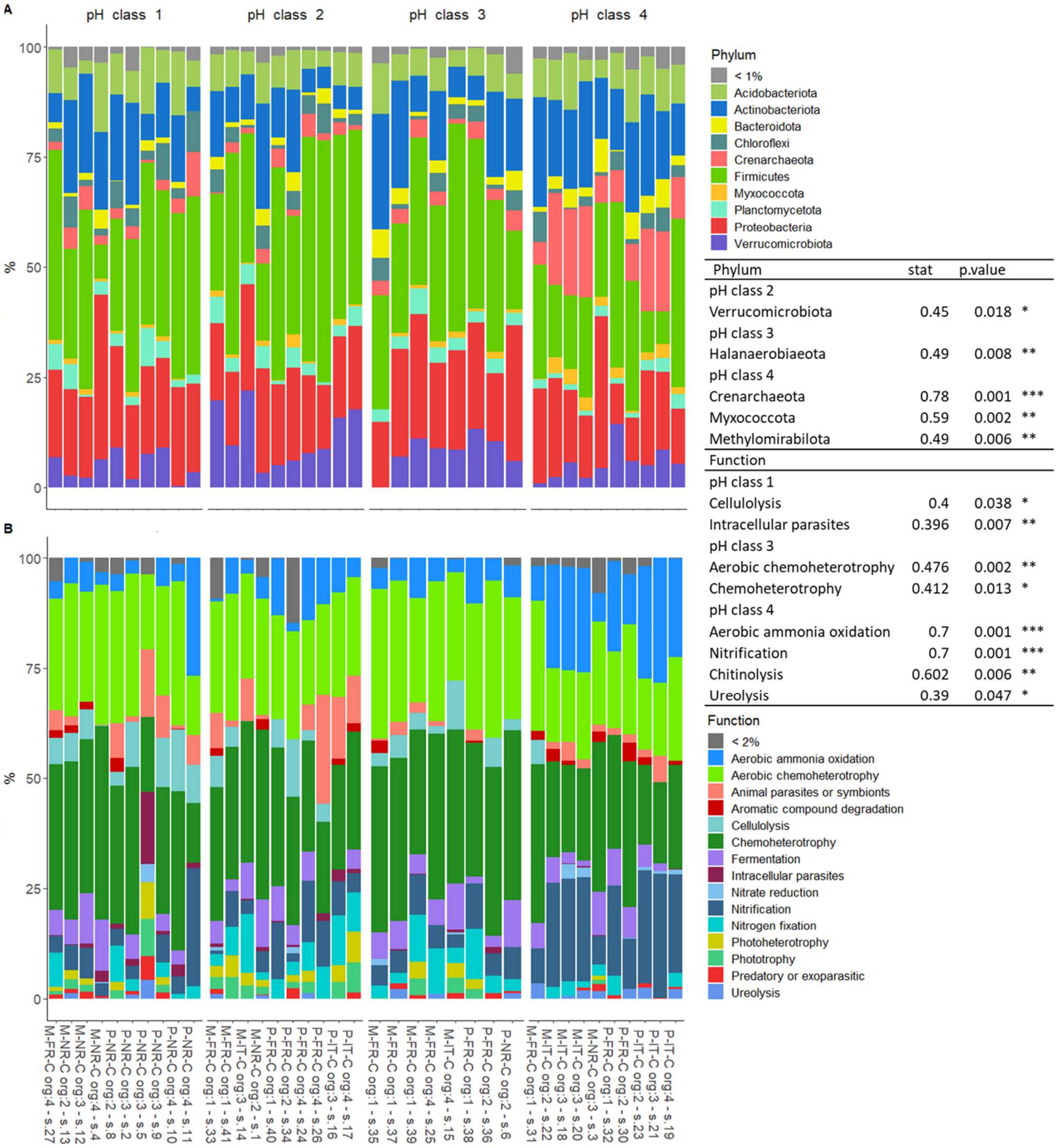
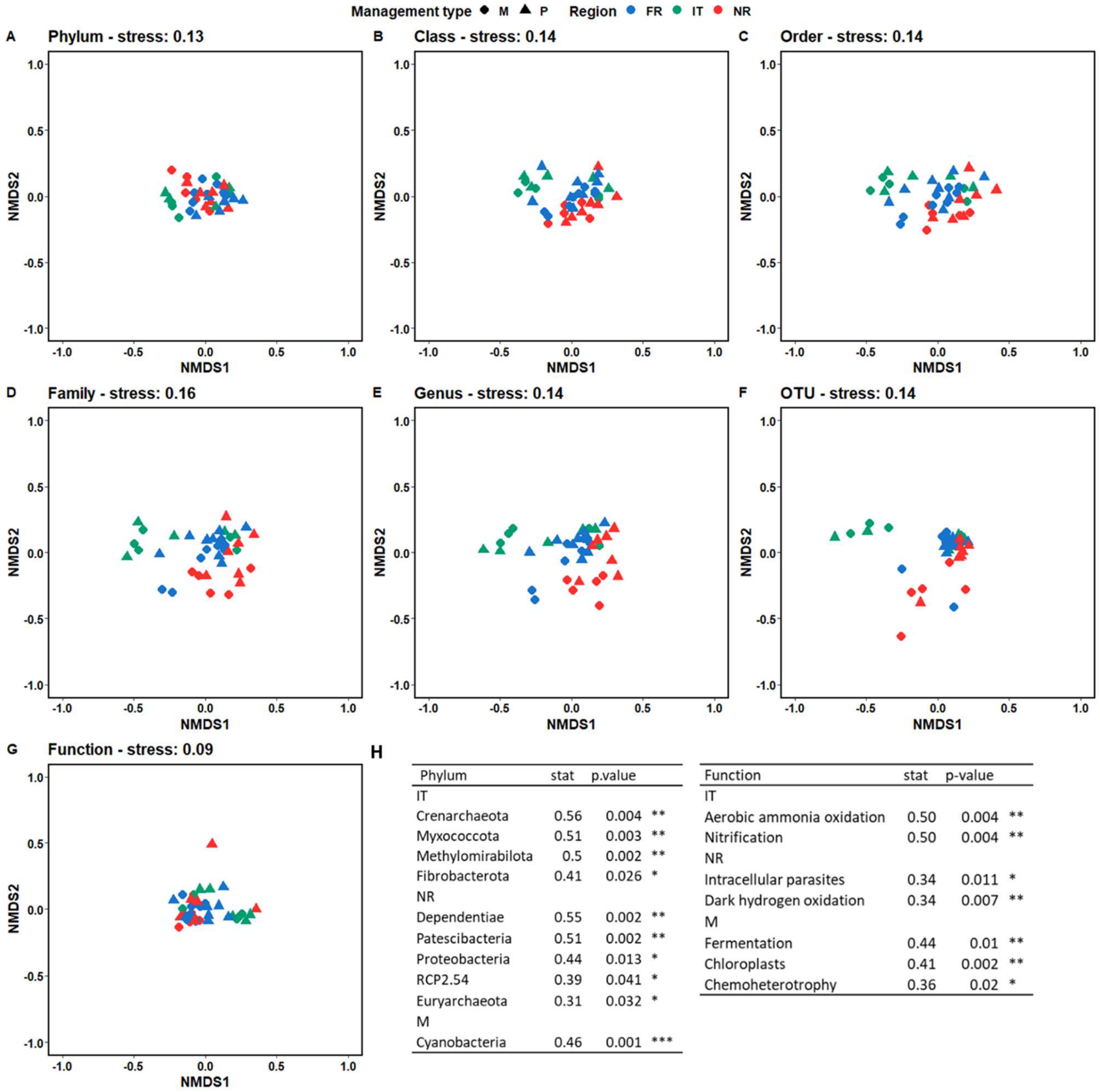


Fig. 4 Barplot chart of the taxonomy results for single samples at phylum rank level (panel A) and the functions identified by FAPROTAX (panel B), distinguished by pH class and ordered by management type, region, and organic C class. Each sample is labelled reporting the management type (M or P), region (FR, IT, NR) and the C org class (1, 2, 3, 4) followed by the sample number. The phyla characterized by relative abundance below 1% were pooled as single group. The pH class and the C org class present 4 levels on quartiles (pH class: 4.5<"1"≤5.4; 5.4<"2" ≤5.8; 5.8<"3" ≤6.4; 6.4<"4" ≤7.6 – C org class: 1.9<"1"≤4.7; 4.7<"2" ≤6.9; 6.9<"3" ≤9.8; 9.8<"4" ≤35.9). The most associated phyla and functions to pH class were reported.



Phylum	stat	p.value	
pH class 2			
Verrucomicrobiota	0.45	0.018	*
pH class 3			
Halanaerobiaeota	0.49	0.008	**
pH class 4			
Crenarchaeota	0.78	0.001	***
Myxococcota	0.59	0.002	**
Methylomirabilota	0.49	0.006	**
Function			
pH class 1			
Cellulolysis	0.4	0.038	*
Intracellular parasites	0.396	0.007	**
pH class 3			
Aerobic chemoheterotrophy	0.476	0.002	**
Chemoheterotrophy	0.412	0.013	*
pH class 4			
Aerobic ammonia oxidation	0.7	0.001	***
Nitrification	0.7	0.001	***
Chitinolysis	0.602	0.006	**
Ureolysis	0.39	0.047	*

Fig. 5 NMDS of taxonomic profiles at different ranks levels and function (panel A-G) and the most associated phyla and functions (panel H) with respect to the region and management type (“M” meadow and “P” pasture). The stress value (goodness of ordination) is reported for each rank level.



Phylum	stat	p.value	
IT			
Crenarchaeota	0.56	0.004	**
Myxococcota	0.51	0.003	**
Methylomirabilota	0.5	0.002	**
Fibrobacterota	0.41	0.026	*
NR			
Dependentiae	0.55	0.002	**
Patescibacteria	0.51	0.002	**
Proteobacteria	0.44	0.013	*
RCP2.54	0.39	0.041	*
Euryarchaeota	0.31	0.032	*
M			
Cyanobacteria	0.46	0.001	***

Function	stat	p-value	
IT			
Aerobic ammonia oxidation	0.50	0.004	**
Nitrification	0.50	0.004	**
NR			
Intracellular parasites	0.34	0.011	*
Dark hydrogen oxidation	0.34	0.007	**
M			
Fermentation	0.44	0.01	**
Chloroplasts	0.41	0.002	**
Chemoheterotrophy	0.36	0.02	*

Taxonomic profiles

The Venn Diagram of taxonomic profiles (Fig. 3) revealed different patterns between phylum and OTU levels with respect to the management type, region, pH class and C org class. The phylum level presented more abundant shared cores for all considered factors than the OTU level, which was characterised by more abundant cores for single factor levels. The differentially featured taxa analysis revealed significant abundances in terms of both OTU (70 OTUs) and phylum (9 phyla) among the three countries (Figure 3 and 4). In particular, France showed significant differences for 19 OTUs, Italy for 31 and Norway for 20, while in terms of phyla only Italy and Norway presented significant differences with 4 phyla for the former and 5 for the latter. With respect to the management type, these taxonomic differences involved only 14 different OTUs, 10 of which were enhanced in the pasture cases and 4 in the meadow, while in terms of phyla, there was only 1 for the meadow (Supplementary Materials Table S4, Supplementary Materials Table S5). The ANOSIM (Supplementary materials Table S3) highlighted significant differences in microbial taxonomy profiles at both the OTU and phylum levels with greater dissimilarity for the former than the latter. The region was the factor which explained mostly the differences among samples at both OTU and phylum levels (OTU region: $p\text{-value}<0.001$ – $R=0.293$; phylum region: $p\text{-value}<0.001$ – $R=0.182$) (Supplementary materials Table S3). Instead, the type of management had significant effects at only OTU level although the dissimilarity between groups was less than that among regions as reported by the R indices (OTU type: $p\text{-value}=0.0393$ – $R=0.0607$). Regarding the soil characteristics, pH had significant effect at both OTU and phylum levels (OTU pH: $p\text{-value}<0.00$ – $R=0.193$; phylum pH: $p\text{-value}<0.00$– $R=0.19$), while organic C showed a significant effect only at OTU level (OTU organic C: $p\text{-value}<0.01$- $R=0.0956$). The stress value of NMDS based on two dimensions was rather constant from phylum to OTU level, passing from 0.13 to 0.14 (Figure 5, Supplementary materials Figure S3).

The Indicator Species Analysis of differentially featured taxa in relation to the variables also confirmed that pH explained more dissimilarities among taxonomic levels and their abundances (60 OTUs, 5 phyla) than organic C (31 OTUs, 0 phyla), where the class 4 of pH presented the most dissimilar taxonomic profiles (Supplementary Materials Table S4, Supplementary Materials Table S5). At OTU level, pH class 1 presented 16 associated OTUs, class 2 had 13 OTUs, class 3 16 and class 4 24 (Supplementary Materials Table S4, Supplementary Materials Table S5), while at phylum level there were significant phyla only among class 2, with 2 cases, class 3, with 1, and class 4, with 3 (Figure 4). Organic Carbon significantly affected OTUs occurrences and abundances, in particular in class 1 with 14 significant OTUs and at class 4 with 12 (Supplementary Materials Table S4, Supplementary Materials Table S5).

The PERMANOVA analysis of the OTU profiles confirmed the results of the ANOSIM, showing a significant stronger effect of region (p-value<0.01) in comparison to the management type (p-value<0.05). In spite of these differences, there were no effects for the interactions (Supplementary Materials Table S6). Regarding the soil characteristics, PERMANOVA confirmed a stronger effect of pH (p-value<0.01) than that of C org (p-value<0.05) to explain the variance of the OTU profiles. In particular, the pairwise comparisons of PERMANOVA revealed the strongest differences between NR and FR (p-value<0.001), IT and FR (p-value<0.001), pH class 1 and 4 (p-value<0.001), and organic C class 1 and 4 (p-value<0.001) (Supplementary Materials Table S6). The dispersion among all factors was not significant, revealing homogeneous communities with respect to the considered factors. The PERMANOVA analysis of phyla profiles also confirmed in part the results of the ANOSIM, showing strong significant effect of region (p-value<0.05) and pH class (p-value<0.01) but no effect of type and C org class (Supplementary Materials Table S3). The analysis of dispersion revealed heterogeneous communities, showing significant differences in beta diversity, with respect to the region, where Italy presented significant differences in terms of distance from the centroids compared to France and Norway (p-value<0.01) (Supplementary Materials Table S7, Supplementary Materials Figure S4).

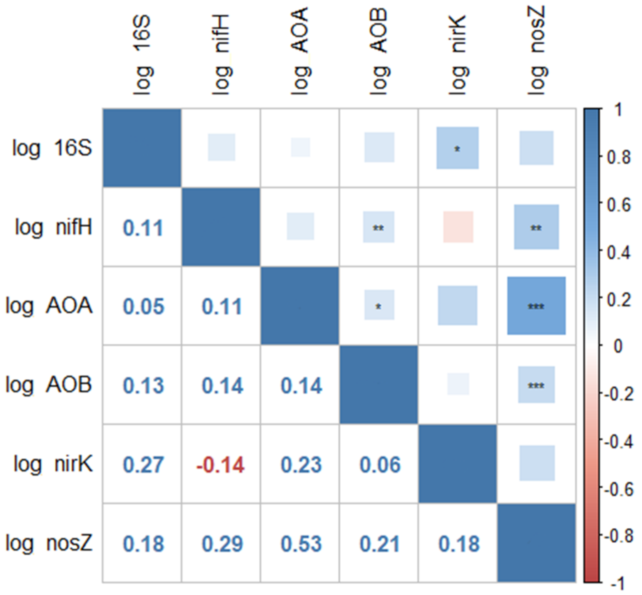
The Mantel test confirmed the significant effect of pH on both OTU and phylum levels with positive linear trends (OTU: p-value<0.001 – r=0.48; phylum: p-value<0.001 – r=0.39) but not for the C org with positive but weak trends (OTU: r=0.093; phylum: r=0.051) (Supplementary Materials Table S8 - Supplementary Materials Figure S6).

