Targeted grazing of robust livestock for the restoration of green alder-encroached pastures

Inaugural dissertation
of the Faculty of Science,
University of Bern

presented by

Mia Svensk

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Supervisors of the doctoral thesis:

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The Dean
Prof. Dr.
Marco Herwegh

List of Abbreviations

ADF_{OM}	Acid detergent fiber
ADL	Acid detergent lignin
$aNDF_{OM}$	Neutral detergent fiber
Ca	Calcium
CH_4	Methane
CO_2	Carbon dioxide
CT	Condensed tannins
Cu	Copper
DM	Dry matter
Fe	Iron
GDD	Growing degree days
HGT	Hohenheim gas test
HNO_3	Nitric acid
HT	Hydrolysable tannins
IVOMD	*In vitro* organic matter digestibility
K	Potassium
LDMC	Leaf dry matter content
MB	Mollasses-based blocks
Mg	Magnesium
Mn	Manganese
N	Nitrogen
NH_3	Ammonia
NH_4^+	Ammonium
NO_3^-	Nitrate
NTP	Non-tannin phenols
OM	Organic matter
P	Phosphorus
PV	Pastoral value
SLA	Specific leaf area
TEP	Total extractable phenols
TT	Total tannins
Zn	Zinc

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CHAPTER I : General Introduction

Alpine and subalpine pasture abandonment

Alpine and subalpine pastures are key providers of ecosystem services, as they benefit society in direct and indirect ways (v. Bieberstein Koch-Weser 2005; Locatelli et al. 2017). For instance, they are valuable semi-natural habitats harbouring high species diversity (Niedrist et al. 2009). These habitats have been shaped by millennia of agro-pastoral activities, with domestic herbivores grazing and trampling on plant communities that became adapted to their pressures, establishing a wider species range than that found in natural habitats (MacDonald et al. 2000; Díaz et al. 2007). Pastures provide valuable forage yield and quality for meat and dairy production, as well as fibre and fuel, and regulate water and carbon balance (Reid et al. 2005). They also have an important role in preserving the aesthetic value of cultural landscapes and they can offer protection from natural hazards (Schirpke et al. 2013). However, mountain ecosystem services are getting degraded through changes in human activities in the form of socioeconomical and climatic factors (Locatelli et al. 2017). Throughout the last decades, European mountain areas have been subjected to such changes, with a drastic reduction of agropastoral activities (Estel et al. 2015; Strebel and Bühler 2015). Marginal mountain agricultural land has been massively abandoned during the second part of the 20th century, due to the lack of economic benefits compared to more favourable sites (MacDonald et al. 2000; Mack et al. 2013). Indeed, mountain areas have lower amount of sunlight, temperatures, and forage yield for livestock, leading to lower production rates compared to lowlands, as well as reduced technical adaptation possibilities due to more difficult accessibility for new machinery (Schirpke et al. 2013; Strebel and Bühler 2015). This has led to a loss in competitiveness for the remote, small and low-productive mountain farms compared to other economic activities, despite the compensatory measures introduced to offset the disadvantages of these areas (Mack et al. 2013). The increase of lowland crop and livestock productivity also participated to the depopulation and land abandonment of these mountain ecosystems (MacDonald et al. 2000).

As a consequence, the abrupt reduction in farming activities in mountain areas have subjected them to environmental changes (Orlandi et al. 2016). Indeed, land abandonment became an important pressure on mountain environments, triggering profound landscape modifications. For instance, the reduction of grazing animals in alpine and subalpine pastures during the summer generated a lower nutrient fertilization input (Mack et al. 2013). Moreover, animal husbandry decline led to a lower species richness, and a homogenization of these heterogeneous ecosystems, with forest re-growth and shrub-encroachment over large areas of abandoned open pastures (Tasser et al. 2007; Niedrist et al. 2009). Shrub-encroachment is a worldwide phenomenon, and it has been massively reported in several European mountain chains (Camacho et al. 2008; Pardini and Nori 2011; Álvarez-Martínez et al. 2016; Skoczowski et al. 2021). For instance, the Swiss Alps have been subjected to a 22% shrub forest cover increase from 1983 to 2017 (Abegg et al. 2020). Therefore, semi-natural open habitats previously associated with low-intensity farming activities have been gradually replaced by dense stands of trees and shrubs that host lower animal and plant biodiversity (Eldridge et al. 2011; Wieczorkowski and Lehmann 2022). Shrub invasion have reduced the amount of valuable forage production areas, leading to lower herbage mass and quality for grass-fed animal products. This loss has also caused problems for tourism attractiveness, as traditional alpine landscapes with open habitats have an increasingly appreciated aesthetic value, specifically in Europe (Buijs et al. 2006). Therefore, these environments are of vital importance for mountain regions economy (Lindemann-Matthies et al. 2010). Moreover, other services are provided by these ecosystems, such as pollination, cultural heritage (Reid et al. 2005), or even albedo effects (Chapin et al. 2005), all of which are threatened by shrub encroachment.

Alnus viridis encroachment

Alnus viridis subsp. *viridis* (Chaix., DC, **Figure 1**) is the most expanding shrub species in all Central Europe, particularly in the Swiss, Slovenian, Austrian, French, and Italian Alps (Anthelme et al. 2002; Camerano et al. 2004; Dakskobler et al. 2013; Caviezel et al. 2014). It is also found in the Balkans and Carpathian mountains (Boscutti et al. 2014; Skoczowski et al. 2021), and has even become an invasive species in New Zealand (Howell 2008). Other *Alnus viridis* subspecies (*A. viridis* subsp. *crispa* and *A. viridis* subsp. *fruticosa*) have also been recorded in northern America (Houle 2001; Lantz et al. 2010). *Alnus viridis* subsp. *viridis* is native to the Alps and is mostly found in west and north-facing steep slopes and in disturbed habitats such as wet ravines or avalanche tracks (Richard 1967). It is a pioneer and heliophilous species, very effective in encroaching open areas, thanks also to its efficient sexual and vegetative reproduction (Caviezel et al. 2017). Indeed, it releases a large number of light-weighted seeds at early stages from the top of its branches, allowing it to easily spread in steep slopes through the wind (Farmer et al. 1985; Houle 2001). At later stages, it displays a high vegetative growth performance with the creation of many sprouts and the ability to grow back from its roots, making it highly difficult to differentiate individual plants (Mallik et al. 1997; Wiedmer and Senn-Irlet 2006). This vegetative growth is rapid, efficient, and creates dense stands reaching up to 4-5m high, with shrubs that can live up to 60 years old (Anthelme et al. 2002). These stands are hardly penetrable, and their flexible branches allow them to resist natural hazards such as avalanches, as they easily bend under snow pressure (Brüchert et al. 2003; Caviezel et al. 2014). This resistance provides a competitive advantage compared to other tree or shrub species that cannot withstand such environmental disturbance.

Figure 1 *Alnus viridis* in subalpine pastures in Western Switzerland, Bovonne, Bex (VD)

In some regions of Switzerland, *A. viridis* was reported to have increased by 50% between 1979 and 2014, and it now represents 70% of the total shrubland cover of the country, with an expansion rate being two to three times faster than tree forest expansion (Bühlmann et al. 2014; Caviezel et al. 2014; Abegg et al. 2020). **Figure 2** illustrates an example of the significant expansion of *A. viridis* in a subalpine pasture used as a study site for this thesis between 1980 and 2017. Nowadays, *A. viridis* is expanding into larger elevation ranges and gentle slopes, as its spreading is not only driven by land use intensity but also accelerated by climate change at higher elevations (Körner 2012; Caviezel et al. 2017; Skoczowski et al. 2021).

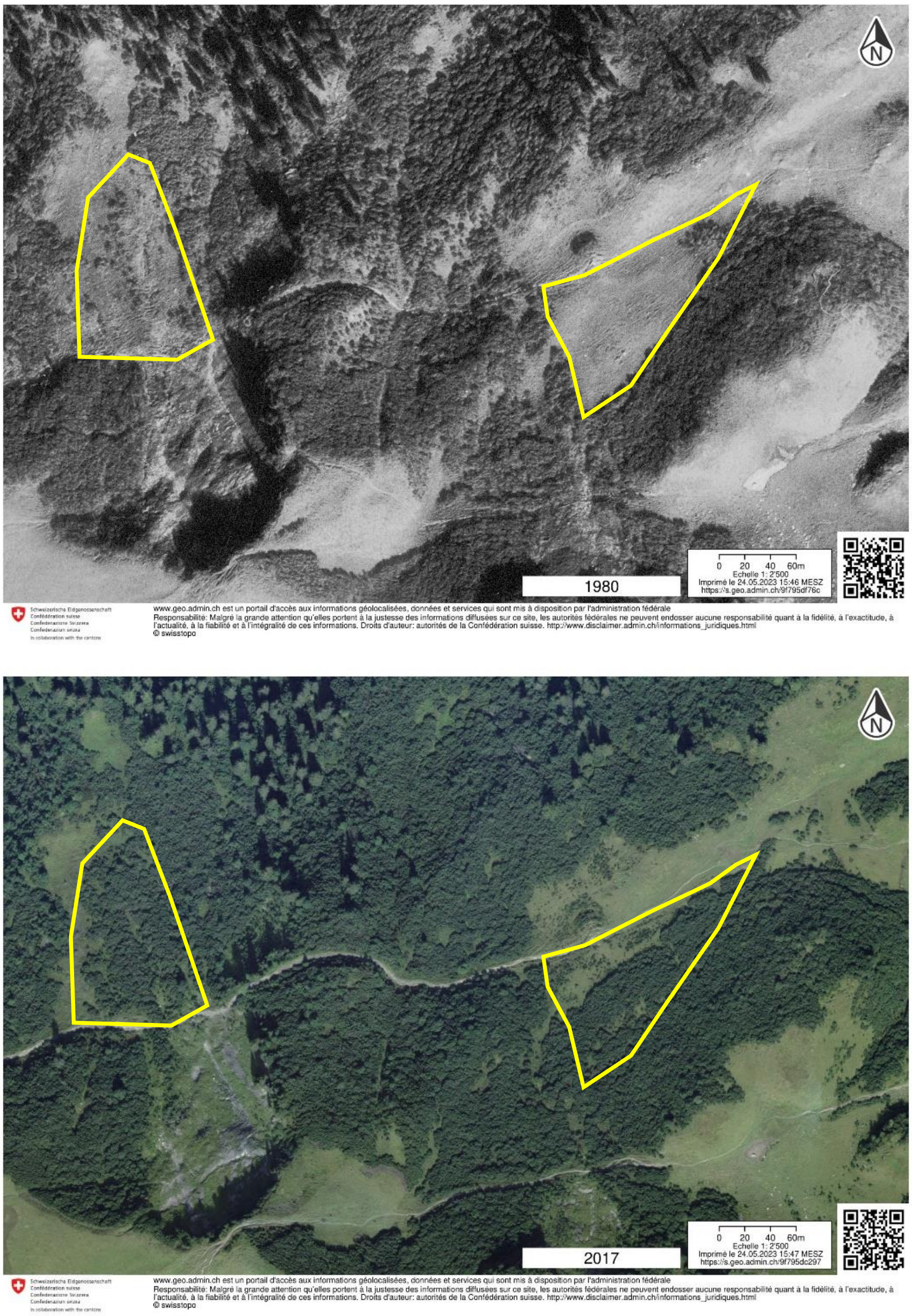

Figure 2 Changes in *Alnus viridis* cover in a subalpine pasture in Western Switzerland, Bovonne, Bex (VD), between 1980 and 2017. Source: Swisstopo. Areas with the most visible increase in *A. viridis* cover are circled in yellow.

A. viridis invasion is of major concern as it creates unfavourable agro-environmental conditions. Indeed, similarly to the case of tree and shrub encroachment in general, *A. viridis* expansion reduces the available areas for agriculture purposes, as well as prevents the provision of several ecosystem services from alpine and subalpine pastures. Among other things, *A. viridis* encroachment increases the risks of natural hazards such as wildfire (Schumacher and Bugmann 2006; Lovreglio et al. 2014) and does not protect from erosion or landslides (Tasser et al. 2003), or even avalanches, which are facilitated due to the elasticity of their branches that allow the snow to slide (Brüchert et al. 2003; Bühlmann et al. 2014; Caviezel et al. 2014).

One of the worrying drawbacks from *A. viridis* encroachment is the reduction of plant and animal biodiversity (Anthelme et al. 2001; Laiolo et al. 2004; Pornaro et al. 2013; Koch et al. 2015; Zehnder et al. 2020). Indeed, the shading created by *A. viridis* stands and the vegetation growing under its canopy reduces the chance of new seedling establishments (Anthelme et al. 2003; Bühlmann et al. 2014). It also creates cold and humid habitats, where a reduced number of tall shade-tolerant plant species can grow, and other plant seedling germination is even hampered by its vegetative basal sprouting. Moreover, *A. viridis* lives in symbiosis with the nitrogen-fixing actinomycete *Frankia alni*, which generates high levels of nitrogen (N) fixation in soils, sometimes leading to eutrophication (Huss-Daniel 1997; Dawson 2008). Indeed, this endosymbiosis is achieved through the formation of N-fixing root nodules (**Figure 3**) in which *Frankia alni* converts the atmospheric N (N_2) into ammonia (NH_3) thanks to nitrogenase enzyme complexes (Huss-Daniel 1997; Schwob et al. 2017). This N fixation creates a N saturation in the soils, resulting into soil acidification through the nitrification of ammonium (NH_4^+) into nitrate (NO_3^-), during which base cations are released, contributing to soil acidity (Verburg et al. 2001; Galloway et al. 2003; Thimonier et al. 2010). Therefore, only a few shade- and N-tolerant plants are able to adapt to these ecological conditions and grow in the understory vegetation of *A. viridis* shrublands (Zehnder et al. 2020). More specifically, the *A. viridis* understory is dominated by a few fast-growing and broad-leaved forbs such as *Adenostyles alliariae* (Gouan) A. Kern., *Peucedanum ostruthium* (L.) W. D. J. Koch, and *Cicerbita alpina* (L.) Wallr., and ferns such as *Dryopteris dilatata* (Hoffm.) A. Gray and *Athyrium filix-femina* (L.) Roth, which take competitive advantage of the high nutrient availability present in the topsoil (Anthelme et al. 2001; Wiedmer and Senn-Irlet 2006). The N accumulation also constrains the re-establishment of natural montane forests and prevents forest succession, by hindering conifers establishment (Hiltbrunner et al. 2014).

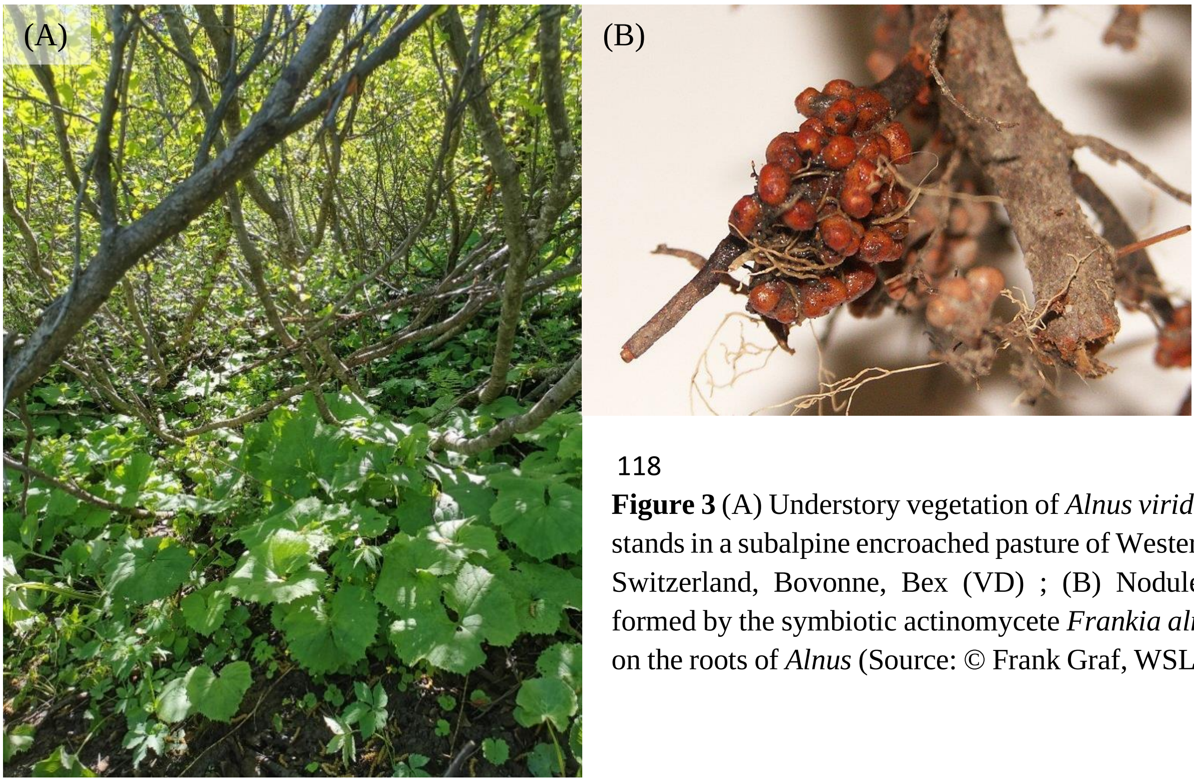

Figure 3 (A) Understory vegetation of *Alnus viridis* stands in a subalpine encroached pasture of Western Switzerland, Bovonne, Bex (VD) ; (B) Nodules formed by the symbiotic actinomycete *Frankia alni* on the roots of *Alnus* (Source: © Frank Graf, WSL).

In addition, the high N concentration in the soil under *A. viridis* stands causes lower C:N ratio, which is indicative of high risks of nitrate leaching (Thimonier et al. 2010). Indeed, when the N storage capacity in the soil is exceeded, and N is not retained by plants anymore, it increases the possibilities of nitrate being leached (Aber et al. 1989; Bühlmann et al. 2014). Therefore, the risk of nitrate contamination in streamlets and groundwater is enhanced. In Switzerland, recent soil water measurements under *A. viridis* stands showed high nitrate concentrations that were reported to be above the freshwater Swiss threshold, while the soil water of adjacent un-encroached pastures was not polluted (Bühlmann et al. 2014, 2016). Similarly, risks of dissolved organic carbon leaching are higher with N saturation. Indeed, Hunziker et al. (2017) demonstrated the negative relationship between dissolved organic carbon concentrations and soil pH, as the acidity created by N saturation under *A. viridis* stands stimulates dissolved organic carbon production. Consequently, there are potentially higher risks of soluble carbon concentration in streamlet water in *A. viridis* stands (Pregitzer et al. 2004; Bühlmann et al. 2014, 2016; Hunziker et al. 2017). These leaching risks created by *A. viridis* are also enhanced by the modification of soil properties, as *A. viridis* expansion is associated with higher soil porosity and lower particle friction, resulting in higher water infiltration capacity, percolation and soil moving processes (Sampò et al. 1997; Caviezel et al. 2014; Alaoui et al. 2014). In addition, the high decomposition rates of N rich compounds under *A. viridis* stands contributes to the release of gaseous N losses into the atmosphere. Indeed, nitrification and denitrification processes are intensified, which results to larger releases of atmospheric N, mostly in the form of nitrous oxide (Bühlmann et al. 2017). This gas, mostly released from agricultural activities, is an important greenhouse gas responsible for ozone depletion and with a higher warming effect than carbon dioxide. Evapotranspiration is also significantly increased under *A. viridis* stands, leading to runoff reduction and related hydro-electric potential reduction at catchment scale (van den Bergh et al. 2018).

By decreasing plant and animal diversity, generating soil and water pollution and releasing greenhouse gases into the atmosphere, *A. viridis* shrubs are direct contributors to the environmental decline in alpine and subalpine ecosystems. Combined with the agronomical losses related to shrub encroachment, the "*A. viridis* encroachment problem" has been increasingly reported in literature, and concerns have risen for grassland conservation and restoration. Therefore, managing the expansion of *A. viridis* is an important goal for the restoration of the ecosystem services provided by alpine and subalpine pastures. However, because *A. viridis* stands are located in remote, steep and hard-to-reach areas, regulating its spread is challenging, and manual removal is often technically difficult, time consuming and expensive. Until the 1950's the manual cutting of *A. viridis* shrubs was regularly carried out for firewood and had an economical purpose (Caviezel et al. 2017). Combined with the high grazing pressure at that time, *A. viridis* spread was then more controlled. Nowadays, the management of its expansion is harder and under several constraints that farmers may not be able to overcome. With the lack of management over several years, some areas have become even harder to reach and the amount of work needed to counteract *A.viridis* encroachment has become more and more significant.

Moreover, *A. viridis* leaves have moderately high contents in cell wall constituents and plant secondary metabolites, such as tannins. Therefore, they may not be palatable to many production-oriented dairy livestock breeds (Papanastasis et al. 2008; Waghorn 2008). While the number of dairy farms have decreased in many alpine regions in the last decades, the majority of alpine farms are still composed by dairy cows (Mack et al. 2013; Battaglini et al. 2014). For instance, Switzerland hosts 7400 alpine summer farms that are mainly oriented for dairy production (Mack et al. 2013). Because these high-production breeds are not able to digest highly lignified plants with high tannin contents, *A. viridis* is not an appreciated feeding resource. Therefore, they are not able to actively reduce shrub stands through grazing pressure only.

Robust livestock

Other livestock species and breeds were reported to have the ability to forage on woody species. Indeed, some robust species and breeds have an adaptative digestive system with bacterial populations able to deteriorate lignified material and detoxify secondary metabolites, as well as providing high resistance to tannic compounds (Manousidis et al. 2016). Therefore, they are better suited to digest lignified plants with low digestibility. For example, goat ability to forage on shrublands is widely recognized and they are commonly used for shrub encroachment management (Álvarez-Martínez et al. 2016; Marques et al. 2017; Pauler et al. 2022b). This performance is also linked to their ability to browse in steep slopes and to easily penetrate dense shrub stands due to their small size (Pauler et al. 2022b). More specifically, the typical alpine breed Camosciata goats have been reported to select several woody plants in their diet, and even eat potentially toxic plants such as ferns (Iussig et al. 2015a). Other goat breeds, such as Boer goats also showed high potential for shrub encroachment regulation as they consumed thorny and spiny invasive shrub species (Elias and Tischew 2016). Some sheep breeds were also reported to actively graze on shrubs, such as Welsh Mountain or Scottish Blackface sheep, as reported by Fraser et al. (2009).

Such robust livestock can therefore have the potential to reduce *A. viridis* encroachment through their browsing. For example, the Swiss local Engadine sheep breed were able to intensely consume *A. viridis* leaves and bark, significantly increasing shrub mortality (Zehnder et al. 2016a, b). Another recent study made by Pauler et al. (2022) compared Pfauen goats, Engadine sheep and Dexter cattle impact on *A. viridis* encroachment and showed that Engadine sheep had the highest effect, as they actively debarked the shrub without damaging other valuable forest species such as *Sorbus aucuparia* L., contrarily to goats. Dexter cattle were the least efficient, but managed to break branches and trample on young shrubs. Indeed, some cattle breeds may also have some potential and could be included in *A. viridis* expansion management. For instance, studies conducted on the Herens breed in Switzerland showed that they were able to constrain the expansion of *A. viridis* cover, spending 14% of their time in encroached areas (Meisser et al. 2014).

Highland cattle (**Figure 4**) could be another promising breed to control *A. viridis*-encroachment. Indeed, this low-productive and rustic breed originating from Scotland displays the ability to forage on very low forage quality vegetation (Pauler et al. 2020a,b). As other robust species, Highland cattle have a tannin-tolerant microbial population in their rumen allowing them to digest plants that are unpalatable for high-production breeds. Feeding in poor nutritional quality pastures does not constrain their weight gain, as they can maintain sufficient nutrient intake from a vegetation with high neutral detergent fibre content and low organic matter digestibility, thanks to their low maintenance energy requirement (Berry et al. 2002). Therefore, this breed may be able to feed on woody species such as *A. viridis*, as they forage less selectively in comparison to high-yielding breeds. Indeed, Pauler et al. showed in 2019 that tree and shrub species cover decreased under Highland cattle grazing in comparison to production-oriented breeds, suggesting their higher feeding preference for these woody species. In 2020, Pauler et al. (2020b) demonstrated that Highland cattle forage behaviour in semi-natural pastures in Switzerland was less influenced by some particular plant functional traits in comparison to Angus and Holstein crossbreeds or Braunvieh breed. More specifically, while high-production oriented breeds avoided plants with higher C:N ratio and leaf dry matter content, Highland cattle did not withdraw them from their diet (Pauler et al. 2020b). This suggests that they excluded a smaller range of plant species and they grazed on less palatable species. Moreover, Highland cattle may be able to break branches through rubbing with their long horns, as well as to apply wounds in trunks and roots through trampling, which could be additional ways to weaken shrub species (Della Marianna et al. 2012). Indeed, trunk, branches and root wounds could promote fungal infection, as fungi species have been recorded to effectively affect alder species. Brasier et al. (2004) described different variants of the pathogen species *Phytophthora alni* that attack alder species across Europe, and Pisetta et al. (2012) identified the spread of *Cryptodiaporthe* (*Valsa*) *oxystoma* (Rehm) Urb in *A. viridis* stands in the Italian Alps, which spreads on necrotic bark along with other fungi species, such as *Peniophora aurantiaca* (Bres.) Höhn. & Litsch. Most of these pathogens spread through water (Bjelke et al. 2016), so that a combination of wet weather conditions and lesions applied to the shrub could help spread the disease to the stands and reduce their expansion. In addition, Highland cattle are characterized by rather large claw size relatively to their body weight, which reduces the pressure they exert on the vegetation (Pauler et al. 2020a). Being among the smallest cattle breed, with 24 kg at birth and generally not exceeding 500 kg at adult age (Pauler et al. 2020a; Radkowski et al. 2022), their low weight allows them to graze in steep and wet areas. As well as their adaptative digestive system, this feature was probably gained from the harsh and wet environmental conditions of the Scottish Highlands (Pauler et al. 2020a). Therefore, this ability could be beneficial in steep slopes where *A. viridis* is usually found, and where the manual cutting is even more hardly feasible. Additionally, their robustness drastically reduces any veterinary care compared to other breeds, which makes them well adapted to remote mountain areas. Finally, their long fur may enable the epizoochoric transportation of seeds which could positively affect the botanical composition and help restore former species-rich grasslands (Pauler et al. 2019).

For all these reasons and since Highland cattle have already been used for the control of woody and shrub-encroachment in several environmental conditions, this cattle breed could become a valuable management tool to reduce *A. viridis* encroachment (Lamoot et al. 2005; Harrington and Kathol 2009; Hedtcke et al. 2009; Della Marianna et al. 2012; Cromsigt et al. 2018; Pauler et al. 2019).

Figure 4 Highland cattle in subalpine pastures in Western Switzerland, Bovonne, Bex (VD).

Alnus viridis forage potential

In order to test whether Highland cattle or other robust breeds could be able to actively forage on *A. viridis* and reduce its cover, assessing forage seasonal changes of *A. viridis* leaves is needed to implement better adapted livestock management systems to control its expansion. Indeed, while *A. viridis* shrub encroachment reduces forage quality in alpine and subalpine grasslands, it could be at the same time an underestimated valuable fodder resource for some robust species.

Indeed, *A. viridis* has a high leaf biomass, and Bühlmann et al. (2016) recorded a leaf production of 311 ± 29 g m^{-2} dry weight (DW) measured across different age stands in Switzerland, while Wiedmer and Senn-Irlet (2006) even measured values up to 380 g m^{-2} DW. Considering the production of both *A. viridis* leaves and understory vegetation, the whole green biomass produced is much higher (566 g m^{-2} DW) than that of adjacent pastures (377 g m^{-2} DW) (Bühlmann et al. 2016). These results are consistent with the case study conducted in the Schächental valley by Wiedmer and Senn-Irlet (2006), who calculated a total amount of green biomass of 575 g m^{-2} DW.

Moreover, many shrub species have interesting protein, fatty acid, and antioxidant compound concentrations that could be beneficial for animal nutrition (Leng 1997). The *A. viridis* foliage presents high N concentrations (average of 19.3 mg N g^{-1} DW), which are generally much higher than that of adjacent pastures. Interestingly, *A. viridis* leaves retain N content along all the vegetative season, since the quantity of N in *A. viridis* litter is only 10% lower than that in leaves at biomass peak (Bühlmann et al. 2016). Moreover, its understory vegetation is composed by protein-rich plants that could be eaten by robust breeds and provide non-negligeable protein supply (Zehnder et al. 2016b, 2017). In a trial carried out on *A. viridis*-encroached pastures in Switzerland, Dexter cattle actively browsed the leaves and buds of *A. viridis* and showed no difference compared to Dexter cattle grazing on adjacent pastures in terms of average daily weight gain, meat and carcass quality (Zehnder et al. 2017).

Finally, shrub species often have a slower temporal decline in the nutritional quality of their foliage if compared to surrounding herbage, especially in late summer season and/or during drought periods, which frequency and intensity are rapidly increasing due to climate change (Papachristou and Papanastasis 1994; Ammar et al. 2004; Moore et al. 2015; Ravetto Enri et al. 2020).

Altogether, all these findings suggest that *A. viridis* leaves could be an underestimated valuable forage resource for robust livestock, and a full evaluation of their temporal nutritional variation is required to implement site specific management systems.

Highland cattle foraging behaviour

Livestock feeding preferences are widely affected by plant species forage quality and availability. Indeed, the content in nutrients, fibre, and phenols are essential factors for leaf palatability and digestibility, and therefore drive herbivore diet selection (Leng 1997; Collins et al. 2017; Ravetto Enri et al. 2020; Mahieu et al. 2021). While woody species such as *A. viridis* could be a good forage resource for robust livestock, its voluntary intake by Highland cattle may not be assured under all conditions. Indeed, the palatability of plant species is relative to their abundance and the abundance of the surrounding plant species (Pauler et al. 2020b). In pastures, cattle choices are primarily driven by good quality forage, as it provides the most important resource for daily needs (Pauler et al. 2020b). Moreover, throughout the season, changes in livestock feeding behaviour in grazing systems can also happen, following the temporal changes in forage yield and quality (Bailey and Brown 2011). Therefore, it is important to estimate the intake of woody plants by Highland cattle to fully understand to what extend they can effectively impact shrub encroachment. In addition to the evaluation of their damage on shrub species through defoliation, the knowledge of their forage preferences under different conditions could be integrated into management strategies and help provide a good quality nutrition for animals, while keeping an efficient impact on shrub encroached areas. Even if the understanding of the feeding preference in woody-dominated areas is rather difficult because of the heterogeneity of the vegetation, direct observations are a good way to understand feeding preferences and behaviour in heterogeneous habitats, and they have indeed been commonly used in shrub encroached environments (Elias and Tischew 2016; Nota et al. 2023).