Functional profiles

The Venn diagram of function profiles (Fig. 3) reflected the patterns found for at phylum level with high number of units shared between levels of management type, region, pH class and C org class. The functional profiles reflected the significant differences found at phylum level through both the ANOSIM, PERMANOVA and Mantel test (Figures 4 and 5; Supplementary Materials Figures S.3, S.5 and Supplementary Tables T.3, T.6, T.8). In particular, the most significant factor for functional profile was the region (ANOSIM p-value<0.001; PERMANOVA p-value<0.01), followed by pH classes (ANOSIM p-value<0.001; PERMANOVA p-value<0.001; Mantel test p-value<0.001 – r=0.37). The interaction between region and management type was not significant in terms of variance (PERMANOVA p-value>0.05). No effects of type management and C org were detected for the functional profiles (Supplementary Table S6). The pairwise comparison of PERMANOVA revealed the strongest differences between FR and IT (p-value<0.01), and between the pH class 4 and 1, 2 and 3 (p-value<0.01). The dispersion test detected homogeneous distribution of samples around the centroids for all factors. Interestingly, the analysis of correlations revealed a significant but negative relation between nitrification and both chemoheterotrophy (r=-0.45) and aerobic chemoheterotrophy (r=-0.43), which were strongly correlated (r=0.69 - Supplementary Materials Figure S6). Nitrification was also significantly correlated to the percentage of OTUs assigned (r=0.25). Nitrogen fixation was significantly correlated with ureolysis (r=-0.47), which was also correlated with methanogenesis (r=-0.29 - Supplementary Materials Figure S6).

The RealTime PCR quantified gene copies of nitrogen cycling reactions showed different correlations and patterns (Figure 6; Supplementary Materials Figure S7 and Supplementary Tables T9): the *nosZ*, *nifH*, AOA and AOB showed significant and positive correlations among them (*nosZ-nifH*: r=0.29 – p-value<0.01, *nosZ*-AOA a: r=0.53 – p-value<0.001, *nosZ*-AOB: r=0.21 – p-value<0.001, AOB-*nifH*: r=0.14 – p-value<0.01), while the *nirK* and 16S were significantly correlated only between them (*nirK*-16S: r=0.27 – p-value<0.05).

Fig.6 Plot of Kendall rank correlations among log transformed gene abundances (*16S, nifH*, AOA, AOB, *nirK* and *nosZ*) considering all sites.

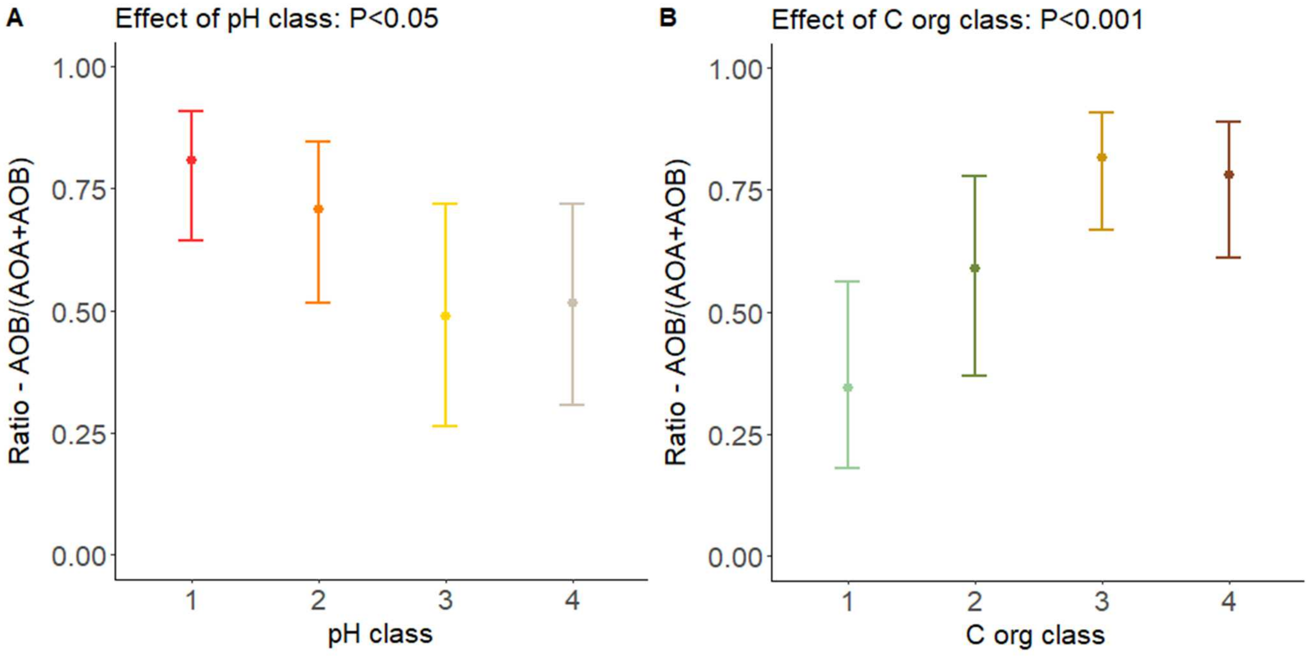


The ANOVA of gene copies revealed the region as the only significant factor among almost all genes (*nosZ*: p-value<0.05, *nirK*: p-value<0.05, *nifH*<0.05, AOA: p-value=0.05), except for 16S and AOB (Supplementary Materials Figure S7 and Supplementary Table T9). The ANOVA as regards pH and organic C classes showed a generally stronger effect of the latter than the former on gene copies except for the *nirK*, which was more shaped by the pH (Supplementary Material Figure S7 and Supplementary Materials Table T9-10). pH affected the same genes on which organic C had effect, but *nirK* presented a stronger effect (p-value<0.001) while AOB did not show one (Supplementary

Material Figure S9 and Supplementary Materials Table T10). The organic C class had significant and negative effect on *nosZ* (p-value<0.01), AOA (p-value<0.001) and AOB (p-value<0.05), confirming the correlation analysis (Figures 6 – Supplementary Materials Figure S9 and Supplementary Materials Table S9). No effects on 16S were found (Supplementary Material Figure S9 and Supplementary Materials Tables T10).

The ratio between AOB and the sum of AOA and AOB did not present a significant effect for both region and management type, and their interaction (Supplementary Table T11). However, the ratio was significantly affected by both pH (p-value<0.05) and organic carbon (p-value<0.001), where the former presented a negative trend and the latter a positive one (Figure 7 – Supplementary Materials Table T11). The effect of organic carbon was stronger than pH (R^2 C org class: 0.28; Deviance explained C org class: 42.9%; R^2 pH class: 0.11; Deviance explained pH class: 27.8%) (Supplementary Materials Table T11).

Fig. 7 Least Square Means of ratio AOB/(AOA+AOB) as function of pH class (panel A) and C org class (panel B). The significant effects were estimated by GAM model based on beta distribution with log link function. The pH class presents 4 levels on quartiles as the C org class (pH class: 4.5< "1" ≤5.4; 5.4<"2" ≤5.8; 5.8<"3" ≤6.4; 6.4<"4" ≤7.6 – C org class: 1.9< "1" ≤4.7; 4.7<"2" ≤6.9; 6.9<"3" ≤9.8; 9.8<"4" ≤35.9). The significant effects are reported in table S.11.



Discussion

The semi-natural grasslands investigated presented appreciably distinct microbial communities in terms of both taxonomic and functional profiles. Communities shared a common core of phyla (Fig.3) in which the majority for all areas was made by Firmicutes, Proteobacteria and Actinobacteriota, (Figure 4) which are typically among the most abundant in both soil and water environments (Bahram et al. 2018). The differences relative to taxonomy increased upon the descending ranks of resolution from phylum to OTU (Figure 5, Supplementary Materials Figure S3), where the common units drastically reduced in number (Fig.3), in line with a common phyletic starting point and with the time-related possibilities of homogenizing dispersion. Given the metrics used as standard to assess microbial identity and phylogeny, i.e. the 16S ribosomal RNA gene (Woese, 1987), its estimated rate of change (Clark et al., 1999) allows to judge phyla as having started their branching in a timeframe of the order of 1-2 billions of years ago, and to species as stemming on average more than 100 millions of years ago. In practical terms, considering the timing of tectonic motions and land emersion, that originated the current position of European regions, and, most critically, the comparatively short duration of soils formation, taking timeframes between decades to thousands of years it can be first commented that, in this sort of surveys, the contribution of in situ bacterial speciation can be regarded as practically irrelevant. Therefore, when commenting data, we shall consider that differences in composition have to be interpreted partly as effect of deterministic forces as local environmental selection, and partly of stochastic ones as dispersal drifts. The existence of the common core composed by the most abundant phyla can reveal an origin shared by microbial communities, which are subsequently diversified by environmental conditions. Notwithstanding the common core, phyla diversity showed a significant tendency to be grouped by the 'region' variable, which is not to be intended as straight geography since, besides latitude, the occurrence in Norway, France or Italy entails also concurrent superimposed differences in terms of pedo-climatic conditions, such as primarily pH or organic C. In fact, at phylum level the beta diversity of microbial communities was driven by region in its variability, but the alpha diversity was instead ruled by pH and organic C (Supplementary Tab.S4, Fig.2), confirming the role of local pedo-climatic conditions. Thus, geographical distances appear to explain community dissimilarities, which are actually shaped by local condition, according to Louca et al. 2016. Inspecting the differences among microbial communities relative to uniqueness or differential abundance of the highest ranks, i.e. phyla, those are due only to the most rare ones and to those containing the lowest abundance of sequence reads.

pH resulted the single significant driver of difference among phyla as PERMANOVA and ANOSIM and Mantel test revealed, and the indicator species analysis confirmed. In fact, the extreme pH class, which corresponds to alkaline pH into the USDA classification, presented three phyla significantly associated with it, Crenarchaeota (within the domain Archaea), and Myxococcota and Methylomirabilota (within the domain Bacteria), which tended to be fewer in the other pH classes. Instead, the intermediate classes were characterised by the highest presence of Verrucomicrobiota. Interestingly, no effects of management type were found. This confirms the above premise about the fact that differences among microbial communities as deeply branched as those that individuate the phyla divergence levels would require processes able to heavily influence environmental conditions in geological time scales (Clark et al., 1999). That chronological framework is thus far longer in the past than those related to European continent shaping, pedogenetic processes, which in turn are themselves definitely much longer than the human practices leading to meadow or pasture management, which can be considered comparatively, as extremely "young" disturbances.

Also, for OTUs, the region was confirmed as the main driver for the diversity among communities along with pH and also organic C. The OTU cores showed higher diversity than those at phylum level, with higher number of unique units and a drastic decrease of the common ones (Fig. 3). OTU level, in line with its definition criterion linked to the lowest cut-off of divergence (2.5%), showed to be the most variable rank as it was also influenced by management type, revealing the tendency of single communities to reflect selection issues linked to animal presence or periodical cutting and fertilization. Presence of animals or humans involves short-ranged temporal disturbance, and a consequent compositional turnover linked to microbial dispersion by wind and water (Griffin et al. 2002; Smith et al. 2012).

Functional profiles presented patterns similar to the taxonomy-related ones at phylum level with respect to region, management type and pH (Figure 3). Also, for the functions there was a common and persistent core (Fig.3), which is not unusual as the large fraction of metabolic genes encoding for functions appeared early in the Earth history and propagated into multiple clades (Falkowski et al. 2008; David and Alm 2011). Nevertheless, a common functional core can be also affected not only by adaptive loss of functions to environmental conditions (Morris et al. 2012) but also by horizontal gene transfer (Falkowski et al. 2008; David and Alm 2011). Being the latter event independent from 16S-based phylogeny on which bacterial taxonomy and metabarcoding assignments are based, bacteria could bear actual functional traits that are invisible to current attempts to infer ecosystem properties when those are based on ribosomal database-dependent annotation which accounts for sharing of traits among OTUs. In our case, the common functional core is constituted by chemoheterotrophy and aerobic chemoheterotrophy, which represent two general metabolisms in microbial communities. The massive presence of chemoheterotrophy and aerobic chemoheterotrophy reflects the attitude of microbial communities to obtain energy not through carbon fixation but though oxidation, revealing a likely emission of green-house gasses such as CO2 (Zhang et al. 2018; Yu et al. 2021). Thus, semi-natural grasslands managed by humans as pasture or meadow seem to present a high potential of cycling carbon, with also possible emission of CO2. Interestingly, the chemoheterotrophy resulted significantly associated to meadow (Supplementary Material Table S.7), revealing a possible contribution of fertilization or cutting to support C cycling, despite the absence of significant effects of management type and organic C amount. The autotrophic activity of nitrification resulted negatively correlated with the heterotrophic ones of chemoheterotrophy (Supplementary Material Figure S6), in either aerobic or anaerobic conditions, indicating that the presence of organic substrates, not needed by the former, is hierarchically more effective than that of oxygen which is needed only by one of the two heterotrophic metabolisms. The uneven requirements of oxygen with respect to respiration and ammonia oxidation contribute to explain this difference. The extreme pH class was associated to a significantly higher presence of nitrification, ammonia oxidation, and ureolysis, whose larger bars width are also appreciable by the graph, while the lower pH class by animal parasites and symbionts (Fig. 4). The inferred higher levels of those ammonium metabolism-related traits are confirmed by the representation of the source data at taxonomy level (Fig. 4), as those functions can be performed by Crenarchaeota and Myxococcota (Weidler et al. 2008; Langwing et al 2022), and we can observe the evidence of higher occurrence of those phyla at the corresponding pH class, and in particular for the Crenarcheota, at the expense of the Firmicutes share. The coherence of results among correlations, phylum-level taxonomy and function strengthens the concept that functional profiles of ecosystems are mainly defined by the phyla. Thus, phylum diversity can be regarded as a better index for ecosystem functional potential in comparison to OTU diversity, despite the positive and significant correlation between number of total OTUs and functions (Fig.2). A high number of OTUs appears to increase the number of both functions and phyla, but only marginally considering the whole community. The marginal contribution of OTU diversity to functional profiles may be due to the fact that a large fraction of functions is not monophyletic and multiple, coexisting distinct OTUs can perform common functions (Aguilar 2004; Martiny et al. 2015; Louca et al. 2018). The presence of distinct OTUs able to perform shared functions provides an ecosystem buffer against taxonomic diversity variation due to local disturbances, making the entire community performance resistant to impacts of a given extent (Jurburg et al. 2015; Louca et al. 2018). Thus, it is reasonable to uncouple considerations on OTU diversity from microbial functional diversity thanks to the existence of functional redundancy across taxonomy (Louca et al 208). It can be also postulated that the current databases used to extract the functional profiles could likely be more influenced by high taxonomy levels, such as phylum, during the assignation. This could be also partly due to the presence of OTUs that lack lineage annotation as their individuation is based only on a concept of shared sequence similarity higher that 97%, but not on taxonomical recognition, as revealed by the negative correlation between function assignation and total OTUs number (Fig.2 and Supplementary Fig. S6).

The absence of significant effects of management type for both phyla and functions (Supplementary Tables T3 and T6, Fig.3) may be due to a resistance of the proportionally dominant common cores of the community structures to reveal quantitatively the effect of disturbances derived from pastures and meadows. A different hypothesis would be that pastures and meadows would tend to equate and compensate their differences because, in spite of the occurrence of animal excreta as fertilizer for both pastures and meadows , the ‘grazing-like’ mowing practice that is applied to those is the main effector, as it simulates the disturbance of animal presence (Schroder et al. 2011; Liu et al. 2014; Mencel et al. 2022), yielding similar community cores. Moreover, the return of animal excreta could barely shift microbial communities’ composition with respect to those of pastures as the faeces also contain DNA from transient taxa that abound in soil and in the browsed vegetation of the environment where animals spend their time (NandaKafle et al. 2017).

Gene pools analysed by quantitative PCR confirmed the functional and phylum-level effects but with an added resolution level, in that only a part of the N cycle genes, in particular nifH (nitrogen fixation) and nirK and nosZ (intermediate and terminal denitrification steps, respectively) were significantly affected by region, while the nosZ and nirK and AOA (archaeal nitrification) were affected by both pH and organic Carbon. nifH and nosZ were more abundant in Italy, which presented the highest values of pH which, as shown above, has been found compliant to the function and taxonomy proportions of nitrifications and ammonia oxidation with respect to the pH classes found. Thus, denitrification, represented by nirK and nosZ, seems to be favoured by neutral soils than by acidic ones. Also, nitrification, represented by AOA and AOB, showed a similar preference despite the absence of significant region effect. In particular, AOA showed greater variability than AOB, which were more constant as a function of region, pH and organic Carbon. This can be due to the different niche between the archaea (AOA) and bacteria (AOB) where the former prefers more nutrient-limited environments than the latter as the analysis of the ratio between AOB and (AOA+AOB) confirmed in agreement with the positive effect of organic C (Baolan et al. 2014; Sun et al. 2019). Moreover, the negative effect of pH on the AOB proportion seems to indicate AOA as more adapted to neutral soil. AOA are part of Crenarchaeota, which were however reported as present in acid pH condition according to the preference of archaea nitrifiers when comparing neutral vs. acidic soil (Lehtovirta et al.2009, Prosser et al. 2012). Nevertheless, our results show a different scenario with a consistent presence of Crenarchaeota and AOA at neutral pH conditions, revealing the possible presence of this phylum across a wide variety of pH conditions (Figure 4). Thus, the niche diversification between AOA and AOB would not be universal, and AOA have indeed been found at both low and neutral pH (Sun et al 2019). The common trend of AOA and nosZ in relation to pH may reveal a likely interdependency between the two genes, also highlighted by the strong and positive relation between nosZ and AOA (Fig.6). The high potential of ammonia-oxidation may support high rates of nitrification and then the total denitrification.

nifH is instead an indicator for nitrogen fixation, either free-living or symbiotic (Shaffer et al. 2000) and it showed an interesting behaviour as it was significantly affected by region but not by pH and organic carbon, suggesting different environmental conditions able to modify its abundance as possibly vegetation and fertilization, irrespective of the hierarchically dominant ones. nifH resulted to be influenced by plant cover during field restoration (Wang et al. 2017) as possible consequence of interactions between the diazotrophic community, i.e. bacteria able to fix N2, and plant species. Within this context, typical of pastures and meadows, acid soil with high amounts of organic carbon can favour functions different from those tied to N cycling, despite the general microbial communities are not influenced by pH and organic carbon as the constant abundance of 16S genes reveals.

Conclusions

Microbial communities of semi-natural grasslands are characterized by common phyla whose deep-level variability mainly depends on pedological conditions, which have also a significant impact on taxonomy-inferred functions. Such taxonomy-predicted functional profiles showed similar behaviour to the corresponding phylum-level ordination profile, testifying the major influence of high taxonomic levels on the assembly of microbial functions. Pasture or meadow management of grasslands instead tended to affect essentially OTU-level diversity, not causing shifts in the higher taxonomic levels and functional profiles. Management type did not affect N cycle-related functions at single gene level as detectable by quantitative PCR. Thus, at the level of intensification that we examined, human management may affect only marginall y the functional biodiversity of semi-natural grasslands, which can have high resistance to local disturbance due to the functional redundancy within guilds of the biogeochemical elemental cycling. Further studies can be envisaged to involve different environmental variables, such as vegetation, soil bulk density and slope, and different management types and intensities, to investigate the consequent microbial patterns and detect different drivers.

Acknowledgments

We thank all the farmers involved during the sampling and their availability, the association Lofotlam and the Parc naturel régional Livradois-Forez for their support.

Disclosure Statement

No potential conflict of interest was reported by the author(s).

Data Availability Statement

The data of this study are freely available from the corresponding author upon request. The data are not publicly available due to the involvement of private partners (farmers).

Funding

The study was funded by the European RISE Project “HIGHLANDS.3” No. 872328, grant “Fondazione Gini 2019” and Cariparo Foundation

References

Aguilar D, Aviles FX, Querol E, Sternberg MJ. 2004. Analysis of phenetic trees based on metabolic capabilites across the three domains of life. J. Mol. Biol. 340(3): 491-512.

Anderson MJ, Ellingsen KE, McArdle BH. 2006. Multivariate dispersion as a measure of beta diversity. Ecol Lett. 9(6); 683-693.

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology. 26: 32–46.

Apprill A, McNally S, Parsons R, Weber L. 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol 75:129–137. doi: 10.3354/ame01753.

Bahram M, Hildebrand F, Forslund SK, Anderson JL, Soudzilovskaia NA, Bodegom PM, Bengtsson-Palme J, Anslan S, Coelho LP, Harend H, Huerta-Cepas J, Medema MH, Maltz MR, Mundra S, Olsson PA, Pent M, Põlme S, Sunagawa S, Ryberg M, Tedersoo L, Bork P. 2018. Structure and function of the global topsoil microbiome. Nature 560: 233–237.

Bahram M, Hildebrand F, Forslund SK, Anderson JL, Soudzilovskaia NA, Bodegom PM, Bengtsson-Palme J, Anslan S, Coelho LP, Harend H, Huerta-Cepas J, Medema MH, Maltz MR, Mundra S, Olsson PA, Pent M, Polme S, Sunagawa S, Ryberg M, Tedersoo L, Bork P. 2018. Structure and function of the global topsoil microbiome. Nature. 560(7717): 233-237.

Bai Y, Cotrufo M. F. 2022. Grassland soil carbon sequestration: Current understanding, challenges, and solutions. Science. 377(6606): 603-608.

Baolan H, Shuai L, Wei W, Lidong S, Liping L, Weiping L, ... & Ping Z. 2014. pH-dominated niche segregation of ammonia-oxidising microorganisms in Chinese agricultural soils. FEMS Microbiol Ecol. 90(1): 290-299.

Baolan H, Shuai L, Wei W, Lidong S, Liping L, Weiping L, Guangming T, Xiangyang X, Ping Z. 2014. pH-dominated niche segregation of ammonia-oxidising microorganisms in Chinese agricultural soils. FEMS Microbiol Ecol. 90(1): 290-299.

Bates D, Machler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. J Stat Softw. 67(1):1–48.

Bunce RGH, Pérez-Soba M, Jongman RH, Gómez Sal A, Herzog F, Austad I. 2004. Transhumance and biodiversity in european mountains. IALE publication series nr 1, Wageningen UR

Burczyk P, Gamrat R, Gałczyńska M, Saran E. 2018. The role of grasslands in providing ecological sustainability of the natural environment. Woda Środowisko Obszary Wiejskie. 18(63): 21-37.

Canfield DE, Glazer AN, Falkowski PG. 2010. The evolution and future of Earth's nitrogen cycle. Science. 330(6001): 192-196.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Gonzalez Pena A, Goodrich JK, Gordon J, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald, D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zeneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. methods. 7(5): 335-336.

Cavicchioli R, Ripple WJ, Timmis KN, Azam F, Bakken LR, Baylis M, Behrenfeld MJ, Boetius A, Boyd PW, Classen AT, Crowther TW, Danovaro R, Foreman CM, Huisman J, Hutchins DA, Jansson JK, Karl DM, Koskella B, Mark Welch DB, Martiny JBH, Moran MA, Orphan VJ, Reay DS, Remais JV, Rich VI, Singh BK, Stein LY, Stewart FJ, Sullivan MB, van Oppen MJH, Weaver SC, Webb EA, Webster NS. 2019. Scientists' warning to humanity: microorganisms and climate change. Nat. Rev. Microbiol. 17: 569–586.

Chadwick DR, Cardenas LM, Dhanoa MS, Donovan N, Misselbrook T, Williams JR, Rees RM. 2018. The contribution of cattle urine and dung to nitrous oxide emissions: Quantification of country specific emission factors and implications for national inventories. Sci. Total Environ. 635: 607-617.

Chang J, Ciais P, Gasser T, Smith P, Herrero M, Havlìk P, Obersteiner M, Guenet B, Goll D.S, Li W, Naipal V, Peng S, Qiu C, Tian H, Viovy N, Yue C, Zhu D. 2021. Climate warming from managed grasslands cancels the cooling effect of carbon sinks in sparsely grazed and natural grasslands. Nat Commun 12: 118.

Chroňáková A, Radl V, Čuhel J, Šimek M, Elhottová D, Engel M, Schloter M. 2009. Overwintering management on upland pasture causes shifts in an abundance of denitrifying microbial communities, their activity and N_2O-reducing ability. Soil Biol. Biochem. 41: 1132–1138.

Clark MA, Moran NA, Baumann P. 1999. Sequence evolution in bacterial endosymbionts having extreme base composition. Mol. Biol. Evol. 16 :1586-1598.

Clarke KR. 1993. Nonparametric multivariate analyses of changes in community structure. Austral Ecol. 18:117–143.

David LA, Alm EJ. 2011. Rapid evolutionary innovation during an Archaean genetic expansion. Nature. 469(7328): 93-96.

De Caceres M, Jansen F, De Caceres M. M. 2016. Package ‘indicspecies’. Indicators. 8(1).

Dexter E, Rollwagen-Bollens G, Bollens SM. 2018. The trouble with stress: A flexible method for the evaluation of nonmetric multidimensional scaling. Limnol Oceanogr Meth. 16:434–43.

Dixon P. 2003. VEGAN, a package of R functions for community ecology. Journal of Vegetation Science. 14(6): 927-930.

Dong S, Li Y, Ganjurjav H, Gao Q, Gao X, Zhang J, Li S. 2020. Grazing promoted soil microbial functional genes for regulating C and N cycling in alpine meadow of the Qinghai-Tibetan Plateau. Agric Ecosyst Environ. 303: 107111.

Du Y, Shu K, Guo X, Pengjin Z. 2019. Moderate Grazing Promotes Grassland Nitrous Oxide Emission by Increasing Ammonia-Oxidizing Archaea Abundance on the Tibetan Plateau. Curr. Microbiol. 76: 620–625.

Dufrene M, Legendre P. 1997. Species Assemblages and Indicator Species: The Need for a Flexible Asymmetrical Approach. Ecological Monographs. 67: 345-366.

Falkowski PG, Fenchel T, Delong EF. 2008. The microbial engines that drive Earth's biogeochemical cycles. Science. 320(5879): 1034-1039.

Fierer N. 2017. Embracing the unknown: Disentangling the complexities of the soil microbiome. Nat. Rev. Microbiol. 15: 579–590.

Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proc. Natl. Acad. Sci. USA 102:14683–14688.

Griffin DW, Kellogg CA, Garrison VH, Shinn EA. 2002. The global transport of dust: an intercontinental river of dust, microorganisms and toxic chemicals flows through the Earth's atmosphere. Am. Sci. 90(3): 228-235.

Henry S, Baudoin E, Lopez-Gutiérrez JC, Martin-Laurent F, Brauman A. 2004. Quantification of denitrifying bacteria in soils by *nirK* gene targeted real-time PCR. J. Microbiol. Met. 59: 327–335.

Johnson JB, Omland KS. 2004. Model selection in ecology and evolution. Trends Ecol. Evol. 19(2):

101-108.

Jurburg SD, Salles JF. Biodiversity in Ecosystems - Linking Structure and Function (eds Lo, Y.-H., Blanco, J. A. & Roy, S.) Ch. 2, 29–49 (INTECH, 2015).

Kuypers MMM, Marchant HK, Kartal B. 2018. The microbial nitrogen-cycling network. Nat. Rev. Microbiol. 16: 263–276.

Langwig MV, De Anda V, Dombrowski N, Seitz KW, Rambo IM, Greening C, Teske AP, Baker BJ. 2022. Large-scale protein level comparison of Deltaproteobacteria reveals cohesive metabolic groups. The ISME Journal. 16(1): 307-320.

Leigh MB, Pellizari VH, Uhlìk O, Sutka R, Rodrigues J, Ostrom NE, Zhou J, Tiedje JM. 2007. Biphenyl-utilizing bacteria and their functional genes in a pine root zone contaminated with polychlorinated biphenyls (PCBs). The ISME Journal (1): 134–148.

Liu X, Zhou J, Li W, Xu J, Brookes P.C. 2014. The combined effects of urea application and simulated acid rain on soil acidification and microbial community structure. Environ. Sci. Pollut. Res. 21(10): 6623-6631.

Lomba A, Guerra C, Alonso J, Honrado J. P, Jongman R, McCracken D. 2014. Mapping and monitoring high nature value farmlands: challenges in European landscapes. J. Environ. Manage. 143: 140-150.

Louca S, Parfrey L.W, Doebeli M. 2016. Decoupling function and taxonomy in the global ocean microbiome. Science 353:1272-1277.

Louca S, Polz MF, Mazel F, Albright MBN, Huber JA, O'Connor MI, Ackermann M, Hahn AS, Srivastava DS, Crowe SA, Doebeli M, Parfrey LW. 2018. Function and functional redundancy in microbial systems. Nat. Ecol. Evol. 2: 936–943.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27: 209–220.

Maron PA, Sarr A, Kaisermann A, Lévêque J, Mathieu O, Guigue J, Karimi B, Bernard L, Dequiedt S, Terrat S, Chabbi A, Ranjard L. 2018. High microbial diversity promotes soil ecosystem functioning. Appl. Environ. Microbiol. 84: 1–13.

Martiny JB, Jones SE, Lennon JT, Martiny AC. 2015. Microbiomes in light of traits: a phylogenetic perspective. Science. 350(6261): aac9323.

Mencel J, Mocek-Płóciniak A, Kryszak A. 2022. Soil Microbial Community and Enzymatic Activity of Grasslands under Different Use Practices: A Review. Agronomy. 12(5): 1136.

Moonen AC, Bàrberi P. 2008. Functional biodiversity: An agroecosystem approach. Agric. Ecosyst. Environ. 127: 7–21.

Morris JJ., Lenski RE, Zinser ER. 2012. The Black Queen Hypothesis: evolution of dependencies through adaptive gene loss. MBio. 3(2): e00036-12.

Muñoz Sabater J. 2019. ERA5-Land monthly averaged data from 1981 to present. Copernicus Climate Change Service (C3S) Climate Data Store (CDS)

NandaKafle G, Seale T, Flint T, Nepal M, Venter S.N, Brözel V.S. 2017. Distribution of diverse Escherichia coli between cattle and pasture. Microbes Environ. 32: 226–233

Parada AE, Needham DM, Fuhrman JA. 2015. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol. 18(5): 1403-1414.

Prosser, J. I., & Nicol, G. W. (2012). Archaeal and bacterial ammonia-oxidisers in soil: the quest for niche specialisation and differentiation. Trends in microbiology, 20(11), 523-531.

Qu T, Du W, Yuan X, Yang Z, Liu D, Wang D, Yu L. 2016. Impacts of Grazing Intensity and Plant Community Composition on Soil Bacterial Community Diversity in a Steppe Grassland. PLoS One. 11: e0159680.

R Core Team. 2016. R: A language and environment for statistical computing. Vienna, Austria: The R Foundation for Statistical Computing

Rocca JD, Hall EK, Lennon JT, Evans SE, Waldrop MP, Cotner JB, Nemergut DR, Graham EB, Wallenstein MD. 2015. Relationships between protein-encoding gene abundance and corresponding process are commonly assumed yet rarely observed. ISME J. 9: 1693–1699.