In addition to forage availability and quality, environmental conditions and management choices might play another important role in Highland cattle feeding behaviour and grazing patterns in *A. viridis* encroached pastures. Indeed, factors such as slope, exposition or presence of water can highly impact grazing behaviour (Kohler et al. 2006; Jewell et al. 2007; Auerswald et al. 2010; Schnyder et al. 2010). Therefore, it is essential to evaluate the concurrent effects of vegetation cover, botanical composition, topographical and management conditions in *A. viridis*-encroached pastures on Highland cattle grazing behaviour and distribution.

Tracking cattle behaviour and distribution using GPS technology

In order to assess livestock grazing patterns, many studies have used Global Positioning System (GPS) technology, mostly in the form of collars, in order to record herd spatial distribution within pastures (Bailey et al. 2001; Bailey and Welling 2007; Probo et al. 2014; Koch et al. 2018). Indeed, in the last three decades GPS tracking has become an indispensable and increasingly precise tool to monitor livestock herds. The GPS collars can record the animal position at a very precise scale and time margin, and some are even able to record the activity of the cows. Indeed, some GPS collars (such as Followit Tellus GPS collars used in this project, see **Figure 5**) can be equipped with activity sensors that record the cow neck movements on two different axes, allowing to identify if a cow is eating or resting. Therefore, GPS collars can provide an accurate information on pasture use and selection by livestock (Ungar et al. 2005). This is of particularly high importance in mountainous regions, where the high topographical and climatic heterogeneity affects livestock behaviour at a very fine scale (Homburger et al. 2015). The GPS tracking offers a 24-hours continuous and systematic tracking of the herd that direct visual observations cannot allow, and provides replicable data that is independent on the observer (Homburger et al. 2014, 2015). Moreover, visual observations may alter the behaviour of the animals, as the presence of the observer has to be at a close range for the data to be recorded. Nowadays, new GPS tracking devices are promoted to farmers in order to facilitate their work and the management of their herds. For example, real-time position monitoring collars, such as AlpTracker devices, allow farmers to have instantaneous information on their herds and control their position and well-being (https://www.alptracker-ag.ch/). These devices work through the direct transmission of data from the collar to a personal device through an antenna. Pittarello et al. (2021) showed that the results from such GPS collars are comparable to conventional GPS collars and provide robust information for scientific purpose. Highland cattle grazing in highly encroached pastures could therefore be assessed through the help of adapted GPS tracking systems.

Figure 5 (A) GPS Tellus collar for livestock (©Followit); (B) Highland cattle wearing a GPS collar.

Targeted grazing

Livestock management systems are essential factors in determining animal behaviour and distribution in pastures. Previous studies, such as Probo et al. (2014), have highlighted the importance of an adapted stocking rate in subalpine pastures, as well as of rotational grazing to avoid the over- and under-grazing situations and ensure a more even herd distribution across heterogeneous alpine and subalpine pastures. The implementation of fences, shaded areas, or the water availability have also an important impact on livestock grazing behaviour (Pittarello et al. 2016b; Carnevalli et al. 2019). The strategic placement of dietary supplements can also be used to attract herds into underused areas (Probo et al. 2013, 2016; Pittarello et al. 2016a). This technique could therefore be introduced in highly *A. viridis*-encroached pastures, where the denser areas may be less visited due to their lower forage quality and harsher accessibility conditions. Indeed, studies conducted in the Western Italian Alps have already shown the potential of phosphate salt blocks to significantly attract robust beef cattle into shrub-encroached, marginal and steep areas that were underused (Probo et al. 2013; Pittarello et al. 2016a). In addition to efficiently change the grazing patterns of the herd, this management resulted in a restoration of the grassland vegetation in the medium-term, as cattle were able to gradually open the shrub stands through defoliation, trampling and mechanical damages, and actively transported seeds of high forage quality species from adjacent pastures. Different types of supplements can be used, such as salt blocks or low-moisture molasses-based blocks, which have also been reported to effectively change cattle grazing patterns in extensive American rangelands (Bailey et al. 2001; Bailey and Welling 2007). In addition to the effects produced on vegetation through the modification of grazing patterns, molasses-based blocks are an additional supply of energy and protein for livestock, which could be beneficial in mountain pastures. They also have an appetizing role which could increase the consumption of less palatable forage. For all these reasons, the strategic placement of attractive points in *A. viridis*-encroached areas could help maximising the restoration effects by Highland cattle on dense shrub stands.

Nitrogen translocation in *A. viridis*-encroached pastures

The targeted grazing of Highland cattle in *A. viridis* encroached pastures could have an additional ecological benefit for the restoration of former open pastures. Indeed, in addition to the slow opening of the canopy leading to a progressive reestablishment of typical pasture species, they could also help to rebalance nutrient levels within pastures. Because *A. viridis* generates a N accumulation in the soil and establishes a habitat where a few nitrophilous species can grow, it is important to assess the effect that a targeted grazing management could produce on N redistribution between shrub stands and adjacent pastures. Indeed, previous studies have assessed the effect of livestock grazing on nutrient translocation within pastures, as livestock can redistribute nutrient through urine and faeces excretions (Haynes and Williams 1993; Schnyder et al. 2010). In particular, Haynes and Williams (1993) described in detail the partitioning and the form of several nutrients excreted in livestock urine and faeces that could be later available for pasture plant species. This fertilization related to livestock activity is an important aspect for the maintenance of pasture vegetation. Because cattle occupation in pastures is heterogeneous, with specific areas dedicated to differentiated activities, above all in highly heterogeneous mountain conditions, this nutrient distribution through cattle excretions is also un-evenly spread (Homburger et al. 2015). Indeed, flat and open areas are usually used as resting areas by cattle, where excretion is at its highest levels, while steeper and denser areas are less exploited (Costa et al. 1990; White et al. 2001; Kohler et al. 2006; Buttler et al. 2008; Koch et al. 2018). This is mostly due to the effect of the topography, which constrains cattle resting and grazing activities, but is also due the available vegetation, which is usually of poorer quality in steep slopes, because of shallow soils and reduced nutrient availability (Auerswald et al. 2010). Moreover, it can also be dependent on management infrastructures, as fences, water trough and shaded areas positions can impact livestock distribution and consequently excreta distribution (Jewell et al. 2007; Buttler et al. 2008). Additionally, cattle will tend to graze less in resting areas as the season advances, as vegetation is gradually trampled and fouled by excretions (MacLusky 1960). Including this knowledge in livestock management strategies is crucial to control the nutrient redistribution and avoid overutilization of specific areas, which can lead to nutrient pollution and negative effects on the botanical composition and forage quality in the long term.

The problem of N accumulation under *A. viridis* stands could therefore be mitigated through Highland cattle grazing, by spatially redistributing N from dense areas to adjacent pastures. Indeed, the high N level in both *A. viridis* leaves and the understory vegetation may lead to a considerable N intake by cattle, which could be then excreted in high proportions (Bühlmann et al. 2016; Zehnder et al. 2017). This nutrient redistribution could impact vegetation dynamics and contribute to restore the heterogeneity of alpine and subalpine pasture vegetation. Moreover, Stević et al. (2010) measured high levels of leaf tannins in *A. viridis* shrubs, which could induce higher levels of N excreted through dung instead of urine. Indeed, tannins can bind with proteins in the digestive system of cows, providing a better protection from rumen digestion, favouring the passage of N in faeces rather than urine (Waghorn 2008; Burggraaf and Snow 2010; Theodoridou et al. 2011; Woodfield et al. 2019). This is of importance, as ammonia volatilization and nitrate leaching is far more important through urea (Tamminga 2006; Angelidis et al. 2019; Longhini et al. 2020), and Highland cattle grazing could therefore have a potential beneficial impact on the reduction of the localised N accumulation induced by *A. viridis* encroachment.

Assessing a N budget in *A. viridis*-encroached pastures under the targeted grazing of Highland cattle is therefore an important step in the implementation of such management, in order to fully understand the effects that Highland cattle grazing can exert on pasture vegetation.

Study sites

This PhD thesis was carried out in the framework of the RosbustAlps project, between 2019 and 2023, in four study sites across the Swiss and Italian Alps, where *A. viridis* encroached former open pastures. The sites corresponded to different pedo-climatic conditions with various degrees of *A. viridis* encroachment. Three of the four sites were grazed by Highland cattle herds, with different paddock sizes but similar livestock stocking rates (**Figure 6**). Indeed, the first paddock was located in Val Vogna, in Northern Italy, in the province of Vercelli. This site was the largest of the project and it was grazed by the largest herd (~ 80 cows) for the whole project lenght. It was moderately encroached by *A. viridis* shrubs and had the lowest *A.viridis* cover (20%). The second paddock (summer pasture of "Champlong") was located in Western Switzerland, in the canton of Valais, municipality of Bourg-Saint-Pierre. It was the smallest paddock of the project and was therefore grazed by the smallest herd (12 cows). *A. viridis* encroachment was of 44%. This paddock was only grazed by Highland cattle during 2019 but served as a sampling site for *A. viridis* leaves throughout the project. Three other paddocks were placed in the site of Bovonne, in Western Switzerland, in the canton of Vaud, municipality of Bex. They were grazed by the same herd of approximately 30 cows every year of the project. Each of these paddocks had a different degree of *A. viridis* encroachment, from 51 to 71%. Finally, the last site "Weissenstein" was located in Eastern Switzerland, in the canton of Grisons, municipality of Bergün. This site was not grazed by Highland cattle and only served as a sampling site for *A. viridis* leaves to provide a larger sample pool across the Alps.

With different degrees of *A. viridis* encroachment, the study sites also had open pasture patches with good forage quality where Highland cattle could graze as well. Some of them, specifically Val Vogna, were also largely encroached by *Rhododendron ferrugineum* L.. Historically, part of these sites had undergone pastoral management changes in the last years/decades, such as the reduction of livestock stocking rates and/or small ruminant grazing abandonment (site of Bovonne). However, Val Vogna was grazed by Highland cattle for several years before the project (from 2008), in order to restore the pastures and for extensive meat production purpose, which led to an observed slight opening of the area. Similarly, in Champlong, a Highland cattle herd was also placed in a neighboring *A. viridis* encroached area, where damages were observed over a period of 5 years before project starting.

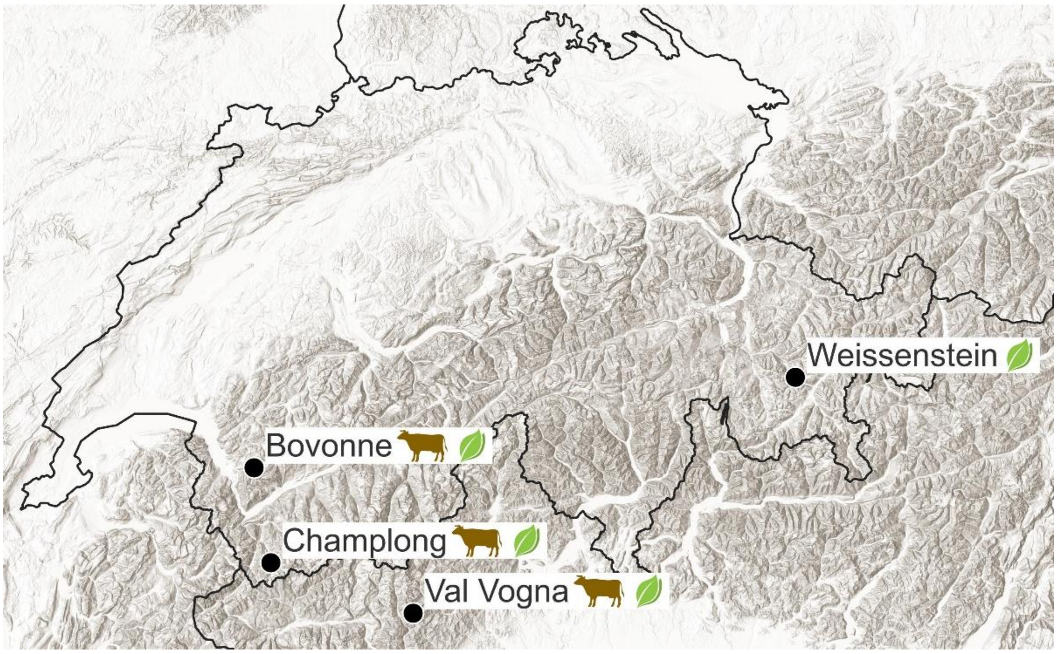

Figure 6 Localisation of the four study sites across Switzerland and Italy. All sites were used for leaf sampling in 2020 and 2021. Paddocks with Highland cattle herds were implemented in Bovonne (3 paddocks, 2019-2023), Val Vogna (1 paddock, 2019-2023) and Champlong (1 paddock, 2019).

Objectives

The main goal of the present doctoral project was to evaluate the potential of targeted Highland cattle grazing to actively counteract the encroachment of *A. viridis*. For this purpose, various objectives were set to draw an ideal management strategy, which are detailed in **Figure 7**. The first objective was to assess the chemical characteristics and digestibility of *A. viridis* leaves along the whole vegetative season (**Chapter II**). The goal of this chapter was to evaluate the shrub forage potential across a range of different alpine pedo-climatic conditions. With this information, an optimal grazing period could be identified to increase *A. viridis* defoliation by Highland cattle, while keeping a proper beef cattle productivity. In **Chapter III**, we assessed the grazing behaviour of Highland cattle to describe their feeding preferences in *A. viridis* and other shrub encroached pastures. Using several sites with contrasting woody vegetation, our objective was to assess Highland cattle feeding behaviour in regard to plant species abundance, by evaluating species consumption and selection and describe animal feeding preferences. In the next chapter, we assessed the main factors impacting Highland cattle occupation patterns in *A. viridis* encroached pastures **(Chapter IV)**. We specifically aimed to assess the spatial distribution of Highland cattle herds thanks to GPS tracking technology and relate it to site-specific topographical, management and vegetation drivers. In **Chapter V**, we assessed the effect of strategically placed attractive points (i.e., molasses-based blocks) on Highland cattle spatial distribution at different scales. In addition, we evaluated the short-term impacts on the surrounding vegetation and *A. viridis* shrubs and compared it control areas. Finally, we assessed the N translocation of Highland cattle in *A. viridis* encroached pastures, to evaluate whether Highland cattle can become a management tool to cope with the N saturation in *A. viridis* stands (**Chapter VI**). We estimated the N fluxes between *A. viridis* encroached areas and adjacent pastures to detect the potential active translocation that could benefit the vegetation on the long term.

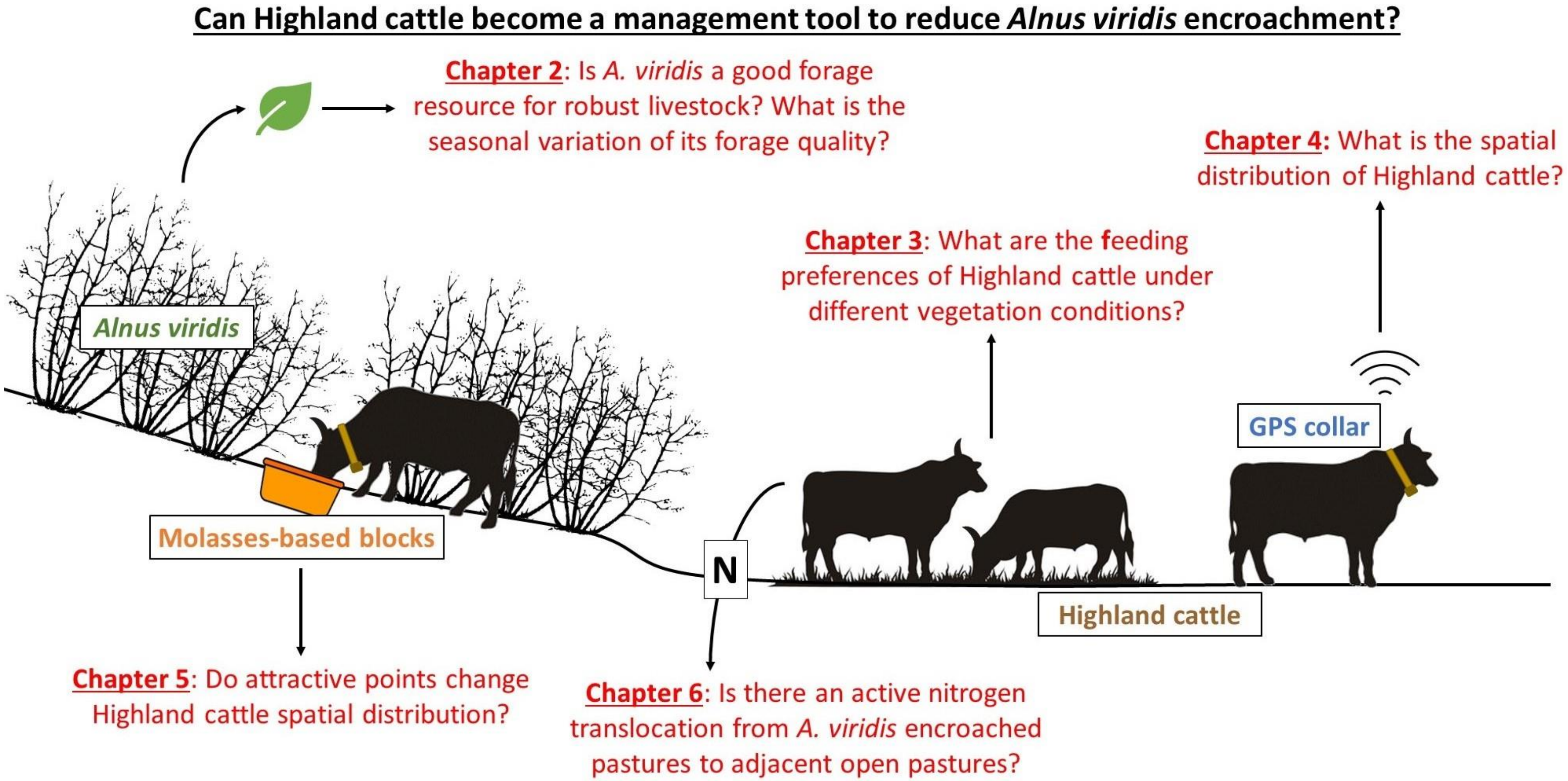

Figure 7 Summary of the objectives of the RobustAlps project

CHAPTER II: *Alnus viridis*: an encroaching species with valuable nutritional value reducing livestock greenhouse gas emissions

Based on: Svensk M, Mariotte P, Terranova M, Pittarello M, Nota, G, Frund D, Dubois S, Manzocchi E, Napoleone F, Meese S, Lombardi G, Allan E, Probo, M. *Alnus viridis*: an encroaching species with valuable nutritional value reducing livestock greenhouse gas emissions. (under review in Agriculture, Ecosystems and Environments).

Abstract

Alnus viridis is a shrub species that has been increasing in many European mountains over the past decades, leading to a loss of agricultural areas and several negative environmental impacts. Recently, targeted livestock management systems have been investigated to reduce its encroachment. This study aims to provide an exhaustive assessment of *A. viridis* leaf composition and its temporal variation across the grazing season. *A. viridis* leaves were collected throughout the summers of 2020 and 2021 in four encroached sites across the Swiss and Italian Alps, characterized by different pedo-climatic conditions. Based on the data collected by meteorological stations, the growing degree days (GDD) were calculated for each site and year. The leaf functional traits (i.e., Leaf Dry Matter Content – LDMC – and Specific Leaf Area – SLA), chemical composition (macro- and micro-elements and fibre content), phenolic content, in vitro organic matter digestibility (IVOMD), and related gas production (CO_2 and CH_4) were assessed. The LDMC significantly increased throughout the season, whereas the SLA decreased. All macro- and micro-elements significantly varied during the season, with leaf nitrogen (N) and phosphorus (P) decreasing. In contrast, leaf fibre contents significantly increased as the season advanced. There was a significant decrease in total phenol and total tannin content during the summer season. Finally, adding *A. viridis* leaves (20% dry matter) to cattle diets significantly reduced IVOMD, methane produced per digested organic matter (CH_4/dOM) and CO_2/dOM, compared to a 100% hay diet. Moreover, CH_4/dOM and CH_4/CO_2 ratio increased during the summer season. These results highlight the potential of *A. viridis* leaves as a valuable forage resource, especially at the beginning of the summer. Such information could be used to optimize grazing of robust livestock in *A. viridis*-encroached alpine pastures in order to reduce its invasion and minimize greenhouse gases production at the same time.

Introduction

Due to agricultural land abandonment in the most marginal areas, tree and shrub-encroachment has strongly increased in European mountains in the last century (MacDonald et al. 2000; Orlandi et al. 2016). This trend has caused the loss of grassland areas in alpine regions, with a reduction in landscape diversity and aesthetic value (Strebel and Bühler 2015; Schirpke et al. 2016). *Alnus viridis* (Chaix) DC is one of the most rapidly spreading shrub species in several European mountain chains (Boscutti et al. 2014; Caviezel et al. 2017; Skoczowski et al. 2021), thanks to high reproduction and growth (Wiedmer and Senn-Irlet 2006). Its expansion can create unfavourable agro-environmental conditions and the reduction of key ecosystem services. Indeed, in addition to the reduction of forage yield due to grassland loss, it also prevents forest succession and does not provide protection from natural hazards such as avalanches (Caviezel et al. 2014; Faccioni et al. 2019). Moreover, its ability to fix nitrogen (N) due to the symbiosis with the N_2-fixing actinomycete *Frankia alni* can lead to N saturation in soils (Bühlmann et al. 2016). This increases risks of nitrate leaching and emissions of greenhouse gasses, such as nitrous oxide, and reduces animal and plant biodiversity (Bühlmann et al. 2017; Zehnder et al. 2020). While *A. viridis* is mostly found on northern and west facing slopes, it is predicted to expand to a wider range of environmental conditions due to its observed occurrence in new habitats (Skoczowski et al. 2021).

Since it is costly and difficult to control its spread through manual cuts, recent studies have investigated the potential of robust livestock to forage on *A. viridis* and reduce its encroachment. For example, (Pauler et al. 2022b) showed that Dexter cattle, Pfauen goats and Engadine sheep can cause significant damage to *A. viridis* shrubs. Indeed, cattle were able to break branches and trample on young shrubs, while goats and sheep foraged on and debarked the plants, with Engadine sheep being the most effective livestock in clearing encroached areas. Other robust breeds, such as Highland cattle, were also evaluated and shown to be efficient in opening dense *A. viridis* stands (Svensk et al. 2022). While it was observed that some species and breeds are thus able to feed on *A. viridis* shrubs, it is essential to assess the full potential of this plant species as a forage resource for livestock. Tree and shrub species can have high potential for ruminant nutrition (Luske and van Eekeren 2017), as they maintain higher nutrient content during the late summer season compared to herbaceous species (Papachristou and Papanastasis 1994; Ravetto Enri et al. 2020). Therefore, shrubs such as *A. viridis* could become an interesting forage supplement and help compensate for animal feeding during the summer and periods with forage shortage. Bühlmann et al. (2016) measured the N concentration of *A. viridis* leaves at different elevations (1650 m and 1950 m) and found slightly higher N concentration at higher elevations. Another study assessed the biomass production of *A. viridis* (Wiedmer and Senn-Irlet 2006), and Stević et al. (2010) measured the total tannin content of *A. viridis* leaves (4.4 ± 0.4%), which is an important factor in leaf digestibility assessment. However, to the best of our knowledge, no study has ever conducted an exhaustive analysis of *A. viridis* leaf functional traits, chemical composition, and nutritive value across the whole summer season. This aspect is crucial to fully understand the forage potential of *A. viridis* and to identify the ideal period for it to be grazed. Several studies have highlighted the importance of assessing leaf characteristics at different development stages to better estimate the nutritive value for livestock. For example, Pauler et al. (2020b) pointed out the significant impact of leaf functional traits, such as specific leaf area (SLA) and leaf dry matter content (LDMC), on cattle foraging behaviour, where larger leaves (i.e., with higher SLA) were preferred for grazing, while leaves with higher LDMC were avoided. Other studies found that these plant traits impacted digestibility, as smaller and denser leaves reduced ruminal degradation (Pontes et al. 2007). Pauler et al. (2020b) also found that leaves with higher N and phosphorus (P) contents were more often selected by cattle, and other research highlighted a significant impact of primary and secondary leaf compounds on forage selection, digestibility, and animal health (Leng 1997; Collins et al. 2017; Ravetto Enri et al. 2020; Mahieu et al. 2021). The impact of phenolic compounds on forage quality is also well known, as high tannin and non-tannin phenolic concentrations tend to reduce palatability and digestibility (Sunnerheim et al. 1988; Kumar and Vaithiyanathan 1990). Finally, leaf neutral detergent fibre (NDF) content is an essential factor for livestock digestibility as well, and has previously been found to increase throughout the growing season for woody fodder species (Papachristou and Papanastasis 1994). Moreover, since livestock production is one of the most important contributors to direct greenhouse gas emissions, especially methane (CH_4) production from ruminants (Slade et al. 2016), it is relevant to investigate the potential mitigating effect of *A. viridis* on cattle digestion and CH_4 emissions. Previous studies investigated the impact of the inclusion of woody plants on forage intake, nutrient digestibility, and gas production (Terranova et al. 2018, 2019), but the impact of *A. viridis* leaves in livestock diet has not been assessed yet, hampering the implementation of livestock management systems to control *A. viridis* expansion.

To fill these knowledge gaps, this study aimed to assess the temporal variation in *A. viridis* leaf functional traits, chemical composition and *in vitro* digestibility and the impact on gas production when they are added to cattle diet. Specifically, the objectives of this study were to assess: (1) the functional traits (LDMC and SLA), (2) the chemical composition (macro/micro- elements and fibre fractions), (3) the phenol composition, (4) the *in vitro* organic matter digestibility (IVOMD) and gas production of *A. viridis* leaves, as well as (5) the relationships among all measured leaf characteristics. We expected that *A. viridis* leaf functional traits, chemical composition, IVOMD, and gas production varied across the season, with a loss of leaf forage quality as the season advanced. We also expected the IVOMD and related gas production of a livestock diet partially composed by *A. viridis* leaves to be lower than that of a control diet purely composed of hay.

Methods

1. Study sites

The study was carried out in 2020 and 2021 in four sites in the Italian and Swiss Alps, characterised by different pedo-climatic conditions (**Figure 8, Table 1**). The first site was located in Val Vogna, in Northern Italy (province of Vercelli), the second and third sites in Western Switzerland, namely in Bovonne (canton of Vaud) and Champlong (canton of Valais), and the fourth one in Bergün (Alp Weissenstein), in Eastern Switzerland (canton of Grisons). In all the sites, areas highly encroached by *A. viridis* were present at comparable elevations (1800-2000 m). At each site, a meteorological station (DWS Decagon weather station from Decagon devices Inc and HOBO Pro v2 U23-00x from Onset Corp., Pocasset, MA) was placed throughout the two years at two meters from ground level to record air temperature every hour.

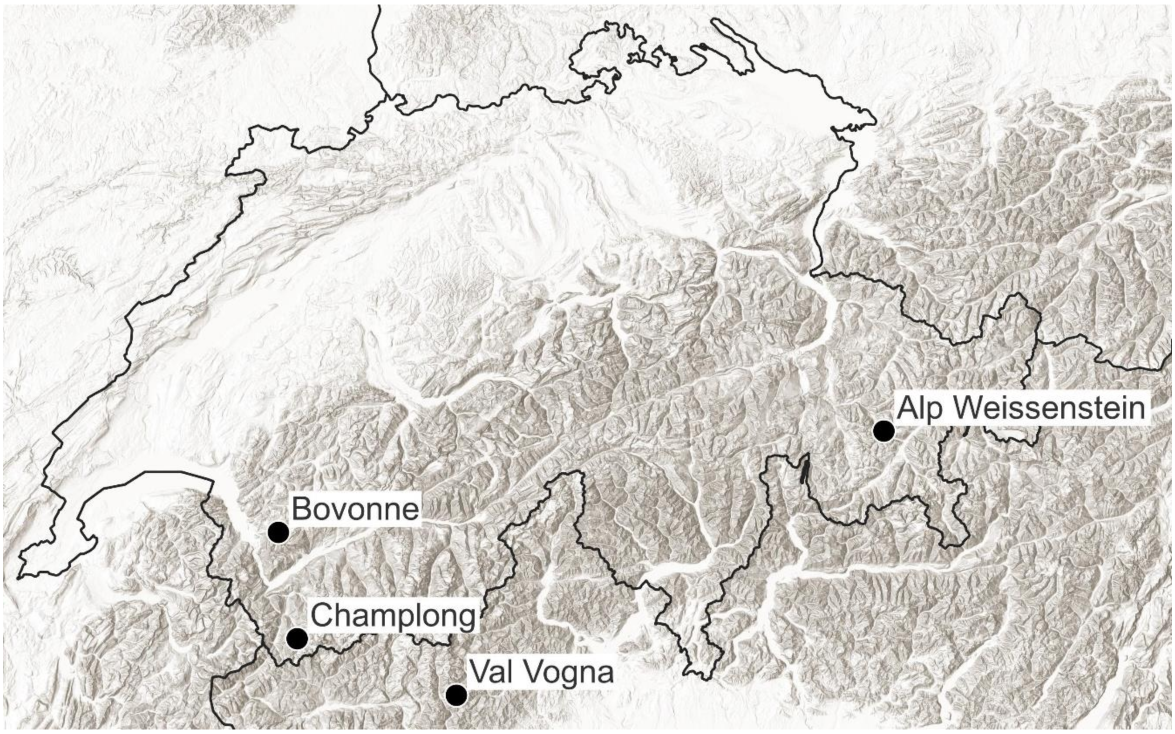

Figure 8 Location of the four sites of the study in the Swiss and Italian Alps. Basemap: ESRI Terrain.