Rosch C, Mergel A, Bothe H. 2002. Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. Appl. Environ. Microbiol. 68: 3818–3829

Rotthauwe JH, Witzel KP, Liesack W. 1997. The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microbiol. 63: 4704–4712

Rubel F, Kottek M. 2010. Observed and projected climate shifts 1901-2100 depicted by world maps of the Köppen-Geiger climate classification. Meteorol. Z. 19: 135-141

Schils RLM, Bufe C, Rhymer CM, Francksen RM, Klaus VH, Abdalla M, Milazzo F, Lellei-Kovács E, ten Berge H, Bertora C, Chodkiewicz A, Dămătîrcă C, Feigenwinter I, Fernández-Rebollo P, Ghiasi S, Hejduk S, Hiron M, Janicka M, Pellaton R, Smith KE, Thorman R, Vanwalleghem T, Williams J, Zavattaro L, Kampen J, Derkx R, Smith P, Whittingham MJ, Buchmann N, Newell Price JW. 2022. Permanent grasslands in Europe: Land use change and intensification decrease their multifunctionality. Agric. Ecosyst. Environ. 330(November 2021):107891

Schroder JL, Zhang H, Girma K, Raun WR, Penn CJ, Payton ME. 2011. Soil acidification from long-term use of nitrogen fertilizers on winter wheat. Soil Sci Soc Am J. 75(3): 957-964.

Shaffer BT, Widmer F, Porteous LA, Seidler RJ. 2000. Temporal and spatial distribution of the *nifH* gene of N2 fixing bacteria in forests and clearcuts in western Oregon. Microbial Ecol. 39(1): 12-21.

Smith DJ, Jaffe DA, Birmele MN, Griffin DW, Schuerger AC, Hee J, Roberts MS. 2012. Free tropospheric transport of microorganisms from Asia to North America. Microbial Ecol. 64(4): 973-985.

Sun R, Myrold DD, Wang D, Guo X, Chu H. 2019. AOA and AOB communities respond differently to changes of soil pH under long-term fertilization. Soil Ecol Lett. 1(3): 126-135.

Sun, R., Myrold, D. D., Wang, D., Guo, X., & Chu, H. (2019). AOA and AOB communities respond differently to changes of soil pH under long-term fertilization. Soil Ecology Letters, 1(3), 126-135.

Uleber E, Hanssen-Bauer I, van Oort B, Dalmannsdottir S. 2014. Impact of climate change on agriculture in Northern Norway and potential strategies for adaptation. Clim Chang. 122(1): 27-39.

Wang H, Deng N, Wu D, Hu S. 2017. Quantitative response relationships between net nitrogen transformation rates and nitrogen functional genes during artificial vegetation restoration following agricultural abandonment. Sci. Rep. 7(1): 1-8.

Wang Z, Jiang S, Struik P.C, Wang H, Jin K, Wu R, Na R, Mu H, Ta N. 2022. Plant and soil responses to grazing intensity drive changes in the soil microbiome in a desert steppe. Plant and Soil. 1-19.

Warton DI, Wright ST, Wang Y. 2012. Distance-based multivariate analyses confound location and dispersion effects. Methods Ecol. Evol. 3(1): 89-101.

Weidler GW, Gerbl FW, Stan-Lotter H. 2008. Crenarchaeota and their role in the nitrogen cycle in a subsurface radioactive thermal spring in the Austrian Central Alps. Appl. Environ. Microbiol. 74(19): 5934-5942.

Wheeler R. E, Torchiano M. 2010. Permutation tests for linear models in R. The Comprehensive R Archive Network. 1(2).

Woese CR. 1987. Bacterial evolution. Microbiol. Rev. 51: 221–271.

Wood S. 2017. Generalized Additive Models: An Introduction with R. 2nd ed. Chapman and Hall/CRC.

Yin M, Gao X, Tenuta M, Li L, Gui D, Li X, Zeng F. 2020. Enhancement of N2O emissions by grazing is related to soil physicochemical characteristics rather than nitrifier and denitrifier abundances in alpine grassland. Geoderma 375: 114511.

Yu Y, Liu L, Wang J, Zhang Y, Xiao C. 2021. Effects of warming on the bacterial community and its function in a temperate steppe. Sci. Total Environ. 792: 148409.

Zhang X, Hu BX, Ren H, Zhang J. 2018. Composition and functional diversity of microbial community across a mangrove-inhabited mudflat as revealed by 16S rDNA gene sequences. Sci. Total Environ. 633: 518-528.

Zhong L, Bowatte S, Newton PCD, Hoogendoorn CJ, Li FY, Wang Y, Luo D. 2015. Soil N cycling processes in a pasture after the cessation of grazing and CO2 enrichment. Geoderma. 259–260: 62–70.

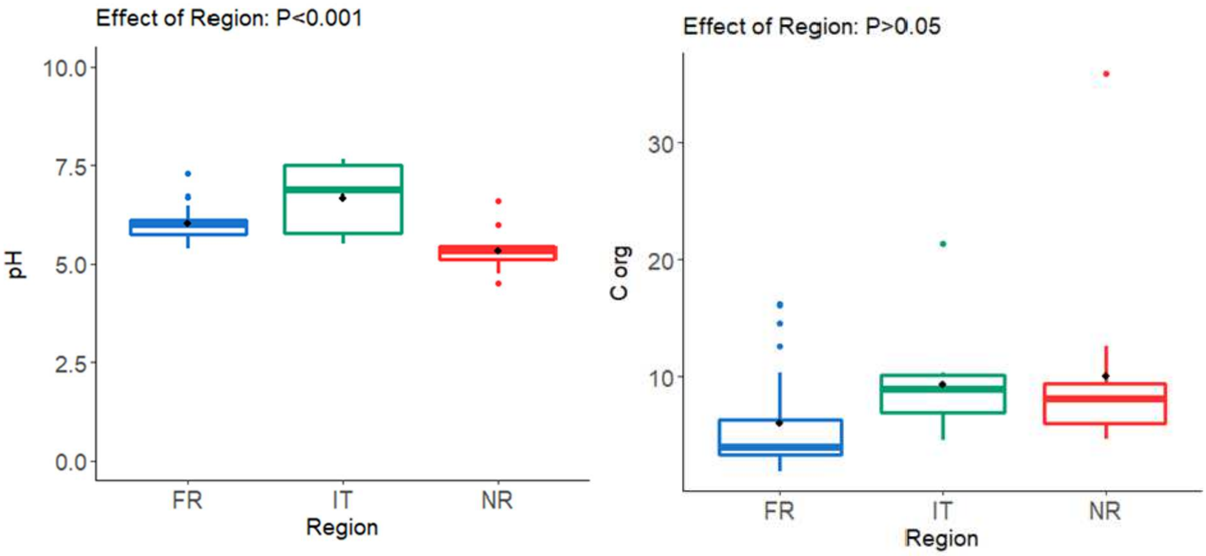
Zhou J, Xue K, Xie J, Deng Y, Wu L, Cheng X, Fei S, Deng S, He Z, Van Nostrand JD, Luo Y. 2012. Microbial mediation of carbon-cycle feedbacks to climate warming. Nat. Clim. Chang. 2: 106–110.

Supplementary Material

Tab.S.1 Results of permutation ANOVA on pH and C org as function of region.

	pH		C org	
	Estimate	Pr(Prob)	Estimate	Pr(Prob)
Management type M	0.11	0.34	0.37	0.80
Region IT	0.64	<0.001	0.71	1
Region FR	0.0047	0.29	-2.54	0.054
Management type M: region IT	0.048	0.78	-1.57	0.21
Management type M: region FR	-0.16	0.30	-0.63	0.43
Residual	0.65		6.14	
R^2	0.43		0.15	

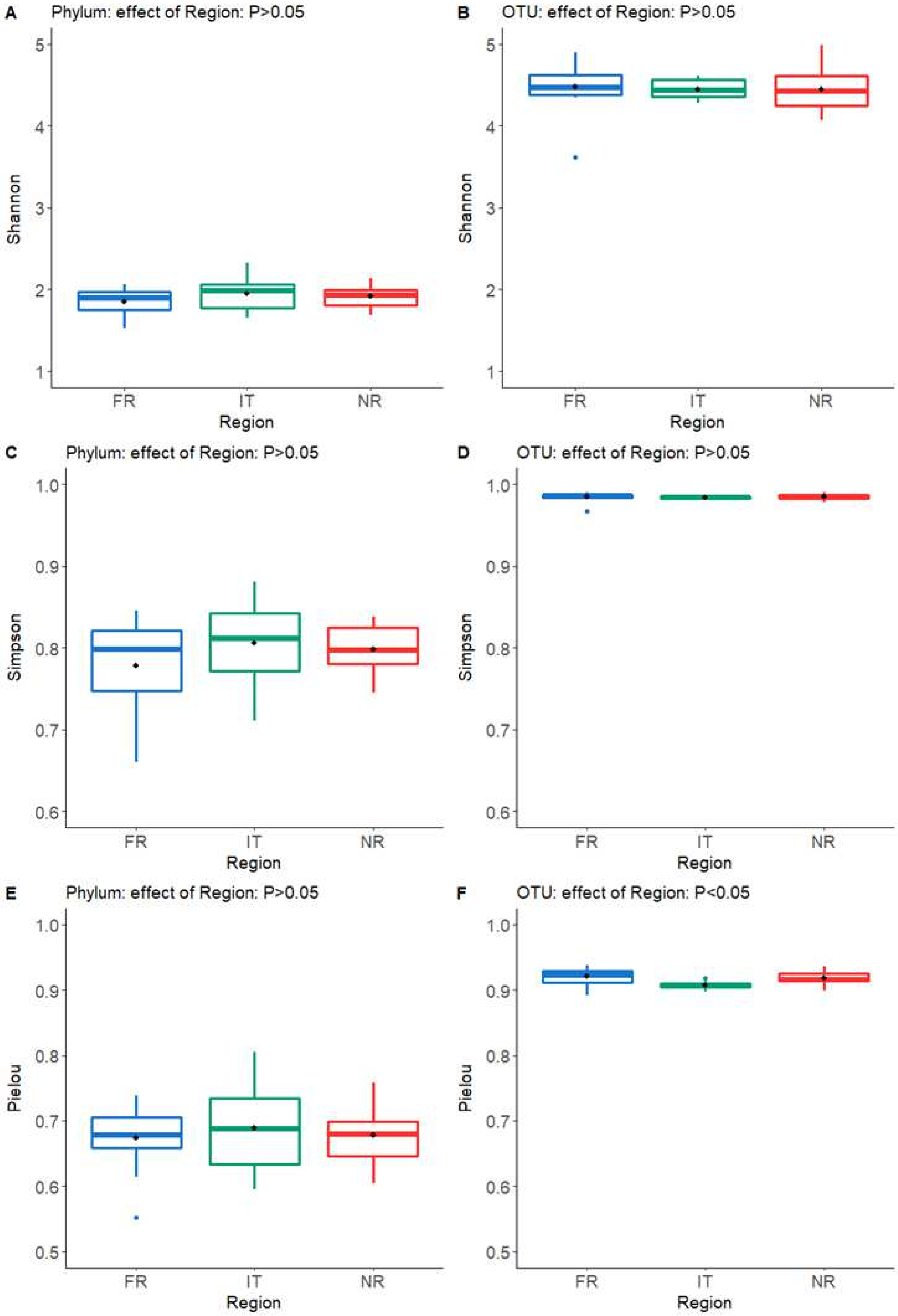
Fig.S.1 Boxplot of pH and C org as function of region. The black dot represents the mean. The significance values of the factor where obtained from the permutation ANOVA reported in table S.1.



Tab.S.2 Result of Permutation ANOVA of Shannon, Simpson and Pielou indices as function on 2-way interaction between type and region, pH class and C org class. The pH class presents 4 levels based on quartiles: “1” from 4.5 to 5.44, “2” from 5.44 excluded to 5.85, “3” from 5.85 excluded to 6.42 and “4” from 6.42 excluded to 7.65. The C org class presents 4 levels based on quartiles: “1” from 1.95 to 4.67, “2” from 4.67 excluded to 6.92, “3” from 6.92 excluded to 9.78 and “4” from 9.78 excluded to 35.89. The statistical significance is expressed with *** for p-value<0.001, ** for p-value<0.01 and * for p-value<0.05.

	Shannon						Simpson						Pielou					
	Phylum			OTU			Phylum			OTU			Phylum			OTU		
	Estimate	Prob		Estimate	Prob		Estimate	Prob		Estimate	Prob		Estimate	Prob		Estimate	Prob	
management type P	0.012	0.69		0.032	0.67		0.0062	0.62		0.0004	0.84		-0.01	0.28		0.0003	0.56	
Region FR	-0.058	0.17		0.023	0.94		-0.016	0.15		0.0004	0.49		-0.01	1		0.0051	0.1	
Region IT	0.042	0.37		-0.013	0.94		0.011	0.38		-0.0002	1		0.01	0.46		-0.008	0.01	*
Management type P: region FR	0.033	0.88		0.101	0.84		0.012	0.28		0.002	0.12		0.02	0.28		0.005	0.69	
Management type P: region IT	-0.058	0.11		-0.1	0.13		-0.0093	0.39		-0.001	0.2		-0.01	0.23		-0.0025	0.38	
R^2	0.17			0.16			0.048			0.15			0.053			0.01		
Residual	0.13			0.24			0.13			0.004			0.078			0.33		
pH class1	-0.042	0.016	*	-0.073	0.71		-0.01	0.043	*	-8.65E-04	0.94		0.0018	0.11		0.0048	0.0074	**
pH class2	-0.091	0.014	*	0.043	0.23		-0.03	0.0054	**	1.85E-05	0.92		-0.03	0.0088	**	0.001	0.52	
pH class3	-0.0023	0.88		0.059	0.41		0.00041	0.88		1.25E-03	0.32		-0.0055	0.65		0.0033	0.32	
Residual	0.15			0.25			0.043			0.004			0.047			0.01		
R^2	0.27			0.048			0.26			0.034			0.21			0.22		
AIC	-30.85			8.04			-125.46			-302.069			-118.07			-230.84		
BIC	-22.66			16.228			-117.27			-293.88			-109.88			-222.65		
C org class1	-0.045	0.34		0.07	0.36		-0.007	0.11		1.50E-03	0.084		-0.013	0.64		0.0049	0.43	
C org class2	0.14	0.0008	***	0.017	0.78		0.037	0.002	**	1.60E-04	0.84		0.028	0.04	*	-0.0046	0.13	
C org class3	0.024	0.92		-0.003	0.94		0.01	0.78		7.00E-05	0.84		0.008	0.94		-0.0001	0.96	
Residual	0.14			0.25			0.04			0.0041			0.049			0.011		
R^2	0.31			0.052			0.35			0.083			0.14			0.079		
AIC	-33.13			7.917			-130.89			-304.061			-115.12			-224.08		
BIC	-24.94			16.105			-122.7			-295.873			-106.94			-215.89		

Fig.S.2 Boxplot of Shannon (panels A and B), Simpson (panels C and D) and Pielou (panels E and F) indices where the left column corresponds to Phylum and right column to OTU. The black dot represents the mean. The significance values of the factor where obtained from the permutation ANOVA reported in table S.2.

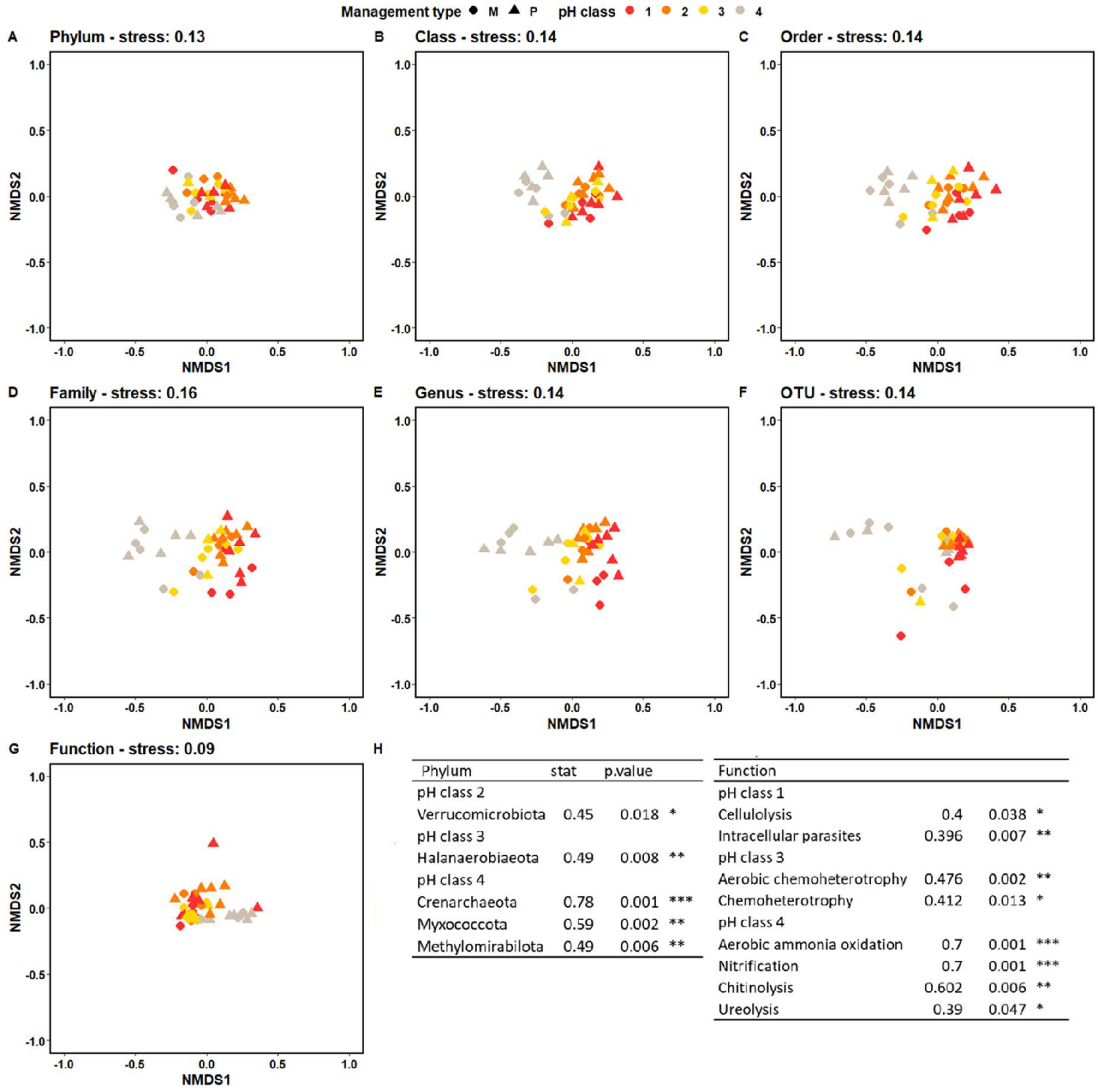


Tab.S.3

Results of ANOSIM at phylum, OTU and Function rank levels as function of region, management type, pH class and C org class. The R is the ratio of mean of ranked dissimilarities between groups to the mean of ranked dissimilarities within groups. An R value close to 0 suggests distribution of high and low ranks within and between groups, while a value close to 1 suggests dissimilarity between groups. Negative R values suggest greater dissimilarities within groups than between groups. The statistical significance is expressed with *** for p-value<0.001, ** for p-value<0.01 and * for p-value<0.05.

	Phylum			OTU			Function		
	R	p-value		R	p-value		R	p-value	
region	0.16	0.01	**	0.28	1.00E-04	***	0.24	5.00E-04	***
management type	0.06	0.07		0.07	0.03	*	0.03	0.18	
pH class	0.20	0.001	**	0.19	3.00E-04	***	0.29	1.00E-04	***
C_org class	0.13	0.01	**	0.11	0.003	**	-0.04	0.82	

Fig.S.3 NMDS of taxonomic profiles at different ranks levels and functions (panel A – G) as a function of pH class and management type. The stress value is reported for each ranks level. The pH class presents 4 levels based on quartiles (pH class: 4.5<"1"≤5.4; 5.4<"2" ≤5.8; 5.8<"3" ≤6.4; 6.4<"4" ≤7.6). The most associated phyla and functions to pH class were reported (panel H).



Phylum	stat	p.value	
pH class 2			
Verrucomicrobiota	0.45	0.018	*
pH class 3			
Halanaerobiaeota	0.49	0.008	**
pH class 4			
Crenarchaeota	0.78	0.001	***
Myxococcota	0.59	0.002	**
Methylomirabilota	0.49	0.006	**

Function			
pH class 1			
Cellulolysis	0.4	0.038	*
Intracellular parasites	0.396	0.007	**
pH class 3			
Aerobic chemoheterotrophy	0.476	0.002	**
Chemoheterotrophy	0.412	0.013	*
pH class 4			
Aerobic ammonia oxidation	0.7	0.001	***
Nitrification	0.7	0.001	***
Chitinolysis	0.602	0.006	**
Ureolysis	0.39	0.047	*

Tab.S.4 Numbers of selected units from Indicator Species Analysis at phylum and OTUs rank levels and function as function of region and pH class. The pH class presents 4 levels based on quartiles as the C org class (pH class: 4.5<“1”≤5.4; 5.4<”2” ≤5.8; 5.8<”3” ≤6.4; 6.4<”4” ≤7.6 – C org class: 1.9<“1”≤4.7; 4.7<”2” ≤6.9; 6.9<”3” ≤9.8; 9.8<”4” ≤35.9).

	OTU	Phylum	Function
Total units	3323	44	44
Selected units	70	9	4
Region FR	19		
Region IT	31	4	2
Region NR	20	5	2
Selected units	14	1	3
Mangement type M	4	1	3
Mangement type P	10		
Selected unit	60	5	8
pH class 1	10		2
pH class 2	8	1	
pH class 3	16	1	2
pH class 4	26	3	4
Selected units	30	5	1
C org class 1	14		
C org class 2	1	1	1
C org class 3	3	1	
C org class 4	12	3	

Tab.S.5 Indicator units (phyla first section of table and functions bottom) resulted from Indicator Analysis as function of region, management type and pH class. The pH class presents 4 levels based on quartiles as the C org class (pH class: 4.5<"1"≤5.4; 5.4<"2" ≤5.8; 5.8<"3" ≤6.4; 6.4<"4" ≤7.6 – C org class: 1.9<"1"≤4.7; 4.7<"2" ≤6.9; 6.9<"3" ≤9.8; 9.8<"4" ≤35.9). The stat value corresponds to the association strength to the group, values close to 1 suggest strong association while values close to 0 suggest weak association. The statistical significance is expressed with *** for p-value<0.001, ** for p-value<0.01 and * for p-value<0.05.

		Factor	stat	p.value	
Phylum	Crenarchaeota	Region IT	0.56	0.004	**
	Myxococcota	Region IT	0.51	0.003	**
	Methylomirabilota	Region IT	0.5	0.002	**
	Fibrobacterota	Region IT	0.41	0.026	*
	Dependentiae	Region NR	0.55	0.002	**
	Patescibacteria	Region NR	0.51	0.002	**
	Proteobacteria	Region NR	0.44	0.013	*
	RCP2.54	Region NR	0.39	0.041	*
	Euryarchaeota	Region NR	0.31	0.032	*
	Cyanobacteria	Management type M	0.46	0.001	***
	Verrucomicrobiota	pH class 2	0.45	0.018	*
	Halanaerobiaeota	pH class 3	0.49	0.008	**
	Crenarchaeota	pH class 4	0.78	0.001	***
	Myxococcota	pH class 4	0.59	0.002	**
	Methylomirabilota	pH class 4	0.49	0.006	**
	Actinobacteriota	C org class 2	0.43	0.012	*
	Firmicutes	C org class 4	0.04	0.026	*
Function	aerobic_ammonia_oxidation	Region IT	0.50	0.004	**
	nitrification	Region IT	0.50	0.004	**
	intracellular_parasites	Region NR	0.34	0.011	*
	dark_hydrogen_oxidation	Region NR	0.34	0.007	**
	Fermentation	Management type M	0.44	0.01	**
	chloroplasts	Management type M	0.41	0.002	**
	chemoheterotrophy	Management type M	0.36	0.02	*
	cellulolysis	pH class 1	0.4	0.038	*
	intracellular_parasites	pH class 1	0.396	0.007	**
	aerobic_chemoheterotrophy	pH class 3	0.476	0.002	**
	chemoheterotrophy	pH class 3	0.412	0.013	*
	aerobic_ammonia_oxidation	pH class 4	0.7	0.001	***
	nitrification	pH class 4	0.7	0.001	***
	chitinolysis	pH class 4	0.602	0.006	**
	ureolysis	pH class 4	0.39	0.047	*
	aromatic_compound_degradation	C org class 2	0.43	0.016	*

Tab.S.6 Results of PERMANOVA at phylum and OTU rank levels and function combined with Pairwise comparison for significant factors. Significant threshold was set at 0.05. Dispersion corresponds to the p-value of beta dispersion on Bray-Curtis dissimilarities and single factors. The statistical significance is expressed with *** for p-value<0.001, ** for p-value<0.01 and * for p-value<0.05

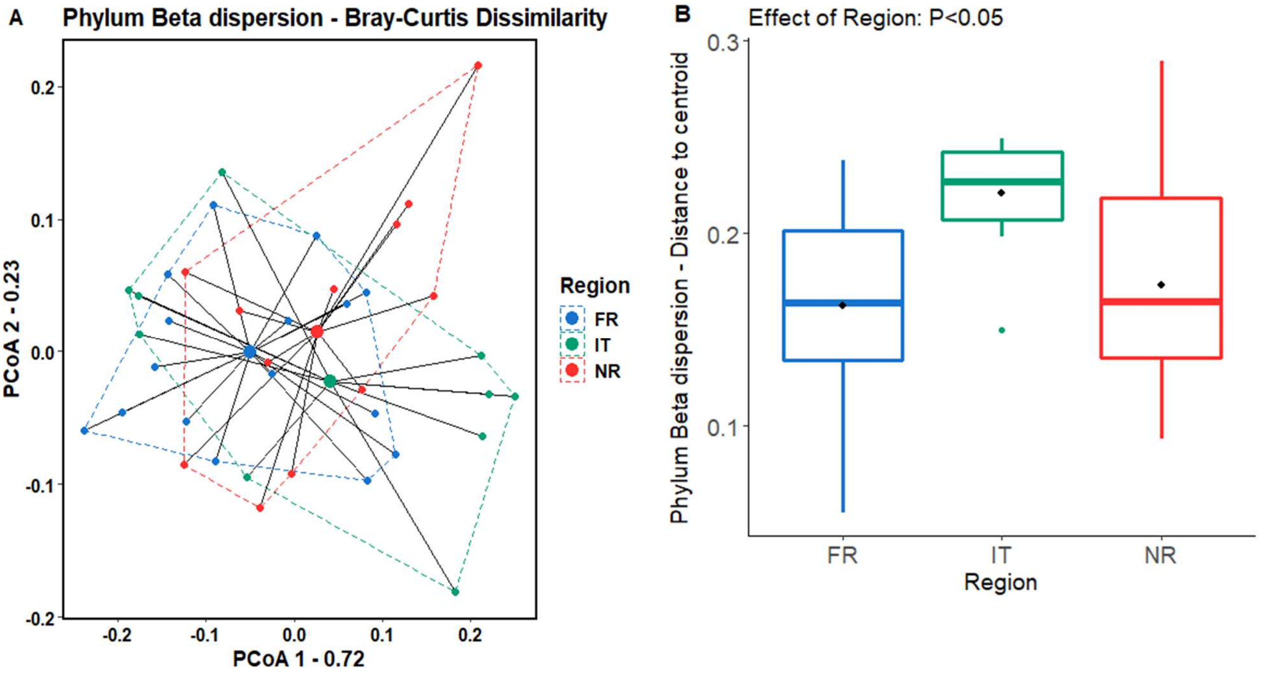
	Phylum					OTU					Function				
	Df	R^2	F	Pr(>F)	Dispersion	Df	R^2	F	Pr(>F)	Dispersion	Df	R^2	F	Pr(>F)	Dispersion
Management type	1	0.06	2.47	0.05 .	0.53	1	0.03	1.37	0.04 *	0.06	1	0.05	2.35	0.07 .	0.06
Region	2	0.11	2.31	0.04 *	0.01 *	2	0.1	2.09	0.001 **	0.13	2	0.18	3.84	0.002 **	0.67
Management type: Region	2	0.03	0.61	0.79		2	0.05	1.04	0.31		2	0.04	0.81	0.58	
Residual	32	0.79				32	0.81				32	0.73			
Total	37	1				37	1				37	1			
pH class	1	0.12	4.97	0.007 **	0.66	1	0.06	2.21	0.002 **	0.07	1	0.16	7.01	0.002 **	0.36
Residual	36	0.88				36	0.94				36	0.84			
Total	37	1				37	1				37	1			
C org class	1	0.04	1.69	0.14	0.22	1	0.04	1.67	0.01 *	0.05 .	1	0.02	0.8	0.48	
Residual	36	0.96				36	0.96				36	0.98			
Total	37	1				37	1				37	1			

Pairwise							
	Phylum	OTU	Function		Phylum	OTU	Function
FR-IT	0.069 .	0.015 *	0.01 **	C org 1-2		0.035 *	
FR-NR	0.068 .	0.001 ***	0.215	C org 1-3		0.04 *	
NR-IT	0.121	0.002 **	0.016 *	C org 1-4		0.001 ***	
pH 1-2	0.089 .	0.008 **	0.292	C org 2-3		0.683	
pH 1-3	0.506	0.014 *	0.191	C org 2-4		0.009 **	
pH 1-4	0.007 **	0.001 ***	0.001 ***	C org 3-4		0.029 *	
pH 2-3	0.248	0.197	0.042 *				
pH 2-4	0.004 **	0.001 ***	0.002 **				

Tab.S.7 Result of ANOVA on distances to centroids obtained from permutation test of Beta dispersion as function of region. The reference level corresponds to FR.

	Estimate	Std.Error	t-value	Pr(>|t|)	
(Intercept)	0.16247	0.01232	13.184	3.88E-15	***
IT	0.0581	0.01987	2.924	0.00603	**
NR	0.01062	0.01882	0.564	0.57639	
Residual error	0.049				
R^2	0.2				

Fig.S.4 PCoA of Beta dispersion at phylum rank level based on Bray-Curtis dissimilarity as function of region (panel A) and boxplot of distances to centroid at phylum rank level as function of region where black dots rapresent the means (panel B). The significant effect of factor where obtain from a parametric ANOVA and reported in table S.8.



Tab.S.8 Result of Mantel Test on Bray-Curtis dissimilarity matrix of phylum and OTU rank levels and function as function of pH and C org. The Mantel r is a correlation measure which ranges between -1 and 1. An r value of 1 suggests a strong positive relationship, 0 suggests absence of relationship and -1 suggests a strong negative relationship. The statistical significance is expressed with *** for p-value<0.001, ** for p-value<0.01 and * for p-value<0.05.

	Phylum			OTU			Function		
	r	Significance		r	Significance		r	Significance	
pH	0.39	1.00E-04	***	0.48	1.00E-04	***	0.37	1.00E-04	***
C org	0.093	0.10		0.051	0.29		-0.0024	0.47	

Fig.S.5 Scatterplot of Bray-Curtis dissimilarity at phylum (panels A and B) and OTU (panels C and D) rank level and function (panels E and F) as function of difference in pH (left column) and C org (right column) expressed as Euclidean distance with linear trend. The significant effects were estimated by Mantel Test and reported in table S.8.