Table 1 Characteristics of the four experimental sites. Soil data refer to the laboratory analyses of the first 10 cm of soil below four different *A. viridis* encroached areas at each site.

	Val Vogna	**Bovonne**	**Champlong**	**Alp Weissenstein**
Coordinates	N45°46’18.815’’ E7°54’9.198’’	N46°16’20.109’’ E7°6’47.327’’	N45°56’2.306’’ E7°12’14.333’’	N46°34’50.711’’ E9°47’58.761’’
Elevation (m)	1897 ± 67	1820 ± 66	2012 ± 36	2033 ± 26
Aspect (°N)	102.15	105.38	111.43	190.00
Mean annual precipitation (mm)	881[a]	1674[b]	1023[b]	1200[b]
Mean annual temperature (°C)	7.46[c]	4.51[b]	4.21[b]	1.79[b]
Soil total organic carbon (TOC mg kg^{-1})	548.53 ± 37.48	282.04 ± 42.45	483.89 ± 70.93	370 ± 33.37
Soil total nitrogen (TN mg kg^{-1})	56.33 ± 4.61	19.48 ± 1.21	49.02 ± 9.41	29.22 ± 3.04
Soil dissolved inorganic nitrogen (DIN mg kg^{-1})	16.74 ± 5.1	12.36 ± 1.1	19.35 ± 4.49	10.44 ± 1.86
Soil available phosphorus (mg kg^{-1})	11.27 ± 5.32	0.56 ± 0.13	9.36 ± 4.47	5.23 ± 1.77
pH (H_2O)	3.84 ± 0.03	6.27 ± 0.28	5.44 ± 0.18	4.71 ± 0.22

[a] Mean annual precipitation (mm) for the period from 1977 to 2007.

[b] Mean annual precipitation (mm) or mean annual temperature (°C) for the period from 1991 to 2021.

[c] Mean annual temperature (°C) for the period from 1977 to 2007, calculated on a 10 km² grid.

2. *Alnus viridis* leaf sampling

During the summers of 2020 and 2021, 1.75 kg of *A. viridis* fresh leaves were collected three times (i.e. in June, July and August) at each site to assess the seasonal changes in leaf functional traits, chemical composition, phenolic contents, and IVOMD. For each sample, *A. viridis* leaves and petioles were hand-harvested from different points in the canopy of five different trees, at a maximum height of 1.80 m to simulate the potential browsing of cattle (Svensk et al. 2022). New trees were selected for each harvest at all four sites to analyze seasonal changes without repeated harvesting, which would imply feeding pressure. Moreover, sampled *A. viridis* shrubs were chosen outside of grazed areas and they were comparable in terms of height (three to five meters high).

3. Leaf functional traits

The LDMC was measured according to the protocol of Cornelissen et al. (2003). For each of the five trees and for each sampling time, 10 leaves were randomly selected and then weighed to record their fresh weight (FW). Afterwards the samples were dried at 60°C for 72 hours and their dry weight (DW) was recorded. The LDMC was then calculated as followed:

$$\text{LDMC (mg g}^{-1}\text{)} = \text{DW(mg)} / \text{FW(g)}$$

The SLA was measured according to Cornelissen et al. (2003) using the leaves collected for LDMC described above. Before drying the leaves, we measured the surface of all 10 leaves of each sample by photocopying them and calculating their area with an image analysis software (ImageJ, Schneider et al. 2012). This allowed us to calculate SLA as followed:

$$\text{SLA (cm}^2\text{ g}^{-1}\text{)} = \text{leaf surface (cm}^2\text{)} / \text{DW (g)}$$

4. Leaf chemical composition

A total of 350 g of fresh leaves were taken from each collection batch and dried at 60°C for 72h. After being ground to pass a 1-mm screen (Brabender rotary mill; Brabender GmbH & Co. KG, Duisburg, Germany), leaf samples were analysed to determine residual dry matter (DM) content by heating at 105˚C for 3h, followed by incineration at 550°C until a stable mass was reached, to determine the ash content according to ISO 5984_2002 (prepASH, Precisa Gravimetrics AG, Dietikon, Switzerland). Mineral content (i.e. P, calcium, potassium, magnesium, copper, iron, manganese and zinc) was analysed according to the EN 15510:2008 by ICP-OES (ICP-OES 5800, Agilent Technologies, Switzerland) after microwave digestion. The samples were dissolved in a glass tube (5ml HNO_3 65% + 3 ml H_2O ASTM Class I) using a microwave digester (UltraClave MLS, Leutkirch, Germany) at 235°C for 60 min (1000 W). If necessary, samples were diluted with HNO_3 2% prior to analysis. The N content was determined by the Dumas method (ISO 16634-1:2008) and crude protein was calculated as N × 6.25.

Fibre fractions were analysed according to Van Soest et al. (1991). The neutral detergent fiber ($aNDF_{OM}$) and acid detergent fiber (ADF_{OM}) contents (ISO 16472:2006 for NDF and ISO 13906:2008 for ADF) were determined gravimetrically (ISO 6865:2000) after acid and alkaline digestions using a fibre analyser (Fibretherm Gerhardt FT-12, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) and were expressed without residual ash after incineration at 600°C for 3 h. The $aNDF_{OM}$ was evaluated with heat stable α-amylase and sodium sulfite. Acid detergent lignin (ADL) was analysed according to ISO 13906:2008. Hemicellulose and cellulose concentrations were estimated as $aNDF_{OM}$ minus ADF_{OM} and ADF_{OM} minus ADL, respectively.

5. Leaf phenolic compounds

Concentrations of total extractable phenols (TEP) and non-tannin phenols (NTP) were determined using a modified Folin-Ciocalteu method according to Makkar (2003). For a detailed description of the method see Terranova et al. (2018). The determination of the condensed tannins (CT) was performed with the butanol-HCl-iron method (Makkar 2003) and the contents were given as leucocyanidin equivalents. Total tannins (TT) and hydrolysable tannins (HT) were calculated as TEP minus NTP and TT minus CT, respectively.

6. Leaf *in vitro* organic matter digestibility and gas production measurements

The assessment of IVOMD and gas production was made using the *in vitro* incubation with the Hohenheim gas test (HGT) method, which was performed as outlined by Menke and Steingass (1988). For each sampling date, the remaining leaves from the five trees were pooled in one sample, ending up in 12 samples per year. *A. viridis* leaves were freeze dried and ground with a centrifugal mill (Model ZM1, Retsch GmbH, Haan, Germany) to pass through a 1 mm sieve. The incubation of *A. viridis* leaves was performed by combining the leaves with hay (ryegrass dominated sward, crude protein = 11.63%; NDF = 48.35%), in a ratio of 1:4 on a DM basis. This proportion was chosen to simulate the diet of Highland cattle in *A. viridis*-encroached pastures, as described in Svensk et al. (2023). A diet of hay (100%) served as the control. For each year, rumen fluid was collected from three ruminally-cannulated multiparous late lactating Original Braunvieh cows, and was collected before morning feeding three times, across three weeks in both 2020 and 2021. It was then transported in a pre-heated thermos flask to the laboratory. Within one hour after collection, rumen fluid was strained through four layers of gauze and added to a buffer solution in a 1:2 ratio according to the protocol of Menke and Steingass (1988). For incubation, modified 100-ml glass syringes with two outlets, one for fluid and one for gas sampling, were used as described in Soliva and Hess (2007). The incubation lasted for 24 h at 39 °C in an incubator with an integrated rotor. After 24 h, the fermentation gas volume was recorded from the calibrated scale printed onto the syringes, and the fermentation was terminated by removing the incubation fluid from the syringes while the gas phase remained inside. Fermentation gas samples of 150 µl were taken from the incubation syringes and injected using a gas-tight Hamilton syringe (Hamilton AG, Bonaduz, Switzerland) into a gas chromatograph (6890N, Agilent Technologies, Wilmington, DE, USA) equipped with a thermal conductivity detector. Concentrations of CH_4 and CO_2 were analysed with this detector, and expressed per digested organic matter (dOM). Each pooled sample and the control were incubated in duplicates per run resulting in a total number of n = 6 per each year. Two syringes filled with only rumen fluid buffer mixture were incubated as blank.

7. Statistical analyses

All statistical analyses were performed using R version 4.2.2 (R Core Team, 2021). The cumulative growing degree-days (GDD) of each site was calculated from the meteorological stations as described in Grigorieva et al. (2010):

$$GDD = \sum_{i=1}^{n} \left[\left(\frac{Tmax + Tmin}{2} \right) - Tbase \right]$$

where i = n are the days with a temperature above 0°C, Tmax and Tmin are the daily maximum and minimum temperature respectively, and Tbase is a threshold temperature defined here at 5°C following the description for pasture grasses from Grigorieva et al. (2010). The GDD was then used as a proxy to represent the seasonal temperature changes, as it allows comparison between sample dates of both years and is commonly used in agro-ecosystems as an indicator of season advancement (McMaster 1997; Grigorieva et al. 2010). We tested the effect of GDD on plant functional traits, leaf chemical composition, and phenolic contents using a Linear Mixed-effect Model (lme, package "nlme"), with GDD as a fixed effect and "year" nested in "site" as random factors. The same model was used to test the effect of GDD on IVOMD and fermentation gas with the "cow" used for the experiment as an additional random factor. When residuals were not normally distributed, a log transformation was applied to the data. Some outliers were also deleted (0.83% of the data was deleted for Fe and Zn and 3.33% for Cu). The marginal and conditional R-squared values were obtained using the "performance" package (function "model_performance"). For IVOMD and gas measurements, a comparison between *A. viridis* treatment (20% DM *A. viridis* leaves, 80% DM hay) and the control (only hay) was made using a Student's test (*t*-test) for independent samples. Finally, a principal component analysis (PCA, FactoMineR package) was performed to assess the relationships among all variables. For this analysis, the leaves of the five trees of each sampling date were pooled in one sample, ending up in 12 samples per year.

Results

1. Leaf functional traits

The GDD were not significantly different between 2020 and 2021 ($P = 0.41$) and ranged from 151.31 to 721.68 in 2020 and from 221.02 to 799.49 in 2021. The LDMC of *A. viridis* leaves significantly increased with GDD, from 258.70 to 483.41 mg g^{-1} (**Figure 9A**, $P < 0.001$, R^2 marginal = 0.49). In contrast, the SLA of *A. viridis* leaves significantly decreased with GDD (**Figure 9B**, $P < 0.001$, R^2 marginal = 0.11) from 348.72 to 104.16 cm^2 g^{-1}.

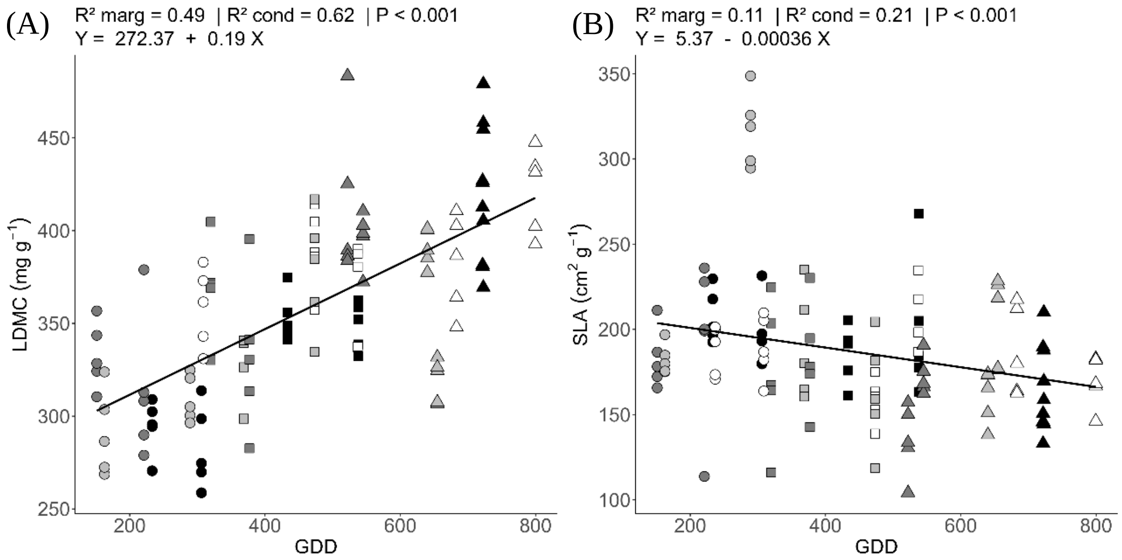

Figure 9 Variation of (A) Leaf Dry Matter Content (LDMC) and (B) Specific Leaf Area (SLA) in relation to growing degree days (GDD) in Val Vogna (white), Bovonne (black), Champlong (dark grey) and Weissenstein (light grey). Sampling periods are represented with different shapes as circles (June), squares (July) and triangles (August).

2. Leaf chemical composition

All macro- and micro-elements of *A. viridis* leaves were significantly affected by seasonal temperature changes. Indeed, N, P, K, Cu and Zn all significantly decreased with increasing GDD (**Figure 10A**, **B**, **D**, **F**, **I**, $P < 0.001$). In contrast, Ca, Mn, Fe and Mg significantly increased with GDD (**Figure 10C**, **E**, **G**, **H**). The N and P contents ranged from 46.98 to 22.62 g kg^{-1} DM and from 5.84 to 0.92 g kg^{-1} DM, respectively. Therefore, leaf crude protein content had minimum and maximum values of 141.37 g kg^{-1} DM and 293.63 g kg^{-1} DM, respectively. Residual ash ranged from 32.07 g kg^{-1} DM to 62.79 g kg^{-1} DM, and increased significantly with GDD ($P < 0.001$).

The fibre content of *A. viridis* leaves, aNDF_{OM}, aADF_{OM}, and ADL concentrations significantly increased with increasing GDD (**Figure 11**, $P < 0.01$), ranging from 202.77 to 454.59 g kg^{-1} DM, from 116.14 to 353.46 g kg^{-1} DM and from 41.61 to 257.28 g kg^{-1} DM, respectively. *A. viridis* leaves had a hemicellulose content of 124.8 g kg^{-1} DM, and a cellulose content of 97.4 g kg^{-1} DM on average.

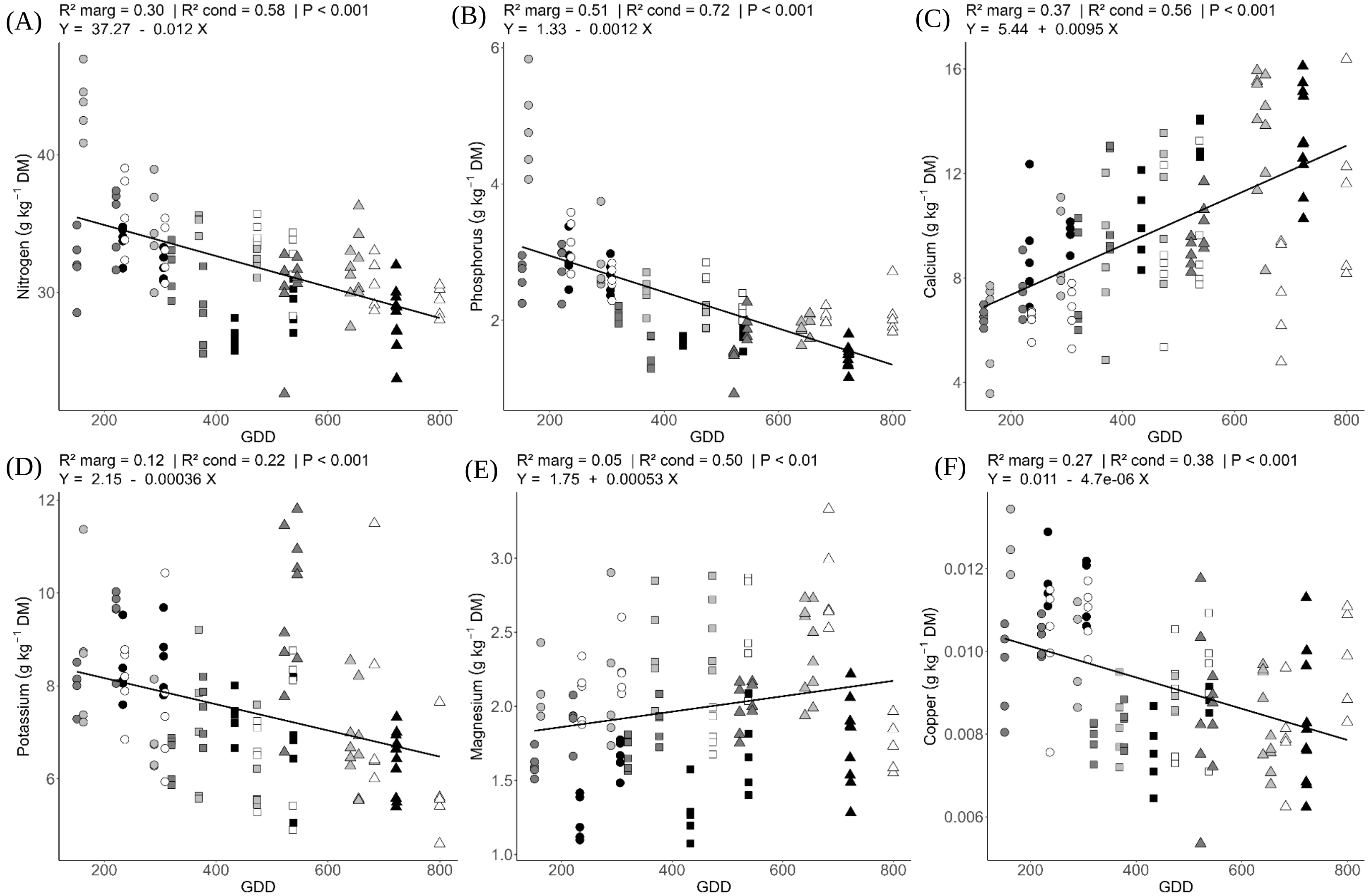

Figure 10 Variation of (A) nitrogen, (B) phosphorus, (C) calcium, (D) potassium, (E) magnesium, (F) copper, (G) iron, (H) manganese and (I) zinc, in relation to growing degree days (GDD) in Val Vogna (white), Bovonne (black), Champlong (dark grey) and Weissenstein (light grey). Sampling periods are represented with different shapes as circles (June), squares (July) and triangles (August).

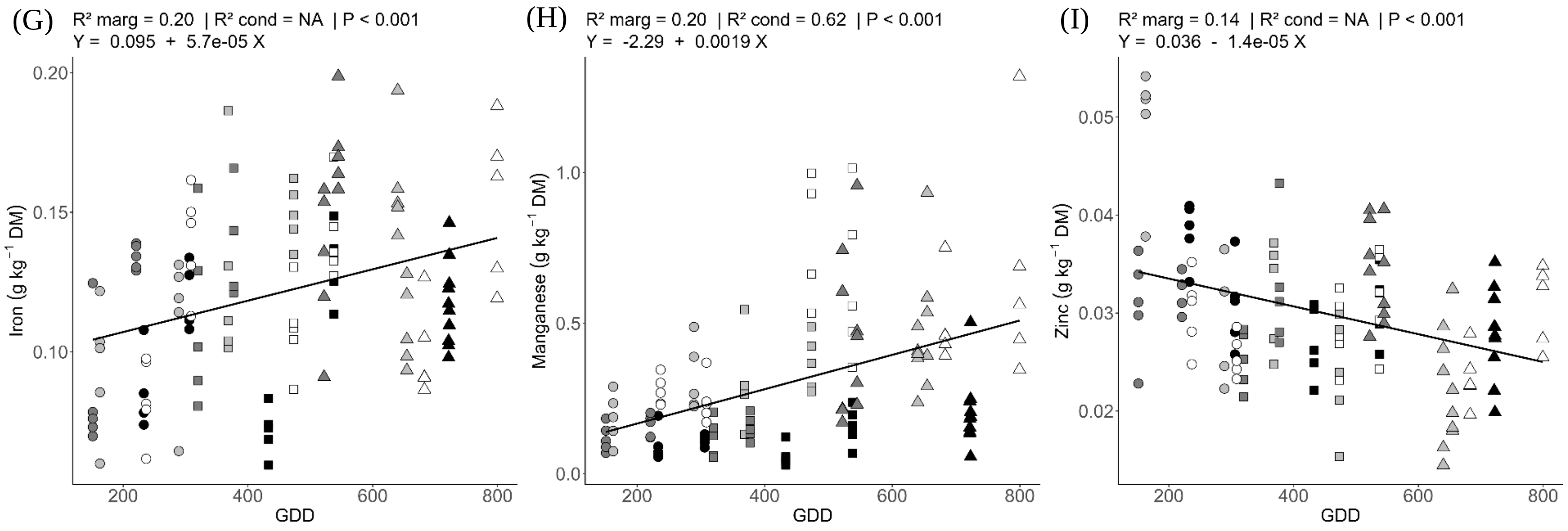

Figure 10 continued

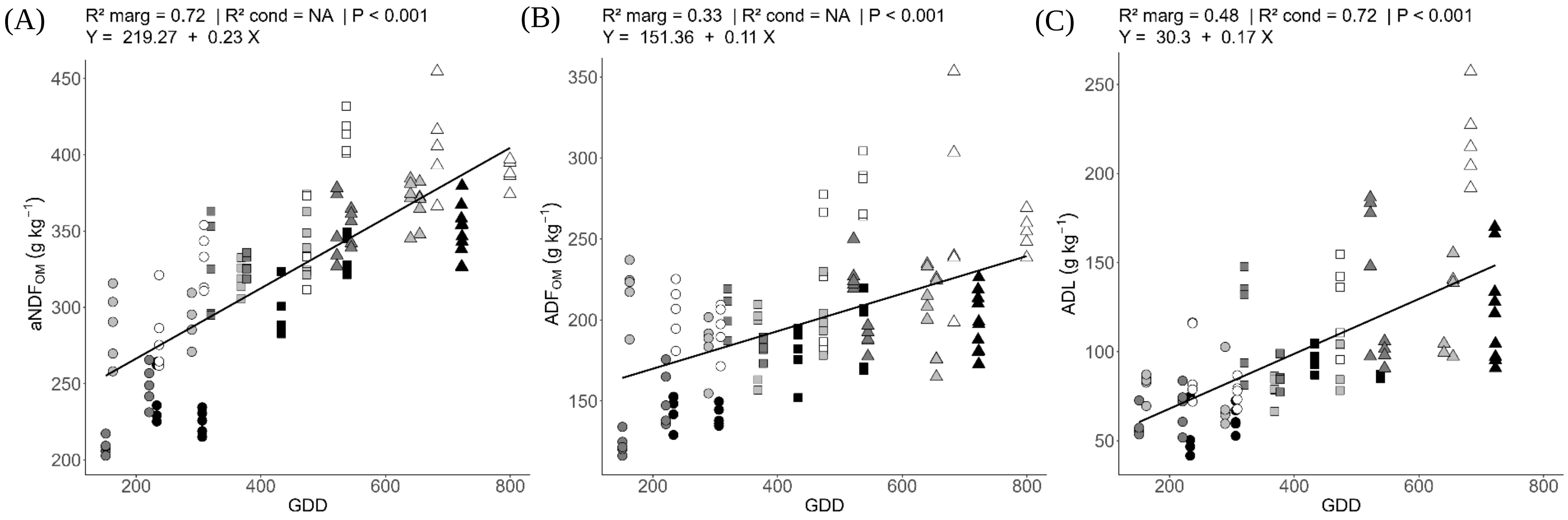

Figure 11 Variation of aNDF$_{OM}$ (A), ADF$_{OM}$ (B) and ADL (C) in relation to growing degree days (GDD) in Val Vogna (white), Bovonne (black), Champlong (dark grey) and Weissenstein (light grey). Sampling periods are represented with different shapes as circles (June), squares (July) and triangles (August).

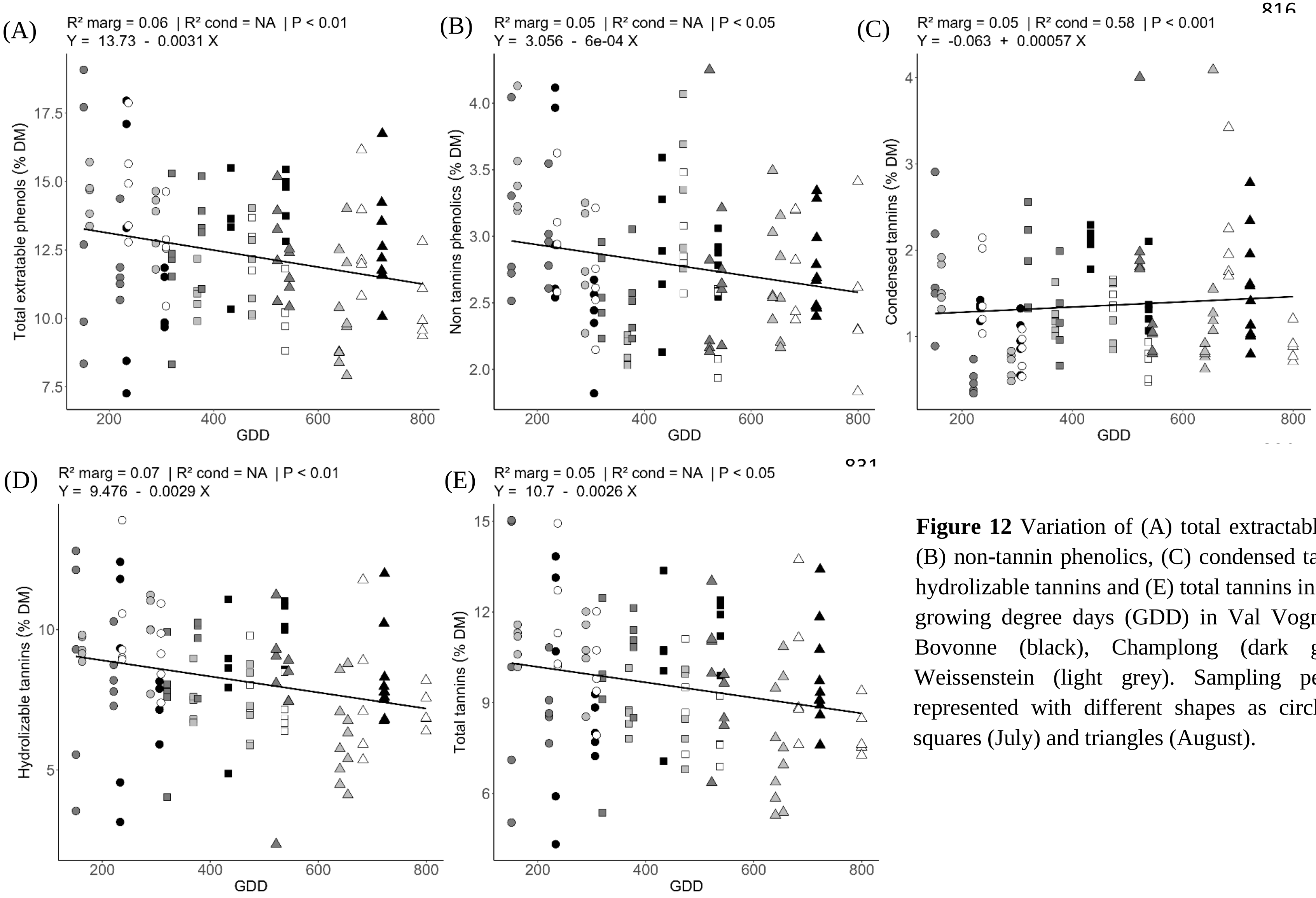

Figure 12 Variation of (A) total extractable phenols, (B) non-tannin phenolics, (C) condensed tannins, (D) hydrolizable tannins and (E) total tannins in relation to growing degree days (GDD) in Val Vogna (white), Bovonne (black), Champlong (dark grey) and Weissenstein (light grey). Sampling periods are represented with different shapes as circles (June), squares (July) and triangles (August).

3. Leaf phenolic compound

While having the weakest relationships with GDD compared to other leaf compounds, all phenolic compounds measured in *A. viridis* leaves significantly changed over the season (**Figure 12**). The TEP significantly decreased with GDD (**Figure 12A**), as did the non-tannin phenolics (NTP, **Figure 12B**). Leaf TEP ranged from 19.08% DM to 7.26% DM, and NTP ranged from 4.25% DM to 1.82% DM. Condensed tannins (CT) slightly increased with GDD (**Figure 12C**), from 0.34% DM to 4.09% DM. In contrast, HT and TT concentrations in the leaves decreased (**Figure 12D, E**), with values ranging from 13.90% DM to 2.36% DM and 15.03% DM to 4.32% DM, respectively.

4. Leaf in vitro organic matter digestibility and gas production

Including 20% of *A. viridis* leaves in the hay diet for in vitro incubation, reduced the IVOMD by 5.80% on average (**Figure 13A**), with an average digestibility of 63.30 ± 0.19% compared to 69.10 ± 0.46% for the control. The IVOMD of the *A. viridis*-composed diet also significantly decreased over the season (**Figure 13A**), from a maximum value of 66.80% to a minimum value of 60.11%.

The amount of CH_4 produced per unit of digestible OM (CH_4/dOM) was reduced on average by 17.73% when adding *A. viridis* leaves (**Figure 13B**, Student's *t*-test $P < 0.01$), with a mean value of 43.83 ± 0.48 ml g^{-1}, compared to 53.27 ± 0.46 ml g^{-1} of the control. It significantly increased with GDD (**Figure 13B**), ranging from 34.54 ml g^{-1} to 49.86 ml g^{-1}.

The amount of CO_2 produced per unit of digestible OM (CO_2/dOM) followed an opposite trend, with a significant reduction with the seasonal increase in GDD **Figure 13C**). It ranged from 305.03 to 259.84 ml g^{-1}, and was also significantly reduced (by 5.84%) compared to the control (**Figure 13C**, Student's *t*-test $P < 0.01$), with average values of 278.74 ± 0.22 ml g^{-1} and 297.03 ± 2.09 ml g^{-1}, respectively.