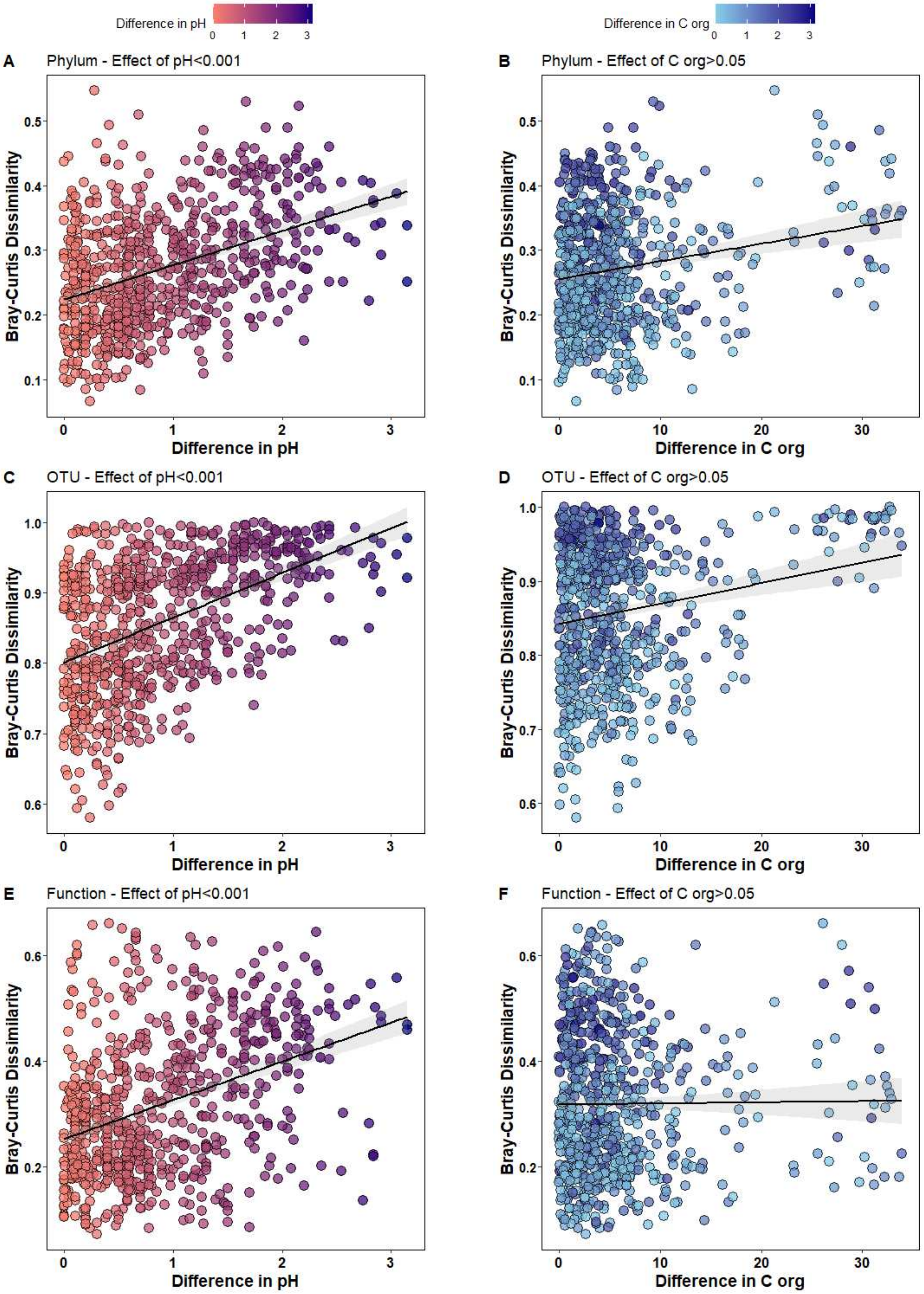
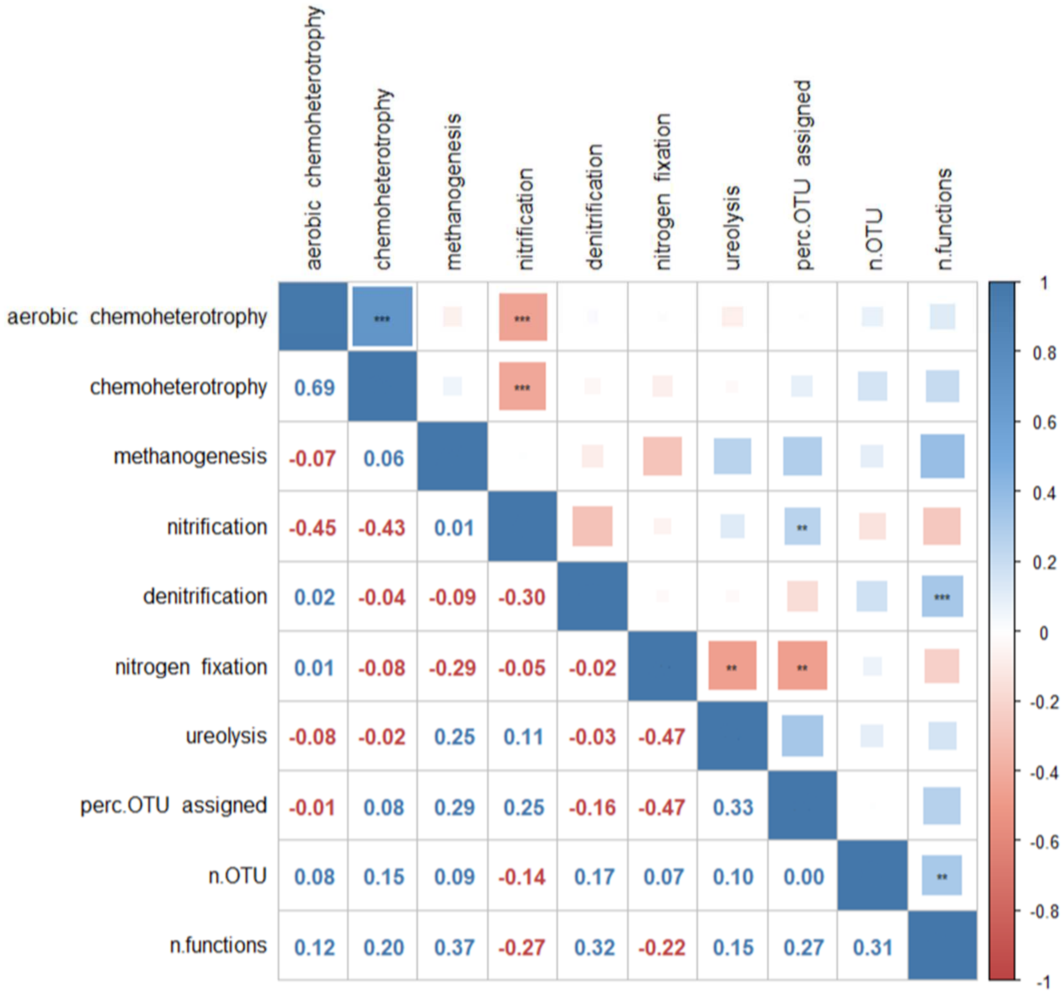


Fig.S.6 Plot of correlations among selected functions (aerobic chemoheterotrophy, chemoheterotrophy, nitrification, denitrification, nitrogen fixation, ureolysis), percentage of OTU assigned by FAPROTAX (perc.OTU assigned), total number of OTUs (n.OTU) and total number of functions (n.functions) considering all sites

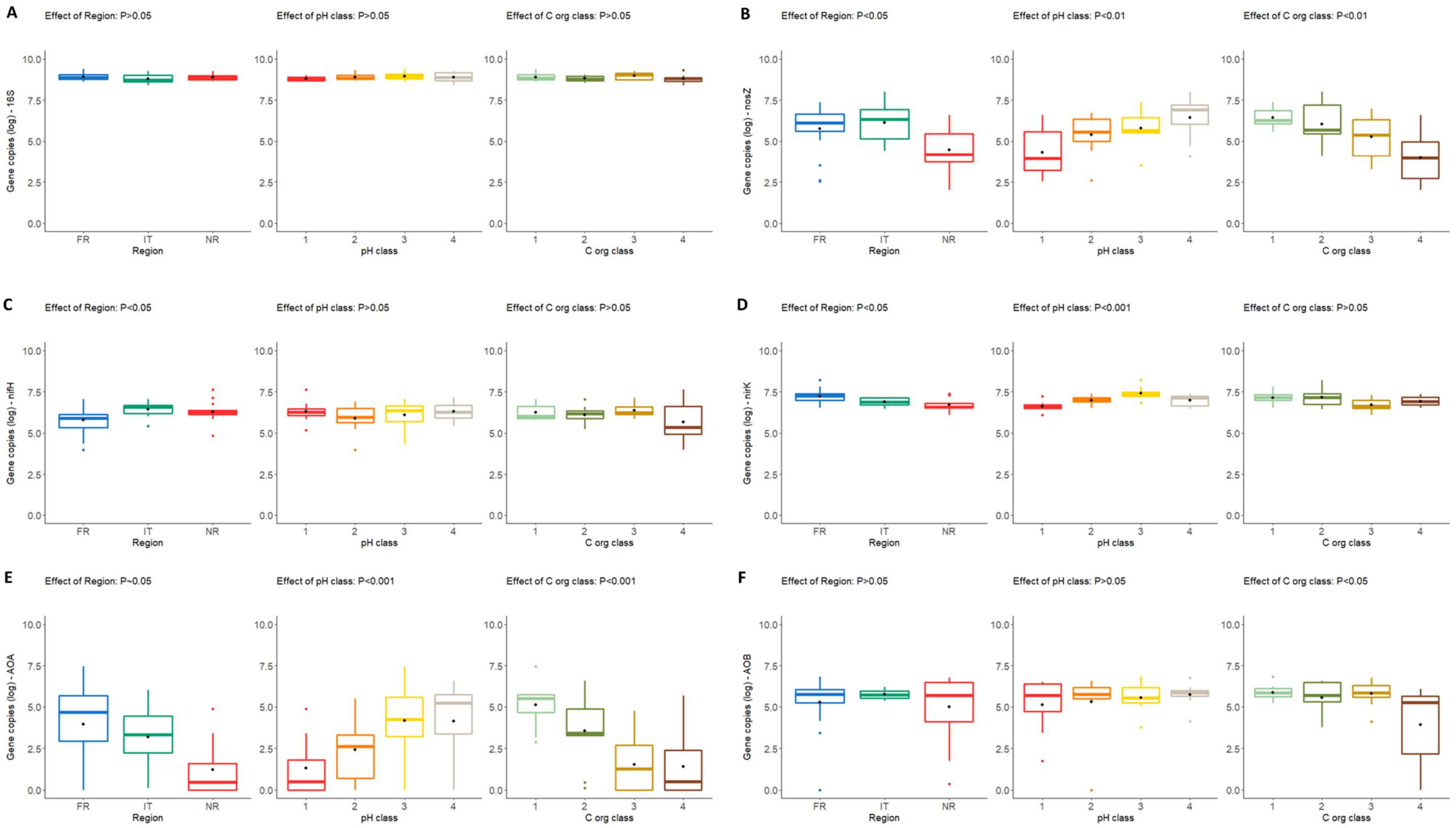


	aerobic chemoheterotrophy	chemoheterotrophy	methanogenesis	nitrification	denitrification	nitrogen fixation	ureolysis	perc.OTU assigned	n.OTU	n.functions
aerobic chemoheterotrophy		***		***						
chemoheterotrophy	0.69			***						
methanogenesis	-0.07	0.06								
nitrification	-0.45	-0.43	0.01					**		
denitrification	0.02	-0.04	-0.09	-0.30						***
nitrogen fixation	0.01	-0.08	-0.29	-0.05	-0.02		**	**		
ureolysis	-0.08	-0.02	0.25	0.11	-0.03	-0.47				
perc.OTU assigned	-0.01	0.08	0.29	0.25	-0.16	-0.47	0.33			
n.OTU	0.08	0.15	0.09	-0.14	0.17	0.07	0.10	0.00		**
n.functions	0.12	0.20	0.37	-0.27	0.32	-0.22	0.15	0.27	0.31	

Tab.S.9 Result of permutational ANOVAs on log transformed gene copies (*16S, nifH,* AOA, AOB*, nirK* and *nosZ*) as function of 2-way interaction between management type and region, pH class and C org class. The statistical significance is expressed with *** for p-value<0.001, ** for p-value<0.01 and * for p-value<0.05.

	16S		log *nifH*			log AOA			log AOB			log *nirK*			log *nosZ*		
	Estimate	Prob	Estimate	Prob		Estimate	Prob		Estimate	Prob		Estimate	Prob		Estimate	Prob	
Management type M	0.0068	0.92	0.20	0.10		-0.29	0.47		0.28	0.22		0.016	0.78		0.087	0.73	
Region IT	-0.064	0.36	0.27	0.22		0.40	0.13		0.42	0.80		-0.072	1.00		0.72	0.02	*
Region FR	0.061	0.20	-0.41	0.03	*	1.19	0.01	**	-0.11	0.90		0.27	0.002	**	0.30	0.08	
Management type M: region IT	0.049	0.30	0.00	0.92		-0.66	0.28		-0.18	0.82		-0.018	0.76		0.37	0.25	
Management type M: region FR	-0.094	0.11	0.06	0.41		0.53	0.45		-0.12	0.50		-0.12	0.08		-0.29	0.86	
Residual	0.23		0.72			2.15			1.61			0.37			1.43		
R^2	0.15		0.24			0.30			0.09			0.33			0.25		
pH class1	-0.079	0.62	0.037	0.51		-1.89	0.0028	**	-0.73	0.061		-0.40	0.014	*	-1.35	<2e-16	***
pH class2	0.012	0.60	-0.21	0.82		-0.53	0.98		0.022	0.64		-0.00031	0.079		-0.036	0.94	
pH class3	0.062	0.36	-0.022	0.96		1.22	0.17		0.26	0.45		0.42	<2e-16	***	0.37	0.23	
Residual	0.23		0.78			2.08			1.57			0.33			1.30		
R^2	0.049		0.040			0.31			0.09			0.45			0.34		
AIC	2.57		95.16			169.08			147.69			28.32			133.54		
BIC	10.75		103.35			177.27			155.88			36.51			141.73		
C org class1	0.057	0.18	0.19	0.10		2.40	<2e-16	***	0.60	0.002	**	0.13	0.35		1.08	<2e-16	***
C org class2	-0.060	0.59	-0.005	0.86		0.59	0.84		0.26	0.383		0.18	0.36		0.58	0.14	
C org class3	0.088	0.13	0.27	0.16		-1.44	0.0012	**	0.50	0.225		-0.25	0.04	*	-0.20	0.88	
Residual	0.22		0.74			1.81			1.40			0.40			1.24		
R^2	0.11		0.146			0.47			0.27			0.16			0.40		
AIC	0.08		90.73			158.83			138.99			44.56			129.86		
BIC	8.27		98.92			167.02			147.18			52.75			138.05		

Fig.S.7 Boxplot of gene copies log transformed as function of region where black dots correspond to the means. The panel A corresponds to *16S* gene, B to *nosZ* gene, C to *nifH* gene, D to *nirK* gene, E to AOA gene and F to AOB gene. The pH class presents 4 levels based on quartiles as the C org class (pH class: 4.5<“1”≤5.4; 5.4<”2” ≤5.8; 5.8<”3” ≤6.4; 6.4<”4” ≤7.6 – C org class: 1.9<“1”≤4.7; 4.7<”2” ≤6.9; 6.9<”3” ≤9.8; 9.8<”4” ≤35.9). The significance values of the factor where obtained from the permutation ANOVA reported in table S.9.



Tab.S.10 Comparison between pH class and C org class permutation ANOVA considering R^2, AIC and BIC indices.

	pH class			C org class		
	R^2	AIC	BIC	R^2	AIC	BIC
log *16S*	0.049	2.57	10.75	0.11	0.08	8.27
log *nifH*	0.04	95.16	103.35	0.146	90.73	98.92
log AOA	0.31	169.08	177.27	0.47	158.83	167.02
log AOB	0.09	147.69	155.88	0.27	138.99	147.18
log *nirK*	0.45	28.32	36.51	0.16	44.56	52.75
log *nosZ*	0.34	133.54	141.73	0.4	129.86	138.05

Tab.S.11 Results of ratio AOB/(AOA+AOB) variability through three GAM models (first model: 2-way interaction between type and region; second model: pH class; third model: C org class) based on beta distribution with log link function. The statistical significance is expressed with *** for p-value<0.001, ** for p-value<0.01 and * for p-value<0.05.

	Estimate	Pr(>|z|)	
(Intercept)	0.058	0.90	
Management type P	1.070	0.14	
Region IT	1.47	0.040	
Region NR	-0.011	0.99	*
Region IT: management type P	-1.047	0.33	
regionNR: management type P	-0.26	0.79	
R^2	0.087		
Deviance	29.6%		
(Intercept)	1.43	0.00014	***
pH class2	-0.537	0.33	
pH class3	-1.48	0.015	*
pH class4	-1.37	0.016	*
R^2	0.14		
Deviance	28%		
AIC	-193.85		
BIC	-185.66		
(Intercept)	-0.65	0.13	
C org class2	1.02	0.10	
C org class3	2.22	0.0001	***
C org class4	1.88	0.001	**
R^2	0.31		
Deviance	44%		
AIC	-199.41		
BIC	-191.22		

4 - GENERAL DISCUSSION

This thesis is composed of three chapters based on different methodologies for monitoring semi-natural grasslands, in particular pastures. The first and second contributions correspond to two distinct applications of GPS telemetry and Remote Sensing to monitor the grazing patterns of lactating cows in two alpine summer pastures in north Italy, while the third contribution corresponds to the application of genetic molecular analyses to assess the functional structure of European seminatural grasslands used as meadows and pastures.

The first contribution integrated the GPS telemetry and the remote sensing to discriminate the effects of farmer conduction, topology, and vegetation on grazing patterns at fine temporal and spatial scales (Cagnacci et al. 2010; Nathan et al. 2022). The results confirmed the hypothesis of significant effects of slope and vegetation productivity in determining grazing patterns that resulted in a highly heterogeneous use of the pasture area, only partially mitigated by the farmer's conduction, which was on the other hand the determinant factor of the daily distances walked by the animals, and hence of the associated costs. Thus farmer conduction, the GPS telemetry has been confirmed to be a technique able to discriminate animal movement patterns at the metric scale and characterise animal activity budget by providing information about the distances travelled, average speeds maintained, and habitat selection (Cagnacci et al. 2010; Nathan et al. 2022). Instead, the use of remote sensing has been confirmed to be a technique able to provide environmental information, such as slope, altitude, and vegetation biomass (Pettorelli et al. 2005, 2014), which are essential to define the environmental context used to analyse GPS data.

The second contribution involved a larger spatial context than the first one, and integrated GPS positioning and remote Sensing, with remote assessment of multiple behaviours displayed by lactating cows of different breeds and parity. The remote identification of multiple behaviours was achieved by the application of a random forest model to classify single GPS positions into a single behaviour (grazing, walking, resting) on the base of movement metrics and accelerometer data obtained from GPS collars (Valletta et al. 2017). The integration of GPS telemetry, remote sensing and random forest allowed to discriminate different behavioural patterns as function of breed-parity and habitat type. The results of the second contributes confirmed the null hypothesis revealing significant differences in behavioural patterns and use of habitats, and between local and common dairy breeds and parities. Thus, the integration of behavioural analysis to the methodology adopted in the first contribution allowed for more detailed characterization of both patterns and budgets of animal activity at pasture.

The first two contributions confirmed the goodness of integration between GPS telemetry and remote sensing to monitor the grazing patterns on alpine pastures, (Homburger et al. 2014) revealing a heterogeneous use of pastures which is influenced by multiple factors, from environmental to animal conditions. Thus, the application of GPS telemetry combined with remote sensing provides qualitative and quantitative information about the local disturbance in the ecosystem, such as the local grazing intensity. This information can constitute the base to assess the possible impact of grazing systems on ecosystem dynamics ruled by microbial soil communities.

The first and second contributions were used to develop an operational framework for integrating and managing GPS telemetry and remote sensing data. GPS telemetry provides an immense number of localizations, which can be integrated with multiple data arrays from remote sensing to link animal positions and movement characteristics to environmental variables (Cagnacci et al. 2010; Urbano and Cagnacci, 2014; Nathan et al. 2022). The integration of the different data generates geodatabases (spatial databases), characterised by millions of records. Thus, geodatabases should be managed by specific softwares such as DBMSs (Database Management Systems), which allows to query, create, and manipulate databases quickly and easily. Among the various DBMSs available, we selected PostgreSQL thanks to its versatile data management in GIS (Geographic Information System) and its PostGIS extension, which implements the possibility to manage georeferenced data, such as GPS locations or georeferenced images from satellites (Urbano and Cagnacci, 2014). However, the use of DBMSs requires computer programming skills making it not accessible to all. The operative framework developed during this thesis is composed by three main steps

1. the validation of geodatabase through the detection and classification of outliers

2. the integration of geodatabase with environmental data from remote sensing and weather stations, and animal data about production, body condition, parity, breed, etc…

3. the statistical analysis of geodatabase in R

The validation of the geodatabase is the most delicate step and requires the greatest effort in terms of time due to the development of a procedure able to detect and classify outlier positions derived from the GPS errors. GPS sensors are characterised errors of <5-10 m (D'Eon et al. 2002; Tomkiewicz et al. 2010; Parraga Aguado et al. 2017; Muminov et al. 2019), which can increase when the sky view is limited due to the presence of obstacles, such as forest canopy (Frair et al., 2004; Janeau et al., 2004; Sager-Fradkin et al., 2007). In the study cases presented in this thesis, GPS sensors decreased their spatial accuracy, also losing the signal, when cows were inside the barn during the milking.

Thus, the increase of errors and lost positions were used as criteria to detect both outliers and milking periods. The GPS errors were also detected during the entire day, especially when animals were lying during the resting. In fact, when animals are resting, the orientation of the GPS antenna can turn toward the ground or be screened by the body with negative impact on spatial accuracy (Jiang et al., 2008; Graves et al., 2013). The detection of this type of GPS error required the development of criteria based on movement metrics as suggested by Urbano and Cagnacci (2014). The second step requires attention to the integration of environmental data through the selection of appropriate spatial resolution and the use of common CRSs (coordinate reference systems) across all data sources. Additional environmental data can be extracted from open geodatabases through tools such as Google Earth Engine, which allows to connect and query databases of ESA and NASA (Gorelick et al. 2017). The third step can involve multiple statistical analysis approaches. For example, in this thesis data were analysed with statistical methods such as General Additive Mixed Model (GAMM; Wood et al. 2017) and Generalized Linear Mixed Model (GLMM; Bolker et al. 2009) in frequentist framework and Integrated nested Laplace approximation (INLA) in Bayesian framework (Rue et all. 2009). Thus, the most difficult aspects of the third step are the correct formulation of null hypothesis and the consequent selection-application of statistical methods. The use of GPS telemetry associated with remote sensing allows a deep understanding of where (what habitats, what slopes, etc.), how (how far, how fast, direction,…) and why (to feed, to go to the milking, to rest during the day, to rest at night,...) the animals move, as confirmed by the first and second contributions. GPS telemetry application is part of the livestock precision farming (LPF; Tullo et al. 2019) and can allow to increase the efficiency of grazing management by improving the use of grazing resources and prevent overgrazing phenomena with positive externalities for ecosystem conservation (Millward et al. 2020). However, GPS telemetry implementation requires advanced skills in different fields, such as computer science and data analysis, as discussed above and it is still expensive in terms of purchasing and maintaining instruments (GPS collars). Thus, the application of GPS telemetry to monitor grazing systems appears currently not accessible to all, but it still requires intermediates.

The third contribution was based on the application of Illumina dye sequencing and real time PCR to characterise the microbial soil communities of pastures and meadows in the European context. The Illumina dye sequencing allowed to define the taxonomic structure of microbial communities, from which the functional profiles were extracted applying the external database of FAPROTAX (Louca et al. 2016). Taxonomical and Functional profiles of microbial soil communities were compared in function of management type, region and two pedological parameters, pH and organic C, which are known as important drivers for microbial communities (Fierer, 2017; Bahram et al., 2018; Kuypers et al., 2018). Instead, the real time PCR was used to define the gene potentials of specific functions through the abundances of marker genes. The null hypothesis of different microbial communities varying in function of management type and regional soil conditions was partially verified. In fact, the results revealed differences between meadow and pasture microbial communities only in terms of OTU profiles, but neither functional profiles or gene abundances were different with respect to management type. Instead, communities, at both all taxonomic levels and functional profile, and gene potentials resulted different with respect to region and pH, suggesting that local pedological conditions are more important drivers for microbial communities than local management. Moreover, the different response of microbial communities in terms of taxonomic and functional diversities may confirm the possible decoupling between them (Louca et al. 2016, 2018). In general, all molecular methods used were able to detect information about soil microbial communities with different resolutions that appears complementary. In fact, the sequencing provides general information about the structure of microbial communities while real time PCR allows to obtain detailed information about selected functions. Thus, both methods should be implemented into a general framework to characterise microbial communities of soil and assess possible impact effects. Application of real time PCR and the use of genes as indicators is used in different field (Baldwin et al. 2003; Mocellin et al. 2003; Wang et al. 2020; Nikitin et al. 2022) while the use of sequencing is used to define microbial diversity in general (Lemos et al. 2011; Thompson et al. 2017; Bahram et al. 2018; Kumar et al. 2019). However, information about taxonomic diversity can be only partially useful to assess impact on the ecosystem as it provides information only on number and relative abundance of OTUs. The real challenge is passing from taxonomic profiles to functional profiles, which give information about the potential of functions in an ecosystem, so about its “operational” state (Louca et al. 2018). This passage is fundamental to assess impacts on ecosystem multifunctionality which not necessarily depends on the pure biodiversity as it directly derives from the functional diversity. In fact, functions are not monophyletic in microbial communities (David et al 2010; Martini et al. 2015) thus taxonomic diversity can bring to functional redundancy, which is important for ecosystem resistance and resilience (Louca et al. 2018). The sequencing provides more information than RealTime PCR, but it is also more expensive in terms of cost and time. For both methods, the extraction and purification of DNA is a fundamental step to obtain robust data as the soil contains organic compounds able to inhibit RealTime PCR (Alaeddini 2012). The use of standard kits ensures an efficient purification, and it makes data comparable to others processed by the same kit, but it can be expensive in terms of costs, thus limiting the number of samples. The possibility of comparing samples from different contexts may be fundamental to correctly assess the possible impacts on microbial communities in order to explore their variability, which is still largely uncharted. This highlights the relevance of developing a standard monitoring framework for monitoring able to involve not only the extraction-purification procedure but also the assessment of environmental conditions such as pedological and vegetation characteristics and local disturbance intensity. Thus, merging the operative framework used for the management and analysis of GPS data and the procedure adopted for the analysis of microbial communities can provide an integrated approach able to characterise grassland conditions from a macro to micro scale. Such an integrated approach can be used to assess the effects of local environment conditions, such as pH and organic C amount, and practices, as animal conductions at pasture or fertilisation of meadow, on ecosystem diversity and multifunctionality through standard procedures.

The contributions of this thesis presented some limitations mainly due to gaps in terms of data which limited the analysis. In general vegetation was not characterised in terms of phytosociological associations, despite they can affect both grazing with different selection (Bailey 1995; Dumont et al. 2002; Rivero et al. 2021) and microbial communities through different structure of rhizosphere and root exudate input (Loranger-Merciris et al. 2006; Delgado-Baquerizo et al. 2018). In the first contribution, the spectral index used (NDVI) gave information only about the biomass amount but not about the phenology of plants (Myneni et al. 1995; Pettorelli et al. 2005; Shariatinajafabadi et al. 2014), which may impact on the resource selection. For example, the GWI index derives from NDVI as ratio between NDVI at specific time minus the annual minimum NDVI and the difference between annual maximum and minimum NDVI (White et al. 1997). GWI index is normalized, ranging from 0% to 100%, and provides information about the green-up, so about plant phenology, expressed as relative variation of biomass, where 50% indicates an intermediate stage of greenness (Myneni et al. 1995; Pettorelli et al. 2005; Shariatinajafabadi et al. 2014). This index can be easily implemented giving additional environmental information useful for further characterization of animal behaviour at pasture. About the third contribution, the use of FAPROTAX to classify functional profiles is depending on the bibliographic knowledge as its database obtains the OTU functions from the literature (Louca et al. 2016; Sansupa et al. 2021). In the third contribution, given also the spatial scale of comparison, we could differentiate management only on terms of use of grasslands as either meadow or pastures. We expect that a more detailed information, including for instance stocking rate, intensity and type of fertilising, number of cuts in meadows, might reveal finer, and local, differentiation between management methods than that observed in this study

Interesting future perspectives for the methods used emerge from the limitations and results of this thesis. A future possible application of GPS telemetry may be the study of the behavioural dynamics within herds from individual inter-distances, in order to understand how animals may influence each other in the choice, thus the use, of the available grazing resources, also in relation to individual variables such as weight, body condition, and production, both in quantitative and qualitative terms. The use of local weather stations, along with the integration of multiple sensors, such as GPS and heart rate sensors, and additional vegetation spectral indices (GWI), can allow to improve understanding of determinants of animal movement and grazing behaviour, considering the effect of current climate change (global warming and pasture drought). All this information can be used on one side to estimate more accurately animals' needs in different grazing contexts, such as alpine pasture, improving their management, and on the other side to model variability of animals' load and hence the associated disturbance, on the grazed area. Regarding the molecular analysis, the increase of sample size considering new grassland systems will be useful to understand knowledge about common characteristics of soil microbial communities. Moreover, the implementation of other environmental variables, such as surface temperature or rain precipitation or soil total N or vegetation type, can reveal new microbial patterns. Implementing the monitoring of greenhouse gas fluxes from soil and correlating them to microbial functional potentials will provide important information in the context of climate change. This will allow to accurately estimate the contribution of grasslands to current climate change, verifying their sink or source nature. Also, considering different management types with variable intensity levels can be important to detect possible drivers for microbial variability. Last but not least, the integration of information about grazing patterns from GPS telemetry and molecular analysis can highlight possible effects of grazing intensity on local microbial communities and their functionalities.

4.1 - REFERENCES

Alaeddini, R. (2012). Forensic implications of PCR inhibition—a review. Forensic Sci Int Genet. 6(3):297-305.

Bahram M, Hildebrand F, Forslund SK, Anderson JL, Soudzilovskaia NA, Bodegom PM, Bengtsson-Palme J, Anslan S, Coelho LP, Harend H, Huerta-Cepas J, Medema MH, Maltz MR, Mundra S, Olsson PA, Pent M, Polme S, Sunagawa S, Ryberg M, Tedersoo L, Bork P. 2018. Structure and function of the global topsoil microbiome. Nature. 560(7717):233-237.

Bailey DW. 1995. Daily selection of feeding areas by cattle in homogeneous and heterogeneous environments. Appl. Anim. Behav. Sci. 45(3-4):183-200.

Baldwin BR, Nakatsu CH, Nies L. 2003. Detection and enumeration of aromatic oxygenase genes by multiplex and real-time PCR. Appl. Environ. Microbiol. 69(6):3350-3358.

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JSS. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24(3):127-135.

Cagnacci F, Boitani L, Powell RA, Boyce MS. 2010. Animal ecology meets GPS-based radiotelemetry: A perfect storm of opportunities and challenges. Philos. Trans. R. Soc. B Biol. Sci. 365(1550):2157–62.