Therefore, the CH_4/CO_2 ratio obtained from the *A. viridis* diet was also significantly reduced compared to the control (**Figure 13D**, Student's *t*-test $P < 0.01$), and increased significantly with GDD, ranging from 121.28 to 181.62 ml l^{-1} (**Figure 13D**).

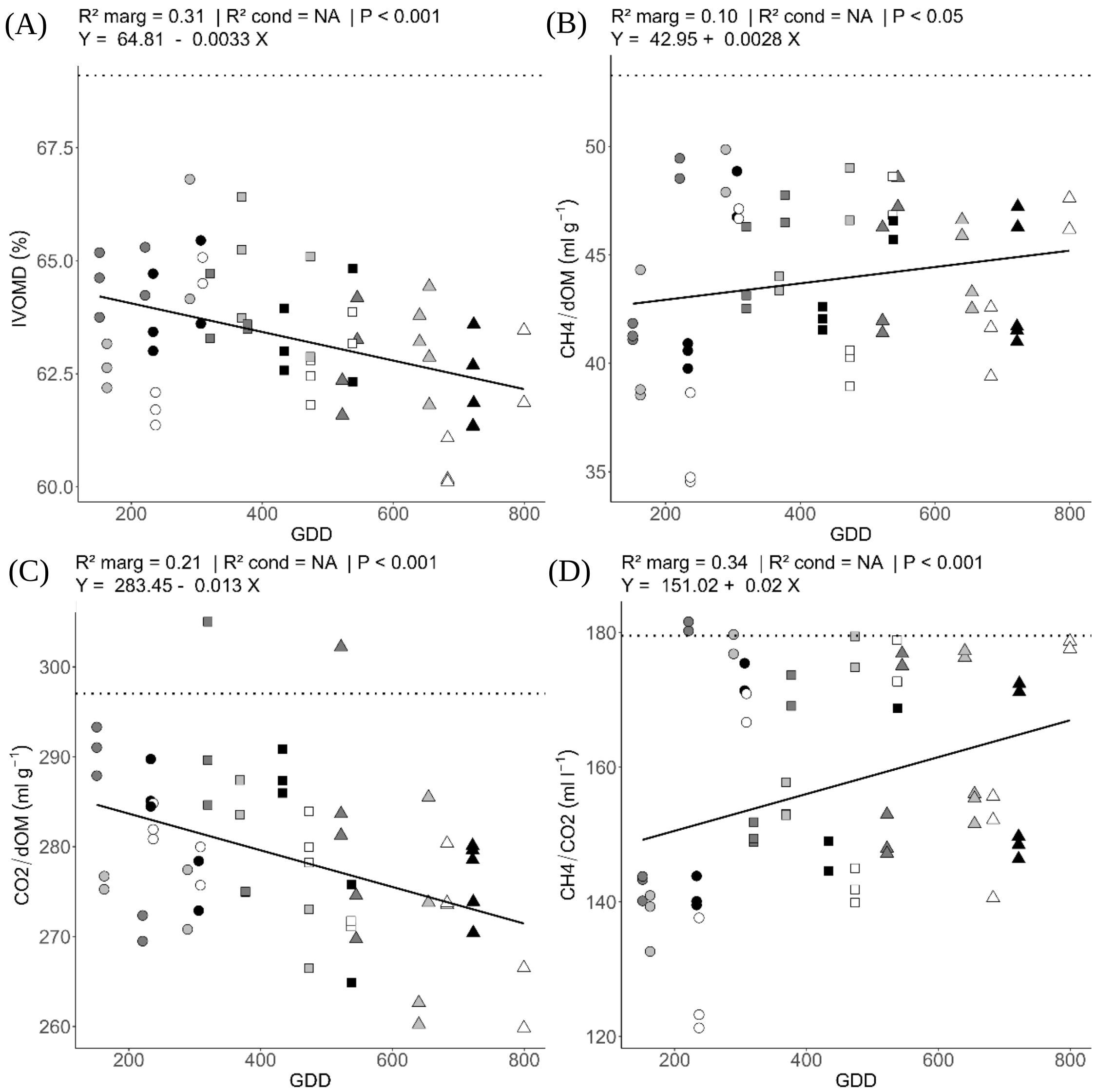

Figure 13 Variation of (A) in vitro organic matter digestibility (IVOMD), (B) methane per digested organic matter (CH_4/dOM), (C) carbon dioxide per digested organic matter (CO_2/dOM) and (D) the ratio between methane and carbon dioxide (CH_4/CO_2) in relation to growing degree days (GDD) in Val Vogna (white), Bovonne (black), Champlong (dark grey) and Weissenstein (light grey). Sampling periods are represented with different shapes as circles (June), squares (July) and triangles (August). The dotted line represents the control for comparison, i.e. the diet composed by 100% hay.

5. Principal component analysis

The results of the PCA are presented in **Figure 14**. The first PCA axis (PC1, accounting for 33.7% of the variance) reflected the seasonal changes in *A. viridis* leaves, with increasing GDD, NDF, LDMC, and Ca, and decreasing P, HT, Zn, Cu and N. The second axis (PC2, accounting for 16.5% of the variance) reflected a gradient of increasing CT, ADL, TEP and TT, and decreasing CH4/dOM, IVOMD, CH_4/CO_2 and Fe. The PCA grouped the different phenolic compounds together, as well as the fibre fractions.

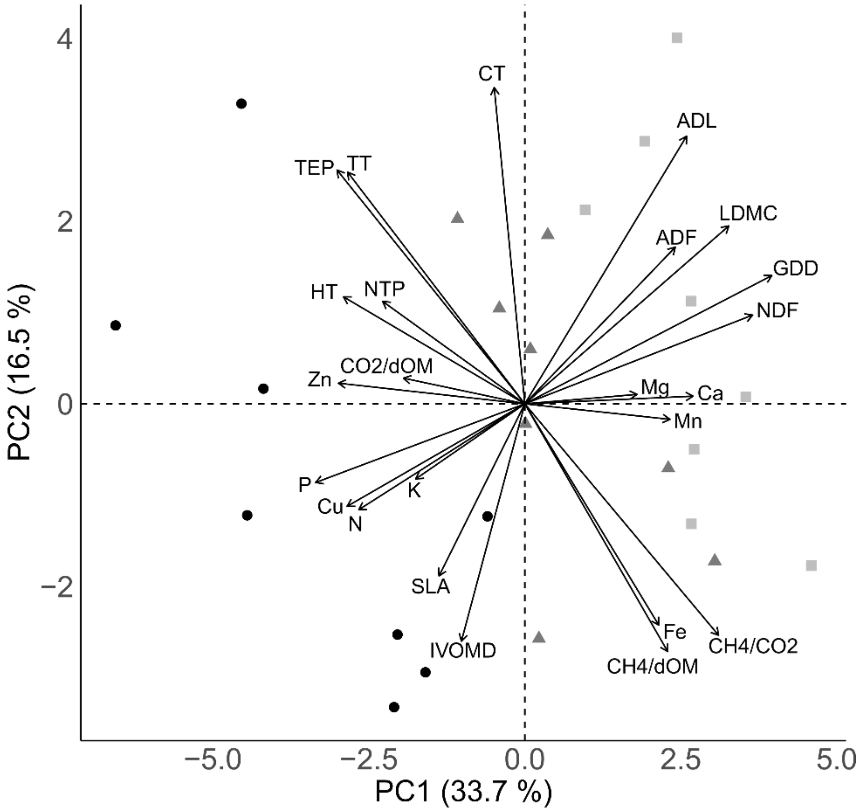

Figure 14 Principal component analysis (PCA) for *A. viridis* leaf functional traits, macro- and micro-elements, fibre fractions, phenolic compounds, gases per digested organic matter, in vitro organic matter digestibility, and the GDD. The samples are represented in black dots (June), dark grey triangles (July) or light grey squares (August).

Discussion

Significant seasonal variations were found in all *A. viridis* leaf components, as all measured variables were significantly affected by GDD. PCA results provided a summary of seasonal changes of leaves, as the first axis represented the season progress.

1. Leaf functional traits

Throughout the season, we found similar SLA and LDMC values for *A. viridis* compared to previous studies (Skoczowski et al. 2021) and compared to other alder species (Pierce et al. 1994). As we expected, *A. viridis* leaf functional traits varied during the season, and leaf SLA and LDMC changed in opposite directions, leading to denser leaves with higher dry matter content as the season advanced. This trend was supported by the PCA that showed these two functional traits following opposite patterns, with LDMC positively correlated to GDD. A study on *A. glutinosa* made by De Kort et al. in 2014 showed similar trends, as the alder had smaller leaves with increasing temperature. Skoczowski et al. (2021) also found variation in *A. viridis* leaf morphology according to elevation, linked to the different climatic conditions and stressful environments. Previous studies on other woody shrubs and trees have also provided similar results (Wilson et al. 1999; Qin et al. 2018). This pattern is explained by tree strategies to acquire or retain resources depending on the environment. SLA and LDMC are directly linked with leaf biomass and nutrient assimilation, and lower SLA and higher LDMC values at the end of the season occur because the plant focuses on conservation of acquired resources (Qin et al. 2018; Ravetto Enri et al. 2020). Consequently, as for other forage plants, *A. viridis* leaves may be less selected by cows at the end of the summer Pauler et al. (2020b).

2. Leaf chemical composition

The chemical composition of *A. viridis* leaves varied significantly as the season advanced. Indeed, we found similar decreasing patterns for many important leaf nutrients (N, P, K, Cu, and Zn) during the summer season. This decrease is in line with the findings on *A. glutinosa* by Rodríguez-Barrueco et al. (1984), who showed that leaf N, P, and K decreased during the vegetative season as well. Other studies on alder species (Dawson and Funk 1981; Chapin and Kedrowski 1983; Luske and van Eekeren 2017) or on other woody plants (James and Smith 1978; Niinemets and Tamm 2005; Gowda et al. 2019) detected similar trends, with the lowest values for these leaf nutrients in autumn. Our results from the PCA also highlighted tight correlations between some of these elements (i.e., positive correlations between N-P and N-K), as also shown by Rodríguez-Barrueco et al. (1984) for *A. glutinosa* leaves. The relationship between leaf N, P and K is well documented, and plays an essential role in the eco-physiological processes of the plant, contributing to photosynthesis, growth and reproduction (Tian et al. 2019). Moreover, in the case of N-fixing trees such as *A. viridis*, the N fixation leads to a higher P demand compared to non-N-fixing plants, thus creating a positive relationship between N and P (Dawson 2008). On the other hand, other *A. viridis* leaf nutrients displayed an increase with leaf senescence, such as leaf Ca, Fe, and Mn, which were also shown to increase in *A. glutinosa* leaves (Rodríguez-Barrueco et al. 1984; Luske and van Eekeren 2017). However, the increase in *A. viridis* leaf Mg was not found in *A. glutinosa* but was found in other woody plants (James and Smith 1978). The increase in *A. viridis* leaf Ca contrasts with herbaceous species, where Ca is generally reported to be stable across the growing season by Schlegel et al. (2016). This increase, as well as the increase in leaf Mn, could be explained by their low mobility in the phloem and the lack of remobilization of these elements from leaves with senescence (White 2012; Maillard et al. 2015).

Despite this significant variation throughout the season, *A. viridis* N leaf concentrations (22.62 to 46.98 g kg^{-1} DM) were similar to previous measurements made on this species, e.g. Bühlmann et al. (2016) measured in late July and early August 28.2 ± 0.2 g kg^{-1} DM and 29.4 ± 0.4 g kg^{-1} DM at low (1650 m) and high (1950 m) elevations, respectively. These results are relatively high in comparison to other woody species, as Tian et al. (2018) found a global mean of 21.13 g kg^{-1} DM when analysing a compiled dataset of several deciduous woody species across continents. This high N concentration in *A. viridis* leaves was expected due to its symbiosis with *F. alni*, which leads to an accumulation of N in the roots and therefore higher N uptake by the plant (Dawson 2008; Luske and van Eekeren 2017). In addition, as they are not N limited, *Alnus* species do not need to resorb leaf N before winter, leading to potentially higher leaf N concentrations at the end of the season (Dawson and Funk 1981; Rodríguez-Barrueco et al. 1984; Han et al. 2013; Maillard et al. 2015). Consequently, as previously found for other alder species (Luske and van Eekeren 2017; Mahieu et al. 2021), *A. viridis* leaf crude protein concentrations, which always exceeded 140 g kg^{-1} DM, were higher than those usually found in temperate green fodder or in permanent grassland at the beginning of the season (Agabriel 2010; Schlegel 2021). Indeed, if compared to typical leguminous forage species found in nutrient-rich grasslands, such as *Trifolium pratense* L. or *Trifolium repens* L., the crude protein content is similar and sometimes higher for *A. viridis* leaves, with values ranging from 141.37 to 293.63 g kg^{-1} DM compared to 161 to 261 g kg^{-1} DM for the two *Trifolium* species (Daccord and Arrigo 2001). Additionally, *A. viridis* leaf N content also mostly exceeded some nutrient optimum requirements for sheep and cattle, as described by Hejcman et al. (2016). Since leaf crude protein concentrations lower than 7% have been shown to decrease forage intake by livestock (Minson and Milford 1967), *A. viridis* could remain a good source of crude protein for livestock throughout the season, despite its seasonal decrease (González-Hernández et al. 2000).

Similarly, high concentrations were found for other nutrients measured in *A. viridis* leaves. For instance, leaf Ca, Mg and P concentrations were similar or higher than that found in typical leguminous forage species. Indeed, *A. viridis* leaf P content ranged between 0.92 to 5.84 g kg^{-1} DM compared to 4.0-4.1 g kg^{-1} DM usually found in *T. pratense* and *T. repens* (Daccord and Arrigo 2001). They were also comparable to the optimum nutritive range, as described by (Hejcman et al. 2016). *A. viridis* leaf P was also similar to that of *A. glutinosa* measured by Luske and van Eekeren (2017), and to other deciduous woody plants (Tian et al. 2018). On the other hand, *A. viridis* leaf K was lower than that of leguminous species but within the range defined by Hejcman et al. (2016). A few out-of-range values for P were found in the samples from Weissenstein at the beginning of the 2020 season, for which the highest N, Cu and Zn concentrations were also recorded. Because macro- and micro- elements are essential for animal health, in terms of growth and reproduction (Mahieu et al. 2021), *A. viridis* leaves could become a significant resource for livestock.

The range of fibre and lignin concentrations of *A. viridis* leaves were very similar to those found for other alder species in France by Mahieu et al. (2021), and were higher than contents found for herbaceous vegetation used as a comparison in the same study. As the season advanced, leaves became more fibrous, as all fibre fractions showed an increase from June to August. This is in line with previous studies on other alder species such as *A. rubra* (González-Hernández et al. 2000) and other deciduous shrubs and trees (Happe et al. 1990; Papachristou and Papanastasis 1994), which showed that NDF, ADF, and lignin leaf content increased from spring to autumn, leading to a lower forage quality at the end of the summer season. Indeed, forage with high fibre contents leads to lower nutrient digestibility and assimilation with plant senescence, as lignin has been described as one of the most important factors limiting the degradation of cell walls in the rumen (Van Soest et al. 1991; Cherney et al. 1993; Mahieu et al. 2021). Moreover, high fibre content tends to reduce voluntary intake from livestock (Allison 1985), which would lead to less selection of the shrub at the end of the summer.

These results show that *A. viridis* leaves decrease in overall nutritive value across the grazing season, which is similar to previous studies on fodder shrubs and trees. Indeed, in 2022 Navale et al. studied the variation in the leaf composition of several fodder trees throughout the season and found that most of the nutritive and mineral content decreased as leaves matured.

3. Leaf phenolic compounds

Leaf total tannin concentrations from our study were higher than what has been previously found for *A. viridis* (as well as for *A. incana*), with values up to 3.5 times higher than the values measured by Stević et al. (2010). Because their results focused on lower elevations, we could hypothesize that *A. viridis* could display higher phenol concentrations at higher elevations, which has been described for other plant species due to their tendency to synthesise phenols to protect against UV radiations that increase with altitude (Alonso-Amelot et al. 2007; Bernal et al. 2013; Zargoosh et al. 2019). Except for leaf CT, the phenolic compounds of *A. viridis* leaves significantly decreased as the season advanced. Previous studies on alder species and on other woody plants have shown a similar phenol decrease during the season, with a higher phenolic allocation to young leaves, while the variation in leaf CT seems to be highly species-dependent (González-Hernández et al. 2000; Gowda et al. 2019). While leaf phenol content has been primarily linked to leaf ontogeny and air temperature, the high phenol content of *A. viridis* leaves in June could also be linked to the seasonal variations of UV radiations that tend to peak in June in Europe (Bernal et al. 2013). The variation in phenols in *A. viridis* leaves during the season may have a significant impact on livestock feeding behaviour, as they are responsible for astringency and affect digestibility. For instance, if consumed in large quantities, HT can be toxic, while CT can negatively impact ruminant digestion rate, leading to lower voluntary feed intake (Waghorn 2008; Piñeiro-Vázquez et al. 2015; Gowda et al. 2019). Indeed, CT create binding complexes with protein that protect them from ruminal degradation and intestinal absorption, leading to a reduced apparent total tract N digestibility (Waghorn 2008; Woodfield et al. 2019). This is supported by the results of our PCA, which showed a negative relationship between CT and IVOMD, On the other hand, this phenomenon may lead to a higher proportion of N excreted through dung instead of urine, which is less subjected to ammonia volatilization and nitrate leaching (Woodmansee et al. 1981; Tamminga 2006; Angelidis et al. 2019). Woodfield et al. (2019) recommended a CT concentration to range between 20 and 40% DM, which aligns well with the concentrations we measured in a diet composed by 20% of *A. viridis* leaves. Including phenols in that proportion in the diet of grazing ruminants might increase energy and N utilisation (Gowda et al. 2019).

4. Leaf *in vitro* OM digestibility and gas production

The IVOMD of *A. viridis* leaves was very similar to the OM digestibility found for *A. glutinosa* leaves by Luske and van Eekeren (2017). As expected, including 20% *A. viridis* leaves in a hay-based diet led to a lower IVOMD when compared to the control. This is most probably due to the higher content of phenols in *A. viridis* leaves compared to hay, which can highly affect digestibility. On the other hand, the IVOMD of the mixed diet, including *A. viridis* leaves, seemed to be at its maximum at the beginning of the season, which is not in line with our results on phenols, except for CT. This is however consistent with previous studies on fodder tree species (Papachristou and Papanastasis 1994) and with our results on functional traits and nutrient variation during the season. Indeed, SLA and LDMC have a positive and negative effect on digestibility respectively (Pontes et al. 2007), while leaf N content may have a positive effect on digestibility (Bumb et al. 2018), and the highest values of N in *A. viridis* leaves at the beginning of the season might thus be one factor for the highest measured digestibility. In addition, the lower fibre fraction found at the beginning of the summer might compensate for the decreased digestibility generated by the phenols.

Interestingly, a diet including 20% of *A. viridis* leaves in the DM significantly decreased *in vitro* total gas production when compared to the control diet consisting of hay only. This is in line with the findings of Terranova et al. (2018), where most of the woody plant leaves tested led to a decrease in CH_4 production in comparison to the control diet. However, CH_4/dOM production from *A. viridis* leaves diet significantly increased as the season advanced, highlighting that leaves have a better CH_4/dOM mitigation effect in the early season. These CH_4/dOM emission values are in line with our results on phenols as high leaf tannin content seems to reduce methanogenesis (Patra and Saxena 2010; Jayanegara et al. 2011; Piñeiro-Vázquez et al. 2015). Therefore, the CH_4/dOM increase during the season is related to decrease in measured total leaf extractable phenols, which is confirmed by the PCA results displaying CH_4/dOM and phenols in opposite positions. Similarly to CH_4 production, CO_2 production from *A.viridis* leaves diet significantly decreased in comparison to the control, showing that the fermentation was generally affected. However, contrary to CH_4, CO_2/dOM decreased with an increasing GDD, which is the consequence of the reduced degradation of the leaves that is in line with the changing chemical composition. Consequently, the CH_4/CO_2 ratio increased with increasing GDD.

Therefore, *A. viridis* leaves tend to have a better mitigation effect on gas emissions at the beginning of the season. According to Tavendale et al. (2005) and Terranova et al. (2018), a CT concentration below 8% in the DM as found in our study could reduce CH_4/dOM production without restraining fermentation rate. Because CH_4 is the most important greenhouse gas emitted in animal production (Slade et al. 2016), making robust livestock graze in *A. viridis*-encroached pastures at the beginning of the season could thus help mitigate greenhouse gas emissions in these mountain livestock production systems, while keeping an appropriate ruminal fermentation and therefore a high animal performance.

Conclusion

This study described the composition of *A. viridis* leaves and their OM digestiblity and related gas production throughout two summer seasons at four different subalpine sites. The high amounts of crude protein and macro- and micro-elements found in *A. viridis* leaves highlighted its value as fodder for robust livestock, offering a complementary forage resource to grass that could be well integrated in an adapted management of encroached mountain pastures. High seasonal variation in leaf chemical composition was found, and because fibre, lignin, crude protein and nutrient contents can impact voluntary intake in foraging behaviour, these results could help defining targeted management strategies for alpine pastures to optimize robust livestock grazing in *A. viridis* encroached areas while increasing beef cattle productivity and reducing the greenhouse gas emissions of CH_4. In particular, the beginning of the summer (June and July) seems to be the best period to graze *A. viridis* leaves, as in this period of the year they have the highest nutritional value and potential to reduce greenhouse gas emissions.

CHAPTER III: Foraging behavior of Highland cattle in silvopastoral systems in the Alps

Based on: Nota G, Svensk M, Barberis D, Frund D, Pagani R, Pittarello M, Probo M, Ravetto Enri S, Lonati M, Lombardi G. Foraging behavior of Highland cattle in silvopastoral systems in the Alps. (under review in Agroforestry Systems).

Abstract

Trees and shrubs expanded in the last decades in European mountains due to land abandonment and the decrease in grazing pressure, and are expected to further spread also due to climate change. As a consequence of low forage quality and topographic constraints, the management of mountain environments dominated by woody vegetation with livestock is often challenging. Silvopastoral systems based on cattle hardy breeds able to forage on woody plants, such as Highland cattle, could be a suitable option for the management and restoration of such environments. In this study, we used direct observations to explore the foraging behavior of Highland cattle in four study areas across the western Alps. In particular, we assessed 1) cattle diet composition, 2) the selection for more than 30 tree and shrub species, and 3) the relationships between species consumption and their abundance in the environment. Highland cattle fed on a mixture of both woody and herbaceous species, including between 15 and 46% of woody plants in the diet. Some trees (e.g., *Celtis australis*, *Fraxinus* spp., and *Populus tremula*) and shrubs (e.g., *Frangula Alnus*, *Rhamnus* spp., and *Rubus idaeus*) were positively selected by cattle, thus could be an important forage supplement to their diet. Moreover, the results highlighted that relative species consumption increased with increasing species abundance in the environment, suggesting that this cattle breed could be suitable to control shrub expansion in highly encroached areas. The outcomes of this study can support the development of targeted silvopastoral systems in the Alps.

Introduction

In European mountains, trees and shrubs expanded in the last decades to the detriment of open habitats (mainly pasturelands) due to land abandonment and grazing pressure decrease, and are expected to further spread due to the additional effect of climate change (Espunyes et al. 2019). Such changes resulted in a general reduction of the ecosystem services associated to mountain agroecosystems (e.g., forage production, protection from natural hazards, and landscape aesthetic value) (Schirpke et al. 2016; Faccioni et al. 2019) and of plant diversity (Maurer et al. 2006; Koch et al. 2015; Orlandi et al. 2016). Mountain environments dominated by tree and shrub species are scarcely exploited by production-oriented livestock, as they provide lower quality forage than open pastures and are usually located in marginal areas with rough topography. Indeed, woody plants are generally characterized by a higher content of lignin and secondary compounds (e.g., tannins, saponins, alkaloids) than the herbaceous forage (Seidavi et al. 2020; Mahieu et al. 2021). Because of these constraints, many mountain shrub-encroached grasslands, shrublands, and forests are left unmanaged and become dense stands characterized by lower biodiversity (Laiolo et al. 2004; Zehnder et al. 2020).

Silvopastoral systems with hardy cattle breeds could be a suitable option for the management of these environments. Indeed, while production-oriented cattle behave as typical grazers, with grass as their main forage source, hardy cattle breeds can include a greater proportion of woody plants in their diet. For instance, Alberes cattle can feed year-round with a predominantly woody diet in Mediterranean forests of the Pyrenees (Bartolomé et al. 2011). In the Alps, Dexter (Pauler et al. 2022b) and Highland (Pauler et al. 2020a; Svensk et al. 2022) cattle are acknowledged to feed on woody species as well. The ability of certain livestock species and breeds to consume woody plants is linked with microbial populations in their rumen able to detoxify secondary metabolites and degrade lignin. This characteristic is well documented in goats (Silanikove 2000; Giger-Reverdin et al. 2020), which are considered as mixed feeders (i.e., they feed on a mixture of both herbaceous and woody species) (Hofmann 1989). In addition to the exploitation of otherwise unused forage resources, livestock grazing can be a valuable ecological restoration tool to contribute in counteracting shrub expansion (Casasús et al. 2007; Öllerer et al. 2019). Moreover, silvopastoral systems can favor a better adaptation of mountain farms to climate change, as woody plants can be an important alternative forage during critical grass shortages (e.g., in summer droughts) and provide shade relief to animals during hot periods (Vandermeulen et al. 2018a, b; Sales-Baptista and Ferraz-de-Oliveira 2021).

Due to their robustness and ability to consume woody plants, Highland cattle were proposed as a tool to control shrub encroachment in different silvopastoral systems and geographic regions, from heterogeneous grass-shrub-woodland communities in The Netherlands (Cromsigt et al. 2018) to coastal dunes in Belgium (Lamoot et al. 2005), North American oak savannas (Harrington and Kathol 2009; Hedtcke et al. 2009), and, more recently, shrub-encroached pastures in the Alps (Pauler et al. 2019; Svensk et al. 2021, 2022). Particularly, in the Alps, Svensk et al. (2022) observed that they could damage *Alnus viridis* (Chaix) DC., which is among the most rapidly expanding shrub species in Central Europe (Anthelme et al. 2007), by the combination of foliage direct consumption, trampling, and mechanical damage to branches. Pauler et al. (2019) observed that grazing by Highland cattle can improve plant diversity in Swiss subalpine pastures and reduce shrub cover more efficiently compared to other cattle breeds.

Despite the fact that the ability of Highland cattle to forage on woody plants in the Alps, as documented by Pauler et al. (2020a) and Svensk et al. (2022), no studies explored their foraging behavior across different mountain silvopastoral systems and assessed their feeding preferences for different tree and shrub species. This information would be essential to support the possible development of silvopastoral systems based on the Highland cattle breed in the Alps. To fill this knowledge gap, we used direct observations to study the foraging behavior of Highland cattle in four study areas in the western Alps characterized by contrasting woody vegetation. Specifically, the aim of this study was to assess the foraging behavior of Highland cattle in the Alps based on their diet composition, feeding preferences, and the influence of species abundance on plant consumption and selection. We hypothesized that: 1) Highland cattle fed on a mixture of both herbaceous and woody plant species, like in a mixed feeder strategy; 2) some woody plants were positively selected, i.e., they were palatable to Highland cattle; and 3) the relative consumption of plant species was influenced by their abundance in the environment.

Methodology

1. Study areas and grazing management

The study was carried out in four paddocks located along an elevation gradient (480-1745 m a.s.l.; Tab. 1) and extensively grazed by Highland cattle herds in the western Alps, i.e, Almese (Piedmont Region, Italy), Torrette (Piedmont Region, Italy), Caldane (Piedmont Region, Italy), and Bovonne (Canton of Vaud, Switzerland) (**Figure 15**). The paddocks were representative of the Alpine encroached pastures, being dominated between 50% and 100% by woody plant communities (shrubs and trees) (Tab. 1). In Almese, the vegetation was a mosaic of small meso-xerophile forests, shrublands, and dry-grasslands. Dominant trees were *Fraxinus ornus* L., *Populus tremula* L., and the alien species *Quercus rubra* L.; dominant shrubs were *Prunus spinosa* L. and *Rubus ulmifolius* aggr., while *Bromus erectus* Huds., *Carex caryophyllea* Latourr., and *Chrysopogon gryllus* (L.) Trin. were the most abundant species in the open grassland patches. In Torrette, the vegetation was a deciduous mesophile forest dominated by *Acer pseudoplatanus* L., *Fraxinus excelsior* L., and *Larix decidua* Mill. The shrub *Rubus idaeus* L. was abundant in the understory and dominated the open clearings, while *Festuca flavescens* Bellardi was the most frequent grass. In Caldane, the vegetation was a mosaic of *F. excelsior* and *Sorbus aria* (L.) Crantz-dominated forests, *P. spinosa* and *Rosa canina* aggr. shrublands, and dry-grasslands dominated by *B. erectus* and *Festuca ovina* aggr. Bovonne was characterized by meso-hygrophile communities dominated by *A. viridis* and by open mesophilous grasslands. In the grassland patches, *Alchemilla xanthochlora* Rothm and *Calamagrostis villosa* (Chaix) J.F.Gmel. were the dominant species, whereas in the *A. viridis* understory, *Adenostyles alliariae* (Gouan) A. Kern. and *Dryopteris dilatata* (Hoffm.) A. Gray were the most abundant species. Aerial photographs (AGEA 2018; SWISSIMAGE 2020) and vegetation maps of the four paddocks are available in Supplementary Materials (**Figures S1-S4**).