D'Eon RG, Serrouya R, Smith G, Kochanny CO. 2002. GPS radiotelemetry error and bias in mountainous terrain. Wildl Soc Bull. 30(2):430–439.

David LA, Alm EJ. 2011. Rapid evolutionary innovation during an Archaean genetic expansion. Nature. 469(7328):93-96.

Delgado-Baquerizo M, Fry EL, Eldridge DJ, de Vries FT, Manning P, Hamonts K, Kattge J, Boenisch G, Brajesh KS, Bardgett RD. 2018. Plant attributes explain the distribution of soil microbial communities in two contrasting regions of the globe. New Phytol. 219(2):574-587.

Dumont B, Carrère P, D'Hour P. 2002. Foraging in patchy grasslands: diet selection by sheep and cattle is affected by the abundance and spatial distribution of preferred species. Anim. Res. 51(05):367-381.

Fierer N. 2017. Embracing the unknown: Disentangling the complexities of the soil microbiome. Nat. Rev. Microbiol. 15:579–590.

Frair J.L., Fieberg J., Hebblewhite M., Cagnacci F., DeCesare N.J., Pedrotti L., 2010. Resolving issues of imprecise and habitat-biased locations in ecological analyses using GPS telemetry data. Philos. Trans. Royal Soc. 365(1550):2187–2200.

Gorelick N, Hancher M, Dixon M, Ilyushchenko S, Thau D, Moore R. 2017. Google Earth Engine: Planetary-scale geospatial analysis for everyone. Remote Sens. Environ. 202:18–27.

Graves, T.A., Waller J.S., 2006. Understanding the causes of missed global positioning system telemetry fixes. J. Wildl. Manage. 70(3):844–85.

Homburger H, Schneider MK, Hilfiker S, Lüscher A. 2014. Inferring behavioral states of grazing livestock from high-frequency position data alone. PLoS One. 9(12):1–22.

Janeau G., Adrados C., Joachim J., Gendner J.P., Pépin D., 2004. Performance of differential GPS collars in temperate mountain forest. C. R. Biol. 327(12):1143–1149.

Kumar S, Suyal DC, Yadav A, Shouche Y, Goel R. 2019. Microbial diversity and soil physiochemical characteristic of higher altitude. PLoS One. 14(3):e0213844.

Kuypers MMM, Marchant HK, Kartal B. 2018. The microbial nitrogen-cycling network. Nat. Rev. Microbiol. 16:263–276.

Lemos LN, Fulthorpe RR, Triplett EW, Roesch LF. 2011. Rethinking microbial diversity analysis in the high throughput sequencing era. J. Microbiol. Methods. 86(1):42-51.

Loranger-Merciris G, Barthes L, Gastine A, Leadley P. 2006. Rapid effects of plant species diversity and identity on soil microbial communities in experimental grassland ecosystems. Soil Biol. Biochem. 38(8):2336-2343.

Louca S, Parfrey L.W, Doebeli M. 2016. Decoupling function and taxonomy in the global ocean microbiome. Science 353:1272-1277.

Louca S, Polz MF, Mazel F, Albright MBN, Huber JA, O'Connor MI, Ackermann M, Hahn AS, Srivastava DS, Crowe SA, Doebeli M, Parfrey LW. 2018. Function and functional redundancy in microbial systems. Nat. Ecol. Evol. 2:936–943.

Martiny JB, Jones SE, Lennon JT, Martiny AC. 2015. Microbiomes in light of traits: a phylogenetic perspective. Science. 350(6261):aac9323.

Millward MF, Bailey DW, Cibils AF, Holechek JL. 2020. A GPS-based evaluation of factors commonly used to adjust cattle stocking rates on both extensive and mountainous rangelands.

Rangelands. 42(3):63-71.

Mocellin S, Rossi CR, Pilati P, Nitti D, Marincola FM. 2003. Quantitative real-time PCR: a powerful ally in cancer research. Trends Mol. Med. 9(5):189-195.

Muminov A, Sattarov O, Lee CW, Kang HK, Ko MC, Oh R, Ahn J, Oh HJ, Jeon HS. 2019. Reducing GPS error for smart collars based on animal's behavior. Appl Sci. 9(16):408.

Myneni RB, Hall FG, Sellers PJ, Marshak AL. 1995. Interpretation of spectral vegetation indexes. IEEE Trans. Geosci. Remote Sens. 33(2):481–86.

Nathan R, Monk CT, Arlinghaus R, Adam T, Alós J, Assaf M, Baktoft H, Beardsworth CE, Bertram MG, Bijleveld AI, Brodin T, Brooks JL, Campos-Candela A, Cooke SJ, Gjelland K Ø, Gupte PR, Harel R, Hellström G, Jeltsch F, Killen SS, Klefoth T, Langrock R, Lennox RJ, Lourie E, Madden JR, Orchan Y, Pauwels IS, Říha M, Roeleke M, Schlägel UE, Shohami D, Signer J, Toledo S, Vilk O, Westrelin S, Whiteside MA, Jarić I. 2022. Big-data approaches lead to an increased understanding of the ecology of animal movement. Science. 375(6582):eabg1780.

Nikitin DA, Semenov MV, Chernov TI, Ksenofontova NA, Zhelezova AD, Ivanova EA, Khitrov NB, Stepanov AL. 2022. Microbiological Indicators of Soil Ecological Functions: A Review. Eurasian Soil Sci. 55(2):221-234.

Parraga Aguado MA, Sturaro E, Ramanzin M. 2017. Individual activity interacts with climate and habitat features in influencing GPS telemetry performance in an alpine herbivore. Hystrix. 28(1):1-7.

Pettorelli N, Laurance WF, O'Brien TG, Wegmann M, Nagendra H, Turner W. 2014. Satellite remote sensing for applied ecologists: Opportunities and challenges. J. Appl. Ecol. 51(4):839–48.

Pettorelli N, Vik JO, Mysterud A, Gaillard JM, Tucker CJ, Stenseth NC. 2005. Using the satellite-derived NDVI to assess ecological responses to environmental change. Trends Ecol. Evol. 20(9):503–10.

Rivero MJ, Grau-Campanario P, Mullan S, Held SD, Stokes JE, Lee MR, Cardenas LM. 2021. Factors affecting site use preference of grazing cattle studied from 2000 to 2020 through GPS tracking: A review. Sensors. 21(8):2696.

Rivero MJ, Grau-Campanario P, Mullan S, Held SD, Stokes JE, Lee MR, Cardenas LM. 2021. Factors affecting site use preference of grazing cattle studied from 2000 to 2020 through GPS tracking: A review. Sensors. 21(8):2696.

Rue H., Martino S., Chopin N. 2009. Approximate Bayesian inference for latent Gaussian models using integrated nested Laplace approximations (with discussion). J R Stat Soc Series B Stat Methodol. 71(2):319-392.

Sager-Fradkin K.A., Jenkins K.J., Hoffman R.A., Happe P.J., Beecham J.J., Wright R.G., 2007. Fix Success and Accuracy of Global Positioning System Collars in Old-Growth Temperate Coniferous Forests. J. Wildl. Manage. 71(4):1298–1308.

Sansupa C, Wahdan SFM, Hossen S, Disayathanoowat T, Wubet T, Purahong W. 2021. Can we use functional annotation of prokaryotic taxa (FAPROTAX) to assign the ecological functions of soil bacteria?. Appl. Sci. 11(2):688.

Shariatinajafabadi M, Wang T, Skidmore AK, Toxopeus AG, Kölzsch A, Nolet BA, Exo KM, Griffin L, Stahl J, Cabot D. 2014. Migratory herbivorous waterfowl track satellite-derived green wave index. PLoS One. 9(9):1–11.

Thompson LR, Sanders JG, McDonald D, Amir A, Ladau J, Locey KJ, Prill RJ, Tripathi A, Gibbons SM, Ackermann G, Navas-Molina JA, Janssen S, Kopylova E, Vàzquez-Baeza Y, Gonzàlez A, Morton JT, Mirarab S, Xu ZZ, Jiang L, Haroon MF, Kanbar J, Zhu Q, Song SJ, Kosciolek T, Bokulich NA, Lefler J, Brislwn CJ, Humphrey G, Owens SM, Hampton-Marcell J, Berg-Lyons D, McKenzie V, Fierer N, Fuhrman JA, Clauset A, Stevens RL, Shade A, Pollard KS, Goodwin KD, Jansson JK, Gilbert JA, Knight R. 2017. A communal catalogue reveals Earth’s multiscale microbial diversity. Nature. 551(7681):457-463.

Tomkiewicz SM, Fuller MR, Kie JG, Bates KK. 2010. Global positioning system and associated technologies in animal behaviour and ecological research. Philos Trans R Soc Lond B Biol Sci. 365(1550):2163–2176.

Tullo E, Finzi A and Guarino M. 2019. ‘Review: Environmental impact of livestock farming and Precision Livestock Farming as a mitigation strategy’. Sci. Total Environ. 650:2751–2760.

Urbano F, Cagnacci F. 2014. Spatial database for GPS Wildlife tracking data. A practical guide to creating a data management system with PostgreSQL/PostGIS and R. 1st ed. Cham: Springer.

Valletta J.J, Torney C, Kings M, Thornton A, Madden J. 2017. Applications of machine learning in animal behaviour studies. Anim. Behav. 124:203-220.

Wang H, Li X, Li T, Zhang S, Wang L, Wu X, Liu J. 2020. The genetic sequence, origin, and diagnosis of SARS-CoV-2. Eur. J. Clin. Microbiol. Infect. Dis. 39(9):1629-1635.

White MA, Thornton PE, Running SW. 1997. A continental phenology model for monitoring vegetation responses to interannual climatic variability. Glob Biogeochem Cycles. 11:217–234.

Wood S. 2017. Generalized Additive Models: An Introduction with R. 2nd ed. Chapman and Hall/CRC.

5 – CONCLUSION

This thesis explores different methods for monitoring grasslands, involving different scales and research fields. The application of GPS systems and remote sensing for monitoring livestock systems was confirmed to be able to provide an enormous amount of data with numerous opportunities for the development of more informed management. In the study cases presented, the use of GPS telemetry made it possible to accurately quantify the areas used by the grazing cows, confirming the presence of mostly extensive grazing and heterogenous habitat use shaped by both human choice and environmental conditions. The geolocation of the animals associated with accelerometer sensors also revealed interesting behavioural aspects, such as distances travelled and speeds, and daily behavioural patterns. This information makes it possible to obtain in-depth knowledge on animal movement patterns and the relative use of pastures, which will allow the development of more efficient management models and sustainable grassland management, both in productive and environmental terms. The use of GPS systems can thus form the basis of a real quantification and qualification of the role of mountain pasture in maintaining ecosystems with high biodiversity, to enhance with greater awareness the role of the breeders in conserving the landscape heritage in its diversity. The application of molecular analysis (real time PCR and amplicon sequencing) for characterising the microbial communities of soil also provided numerous data useful to assess grassland multifunctionality. It was possible to quantify and qualify microbial diversity and functions associated at community and gene levels, thanks to the complementary use of sequencing and real time PCR. Molecular analysis revealed the significant dissimilarities among microbial communities of grasslands at different levels as a function of pedological condition but not of local management. Molecular analyses make possible the characterization of both taxonomic and functional diversity of soil microbial communities with important implications on the assessment of local disturbance effects and conditions on the ecosystem and its multifunctionality. In conclusion, both the methods used in this thesis were able to detect variability of grasslands at fine scale, thus making their future integration feasible, in order to develop an integrated approach for monitoring grasslands considering macro and micro characteristics. Thus, this integrated approach can provide a useful tool to extract the base information to achieve a real sustainable management of grasslands considering these systems in holistic terms.

6 – ACKNOWLEDGEMENTS

This thesis is the product of a wonderful journey full of discoveries started three years ago thanks to a deep curiosity and the support of few Professors who induced me to take the PhD. I must thank many people who made these last three years full of memories and emotions.

First, I want to thank my family which supported me during both the good and difficult periods of this PhD. A special thanks is dedicated to my sister Laura who corrected the English of this thesis. Then I want to thank Professor Enrico Sturaro, Maurizio Ramanzin and Andrea Squartini who are my Mentors, not only into academic context, and always cultivated my curiosity inducing me to go further, without them my PhD should be barren (and this thesis should never have been concluded). I also want to thank Vibeke Lind, who shared many precious teachings with me during my second period in Norway, and Audrey Michaud, who supported me during my period in France.

I want to thank Marta Teston, a partner of multiple adventures and a friend with a rare listening skills (without her this thesis should not be uploaded due to the death of my notebook so she deserves at least one bear…), Elena Benedetti Del Rio, a partner without a great sense of direction but able to instil energy, Vittoria Bisutti, my desktop-partner who makes always my office days Jurassic, and Marco Berton, who shared pills in the right moments. Last but not for the least, I want to thank my cousin Leonardo, who is always ready to share his time with evening precious walks, Emanuele (Lelle Puddu), who helps me to find always a human balance, Elia, who is always ready to follow me in my mountain trip, Marco, who is always ready to run, Federico and Andrè, my pillars.

I also want to thank Luca Carraro who always makes our missions memorable adventures and shares precious suggestions. A special thank is also dedicated to Gustav and Gerbrand, who supported me during my period at the Lofoten, and to all farmers who shared their cows and pastures making possible all my research.

Thanks, Grazie, Merci and Tak

Salvatore, Riccardo Raniolo

7 - OTHER PUBBLICATIONS AND DISSEMINATION ACTIVITIES

7.1 - PAPERS IN SCIENTIFIC JOURNALS

Teston M, Ramanzin M, Bittante G, Gallo L, Gatto P, Orsi M, Raniolo S, Tormen A, Sturaro E. 2022. Added value of local sheep breed in alpine agroecosystems. Sustainability. 14(8): 4698.

7.2 - PRESENTATIONS AT NATIONAL AND INTERNATIONAL CONGRESSES

Raniolo S, Squartini A, Ramanzin M, Sturaro E. Relationships between dairy cattle grazing, soil microbiology and nutrients cycle in highland pastures. In: EAAP 2020 Virtual Meeting, 1st–4th December 2020.

Raniolo S, Ramanzin M. Human choices, slope, and vegetation productivity determine pattering of traditional Alpine summer grazing. In: ASPA 24rd CONGRESS; Padova, 21–24 September 2021. (Oral communication).

Raniolo S, Ramanzin M, Sturaro E, GPS tracking indicates high variability in grazing patterns of lactating cows in Alpine summer farms. In: EAAP 72rd CONGRESS; Davos, 30 August - 3 September 2021. (Oral communication).

Raniolo S, Cournut S, Cournut E, Michaud A, Cremilleux M, Lind V, Karlsen G, Squartini A, Benedetti Del Rio E., Ramanzin M., Sturaro E. Sustainable management of highland grasslands: an integrated methodological approach from HIGHLANDS.3. In: IMC Congress, oral communication. Accepted. Innsbruck 11th September – 15th September 2022. (Oral communication).

7.3 - OTHER PUBLICATIONS

Raniolo S, Squartini A, Ramanzin M, Sturaro E. “Relazione tra il pascolamento bovino, microbiologia del suolo e cicli dei nutrienti nei pascoli di alta quota”, 10th book of SooZooAlp, 2020.

Raniolo S, Ramanzin M, Sturaro E. Dalle vacche al pascolo: applicazione delle tecnologie GPS per il monitoraggio comportamentale delle vacche e l’uso dei pascoli alpini. Quaderni del Parco 17 – SmartAlp: un progetto per valorizzare il sistema alpicolturale. Ente Parco Paneveggio Pale di San Martino. 2022.

Vescovo L, Maffei T, Sassi R, Dalponte M, Frizzera L, Sturaro E, Zampedri R, Raniolo S, Gianelle D. Il telerilevamento satellitare applicato alla gestione sostenibile dei pascoli di montagna. Quaderni del Parco 17 – SmartAlp: un progetto per valorizzare il Sistema alpicolturale. Ente Parco Paneveggio Pale di San Martino. 2022.