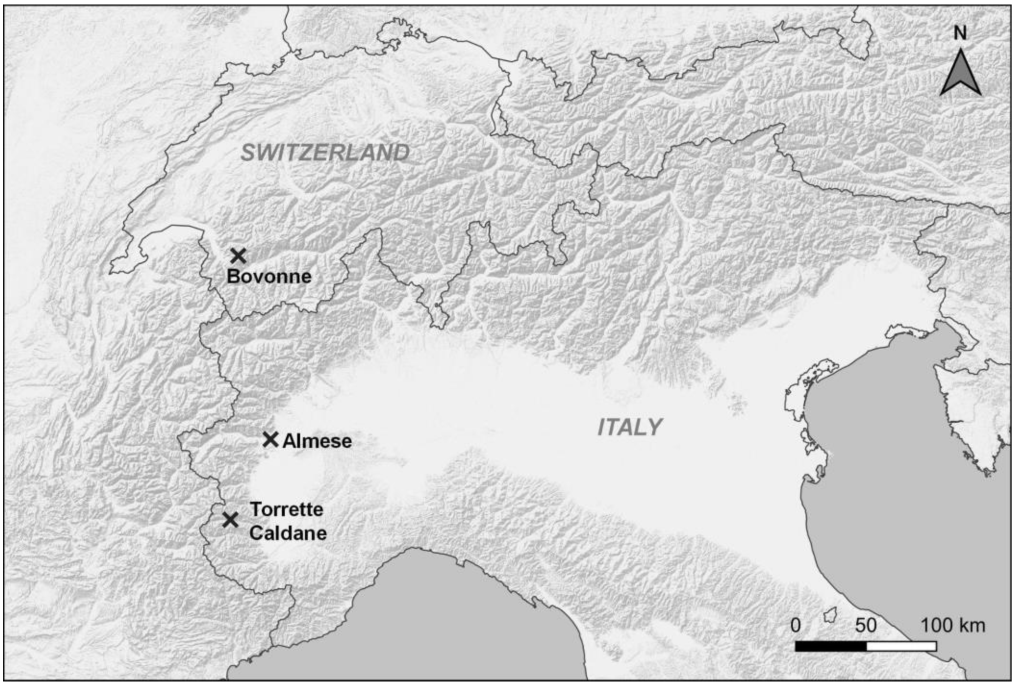

Figure 15 Location of the four study areas (Almese, Torrette, Caldane, and Bovonne) in the western Alps. Basemap: ESRI Terrain.

The study was carried out in the grazing season 2021. The paddocks Almese, Torrette, and Caldane were managed by the same farm. From late April to mid-June, the herd grazed within the Almese paddock. The animals were then moved to summer pastures in the municipality of Casteldelfino, where the herd was divided into two groups: a larger group grazed within the Caldane paddock from late June to the end of August while a smaller group grazed within the Torrette paddock from mid-August to mid-September. Bovonne paddock was grazed for three weeks in July by a different herd. Paddock size, livestock units, herd composition, and grazing season are reported in **Table 2**.

Table 2 Characteristics of the four paddocks used in Chapter III (Almese, Torrette, Caldane, and Bovonne).

	Almese	**Torrette**	**Caldane**	**Bovonne**
Coordinates (Datum: WGS84)	45°06'25.2"N, 7°26'32.9"E	44°34'53.8"N 7°05'15.5"E	44°35'14.0"N, 7°05'39.2"E	46°16'9.86"N, 7°6'44.25"E
Elevation (m a.s.l.)	480	1250	1380	1745
Paddock size (ha)	16.3	14.8	19.0	8.3
Cover of woody plant communities (%)	50.4	100.0	56.4	61.0
Livestock Units*	15.4	4.6	12.2	20.7
Herd composition	13 cows, 10 calves, 1 bull	3 cows, 2 heifers, 2 calves	10 cows, 8 calves, 1 bull	9 cows, 9 heifers, 5 calves, 5 young bulls
Grazing season	late April - mid-June	mid-August - mid-September	late June - August	July

* According to EU Regulation 2018/1091 of the European Parliament and of the Council, Annex 1

2. Direct observations on livestock foraging behavior

To study the foraging behavior, we used direct observations adapting the methodology proposed by Nota et al. (2023) to Highland cattle. Each observer randomly chose an adult cow (focal animal) and recorded its foraging behavior during 15 sec. observation sessions followed by 20 sec. breaks between each observation. Each cow was continuously observed for two hours, on average, before the observer moved to another focal cow. The identification number of the cow was recorded. We used the feeding station as the spatial scale where decisions on plant selection were made by the cow. According to Bailey et al. (1996) classification, the feeding station is the front feet placement explored by grazing animals during a 5-100 second temporal period. Specifically, we spatially delimited the feeding station as a buffer area having a height of 1.5-m from the ground level and a 50-cm radius around the head of the cow. We assumed that all the plants available in this buffer layer were detectable by the cow and that 1.5-m above ground corresponded to the maximum height that animals could exploit (threshold set according to our field observations). For each observation session, two variables were recorded: (1) the plant species relative abundance (SA) and (2) the plant species relative consumption (SC). The SA represented the proportion of biomass (ranging from 10 to 100% and visually estimated with a pace of 10%) of each species available in the feeding station. The SC represented the proportion of biomass (ranging from 0 to 100% and recorded with a pace of 10%) of each species consumed during the 15 sec. session. All woody plant species, tall herbs and ferns were identified at the species level. All other herbaceous species were grouped in a broad category ‘herbage’, as the identification at the species level through direct observations could be extremely difficult for such plants, especially in grassland patches. The nomenclature of plant species followed Landolt et al. (2010).

The direct observations were performed during four days in Almese, Torrette, and Caldane, and three days in Bovonne, for a total of 11’286 observation sessions, corresponding to 153 monitoring hours. The monitoring days were performed approximately once every week to be representative of cattle foraging behavior during the whole grazing period in the paddocks.

3. Data and statistical analysis

a. Diet composition and Jacobs’ Selection Index

All analyses were performed separately for the four study areas.

The proportion in the feeding stations (%FS_i) of each woody, tall herb and fern species, and of the ‘herbage’ category was calculated as follows

$$\%FSi = \frac{\sum SA_i}{\sum_{i=1}^{n} SA_i} \times 100 \tag{1}$$

where SA_i is the abundance of the species *i* or of the ‘herbage’ category at each observation session.

Then, the overall proportion of woody species in the feeding stations was obtained by summing the proportions of all woody plant species. Likewise, the overall proportion of herbaceous species in the feeding stations was obtained by summing the proportions of all tall herbs and ferns, and the ‘herbage’ category.

The proportion in the diet (%$DIET_i$) of each woody, tall herb and fern species, and of the ‘herbage’ category was calculated as follows:

$$\%DIET_i = \frac{\sum SC_i}{\sum_{i=1}^{n} SC_i} \times 100 \qquad (2)$$

where SC_i is the consumption of the species *i* or of the ‘herbage’ category at each observation session.

Then, the overall proportion of woody species in the diet was obtained by summing the proportions of all woody plant species. Likewise, the overall proportion of herbaceous species in the diet was obtained by summing the proportions of all tall herbs and ferns, and the ‘herbage’ category.

To assess plant species selection, we calculated the Jacobs’ Selection Index (Jacobs, 1974) according to the following formula:

$$Jacobs'Selection\ Index_i = \frac{\%DIET_i - FS_i}{\%DIET_i + FS_i - 2 \times \%DIET_i \times FS_i} \qquad (3)$$

Jacobs’ Selection Index ranges between -1 and +1, with positive values representing preference (i.e., plant species consumed proportionally more than their abundance in the environment), values close to zero representing indifference (i.e., plant species consumed according to their abundance in the environment), and negative values representing avoidance (i.e., plant species consumed proportionally less than their abundance in the environment). The Index was computed only for plant species being recorded in at least 20 observation sessions and encountered by at least three different cows, to ensure reliability of the results. For the computation of the Index, $\%FS_i$ and $\%DIET_i$ were rescaled to a 0-1 range.

b. Relationships between species consumption and abundance

The relationships between species relative consumption and abundance were scrutinized for two groups of plants: 1) plant species having the upper range of recorded SA values ≥ 80%; and 2) plant species having the upper range of recorded SA values between 50 and 70%. For the first group (= group 1), the data encompassed a large range of SA values, i.e., from sporadic presence to dominance in the feeding station. The second group (= group 2), instead, included species with lower dominance at the feeding station scale.

For both groups, we modeled the SC of plant species as a function of SA through Generalized Additive Models (GAM) assuming a Gaussian distribution for the response variable. Then, for each GAM curve, we predicted SC values at SA = 10, 20, 30, 40, 50, 60, 70, and 80% for group 1, and SA = 10, 20, 30, 40, and 50% for group 2. For three species which were never consumed by cattle, we could not model their SC because of the absence of variance, thus we attributed zero to all predicted values. To identify different clusters of plant species based on their consumption-abundance relationships, we used the species as response variables and the values predicted with GAMs as explanatory variables to perform a hierarchical cluster analysis (distance matrix: Euclidean; algorithm: Unweighted Pair Group Method with Arithmetic mean, UPGMA). Cluster analyses were performed separately for group 1 and group 2. Finally, for each cluster of species resulting from the cluster analyses, we averaged the predicted values of plant species consumption and plotted the average consumption-abundance relationships.

The analyses were performed with R Software (R Core Team, 2018). The 'mgcv' package (Wood 2011) was used to fit the GAMs and the 'vegan' package (Oksanen et al., 2020) was used to perform the cluster analyses.

Results

1. Diet composition and plant species selection

Thirty different woody plant species were recorded in Almese, 24 in Torrette, 21 in Caldane, and six in Bovonne. About tall herbs and ferns, one species was recorded in Almese, six in Torrette, none in Caldane, and 18 in Bovonne.

The proportion of woody species in the feeding stations used by Highland cattle ranged from 14.8% in Bovonne to 44.6% in Torrette (**Figure 16a**). The proportions in the diet showed similar patterns to those in the feeding stations, with the lowest proportion of woody species in Bovonne (15.1%), intermediate proportions in Almese and Caldane (28.6% and 26.2%, respectively), and the highest in Torrette (45.8%) (**Figure 16b**).

In Almese, the most consumed woody species were *Frangula Alnus* Mill. (3.3% of the diet), *P. spinosa* (2.9%), and *F. ornus* (2.9%). *Rubus idaeus* accounted for about 40% of the diet in Torrette, while *P. spinosa* (4.8%) and *R. ulmifolius* aggr. (2.7%) were the main woody plants browsed in Caldane. In Bovonne, *A. viridis* accounted for about 12% of cattle diet. The complete list with all recorded woody and tall herb and fern species, the corresponding number of observations, number of cows that encountered the species, and the proportions in the feeding stations and in cattle diet is reported in the Supplementary Materials (**Table S1**).

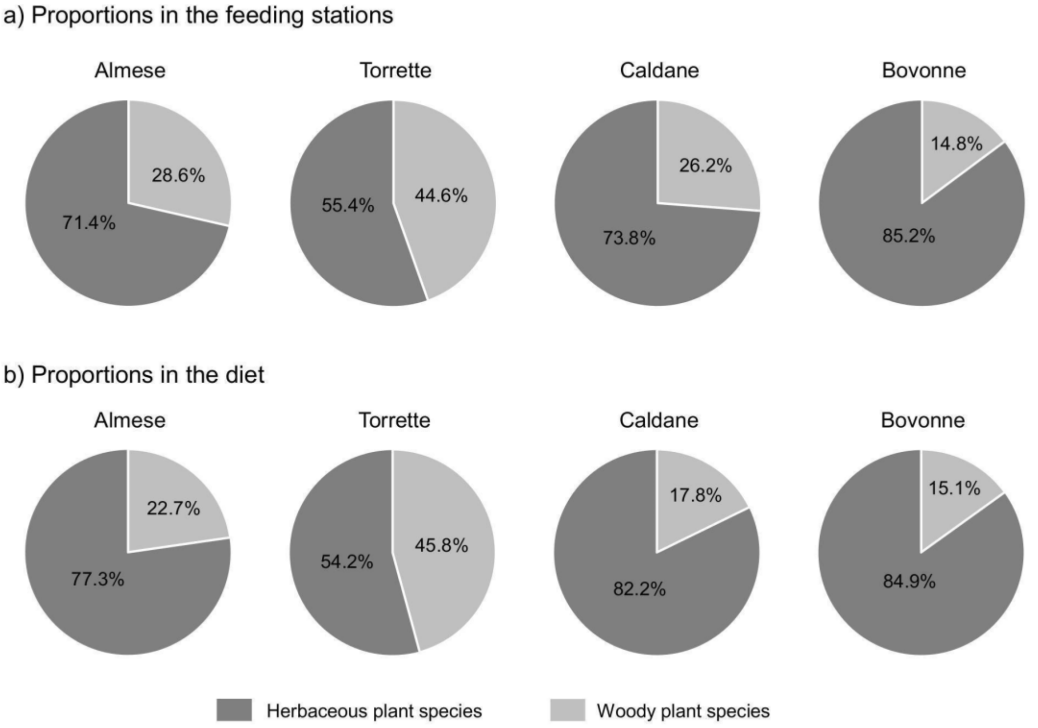

Figure 16 Proportion of woody and herbaceous plant species in a) the feeding stations and b) in Highland cattle diet in the four study areas.

According to Jacobs' Selection Index, plant species selection showed a large variability depending on species identity (**Figure 17a-d**). Cattle expressed preference for broadleaf trees such as *Celtis australis* L., *P. tremula*, *F. ornus*, and *Quercus* species (i.e., *Q. rubra* and *Q. pubescens/petraea*), and shrubs such as *F. Alnus*, *Sambucus nigra* L., *R. idaeus*, and *Rhamnus* species (i.e., *R. alpina* L. and *R. cathartica* L.). Instead, they were rather indifferent (i.e., the relative consumption was equal to their abundance) towards *A. pseudoplatanus*, *A. viridis*, *Picea abies* (L.) H. Karst., and the alien tree *Robinia pseudoacacia* L. Spiny shrubs (i.e., *Crataegus monogyna* Jacq., *P. spinosa*, and *R. canina* aggr.) were moderately avoided, while *Calluna vulgaris* (L.) Hull., *Laburnum alpinum* (Mill.) Bercht. & J. Presl, and the alien tree *Ailanthus altissima* (Mill.) Swingle were strongly refused. For some species, the selection differed depending on the study area, such as for *Corylus avellana* L. (avoided in Torrette and indifferently consumed in Caldane), *F. excelsior* (preferred in Almese and Caldane and avoided in Torrette), *R. ulmifolius* aggr. (avoided in Almese and indifferently consumed in Caldane), and *Ulmus minor* Mill. (preferred in Almese and avoided in Caldane). Among tall herbs, preference was expressed, for instance, towards *Alchemilla xanthochlora* Rothm., *Cicerbita alpina* (L.) Wallr, and *Ranunculus aconitifolius* L., whereas *Aconitum napellus* L., *A. alliariae*, *Gentiana lutea* L., and *Veratrum album* L. were strongly avoided (**Figure 17d**). About ferns, *Pteridium aquilinum* (L.) Kuhn and *Dryopteris filix-mas* (L.) Schott were strongly and moderately avoided, respectively, while *D. dilatata* was positively selected.

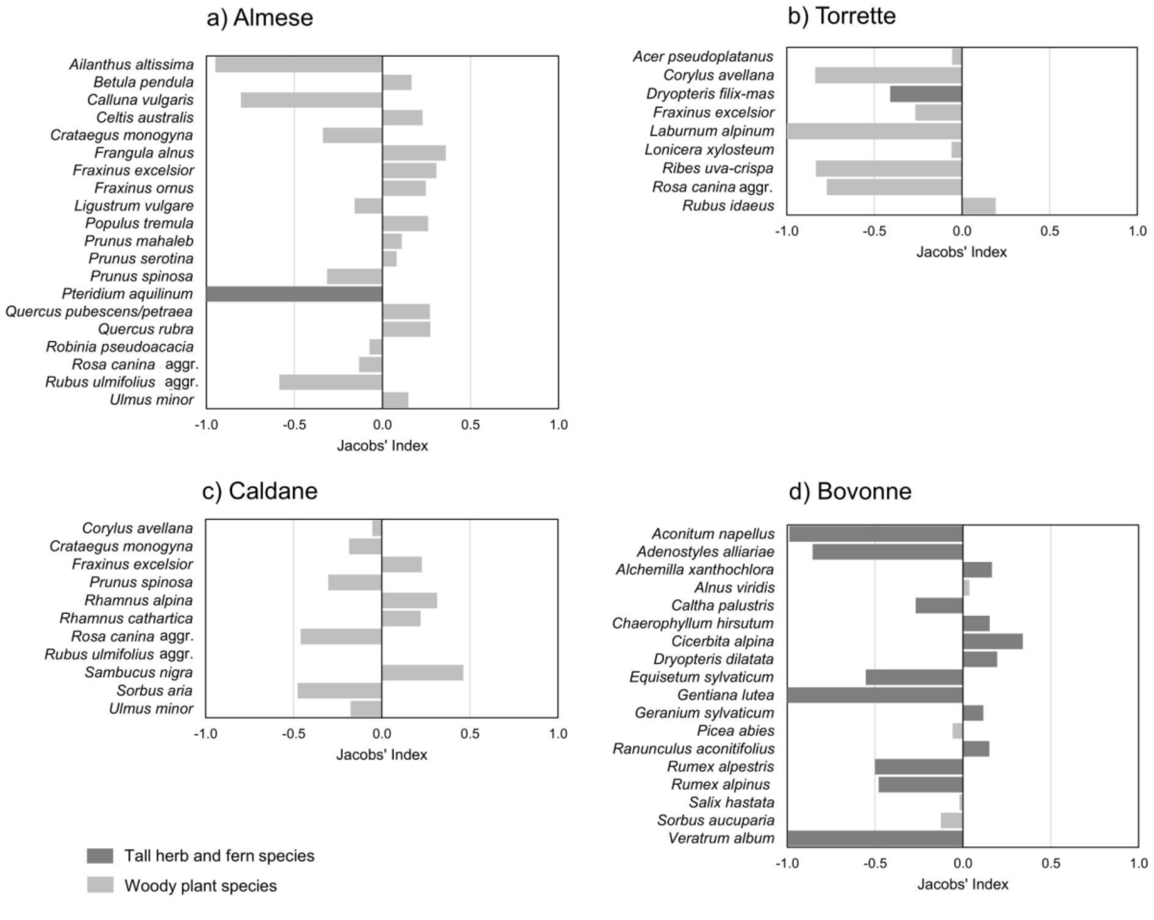

Figure 17 Jacobs' Selection Index of woody and tall herb and fern species in a) Almese, b) Torrette, c) Caldane, and d) Bovonne study areas. Positive values indicate preference, values close to zero indicate indifference, negative values indicate avoidance.

2. Relationships between species consumption and abundance

Group 1 and group 2 of plant species included 30 and 18 plants, respectively. The relationships between species consumption and abundance resulting from the GAMs for each plant are shown in **Figures 18** and **19**. For most of the species, the relative consumption increased with increasing abundance in the feeding station, although the shape and slope of these relations differed among plants.

The cluster analyses performed with the values predicted with GAMs showed four clusters of species for both group 1 and group 2: 1A, 1B, 1C, and 1D for the first group (**Figure 20a**) and 2A, 2B, 2C, and 2D for the second one (**Figure 21a**). Each cluster of species was characterized by a distinct relationship between species consumption and abundance and represented a different level of selection by cattle (**Figure 20b** and **21b**). Particularly, for the plants belonging to clusters 1A (e.g., *F. ornus* and *Q. rubra*) and 2A (e.g., *D. dilatata*), the relationship showed the greatest slope in the first part of the curve compared to all other curves. These species were consumed more than proportionally to their abundance in the feeding station (i.e., they were preferred), also at low SA values. The species belonging to clusters 1B (e.g., *A. viridis* and *R. idaeus*) and 2B (e.g., *R. pseudoacacia*) were consumed less than proportionally to their abundance at low SA values, then more than proportionally when SA were ≥ 20-30%. For the species belonging to clusters 1C (e.g., *C. avellana* and *P. spinosa*) and 2C (i.e., *R. canina aggr*. and *S. aria*), species consumption was less than proportional to their abundance, except at very high SA values (≥ 70%). The species belonging to clusters 1D and 2D showed a very low consumption (< 10%) and were consistently avoided by cattle regardless of their abundance in the feeding station. Among these species, *G. lutea*, *L. alpinum*, and *P. aquilinum* showed no consumption (**Figures 18** and **19**). The selection of plant species by cattle as resulting from the consumption-abundance relationships was coherent with Jacobs' Selection Indices.

Discussion

This study allowed to explore the foraging behavior of Highland cattle in contrasting encroached pastures in the Alps characterized by abundant woody vegetation. Based on diet composition, we accept our first hypothesis that Highland cows fed on a mixture of herbaceous and woody plants in all study areas, indicating they behaved as mixed feeders in these environments. Our data of woody plants proportion in cattle diet (15-46%) fall within the range reported by other studies performed with the Highland breed in silvopastoral systems worldwide (14-21% in Lamoot et al. 2005; 21-60% in Hedtcke et al. 2009; around 20% in Cromsigt et al. 2018; around 10% in Pauler et al. 2020a). Interestingly, in our study such proportion of woody plants eaten (15-46%) mirrored their proportion in the feeding stations (15-45%) and suggested that cows adapted the diet to the vegetation available in the foraging areas they encountered while grazing. The results also showed that diet composition varied among the different paddocks, likely because of differences in terms of resource availability and forage quality (Bartolomé et al. 2011; Mandaluniz et al. 2011; Iussig et al. 2015b; Pauler et al. 2020a). For instance, we suggested that cows consumed the largest proportion of trees and shrubs in Torrette because they were very abundant in the paddock (100% of the paddock dominated by woody plants), with *R. idaeus* being a good forage resource (Mahieu et al. 2021), whereas available herbaceous plants (e.g., F. flavescens) were less palatable. Contrarily, in Bovonne, trees and shrubs accounted for the lowest proportion of the diet (15%) probably because cows preferred to spend more time in open areas to graze on high quality herbaceous forage (with abundance of e.g., *Festuca nigrescens* and *Phleum rhaeticum*) compared to the other sites where grasses were of poorer forage quality (e.g., *F. ovina* aggr. and *B. erectus* in Almese and Caldane).

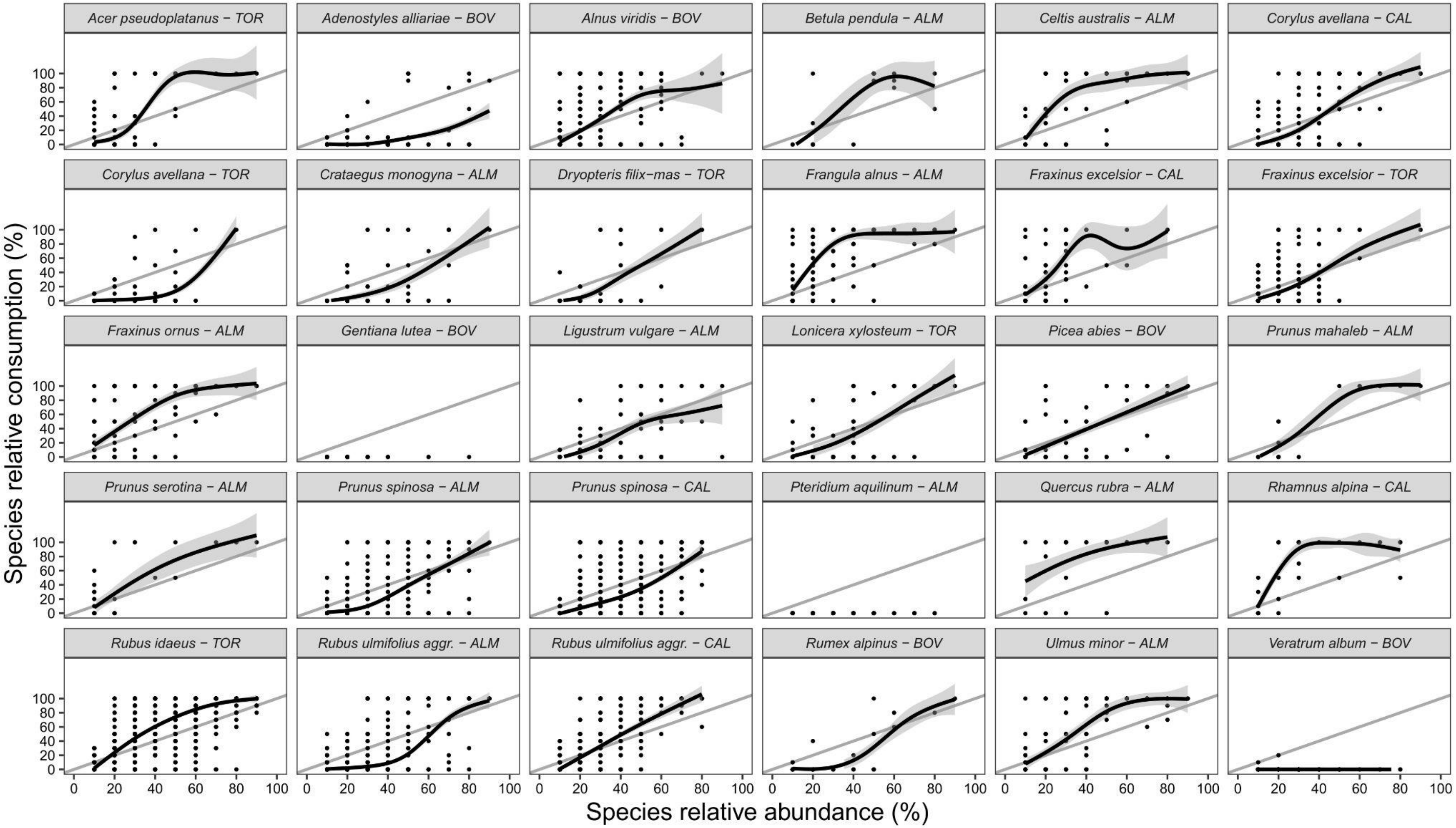

Figure 18 Relationships between species relative consumption and species relative abundance modeled with Generalized Additive Models for group 1 (upper range of species abundance values ≥ 80%) of plant species. *Gentiana lutea* and *Pteridium aquilinum* showed no consumption and thus were not modeled due to the variance equal to 0. The gray line is the identity line. Study areas: ALM = Almese, TOR = Torrette, CAL = Caldane, BOV = Bovonne.

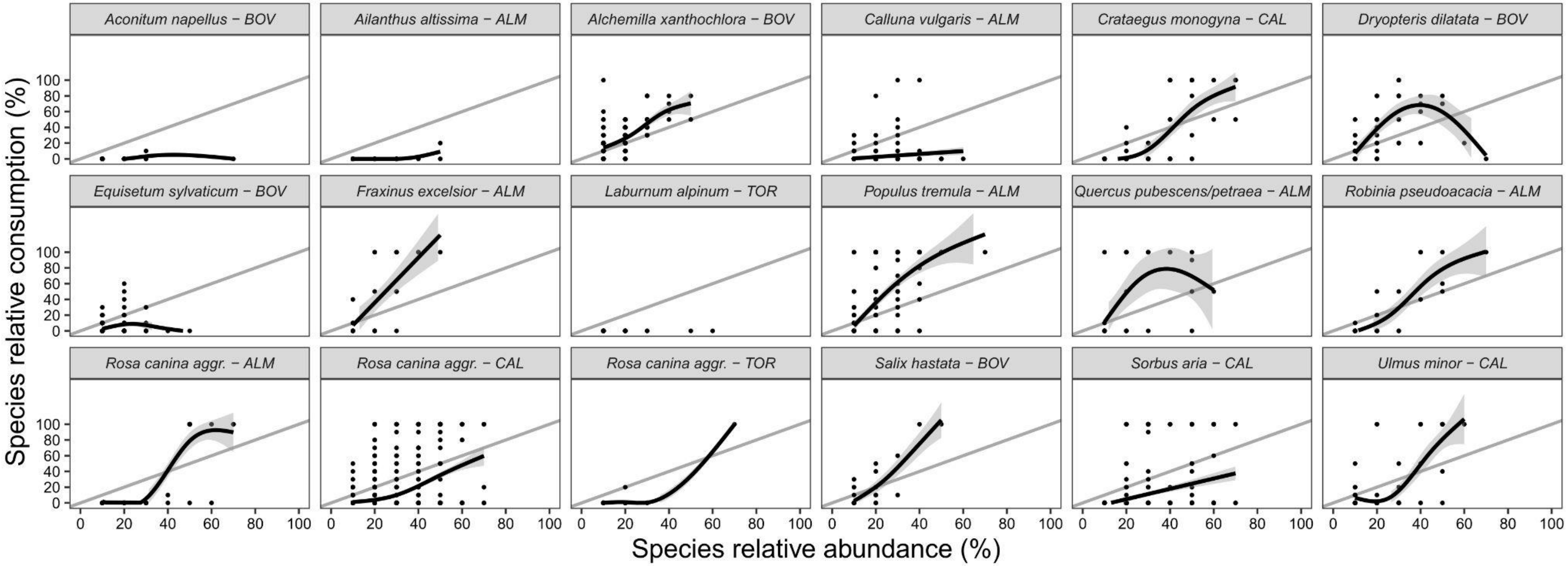

Figure 19 Relationships between species relative consumption and species relative abundance modeled with Generalized Additive Models for group 2 (upper range of species abundance values between 50 and 70%) of plant species. *Laburnum alpinum* showed no consumption and thus was not modeled due to the variance equal to 0. The gray line is the identity line. Study areas: ALM = Almese, TOR = Torrette, CAL = Caldane, BOV = Bovonne.

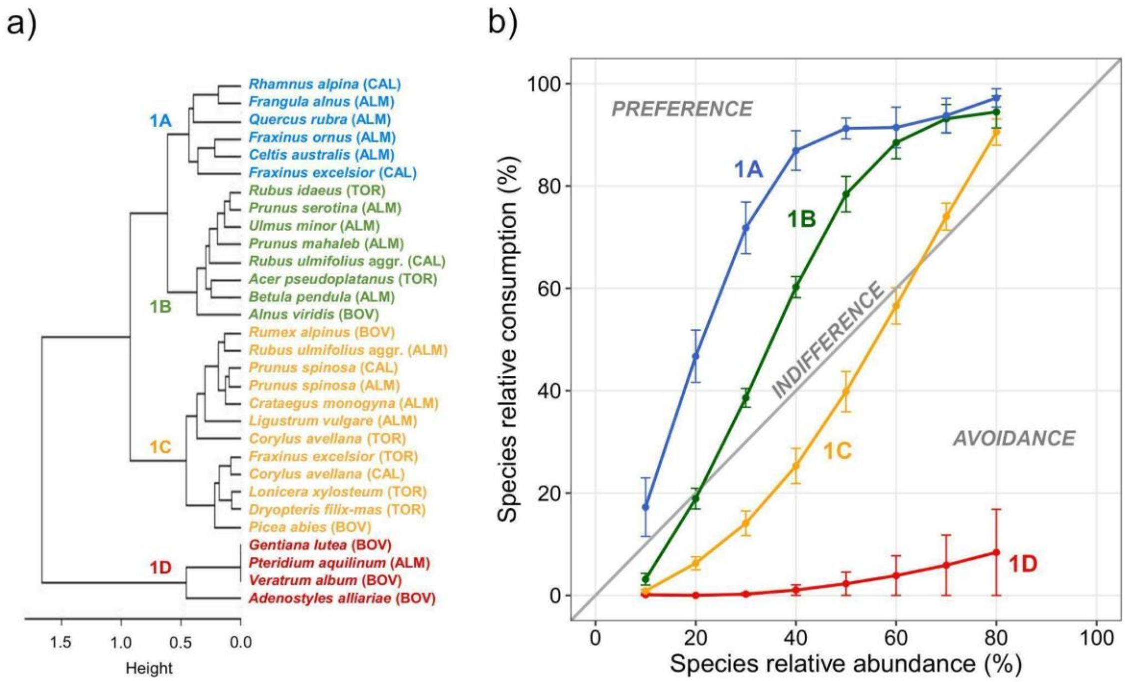

Figure 20 a) Dendrogram and b) relationships between species relative consumption and abundance for group 1 (upper range of species abundance values ≥ 80%) of plant species. Different colors highlight different clusters of species (i.e., 1A, 1B, 1C, and 1D). In panel b, values are means and bars are standard errors, and the plot areas above, close to, and below the identity line indicate preference, indifference, and avoidance by cattle, respectively. Study areas: ALM = Almese, TOR = Torrette, CAL = Caldane, BOV = Bovonne.

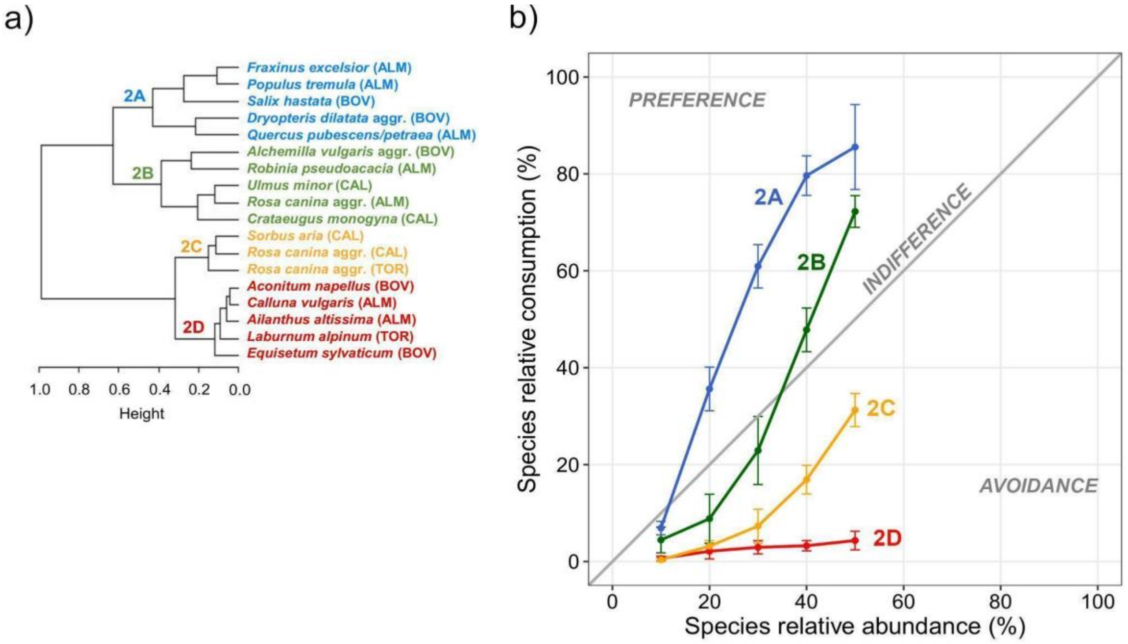

Figure 21 a) Dendrogram and b) relations between species relative consumption and abundance for group 2 (upper range of SA values ≥ 50% and ≤ 70%) of plant species. Different colors highlight different clusters of species (i.e., 2A, 2B, 2C, and 2D). In panel b, values are means and bars are standard errors, and the plot areas above, close to, and below the identity line indicate preference, indifference, and avoidance by cattle, respectively. Study areas: ALM = Almese, TOR = Torrette, CAL = Caldane, BOV = Bovonne.

Results based on Jacobs' Selection Index and consumption-abundance relationships were coherent and confirmed our second hypothesis that some woody plants were palatable to Highland cattle and could represent an important forage resource in silvopastoral systems. For instance, leaves of *C. australis*, *P. tremula*, and *F. ornus* were positively selected by cows. *Celtis australis* is considered a nutritious and high palatable forage species (Singh et al. 2010), while *P. tremula* is of intermediate quality (Hejcmanová et al. 2014). *Fraxinus ornus* is acknowledged as an important browse species for goats in Mediterranean environments (Papachristou and Papanastasis 1994; Papachristou et al. 1999). *Fraxinus excelsior*, which has high forage quality (Hejcmanová et al. 2014; Ravetto Enri et al. 2020), was positively selected by cows too, except in Torrette study area. The avoidance for this species observed in Torrette may be explained by the age of the plants, as in this paddock there were abundant *F. excelsior* seedlings about 20-cm tall, whereas in the other sites the trees were mainly adult and the cows fed on their lowest branches. The reduction in plant chemical defenses and increase in herbivory with increasing plant age has been documented for some trees (Boege and Marquis 2005). Other plants largely appreciated by cows as browse species were the shrubs *F. Alnus*, *S. nigra*, and *R. idaeus*, in line with their high nutritional quality (Mahieu et al. 2021). The leaves of *Rhamnus* species (*R. alpina* and *R. cathartica*) were positively selected too, despite their bark and berries are acknowledged for the presence of toxic compounds (e.g., anthraquinones; Wink 2010). Interestingly, the alien invasive tree *Q. rubra* was palatable to Highland cattle, whereas cows expressed indifference for the alien invasive tree *R. pseudoacacia,* and totally refused the alien invasive tree *A. altissima*, which is rich in secondary compounds (Kowarik and Säumel 2007). Selection of *A. pseudoplatanus* and *C. avellana*, typical species of European temperate forests, ranged from indifference to avoidance. This result agrees with the low forage quality of their leaves (i.e., low digestibility and high phenols concentration) as documented in literature (Papachristou and Papanastasis 1994; Ravetto Enri et al. 2020; Mahieu et al. 2021). As recently observed by Svensk et al. (2022), Highland cattle fed on *A. viridis* leaves, which were consumed according to their abundance in the environment. Despite its moderately high tannin concentration (Stević et al. 2010), this shrub's foliage is rich in protein (Bühlmann et al. 2016; Pauler et al. 2022a) and could represent an important constituent of cattle diet in subalpine pastures. When foraging in *A. viridis* shrublands, cows expressed a strong selection towards understory species, as they preferred to graze *C. alpina* and the fern *D. dilatata* while completely avoiding other frequent tall herbs such as *A. alliariae* and the toxic *A. napellus* and *V. album*. Additionally, according to its well-known poisonous effects for livestock species (Marrs and Watt 2006), the fern *P. aquilinum* was totally refused in Almese. Finally, cows expressed neutral to negative selection for spiny shrubs such as *C. monogyna*, *P. spinosa*, *R. canina* aggr., and *R. ulmifolius* aggr. Despite the leaf quality of such shrubs is rather high (excellent in *P. spinosa*; Mahieu et al. 2021), spines represented an important deterrent to browsing.

The information about cattle feeding preferences obtained with this study could contribute to a better definition of the forage provision and the vegetation carrying capacity in silvopastoral systems managed with Highland cattle. Indeed, in the Alps, the computation of the vegetation carrying capacity has been developed for pastures and is based on the quality indices of herbaceous plants, which represent the main forage source for conventional cattle breeds, whereas trees and shrubs are generally considered with a low nutritional value for domestic ruminants. For instance, several indices of specific quality targeted for herbaceous plants (Roggero et al. 2002; Cavallero et al. 2007) have been defined for the calculation of the pastoral value sensu Daget and Poissonet (1971). The inclusion of woody plants in the estimation of the vegetation carrying capacity, such as through the definition of their specific quality indices (see for example the study by Gusmeroli et al. 2007), could help a better and sustainable management of these mountain environments.

Finally, based on consumption-abundance relationships, we accept our third hypothesis that species consumption was influenced by their abundance in the feeding station. Particularly, most of the species were increasingly consumed when they were increasingly available to cows. These results agree with other studies on grass (Agnusdei and Mazzanti 2001; Chen et al. 2015) and woody (Elias and Tischew 2016) species performed at the pasture scale. Exceptions to this trend were the strongly avoided species, i.e., those of clusters 1D and 2D, which were seldom or never eaten regardless of their abundance, probably because of high unpalatability and toxicity issues. Interestingly, consumption-abundance relationships showed that the selection by cattle for some species (i.e., clusters 1B, 2B, and 1C) varied from avoidance to preference along the gradient of increasing species abundance. This trend may be explained in the context of momentary maximization theories, which assume that animals select the best available alternative at any given time (Senft et al. 1987; Bailey et al. 1996). Particularly, the most palatable plants in the feeding station are selected until palatability of remaining forage decreases to a threshold value, then cows move to another feeding station. The threshold value is not fixed but increases when animals encounter high quality plants and decreases with lower quality plants (Senft et al. 1987; Bailey et al. 1996). According to this interpretation, when cows are foraging in a feeding station highly encroached by tree and shrub species of medium-low palatability (i.e., those of clusters 1B, 2B, and 1C), the threshold decreases and the consumption and selection on these plants increase. Conversely, when these species are occasionally present in the feeding station, they are avoided as cows prefer to forage on more palatable species. In this regard, we highlight that Jacobs' Selection Index was ineffective to detect changes of species selection along the gradient of increasing abundance as it only provides an overall selection value. Consumption-abundance relationships, instead, allowed to scrutinize more thoroughly feeding preferences, highlighting how some generally avoided plants can be positively selected when their abundance is high. This behavior has implications especially for species like *A. viridis*, *P. spinosa*, *R. canina* aggr., and *R. ulmifolius* aggr., whose encroachment into open grasslands represents a crucial issue in European mountains due to land abandonment (Casasús et al. 2007; Svensk et al. 2021; Verdinelli et al. 2022). Our results highlighted that browsing on these species is more effective when cows are foraging in highly encroached patches rather than when these species are sparsely distributed within a matrix of more palatable species. Silvopastoral systems with the Highland breed may thus represent a management tool to control undesirable woody plant species, particularly when cows exploit highly encroached areas. Increases in the animal stocking rates (Pauler et al. 2022b) and the use of attractive points (Pittarello et al. 2016b; Svensk et al. 2022) could further contribute to intensify the impact of cattle on target trees and shrubs.

Conclusion

This study showed that Highland cattle had a mixed diet consisting of both woody and herbaceous plants, suggesting that silvopastoral systems based on this breed could be a valuable option for the management and restoration of abandoned environments in the Alps. Some trees (e.g., *Celtis australis*, *Fraxinus* spp., and *Populus tremula*) and shrubs (e.g., *Frangula Alnus*, *Rhamnus* spp., and *Rubus idaeus*) were very palatable to Highland cattle, thus can be an important forage resource and a supplement to cattle diet. In addition, our results highlighted that cows increased the consumption of plant species with their increasing abundance in the feeding station, suggesting that this breed may be suitable to control shrub expansion in highly encroached areas. Further research should integrate foraging behavior evaluations with tree and shrub forage quality and animal performance analyses. Moreover, the effects of the grazing management with Highland cattle on the restoration of shrub-encroached grasslands, shrublands, and forests should be assessed in the long term.

CHAPTER IV: Spatial distribution of Highland cattle in *Alnus viridis*-encroached subalpine pastures

Based on: Svensk, M., Pittarello, M., Nota, G., Schneider, M. K., Allan, E., Mariotte, P., Probo, M. (2021). Spatial Distribution of Highland Cattle in *Alnus viridis* Encroached Subalpine Pastures. Frontiers in Ecology and Evolution. 9:626599. doi: 10.3389/fevo.2021.62 6599

Abstract

Green alder (*Alnus viridis*) is a shrub species that has expanded over former pastures in Central Europe due to land abandonment, leading to negative agri-environmental impacts, such as a reduction in forage yield and quality and an increase in nitrate leaching. Robust livestock breeds such as Highland cattle could be used to control *A. viridis* encroachment. The objectives of this study were to investigate the impact of *A. viridis* encroachment on plant community composition and diversity and to map the spatial distribution of Highland cattle in *A. viridis*-encroached pastures. During the summer of 2019, three different Highland cattle herds were placed along an *A. viridis* encroachment gradient. A total of 58 botanical surveys were carried out before grazing to assess plant community composition, pastoral value, and ecological indicator values. The spatial distribution of cattle was studied during the whole grazing period by monitoring six to eight cows equipped with GPS collars in each herd. Plant species associated with higher pastoral values of the vegetation were found in areas with lower *A. viridis* cover, while highly encroached areas were dominated by a few nitrophilus and shade-tolerant broad-leaved species and by ferns. Cattle spent more time in areas with higher pastoral value but did not avoid areas with high cover of *A. viridis*, on steep slopes or far from water. These results show that Highland cattle are able to tolerate harsh environmental conditions and that they can exploit *A. viridis*-encroached pastures. This suggests that they have a high potential to reduce *A. viridis* encroachment in the long-term.

Introduction

Throughout the last decades, socioeconomic changes have led to a reduction in agropastoral activities in European mountain areas, triggering profound landscape modifications, and widespread natural reforestation and shrub encroachment in former pastures (Estel et al. 2015). For instance, from 1985 to 2013, shrublands and forests have increased by 10.6% across Switzerland, with the largest increases in the Alps (Abegg et al. 2020). Shrublands now cover an area of 679 km² in Switzerland, with about70% of them dominated by pure stands of green alder [*Alnus viridis* (Chaix.) D. C.], which is the most rapidly expanding shrub species in Central Europe (Anthelme et al. 2007). *A. viridis* is a pioneer species, which lives in symbiosis with the N_2-fixing actinomycete *Frankia alni* (Huss-Daniel 1997), and its presence is determined by land use intensity (Caviezel et al. 2017). Encroachment by *A. viridis* can have several negative agri-environmental impacts: in particular, *A. viridis*-dominated shrublands have lower plant and animal diversity and forage quality than open grazed areas (Anthelme et al. 2001). A recent study showed that *A. viridis* encroachment resulted in a rapid decline in plant species richness, with dense *A. viridis* patches hosting 62% less species than adjacent open pastures (Zehnder et al. 2020). Moreover, there is high nitrate and dissolved organic carbon leaching from *A. viridis* shrublands, which results in water pollution, soil acidification, hydrological drawbacks, and gaseous nitrogen losses (Bühlmann et al. 2016; van den Bergh et al. 2018). It is therefore important to find methods that control *A. viridis* encroachment in order to reverse biodiversity losses and to protect key ecosystem services.

Controlling shrub encroachment is challenging because mechanical removal is extremely difficult and expensive in harsh and marginal mountain conditions due to steep slopes and a lower density of road network. Moreover, many shrub species are not palatable to grazing animals, since their foliage has low digestibility, mostly due to high tannin concentrations (Waghorn 2008). However, some robust livestock species and breeds can forage on shrubs and digest their leaves, thanks to ruminal bacterial populations, which can degrade lignified material and which have high resistance to tannins (Manousidis et al. 2016). Highland cattle are a robust breed that are increasingly being reared in different European alpine countries due to their ability to forage on poor quality and shrub-encroached pastures (Pauler et al. 2020b, a). As a consequence, the particular grazing behavior of Highland cattle can also result in distinct pasture botanical composition, with reduced woody plant species cover in the long term (Pauler et al. 2019). However, the impact of robust livestock on shrub encroachment may depend on environmental and management conditions, such as slope, pastoral value of the vegetation, and distance to water sources and attractive supplements for livestock (Probo et al. 2014; Homburger et al. 2015; Pittarello et al. 2016a). It is therefore important to evaluate the conditions under which robust livestock breeds are able to effectively reduce shrub encroachment in alpine pasture.

However, to the best of our knowledge, no study has assessed in detail the drivers related to plant community composition and diversity in *A. viridis*-encroached pastures nor the potential of Highland cattle to reduce *A. viridis* encroachment. For this reason, in the present study, we aimed (i) to disentangle the topographic and ecological drivers of plant community composition and diversity in *A. viridis*-encroached pastures, from Northern to Southern Alps and (ii) to measure the spatial distribution of Highland cattle in *A. viridis*-encroached pastures to determine whether they are attracted to or avoid *A. viridis* patches. Here, we hypothesized that spatial distribution of Highland cows would vary depending on *A. viridis* cover and other factors such as distance to water, slope, and the pastoral value of the vegetation. More specifically, we expected cows to spend more time in areas around water sources and in more valuable pasture and to avoid steep slopes.

Methods

To investigate vegetation features and cattle spatial distribution, three Highland cattle herds were placed in four different *A. viridis*-encroached paddocks in the Swiss and Italian Alps in the summer of 2019. Three paddocks were located in Switzerland: Bovonne 1 and Bovonne 2 (in the canton of Vaud) and Champlong (in the canton of Valais). Bovonne 1 and Bovonne 2 were adjacent paddocks, grazed one after the other by the same herd. The fourth paddock was located in Val Vogna (in the province of Vercelli) in the Italian Alps (**Table 3**). Highland cattle grazed each paddock for approximately 1 month: in July (Bovonne 1), from mid-July to late-August (Champlong), and in August (Bovonne 2 and Val Vogna). All the herds included suckler cows, heifers, and calves, varying in age from 6 months to 17 years. The paddocks had similar environmental conditions in terms of elevation and slope, were grazed at comparable livestock stocking rates, and were representative of a gradient of *A. viridis* encroachment, i.e., with *A. viridis* cover values ranging from 20 to 70% (**Table 3**). A water trough was installed in each of the two paddocks in Bovonne, while many natural streams were present both in Val Vogna and Champlong paddocks.

Table 3 Topographical, management, and vegetation characteristics of the four *A. viridis*-encroached paddocks used in Chapter IV.

	Bovonne 1	**Bovonne 2**	**Champlong**	**Val Vogna**
Coordinates	N46°16'9.857 E7°6'44.252	N46°16'12.118 E7°6'58.814	N45°56'2.306 E7°12'14.333	N45°46'18.815 E7°54'9.197
Average elevation (m a.s.l)	1,745 ± 46	1,789 ± 32	2,012 ± 36	1,897 ± 67
Average slope (°)	23 ± 8	21 ± 10	22 ± 8	21 ± 6
Average distance to water (m)	116 ± 44	156 ± 3	18 ± 19	107 ± 75
Grazable area (ha)	8.26	7.67	5.99	17.88
Grazing days	28	24	40	28
Number of livestock units	23.5	23.5	11.3	71.2
Stocking rate (livestock units ha^{-1} $year^{-1}$)	0.22	0.20	0.21	0.30
Number of GPS-equipped cows	8	8	7	6
Cover of *Alnus viridis* (%)	61 ± 32	71 ± 28	44 ± 3	20 ± 34
Number of vegetation transects	11	11	12	24
Average vegetation patch size (ha)	0.69 ± 0.12	0.59 ± 0.08	0.50 ± 0.09	1.87 ± 0.53
Pastoral value (PV)	6.5 ± 6.1	5.6 ± 6.5	10.4 ± 6.3	18.3 ± 11.9
Species richness of the paddocks	71	96	123	157

Cover of *Alnus viridis*, distance to water, elevation, slope, and pastoral value of the vegetation are mean values (± SD) measured at a 10-m × 10-m cell scale over the whole paddock area. One livestock unit = 1 animal of 500 kg.

In order to characterize the drivers of plant community composition and diversity within each paddock, the botanical composition was determined along 12.5-m transects. Transects were placed in patches (1.08 ± 0.22 ha, **Table 3**) with homogeneous botanical composition and vegetation structure, and the vertical point-quadrat method was used to record plant species (Daget and Poissonet 1971). At 50-cm intervals along each transect, the plant species touching a steel needle were identified and recorded (i.e., 25 points per transect). Since rare species are often missed by this method, all other species within a 1-m buffer area around the transect were also recorded (Kohler et al. 2004). A total of 58 vegetation transects were surveyed in the four paddocks (**Table 3**). Plant species nomenclature followed Aeschimann et al. (2004). The relative abundance of each plant species was computed by dividing species frequency of occurrence by the sum of frequency of occurrence values for all species in the transect and multiplying it by 100. A species relative abundance value of 0.3% was assigned to all the species found only in the buffer zone and not in the main transect, following (Pittarello et al. 2016a). We then calculated the pastoral value (PV) of the vegetation, based on the species composition. The PV is a synthetic index summarizing forage yield, quality, and palatability for livestock (Pittarello et al. 2018). To estimate PV, we attributed each species an index of specific quality (Cavallero et al. 2007). The index of specific quality depends on the preference, morphology, structure, and productivity of the plant species, and it ranges from 0 (low) to 5 (high). The PV, which ranges from 0 to 100, was calculated as follows (Daget and Poissonet 1971):

$$PV = \sum_{i=1}^{n} (SRA_i \times ISQ_i) \times 0.2$$

where SRA_i is the species relative abundance, and ISQ_i is the index of specific quality value of the species i.

Moreover, we used the indicator values of Landolt et al. (2010) for each plant species. These indicate the environmental conditions in the habitats in which the given species occurs and are a measure of its environmental preferences. We used the light (L), soil moisture (F), and nutrient value (N) (nitrogen) indicators. We then determined the mean ecological conditions of each vegetation patch, as the mean Landolt value for L, F, and N by multiplying each species indicator value by its relative abundance. For each vegetation transect, two plant diversity indexes were computed: species richness and Shannon diversity index Hr (Magurran 1998). *A. viridis* canopy cover (%) was visually estimated within each vegetation patch.

Each paddock was subdivided into 10 × 10-m grid cells, and the distance to water sources (i.e., streams and water troughs), the aspect, elevation, and slope were calculated for the centroid of each cell using (QGIS/ArcView/SAGA/R). Moreover, *A. viridis* cover and PV were calculated for each grid cell using the values of the corresponding vegetation patch. If a cell covered more than one vegetation patch, the patch accounting for most of the cell area was considered. To avoid issues with circular variables (where high and low values are close together), aspect was transformed into southness (180 − | aspect −180 |) (Chang et al. 2004). In each herd, six to eight cows were equipped with GPS collars (Followit Tellus GPS collars) that recorded their position every 10 min during the whole grazing period, with an average accuracy of 2–5 m (**Table 3**). The number of GPS fixes was calculated for each grid cell.

All statistical analyses were performed using R version 3.4.4. A partial canonical correspondence analysis (CCA, vegan package), taking into account the spatial distance between botanical surveys, was performed to describe how PV, biodiversity indices, ecological indicators (i.e., Landolt indexes), and topography (i.e., slope and aspect) were related to plant community composition (after Hellinger transformation). Moreover, relationships between *A. viridis* cover and diversity indexes and PV were analyzed with generalized linear mixed models with Poisson (species richness and PV) and quasi-Poisson (Shannon diversity index Hr) distributions, with paddock considered as a random factor (lme4 package). The spatial distribution of cattle calculated from GPS data (i.e., the number of GPS fixes counted in each grid cell) was investigated separately for three distinct periods within each paddock, i.e., the beginning (P1), middle (P2), and end (P3) of the grazing period. These periods had equal lengths within each paddock and varied between 1 and 2 weeks depending on the length of the grazing period

(**Table 3**). The impacts of slope, *A. viridis* cover, distance to water, and PV on the number of GPS fixes were assessed using linear regression models with zero-inflated negative binomial likelihood, as this error distribution accounted for overdispersion in the response variable (tested using the AER package). Homburger et al. (2015) found this likelihood structure to be appropriate for GPS counts in zones incompletely exploited by cattle. In order to account for the spatial autocorrelation of data, a spatial structure error term (using a two-dimensional random walk of second order) was included, and the model was fitted by Integrated Nested Laplace Approximation (INLA).

Results

We found a total of 252 plant species in all the paddocks, with the highest species richness detected in Val Vogna (157 species, **Table 3**). The results of the CCA analysis highlighted different ecological, topographical, and vegetation impacts on plant community composition (**Figure 22**), which significantly explained the variation in community composition across the paddocks (CCA 1 = 20.9%, CCA2 = 19.0%, CCA3 = 12.9%, CCA 4 = 10.9%, and total = 84.6%). Vegetation patches within the two Bovonne paddocks were characterized by higher *A. viridis* cover and nitrogen enrichment (i.e., higher values for N indicator). In contrast, the Champlong paddock had many open pasture patches (with higher light availability), while Val Vogna patches were more heterogeneous. Four patches in Val Vogna were well distinguished and corresponded to vegetation dominated by dwarf shrubs, such as *Vaccinium vitis-idaea*, *Juniperus nana*, and *Rhododendron ferrugineum*, with low PV (**Figure 22B**). *A. viridis* cover had a significant negative effect on plant species richness, Shannon diversity index Hr, and PV ($p < 0.001$). Indeed, plant species associated with higher PV (e.g., *Festuca pratensis* and *Trifolium pratense*) were often found in the most open areas, characterized by a lower *A. viridis* cover, as well as typical small sized pasture species (e.g., *Medicago lupulina* and *Polygala alpestris*), which were associated with the highest light availability and biodiversity (both in terms of species richness and Shannon diversity index Hr). Conversely, areas with high levels of *A. viridis* encroachment and with high indicator values for N were dominated by a few tall broad-leaved species, such as *Adenostyles alliariae* and *Cicerbita alpina*, together with ferns (mainly *Athyrium filix-femina* and *Dryopteris dilatata*).

Estimates of linear regression models showed that livestock spatial distribution was often positively influenced by PV. Cattle spent more time in high PV areas in Bovonne 1 (during periods 1 and 2), Champlong (period 3), and Val Vogna (periods 2 and 3) (**Figure 23**). In Bovonne 2, the PV did not influence cattle spatial distribution. The cover of *A. viridis*, slope, and distance to water sources did not generally affect livestock spatial distribution. *A. viridis* cover and slope only had significant effects in the Champlong paddock during the third and the first grazing period, respectively; in both cases, cattle spent more time in areas with high *A. viridis* cover and on steeper slopes. Distance to water had a negative effect only in periods 1 and 2 in Bovonne 1 paddock, where water sources were less abundant, meaning cattle spent more time close to water.

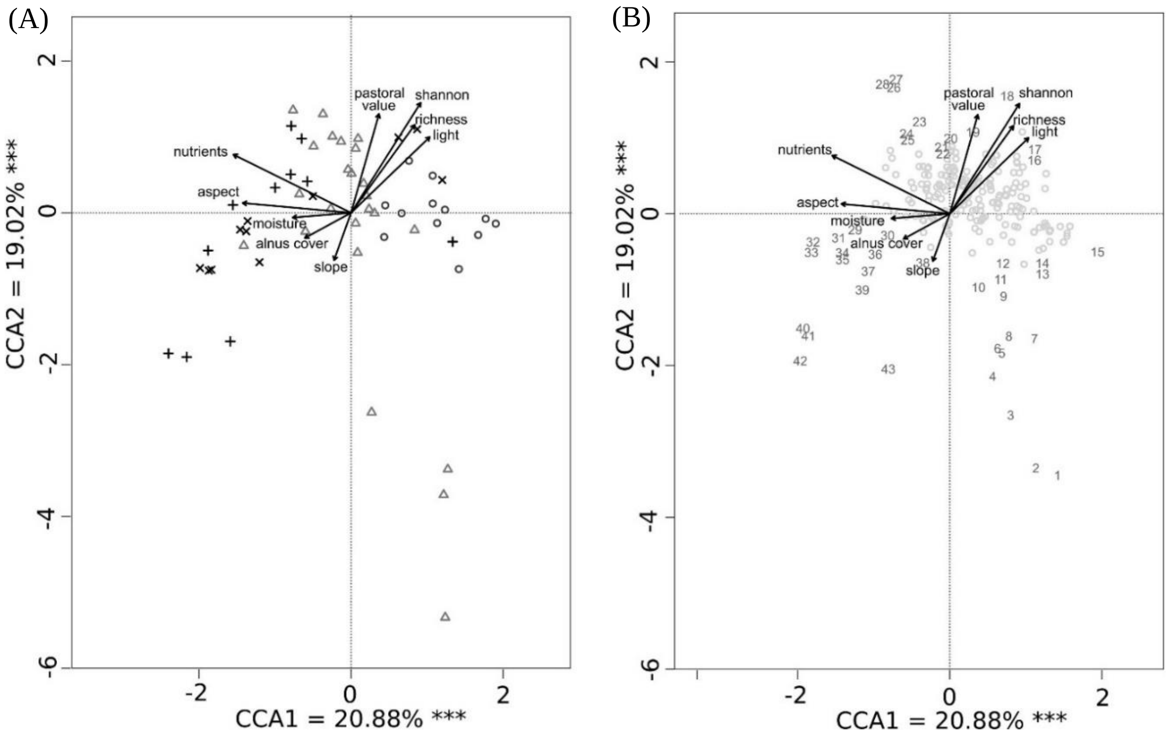

Figure 22 Partial canonical correspondence analysis (CCA) showing plant community composition explained by *A. viridis* cover, slope, aspect, pastoral value, species richness, Shannon index, and ecological indicators for nutrients, light, and moisture (Landolt indexes). Both dimensions presented are significant (∗∗∗p < 0.001). (A) Botanical surveys are represented for Bovonne 1 (+), Bovonne 2 (x), Champlong (o), and Val Vogna (Δ). (B) Plant species are represented by numbers (detailed below) for the most important species and by dots for the remaining species. (1) *Vaccinium vitis-idaea*, (2) *Juniperus nana*, (3) *Cryptogramma crispa*, (4) *Gymnocarpium Dryopteris*, (5) *Astrantia minor*, (6) *Rhododendron ferrugineum*, (7) *Arnica montana*, (8) *Avenella flexuosa*, (9) *Festuca scabriculmis*, (10) *Rosa pendulina*, (11) *Vaccinium myrtillus*, (12) *Calamagrostis villosa*, (13) *Melampyrum sylvaticum*, (14) *Plantago lanceolata*, (15) *Epilobium montanum*, (16) *Medicago lupulina*, (17) *Thlaspi montanum*, (18) *Polygala alpestris*, (19) *Molinia arundinacea*, (20) *Hypericum richeri*, (21) *Festuca pratensis*, (22) *Trifolium pratense*, (23) *Arabis ciliata*, (24) *Rumex alpestris*, (25) *Campanula latifolia*, (26) *Capsella bursa-pastoris*, (27) *Chenopodium bonus-henricus*, (28) *Trifolium badium*, (29) *Cardamine resedifolia*, (30) *Adenostyles alliariae*, (31) *Acer pseudoplatanus*, (32) *Rubus saxatilis*, (33) *Thalictrum aquilegiifolium*, (34) *Saxifraga rotundifolia*, (35) *Cicerbita alpina*, (36) *Urtica dioica*, (37) *Dryopteris dilatata*, (38) *Rubus idaeus*, (39) *Athyrium filix-femina*, (40) *Prenanthes purpurea*, (41) *Sorbus aucuparia*, (42) *Picea abies*, and (43) *Oxalis acetosella*.

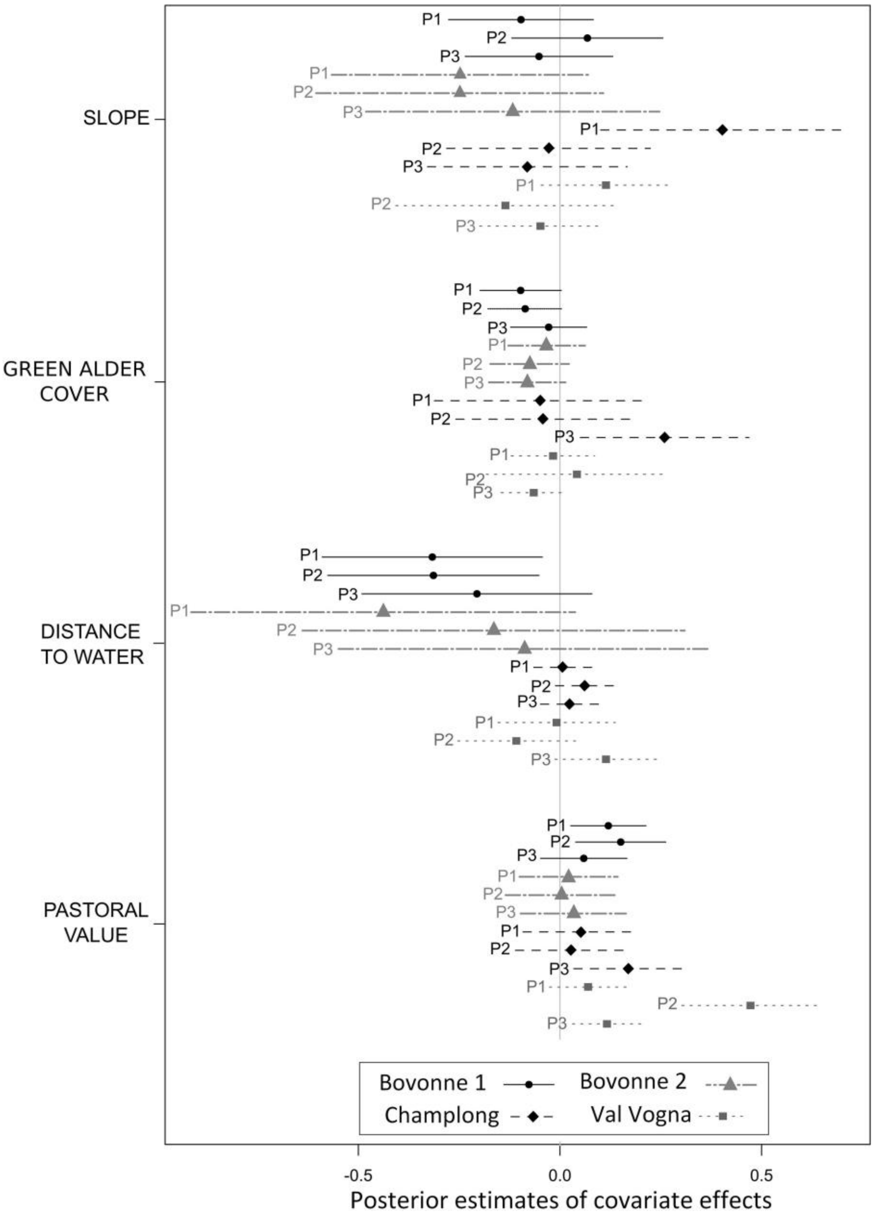

Figure 23 Effects of four covariates on livestock spatial distribution. The dots show the mean estimated effects and the line the 95% confidence interval, in the paddocks of Bovonne 1 (black line), Bovonne 2 (gray dashed line), Champlong (black dashed line), and Val Vogna (gray dotted line) for the three grazing periods (P1, P2, and P3).

Discussion

Overall, plant communities with higher *A. viridis* cover had lower plant diversity and were dominated by a few broad-leaved species, like *A. alliariae* and *C. alpina*, which prefer N-enriched conditions. These results support the findings of Anthelme et al. (2001) and Zehnder et al. (2020), who also showed a decline in alpine plant diversity with *A. viridis* encroachment. The few species that survive in *A. viridis*-dominated areas are able to take advantage of the N-enrichment and increased shading, and they replace many typical pasture species that are highly light demanding. Interestingly, other than broad-leaved species, the understory vegetation of many *A. viridis* patches was dominated by ferns (in particular, *A. filix-femina* and *D. dilatata*). These ferns are also highly shade tolerant, but they typically prefer areas with lower N levels, compared to the broad-leaved species (Landolt et al. 2010). They may therefore be more abundant in areas recently invaded by *A. viridis* where N levels have not increased so much. These results show that encroachment by *A. viridis* threatens the diversity of Alpine pastures and leads to a loss of typical pasture species.

The Highland cattle did not avoid patches with a high density of *A. viridis*. In fact, they were able to graze in most *A. viridis*-covered areas, therefore indicating that they could be valuable in grazing and reducing *A. viridis* patches. In contrast to many other breeds (e.g., Homburger et al. 2015), Highland cattle were also capable of grazing on the steepest slopes and were able to spend time in areas further from water sources, as they were not significantly influenced by these harsh conditions. This is in line with the observations of Pauler et al. (2020a) who found that Highland cattle used space more evenly and avoid steep slopes less than other breeds. Moreover, cattle even preferred *A. viridis* patches and steep slopes in Champlong, during certain grazing periods, which shows that cattle are able to move to areas with the most unfavorable conditions and can stay there for relatively long periods (approximately 2 weeks). Cattle also responded differently to the various factors during the time they were on the paddocks. This indicates that the herds may explore the paddock intensively at the beginning of the grazing period before selecting particular areas later on. However, despite the fact that Highland cattle graze less selectively on plants compared to other breeds (Pauler et al. 2020b), they were still mostly attracted to valuable pastures. Thus, providing a mosaic of open pasture areas with higher quality forage in *A. viridis*-encroached paddocks may be a good solution to respect grazing preferences while increasing grazing pressure on *A. viridis*-encroached patches. Moreover, this type of setting would enhance the potential for seed translocation through endo- and epi-zoochory by Highland cattle (Mouissie 2004; Cosyns et al. 2005). Indeed, in the study of Pauler et al. (2019), plant species relying on epizoochory were significantly more frequent in pastures grazed by Highland cattle than in paddocks grazed by other breeds, probably because the long fur of this breed is ideal for transporting seeds. Animal movements might thus provide additional seed translocation fluxes from pastures to encroached areas to facilitate the restoration of typical pasture vegetation in the long-term (Pittarello et al. 2016a). However, future research is needed to assess the simultaneous effects of cattle grazing, trampling, and seed transportation on the restoration of pasture vegetation in *A. viridis*-encroached areas. We observed considerable impact of the cattle on vegetation within the encroached areas (data not shown), including intense defoliation of *A. viridis* shrubs and a reduction of their canopy cover, due to browsing, as well as damage to branches due to cattle movement and scratching. Moreover, an increase in bare soil due to livestock trampling and a considerable number of dung pats was observed within *A. viridis*-encroached areas. If repeated for several years, these effects of the cattle could provide favorable conditions for the recolonization of typical pasture vegetation in formerly encroached areas. The repetition of the botanical surveys along the fixed vegetation transects installed during this study could allow us to assess the medium- and long-term impacts produced by Highland cattle on vegetation. In addition, testing improved techniques to attract livestock to the most encroached areas, such as the placement of specific attractive points (Pittarello et al. 2016a; Bailey et al. 2019), might be relevant to further increase the grazing pressure and thus livestock impacts on *A. viridis*-encroached subalpine pastures. In conclusion, our results show that Highland cattle can graze in harsh environmental conditions and exploit *A. viridis*-encroached pastures. This suggests that they have a high potential to reduce *A. viridis* encroachment in the long-term.

CHAPTER V: Use of molasses-based blocks to modify grazing patterns and increase Highland cattle impacts on *Alnus viridis*-encroached pastures

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Abstract

Alnus viridis is a pioneer species that has expanded in Central Europe in the last decades, causing a series of negative agro-environmental impacts. Robust livestockgrazing could be used as a targeted tool to reduce its encroachment, but more information is needed to find the best approach to achieve this goal. In this study,we assessed the potential of molasses-based blocks (MB) to lure Highland cattle into *A. viridis*-encroached areas and monitored impacts on the vegetation after grazing. In 2019 and 2020, two Highland cattle herds equipped with GPS collars were placedin three paddocks in the Swiss and Italian Alps, differing in the degree of *A. viridis* encroachment. In 2020, MB were added to highly encroached areas within each paddock to attract the herds to feed on *A. viridis*. Botanical surveys were carried out before and after grazing, around MB and control areas. Highland cattle grazed significantly more around MB (up to 50 m from the MB) compared to the previous year(i.e., same area without MB) and compared to control areas. The increased targeted grazing around MB led to a significant decrease in herbaceous cover and an increase in bare soil compared to control areas. Livestock grazing and trampling significantly reduced the cover of ferns, tall herbs, medium and small herbs, and woody species around MB compared to control areas. *A. viridis* leaves and branches were significantly removed and damaged up to 10 m from the MB, due to the more intense livestock grazing. Such results highlight the potential of this management regime to effectively reduce *A. viridis* encroachment in montane grasslands.

Introduction

During the last century, socio-economic transformations have led to a large-scale decrease in agro-pastoral activities across the mountain areas of Europe, resulting in many challenges for grassland management and biodiversity conservation (MacDonald et al. 2000; Valkó et al. 2018). For instance, in Switzerland, the last 30 years have witnessed a significant decrease in grassland area in alpine regions (Strebel and Bühler 2015; Zehnder et al. 2016a). The reduction of livestock farming has caused profound landscape modification and widespread shrub and tree encroachment in former meadows and pastures (Estel et al. 2015). For example, from 1985 to 2013, shrublands and forests increased by 10.6% across Switzerland, with the largest increases in the Alps (Abegg et al. 2020). Encroachment of montane grasslands by woody species has been even more pronounced in the Italian Alps (Orlandi et al. 2016). Shrublands now cover an area of 679 km² in Switzerland (Abegg et al. 2020), with about 70% of them composed by green alder [*Alnus viridis* (Chaix.) D. C.], which is among the most rapidly expanding shrub species in Central Europe (Anthelme et al. 2007). *Alnus viridis* is a pioneer shrub species that lives in symbiosis with the N_2-fixing actinomycete *Frankia alni* (Huss-Daniel 1997). It is found mostly in steep, north and west-facing slopes, but it can easily expand into other habitats in montane environments, thanks to its efficient colonization ability and substantial seed production (Farmer et al. 1985; Caviezel et al. 2017). Its presence is strongly affected by land-use intensity, as land-abandonment is a key driver of its spread (Caviezel et al. 2017).

Encroachment by *A. viridis* can have several negative agro-environmental impacts on montane grasslands, which in turn reduce the provision of key ecosystem services for society. For instance, *A. viridis* encroachment prevents forest succession, and causes nitrogen enrichment in soils leading to increased nitrate leaching and higher risk of dissolved organic carbon leaching (Bühlmann et al. 2016; Hunziker et al. 2017). This can result in soil acidification, water pollution and gaseous nitrogen losses (Caviezel et al. 2014; Bühlmann et al. 2017). van den Bergh et al. (2018) also showed that *A. viridis*-encroachment increases the evaporative water loss. Moreover, *A. viridis*-dominated shrublands are characterized by a lower forage quality and host lower animal and plant diversity than adjacent grasslands (Anthelme et al. 2001; Bühlmann et al. 2014; Svensk et al. 2021). A recent study by Zehnder et al. (2020) showed that *A. viridis* encroachment resulted in a rapid reduction in plant species richness and grassland specialist species, with dense *A. viridis* patches hosting 62% less species than nearby open pastures. This decrease in biodiversity is linked to reduced light levels in highly encroached areas, as well as to soil nitrogen enrichment. Indeed, only a few tall and shade-tolerant plants with broad leaves, such as *Adenostyles alliariae* (Gouan) A. Kern and *Cicerbita alpina* L. (Wallr.), together with a few fern species, are able to adapt to these ecological conditions and dominate the understory vegetation of *A. viridis* shrublands (Svensk et al. 2021). Furthermore, in contrast to coniferous forests, *A. viridis* stands do not provide protection against erosion and avalanches on steep slopes, mostly due to the elasticity of their branches that easily bend under snow pressure (Caviezel et al. 2014). Their resistance to this environmental pressure provides an advantage for *A. viridis* in these areas, compared to other shrubs or trees that can easily break under such disturbance. Finally, the encroachment by *A. viridis* can also adversely impact landscape quality in montane areas, resulting in reduced attractiveness for tourists.

Because *A. viridis* predominantly establishes on steep slopes and marginal locations, with few roads, the mechanical removal of this shrub species can be technically difficult, expensive and time consuming. One alternative and sustainable nature-based solution to counteract shrub encroachment could be the use of targeted grazing (Elias and Tischew 2016; Elias et al. 2018; Pauler et al. 2022b). Meisser et al. (2014) monitored the grazing behavior of Hérens cows in *A. viridis* dominated pastures and showed that they did spend time in half or more encroached areas, despite their usual preference for open areas. However, many shrub species like *A. viridis* are not palatable for production-oriented livestock due to their low foliage digestibility. Stević et al. (2010) found that *A. viridis* leaves contained an average of 4.4% of tannins, which could eventually cause an astringent taste and reduce palatability (Kumar and Vaithiyanathan 1990). Nevertheless, some robust livestock species and breeds have higher resistance to tannins, with ruminal bacterial populations that can better degrade lignified material, allowing them to feed on shrubs and digest their leaves (Berry et al. 2002; Marques et al. 2017). For instance, previous studies have shown that sheep and goats can efficiently feed on woody plants and significantly reduce their cover (Iussig et al. 2015a; Álvarez-Martínez et al. 2016; Pauler et al. 2022b). Some cattle breeds can also feed on shrub species (Zehnder et al. 2016a; Pauler et al. 2019). For example, Highland cattle, a robust breed originating from Scotland, are able to graze on low quality shrub foliage (Pauler et al. 2020a) due to their low maintenance energy requirements and their more efficient use of nutrients from the vegetation (Berry et al. 2002). This has recently led, together with the low costs of their maintenance and care, to an increased rearing of this livestock breed in alpine regions (Pauler et al. 2020b). Previous studies have demonstrated their capacity to reduce woody plant species cover over time, with a turnover in plant diversity (Pauler et al. 2019, 2020a). In addition to the direct grazing of the leaves, Highland cattle are also able to damage shrub branches and trunks thanks to their long horns (Svensk et al. 2021), even if they do not directly debark trees as some robust goat or sheep breeds do (Pauler et al. 2022b). Recently, Svensk et al. (2021) showed that Highland cattle can graze on steep montane pastures characterized by high *A. viridis* cover and associated low forage quality vegetation in the understory. Therefore, Highland cattle grazing could be an efficient and sustainable tool to reduce *A. viridis* encroachment and restore encroached pastures in the long-term.

Together with cattle feeding behavior, livestock management techniques are also key elements to increase livestock impacts on targeted shrub-encroached vegetation. For instance, livestock supplements could be used to attract them to underused and shrub-encroached locations (Probo et al. 2013, 2016). Different types of dietary supplementation exist, mainly composed of salt (i.e., mineral mix supplements) or sugar (i.e., molasses-based blocks, MB). Previous studies have already demonstrated the efficacy of mineral supplements in attracting beef cattle herds within steep montane shrub-encroached areas (Probo et al. 2013; Pittarello et al. 2016a). For example, in a 5-year study, Probo et al. (2016) highlighted a significant reduction in shrub cover, together with the establishment of typical pasture species with higher forage quality, thanks to the effects produced by grazing, trampling, seed and dung translocation at mineral supplement locations. Dehydrated MB were also proven to be efficient in influencing cattle grazing patterns in unfavorable field conditions (i.e., steep slopes, far from water sources and usually undergrazed zones) and across large areas in the North American steppes (Bailey and Welling 1999; Bailey et al. 2001). However, they have never been tested in montane shrub-encroached areas, despite their lower cost and labor compared to fencing and herding. The MB for cattle grazing management provides different advantages, such as an enrichment of cattle diets through mineral supplementation, that can also reduce the risks of foot pathology and mycotoxicosis, and an enhancement of the intake of forage with low palatability, thanks to their appetizing role (Mordenti et al. 2021). However, more information is needed on the efficiency of molasses-based supplements in changing livestock spatial distribution and increasing the cover of target plant species of functional groups, in montane environments. Indeed, previous studies (e.g., Tocco et al. 2013), showed that the analysis of functional group cover in the short-term can be essential to assess the effectiveness of targeted grazing techniques in a longer term.

In the present study, we placed MB on *A. viridis*-encroached pastures to attract Highland cattle herds and increase their effects on shrub vegetation. Specifically, we aimed to investigate: (i) the effect of strategically placed MB on Highland cattle spatial distribution and (ii) the impacts of targeted grazing by livestock around MB on *A. viridis* shrubs, understory plant functional groups and soil cover. We hypothesized that (i) Highland cattle would significantly use more the areas around MB, and (ii) their targeted grazing and trampling pressure would have a higher impact on *A. viridis* shrubs around MB, with an increased removal of leaves and damage on trunks and branches compared to control areas. Simultaneously, we expected (iii) the understory vegetation to be more affected around MB, with an increase in bare soil due to livestock grazing and trampling, which could lead to a potential re-colonization of those areas by typical pasture species in the long-term.

Methods

1. Study areas and grazing management

During the summer seasons of 2019 and 2020, two Highland cattle herds were placed in three *A. viridis*-encroached paddocks in the Swiss and Italian Alps. The first one (paddock 1, 17.88 ha) was located in Val Vogna, in the province of Vercelli (Italy). The other two paddocks (paddock 2, 8.26 ha and paddock 3, 7.67 ha) were located in Bovonne, in the canton of Vaud (Switzerland) and were grazed by the same herd. All paddocks were grazed at a comparable stocking rate between years (**Table 4**) and had similar topographical conditions, with an elevation of 1,861 45 m a.s.l (mean ± s.e.) and slope of 23 ± 8° (mean ± s.e.). The three paddocks were representative of an *A. viridis* cover gradient, with an average cover of 20, 61, and 71%, respectively, in paddock 1, 2, and 3. The herds grazed in the summer pastures from the middle of June to the beginning of September (**Table 4**). All the herds included cow/calf pairs and heifers, varying in age from 6 months to 17 years (with an average of 5 years for paddock 1 and 4 years for paddock 2 and 3) and about 70% of the animals were present in both years at the same site. A water trough was installed in paddocks 2 and 3, while natural streams were present in paddock 1. In each herd and during both years, six to ten cows (**Table 4**) were equipped with GPS collars (Followit AB, Tellus GPS System collars, Sweden) that recorded their position every 10 min during the whole grazing period, with an accuracy of 2–5 m.

Table 4 Grazing periods, grazable area, livestock stocking rates and number of GPS collars in the study areas in 2019 and 2020.

Year	Paddock	Grazing period	Number of grazing days	Livestock units (LU[a])	Grazable area (ha)	Stocking rate (LU/ha × year)	Number of GPS collars
2019	Paddock 1	July 19th to September 3rd	44	45.4	17.88	0.31	6
	Paddock 2	July 2nd to 19th	17	29.8	8.26	0.17	8
	Paddock 3	July 30th to August 17th	19	29.8	7.67	0.19	8
2020	Paddock 1	July 20th to August 18th	29	70.4	17.88	0.31	8
	Paddock 2	June 15th to July 2nd	17	29.6	8.26	0.17	10
	Paddock 3	July 2nd to 20th	18	29.6	7.67	0.19	10

[a] LU, Livestock Unit. One livestock unit = 1 animal of 500 kg.

In 2020, five dehydrated MB (with 2–3% of residual moisture) of 22.5 kg each were added to each paddock as attractive points. They were provided within small boxes of 40 × 28 × 20 cm. They were mostly composed of sugar (40%), which has an appetizing effect and fosters the intake of low forage quality vegetation (Mordenti et al. 2021), and contained mineral supplements which are often lacking in natural montane environments (Schlegel and Kessler 2001), thus complementing cattle feeding (detailed composition available in **Table S2**). The number of blocks was defined based on the average consumption by cattle given by the producer (i.e., 35–50 g/calf × day, 100 g/heifer day, and 150–200 g/cow × day). The consumption of MB was monitored in each paddock every 2 days to check that enough molasses was available during the grazing period. At the end of the grazing period, the MB were weighed and the average consumption per animal was estimated by dividing the total amount consumed by the number of animals and grazing days. The MB were placed at five points along a 40-m line, lying along a contour line, and separated by 10 m from each other (**Figure S5**), in highly *A. viridis*-encroached areas (i.e., areas with more than 2/3 of *A. viridis* cover). A control line was also established in each paddock in areas with comparable *A. viridis* cover, slope, botanical composition and distance to water sources. These two similar zones (190 ± 72 m apart) were identified in each paddock and assigned randomly to either control or MB areas before the grazing season of 2020. Moreover, to avoid any pre-existing effect of the area on cattle distribution, we also checked that the MB and control locations were similarly exploited by livestock in 2019, before the MB were established in 2020. Around control and MB lines, buffer areas with a radius of 10 and 50 m were created in a GIS environment, and the number of GPS positions was calculated for each cow within each buffer (using QGIS 3.6 software). As one GPS location was recorded every 10 min, we calculated the average number of minutes a cow spent per day in every buffer area. The 10-m buffer was used to assess the attractive effect in a small area, in which livestock were likely present to actively consume MB, while the 50-m buffer was used to estimate the attractive effect on a larger scale, i.e., including areas where livestock grazed and walked around MB locations.

2. Vegetation surveys

Around both MB and control points, botanical surveys were carried out in 2020 both before grazing (June-July) and after grazing (August), in cross-shaped vegetation transects of 10 meters (see **Figure S5**), using the vertical point-quadrat method (Daget and Poissonet 1971). At 50 cm intervals along each transect, the vascular plant species touching a steel needle up to a grazable height (i.e., 1.8 m) were identified and recorded. To account for rare species around the transect, all other species within a one meter buffer around the transect were also recorded (Kohler et al. 2004). Each line of MB and control points in all three paddocks was made up of 16 transects (i.e., 48 vegetation transects in control areas and 48 in MB areas in total). The percentages of herbaceous cover and bare soil were visually assessed before and after grazing within a one-meter buffer around each transect, to assess the changes in vegetation cover produced by livestock targeted grazing and trampling. Plant species nomenclature followed Aeschimann et al. (2004).

Along the vegetation transect, the percentage species cover (%SC) was calculated by converting the recorded frequency of occurrence of each plant species to 100 measurements. A %SC of 0.3% was assigned to the species found only in the 1-m buffer zone and not along the transect, following Pittarello et al. (2016a). Species were grouped in three main functional groups (see **Table S3**) that were common among all paddocks and typical of the understory of *A. viridis*-encroached pastures (Svensk et al. 2021): (1) Ferns and tall herbs, i.e., all ferns and tall broad-leaved forbs having a leaf diameter greater than 10 cm and taller than 40 cm, following Pignatti (1982); (2) medium and small herbs, i.e., other non-graminoid species that were not classified as tall herbs; and 3) woody, i.e., all woody species (including *A. viridis*). Graminoids were not taken into account as they were a minor component of the understory vegetation, representing only 5.9 ± 3.1% on average (mean ± SE) of the vegetation cover in each paddock (with a maximum of 11.5% in paddock 1).

Along each transect three *A. viridis* shrubs were selected every 3–4 m and marked. We did not include the central transect in this selection as the central transects had two MBs close to them. One branch from each tree was selected to assess the number of living leaves before and after grazing, and thus estimate the removal of *A. viridis* leaves by Highland cattle, and the damage done to the observed branch. Whenever the branch was totally broken and untraceable after grazing, the number of remaining living leaves was considered equal to zero. The damage made on *A. viridis* branches through grazing, scratching or trampling was also visually assessed with the following scale: 0 (no damage), 1 (moderate damage), 2 (intense damage) and 3 (branch totally damaged or broken). The maximum height at which damage was observed on each branch was also recorded.

For the analyses of soil and functional group cover as well as leaf removal, a response ratio (RR) was computed for all the assessed variables according to the following formula:

$$RR = \frac{Value_{Post} - Value_{Pre}}{Value_{Pre}}$$

where “ValuePost” is the value measured after grazing and “ValuePre” the value measured before grazing. This formula provides an indication of the effect size, which is not biased by the initial (before grazing) differences among replicates. A negative RR indicates that the value decreased after grazing, while a positive RR indicates that the value increased after grazing.

3. Statistical analysis

All statistical analyses were performed using R version 3.4.4 (R Core Team, 2017). The effects of treatment (control vs. MB), the year (2019, 2020) and their interactions on the spatial distribution of cows were tested separately for the 10- and 50-m buffers by using a linear mixed-effect model (lme, package “nlme”), with “paddock” as a random factor. Spatial distribution of cows was measured as the average time spent by a cow at the herd level within the buffer (minutes day^{-1} cow^{-1}, obtained from GPS location counts), i.e., the sum of the minutes spent by all GPS-equipped cows in the considered buffer, divided by the number of GPS-equipped cows. The effects of treatment (control vs. MB) on 2020 soil cover (RRs of herbaceous cover and bare soil) and on functional group cover (RRs of ferns and tall herbs, medium and small herbs, and woody species cover) were tested using generalized least square models (GLS), with treatment (control vs. MB) as fixed factors and coordinates of the transects, nested in paddock, as random effects. This random effect structure takes into account the nested structure of the data and accounts for any residual spatial autocorrelation among neighboring transects. The effects of MB, distance of shrubs along the transect, and their interactions, on the RR of *A. viridis* leaves were tested using the same type of GLS model, again with the coordinates of the transects nested in paddock. Although the GLS analysis takes into account the potential spatial autocorrelation and pseudoreplication, we carried out an additional, conservative analysis in which we analyzed only the average RR in each area of the paddocks (i.e., 3 control points vs. 3 MB, one control and one MB from each paddock: 6 data points in total), using ANOVA. The ANOVA has much lower power than the GLS model but is completely free from any spatial autocorrelation or pseudoreplication issues. *Post hoc* tests were performed for the models when significant effects were detected (Tukey's test, $P < 0.05$), and additional Student's test (*t*-test) were performed to assess the difference of RRs from zero.

Results

1. Effects of molasses-based blocks placement on livestock spatial distribution

Based on the remaining molasses after grazing, we calculated that each animal consumed 134 g of molasses per day on average. All MB (except two placed in paddock 3) were completely consumed at the end of each grazing period.

There were no significant differences in the average number of minutes spent by cows between control and MB areas in 2019, in both 10 and 50-m buffers ($P = 0.99$ and $P = 0.78$, respectively; **Figure 24** and **Table S4**), indicating that the locations chosen were equally attractive to livestock, before the MB were placed in 2020. There was a significant increase of livestock use in MB areas in 2020 compared to 2019 for both 10-m (MB Y, $P < 0.001$, + 771% on average; **Figure 24A**) and 50-m (MB Y, $P < 0.05$, + 166% on average; **Figure 24B**) buffers.

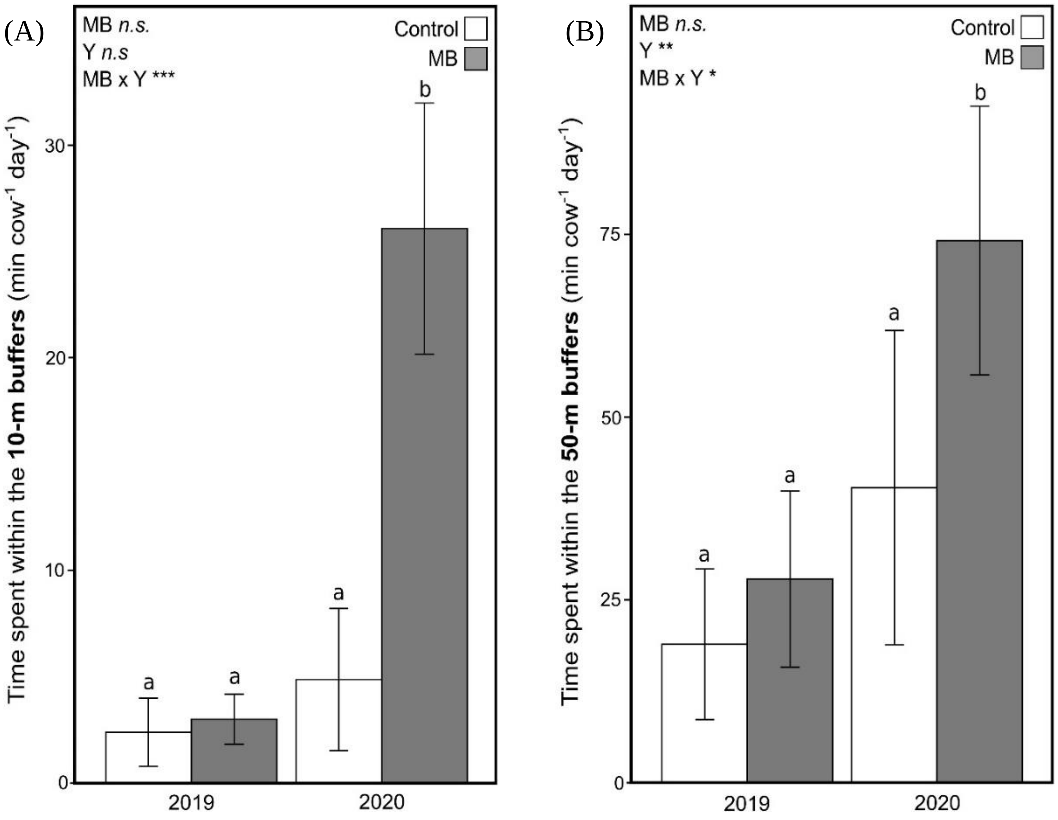

Figure 24 Number of minutes spent (mean ± SE) per day per cow within the herd in 10-m buffers (A) and 50-m buffers (B) in 2019 and 2020 for both molasses-based blocks (MB, gray) and control (white) areas. Significance of treatment (MB), year (Y) and their interaction (MB × Y) is reported on the graph ("n.s" as non significant, "*" for $p < 0.05$, "**" for $p < 0.01$, "***" for $p < 0.001$). Different letters indicate significant differences (Tukey's test, $P < 0.05$) between areas for both years.

2. Effects of livestock on soil cover

The percentage of bare soil was highly impacted by livestock targeted grazing and trampling. There was a significant decrease in herbaceous cover and a significant increase in bare soil after grazing, in both control and MB areas, with response ratios always significantly different from zero ($P < 0.001$, **Figure 25** and **Table S4**). The impacts of livestock around the MB always had a significant effect on both herbaceous cover and bare soil ($P < 0.001$, **Figure 25**), with a higher impact on bare soil around MB compared to control areas. The herbaceous cover had an average RR of –0.77 around MBs, and decreased from 73.5% before grazing to 16.2% after grazing. In control areas, herbaceous cover had an average RR of –0.39 and decreased from 61.9% before grazing to 35.5% after grazing. On the other hand, the percentage of bare soil had an average RR of 4.23 around MB, and represented 17.1% before grazing and 76.2% after grazing. In control areas, bare soil had an average RR of 1.68, and represented 29.2% before grazing and 56.7% after grazing. The ANOVA analysis performed on the average RR cover per paddock provided the same results, except for percentage of bare soil for which the effect of MB was marginally significant (see **Table S5**).

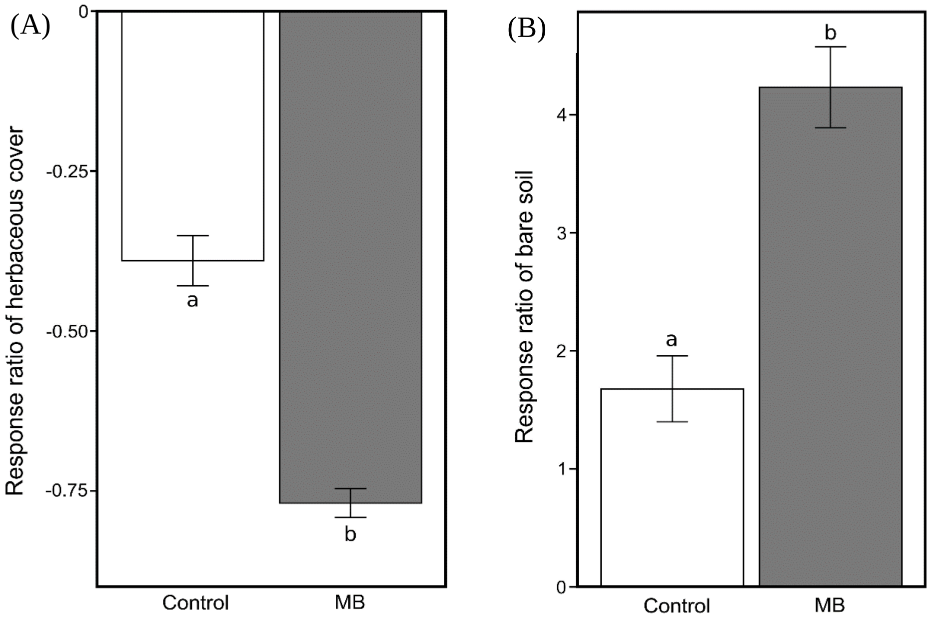

Figure 25 Response ratio (mean ± SE) of herbaceous cover (A) and bare soil (B) in both molasses-based blocks (MB, gray) and control (white) areas. Different letters indicate significant differences (Tukey's test, $P < 0.05$) between areas. Response ratios from both control and MB areas were significantly different from zero for both herbaceous cover and bare soil (*t*-test, $P < 0.001$).

3. Effects of livestock on *Alnus viridis* shrubs

The *A. viridis* shrubs were highly damaged by Highland cattle targeted grazing. There was a significant decrease in the number of *A. viridis* leaves after grazing in both control and MB areas, with a higher decrease around MB and negative response ratios significantly different from zero ($P < 0.001$, **Figure 26** and **Table S4**). The interaction between MB and distance was significant ($P < 0.05$), with the removal being higher on shrubs closer to the MB compared to further shrubs, while in control areas the effect was the same independent of the distance. In addition to leaf removal, damage on *A. viridis* branches was assessed at MB locations at an average height of 1.48 ± 0.53 m (mean SE), with 68% of branches having damage scores between 1 and 2 (moderate to intense damages) and 10% with damage scores of 3 (very severe damage or broken branches). Reducing our dataset to 6 data points and analyzing the average RR of number of *A. viridis* leaves showed that the effect of MB was also significant (see **Table S5**).

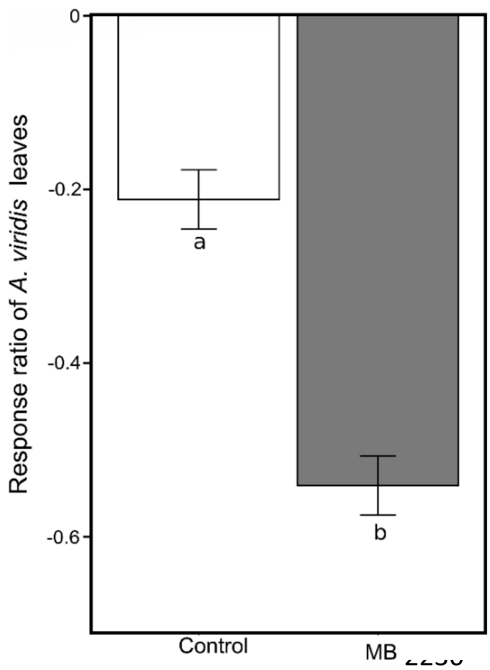

Figure 26 Response ratio (mean ± SE) of *Alnus viridis* remaining living leaves in both molasses-based blocks (MB, gray) and control (white) areas. Different letters indicate significant differences between areas (Tukey's test, $P < 0.05$). Response ratios from both control and MB areas were significantly different from zero (*t*-test, $P < 0.001$).

4. Effects of livestock on understory plant functional group cover

A total of 149 plant species were recorded during the botanical surveys in all paddocks (the species list and their corresponding functional group are available in **Table S3**). Ferns and tall herbs included 37 species and had the highest % SC (44.4%), with *Adenostyles alliariae* (14.1%), *Athyrium filix-femina* (L.) Roth (10.4%) and *Dryopteris dilatata* (Hoffm.) A. Gray (4.3%) being the most dominant species. Most species (78) were medium and small herbs (23.5%), with *Ranunculus aconitifolius* L. (8.7%) *Stellaria nemorum* L. (2.7%) and *Viola biflora* L. (2.2%) being the most dominant. Woody species (26.1%) comprised 14 species, including *Alnus viridis* (18.5%), *Vaccinium myrtillus* L. (2.0%) and *Rhododendron ferrugineum* L. (1.5%). Graminoids were not abundant in the botanical surveys (20 species and 5.9%).

The increased targeted grazing and trampling around MB had a significant impact on understory plant functional groups. There was a significant decrease in fern and tall herb cover and in medium and small herb cover after grazing in both control and MB areas, with negative response ratios always significantly different from zero ($P < 0.001$, **Figure 27A**, **B** and **Table S4**). The presence of MB always had a significant effect on both groups, leading to a higher decrease compared to control areas ($P < 0.001$, **Figure 27A**, **B**). Woody species decreased significantly more around MB than in control areas ($P < 0.001$; **Figure 27C**), in which they were not significantly damaged, with response ratios not different from zero (–0.11 ± 0.07, $P = 0.10$). Reducing our dataset to only 6 data points and analyzing the average RR of functional groups showed the same results, except for fern and tall herb cover for which the effect of MB was not significant (see **Table S5**).

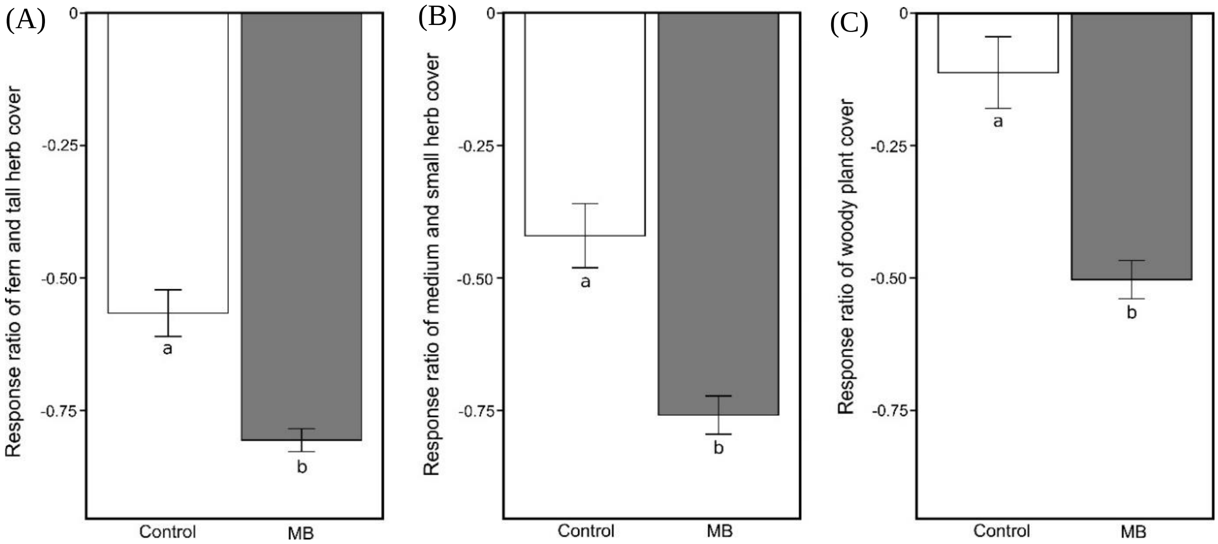

Figure 27 Response ratio (mean ± SE) of the cover of ferns and tall herbs (A), medium and small herbs (B) and woody species (C) for both molasses-based blocks (MB, gray) and control (white) areas. Different letters indicate significant differences (Tukey's test, $P < 0.05$) among areas. Response ratios from both control and MB areas were significantly different from zero for all functional groups (*t*-test, $P < 0.001$), except for woody plants where the control area showed an average response ratio not different from zero (*t*-test, $P = 0.104$).

Discussion

Overall, the strategic placement of MB significantly altered Highland cattle spatial distribution, and increased livestock use of areas around MB locations, compared to the previous year and to control areas. This result confirmed our first hypothesis that Highland cattle were attracted to MB and that they were able to use steep and highly encroached *A. viridis* areas, as recently pointed out by Svensk et al. (2021). The results on the effectiveness of MB are thus consistent with those found by Bailey and Welling (1999), who showed that they could attract herds into underused rangelands with poor quality forage. Those authors assessed the efficiency of dehydrated blocks in un-encroached zones with gentle to moderate slopes, while our study showed that such strategic placements can attract herds even in areas with steeper slopes and high levels of woody species encroachment, where grazing conditions could be more challenging. When analyzing the attractive effect at different spatial scales, a significant increase in the use of areas up to 50-m from MB locations, compared to control areas, was detected. This 50-m scale effect of MB underlines the potential of such management techniques to increase livestock use over large *A. viridis* encroached patches. The effect of MB within 10-m buffers was even more significant, with substantially increased livestock use at a close range near the molasses-based blocks. Such measurements at different spatial scales highlight the influence of MB on Highland cattle spatial distribution, with this type of management enabling cattle to increase their visits to the surrounding encroached zones once they are attracted by the molasses. Other studies have also shown high potential for different strategic placements to reduce shrub-encroachment at different scales, and to attract herds into under-grazed areas (Bailey and Welling 2007; Bailey and Jensen 2008; Pittarello et al. 2016a). In particular, Pittarello et al. (2016a) found a significant attractive effect at 10- and 50-m around supplement blocks in dwarf-shrub encroached montane pastures, using a different supplement type (mineral mix supplements) for another cattle breed (Piedmontese breed). In our study, under comparable mountain topographic conditions, livestock use and supplement consumption were much higher, with 134 g of molasses consumed per animal per day, corresponding to ca. 80 g of minerals without taking into account the 40% of sugar from molasses content (see **Table S2**) compared to 13.7 g of mineral mix supplement in Pittarello et al. (2016a), suggesting a higher attractiveness and efficiency of molasses-based than mineral mix blocks in modifying livestock spatial distribution. Indeed, the average time spent around MB (i.e., 74 min cow^{-1} day^{-1}) was much higher than the 18 min cow^{-1} day^{-1} around mineral mix supplements in a 50-m buffer found by Pittarello et al. (2016a). Furthermore, the familiarity of the cattle with the paddock characteristics might be an important factor to consider when implementing such management in the long term, as the exploration behavior could increase over time and have an enhanced effect on vegetation.

Overall, the increased use by Highland cattle of areas with MB led to a significant removal of leaves and damage to the branches of *A. viridis* shrubs. Moreover, it resulted in a decrease in herbaceous cover and an increase in bare soil after grazing, compared to control areas. Further, the cover of medium and small herbs and woody plants was significantly reduced by the increased livestock use of MB areas. These impacts were related both to targeted grazing and to the mechanical damage caused by Highland cattle once attracted to the MB areas. Such damage may result from trampling, scratching and breaking branches while walking (see **Figure S6-10**), as recently observed by Svensk et al. (2021). The significant effect of the interaction between MB and the distance of shrubs suggests a different effect of the distance between control and MB areas. Indeed, large paths created by the animals were observed between MB and the surrounding area, due to grazing activity and movement from MB to water sources. While we could not differentiate between effects of grazing and trampling on the vegetation, Highland cattle were observed to graze on species with particularly low forage quality, such as ferns (*A. filix-femina*, *D. dilatata*) and tall herbs (*A. alliariae*). This demonstrates the ability of this breed to feed not only on *A. viridis* shrubs but also on other unpalatable understory species, which is consistent with our second and third hypotheses. Previous studies have also shown that Highland cattle tend to be less selective with regard to forage quality compared to other breeds, and are able to feed on woody plants (Pauler et al. 2020b, a). Moreover, they tend to be more adapted to low quality vegetation and lower nutrient intake, as they continue to gain weight under such constraining conditions (Berry et al. 2002). *A. viridis* leaves and the understory vegetation have also proven to be a valuable fodder resource, but only for certain robust cattle breeds, such as Dexter cattle or Hérens cattle (Meisser et al. 2014; Zehnder et al. 2016a). Even if only measured in the short-term, the intense impacts exerted on *A. viridis* shrubs and other woody species indicate the potential for Highland cattle to reduce woody species encroachment in the medium- and long-term.

Maintaining Highland cattle grazing and the strategic placement of MB over the long term could enable typical pasture species to colonize areas where vegetation cover was highly impacted and new gaps were created. Indeed, several studies have shown that long-term targeted grazing by goats led to increased light conditions in formerly highly shrub-encroached pastures, with the establishment of light-demanding, low-growing and less competitive grassland species (Elias et al. 2018; Silva et al. 2019; Köhler et al. 2020). Similarly, we can expect cows to alleviate light competition for the understory vegetation cover (thanks to the combination of increased bare soil and removed of *A. viridis* leaves) likely producing a shift in plant functional groups in the long term, and an increase in plant diversity (Hautier et al. 2009). Moreover, to avoid possible risks of localized erosion linked to excessive livestock trampling, it would be advisable to regularly move MB to different shrub-encroached areas during the grazing season and throughout the years. This would also allow the expansion of the livestock impacts on vegetation to a wider area (Probo et al. 2013). While our paddocks always had comparable stocking rates between years, they did not always have exactly the same stocking densities (e.g., paddock 1, in which in the second year a bigger herd grazed for a shorter period compared to the first year). Therefore, future studies with similar stocking densities among years would be useful to confirm the present findings. The assessment of the potential effect of social interactions within the herd (Stephenson et al. 2016) could also be useful to refine the effect of MB in the herd at an individual level. Moreover, as Highland cattle periodically traveled from open pastures to highly encroached zones (Svensk et al. 2021), the restoration of former pastures could be enhanced by seed translocation through endo- and epi-zoochory. Indeed, a recent study (Pauler et al. 2019) demonstrated that a higher number of epizoochoric plant species were found in pastures grazed by Highland cattle compared to pastures grazed by production-oriented cattle breeds, since the long fur of the Highland cattle resulted in more efficient seed dispersal. The same study also found that the less selective grazing behavior of this breed led to higher plant species richness in pastures, as Highland cattle consumed a larger range of species compared to other breeds. They were thus able to graze on less palatable dominant species and reduce their abundance for the benefit of other herbaceous species. Moreover, a high number of dung droppings was already observed around MB after grazing, which could further promote seed dispersal from adjacent pastures through endozoochory (Cosyns et al. 2005). Indeed, even if other management strategies such as controlled fires or clear-cutting have proven effective at reducing shrub encroachment in montane environments, targeted grazing seems to be best suited to restore montane pastures, especially considering the enhanced plant dispersion by livestock (Alados et al. 2019). In addition, the mechanical damage caused to *A. viridis* branches by livestock might increase pathogen attacks on this shrub species and therefore reduce its spread. For example, fungi species of the genus *Phytophthora* can spread through water and have the potential to infect bark lesions of a plant, and have been reported to have severe impacts on alder shrubs including *A. viridis*, sometimes leading to mortality (Pisetta et al. 2012; Bregant et al. 2020).

Conclusion

In conclusion, our findings demonstrate that the strategic placement of molasses-based blocks was efficient in attracting Highland cattle toward areas highly invaded by *A. viridis*, despite the harsh terrain (steep slopes). Highland cattle successfully fed on, and damaged, *A. viridis* shrubs around the MB, thus significantly altering the understory vegetation through grazing and trampling, in just one grazing season. Highland cattle could thus have the potential to effectively reduce *A. viridis*-encroachment in the long term, and, by increasing light conditions for understory species and translocating seeds, they could promote montane pasture restoration.

CHAPTER VI: Nitrogen translocation by Highland cattle grazing in *Alnus viridis*-encroached pastures

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Abstract

During the last decades, *Alnus viridis* has expanded over former montane pastures and meadows, due to land use and abandonment. This nitrogen-fixing woody species has triggered negative agro-environmental impacts, such as nitrogen (N) leaching, soil acidification and a reduced biodiversity. The aim of this study was to estimate the N translocation from *A. viridis*-encroached areas to adjacent open pastures by Highland cattle. In 2019 and 2020, Highland cattle herds equipped with GPS collars were placed in four *A. viridis*-encroached paddocks across Italy and Switzerland. The N content was measured in *A. viridis* leaves, herbaceous vegetation, and cattle dung pats, which were collected throughout the grazing season. Using GPS locations and collar activity sensors, livestock activity phases were discriminated. The N ingested by cattle was estimated through the N content of herbaceous vegetation and *A. viridis* leaves of vegetation patches visited by cattle during 24 h before dung sampling (N_{24H}). The N content of herbaceous vegetation significantly increased with increasing *A. viridis* cover. The average N content in dung pats (31.2 ± 3.4 g kg^{-1} DM) was higher than average values from literature on grazing cattle. Moreover, it was positively related to the N_{24H}. Most of this N (29.5 ± 10.3 kg ha^{-1} yr^{-1}) was translocated towards resting areas, which generally occurred on flat open pastures. Our results highlight the potential of Highland cattle to effectively translocate part of the ingested N from *A. viridis*-encroached towards targeted open areas, thus bringing new perspective for forage yield and quality improvement in the long-term.

Introduction

Encroachment of grasslands by woody species is a worldwide phenomenon (Eldridge et al. 2011; Wieczorkowski and Lehmann 2022). During the last decades, European mountain chains have faced major socio-economic transformations, above all on marginal areas, such as a decrease in agro-pastoral activities and land abandonment, with a consequent increase in shrub encroachment (MacDonald et al. 2000; Strebel and Bühler 2015; Orlandi et al. 2016). For instance, in Switzerland, shrub forests have increased by 22% between 1983 and 2017, with the greatest expansion recorded in the Alps (Abegg et al. 2020). In Central Europe, *Alnus viridis* (Chaix., D.C.) is the most rapidly expanding shrub species, as it is a pioneer species that has efficient sexual and vegetative reproduction traits (Farmer et al. 1985; Mallik et al. 1997; Caviezel et al. 2017). In Switzerland, its expansion rate is two to three times faster than the forest, and nowadays it represents 70% of the Swiss shrubland cover (Anthelme et al. 2007; Bühlmann et al. 2014; Abegg et al. 2020). This shrub can be found mostly in north and west facing steep slopes, but has recently started to expand in more gentle slopes (Caviezel et al. 2017). Its presence has led to many agro-ecological issues, since it can reduce some of the ecosystem services provided by montane grasslands. Indeed, its encroachment into open grasslands has led to a loss of potential agricultural production and has reduced landscape attractiveness, which has an important economic value for tourism (Ewald 2001). *A. viridis* encroachment also prevents forest succession by hindering montane conifers' establishment (Hiltbrunner et al. 2014).

Because of its symbiosis with the N_2-fixing actinomycete *Frankia alni* (Huss-Daniel 1997; Dawson 2008), the presence of *A. viridis* is leading to nitrogen enrichment in soils (Bühlmann et al. 2016). This creates a nitrogen saturated habitat which, combined with the reduction of light, temperature and the increased humidity under *A. viridis* canopy, facilitates the growth and dominance of a few shade-tolerant and nitrophilous species, such as *Adenostyles alliariae* (Gouan) A. Kern, *Cicerbita alpina* L. (Wallr.) and ferns (e.g. *Athyrium filix-femina* (L.) Roth and *Dryopteris dilatata* (Hoffm.), having low forage quality (Svensk et al. 2021). Therefore, encroached areas have lower plant and animal species richness if compared to adjacent open grasslands (Anthelme et al. 2001; Susan and Ziliotto 2004; Bühlmann et al. 2014; Cislaghi et al. 2019; Zehnder et al. 2020; Svensk et al. 2021).

Nitrogen fixation by *A. viridis* increases nitrification and thus enhances possibilities of nitrate and dissolved organic carbon leaching, leading to the pollution of streamlets and groundwater (Bühlmann et al. 2014, 2016; Hunziker et al. 2017). Consequently, *A. viridis*-encroached habitats display higher risk of soil acidification, through the loss of base cations leached along with nitrates (Galloway et al. 2003; Bühlmann et al. 2016). Moreover, high nitrogen availability increases gaseous nitrogen loss such as the greenhouse gases NO and N_2O (Galloway et al. 2003; Caviezel et al. 2014; Hiltbrunner et al. 2014).

Managing the expansion of *A. viridis* is thus an important goal for the restoration of the ecosystem services provided by montane pastures. Historically, until the 1950's, the mechanical cutting of *A. viridis* for firewood combined with the higher grazing pressure at the time helped in controlling *A. viridis* spread (Caviezel et al. 2017). Nowadays, because of the loss of its economic benefit and the reduced workforce available in mountain areas, other more economically sustainable solutions have to be found to restore open pastures. Moreover, single cuts of *A. viridis* shrublands may lead to sprouting and thus denser stands, and may not be efficient to restore the below-ground conditions, as they are highly altered by the shrub encroachment (Schwob et al. 2017).

The use of targeted grazing to reduce tree and shrub-encroachment has already been recognized as being efficient and promising in the long-term (Mitlacher et al. 2002; Meisser et al. 2014; Elias et al.

2018). Because *A. viridis* leaves contain moderately high tannins levels (Stević et al. 2010), they can have low digestibility and palatability, preventing their use as a forage resource for production-oriented livestock (Kumar and Vaithiyanathan 1990; Besharati and Taghizadeh 2011). However, robust livestock breeds have the ability to digest lignified and tannin-rich vegetation through their tannin-tolerant rumen bacteria (Manousidis et al. 2016). Previous studies have shown that robust cattle breeds, such as Highland cattle, are able to feed on shrub species foliage with low forage quality (Pauler et al. 2020b; Svensk et al. 2021). The exploitation of woody species-encroached areas by Highland cattle can help controlling shrub and tree cover over time, by combining the effect of direct foliage consumption, trampling, and the mechanical breaking of branches, thanks to their long horns, and could potentially lead to the slow opening of the canopy, allowing the recolonization of typical pasture species in the long-term (Probo et al. 2016; Pauler et al. 2019, 2020a; Svensk et al. 2022).

In addition to their use to limit *A. viridis* encroachment, robust livestock could be used to balance the level of nitrogen in shrub-encroached areas and adjacent pastures. Indeed, nutrients can be transported through animal excretions, by taking in nutrients while foraging, and returning them through urine and dung excretions (Haynes and Williams 1993; Schnyder et al. 2010). Up to 95% of the nitrogen ingested by grazing animals can be excreted, mostly in urine (Whitehead 1970; Burggraaf and Snow 2010). Cattle activity (e.g., grazing, resting) is usually affected by topographic, vegetation and management factors (Probo et al. 2014; Homburger et al. 2015). Thus, the spatial distribution of dung pats and urine is not uniform and excretions are mainly deposited in resting areas, which are usually flat areas with low shrub cover (Costa et al. 1990; White et al. 2001; Kohler et al. 2006; Buttler et al. 2008; Koch et al. 2018). As a consequence, nutrients can be spatially redistributed from grazing areas, where they are taken in, to resting areas, where they are deposited (Kohler et al. 2006). Therefore, in *A. viridis* encroached pastures subjected to targeted grazing with Highland cattle, we expect an active N translocation from shrub-encroached to open and flat areas. *A. viridis*-encroached areas can have a high N level in the vegetation, due to the high N content of *A. viridis* leaves (Bühlmann et al. 2016) and that of the understory herbaceous vegetation. Indeed, the understory herbaceous vegetation is characterized by a significantly higher N content than that of both nutrient-rich and nutrient-poor pastures in the surroundings (Zehnder et al. 2017). Moreover, condensed tannins rich-species, such as *A. viridis*, may induce proportionally higher nitrogen excreted through dung than through urine (Burggraaf and Snow 2010), thus limiting ammonia volatilization from urea, and making nitrogen more available for plant utilization on the long-term (Lantinga et al. 1987; Berry et al. 2001).

The aim of this study was to evaluate whether Highland cattle grazing in *A. viridis*-encroached pastures can become a management tool to translocate N from shrublands to adjacent open pastures, and thus help reduce the negative environmental impacts of *A. viridis* expansion. Therefore, we studied Highland cattle herds grazing in Swiss and Italian *A. viridis*-encroached pastures and we measured the N content of the herbaceous vegetation, green alder leaves and cattle dung pats during two grazing seasons. Specifically, we aimed to: (i) measure the N content in Highland cattle dung pats and compare with literature data on grazing cattle dung pats; (ii) assess its relationship with the ingested N content during 24 h prior dung deposition; and (iii) estimate the N import–export fluxes within *A. viridis*-encroached areas and adjacent open pastures. We hypothesized that: (i) the N content in Highland cattle dung pats is higher than in dung pats from cattle grazing on open pastures and/or with similar crude protein-rich diets; (ii) the more often cattle have grazed in *A. viridis* encroached areas during 24 h before dunging, the more nitrogen-rich the dung pats are; and (iii) *A. viridis*-encroached areas have negative N fluxes (i.e., N is exported from these areas), while adjacent open pastures have positive N fluxes (i.e., N accumulates).

Methods

1. Study areas and grazing management

The study was conducted in 2019 and 2020 on four *A. viridis*-encroached paddocks grazed by two Highland cattle herds in the Swiss and Italian Alps (**Table 5**). The first paddock (paddock 1, 30.86 ha) was located in Val Vogna, Italy (province of Vercelli). The other three paddocks (paddock 2, 8.26 ha; paddock 3, 7.67 ha; paddock 4, 7.04 ha) were located in Bovonne, Switzerland (canton of Vaud) and were grazed in rotation by the same herd. All paddocks were grazed at a comparable stocking rate and had similar topographical conditions (**Table 5**). The four paddocks were representative of an *A. viridis* cover gradient, with an average cover of 26%, 51%, 61% and 71% respectively in paddock 1, 4, 2 and 3. Paddock 1, 2 and 3 were grazed during two summer seasons (2019 and 2020), while paddock 4 was grazed during one summer season (2020). More detailed information on the vegetation characteristics of paddocks 1, 2 and 3 can be found in Svensk et al. (2021). Each year, the herds grazed from the middle of June to the beginning of September. All the herds included cow/ calf pairs and heifers, varying in age from 6 months to 17 years (with an average of five years for paddock 1, and four years for paddock 2, 3 and 4) and about 70% of the animals were present in both years at the same site. A water trough was installed in paddocks 2, 3 and 4, while natural streams were present in paddock 1 for the entire grazing period. As described in Svensk et al. (2022), in the second year of the project (2020), five molasses-based blocks were installed in small highly encroached areas of paddocks 1, 2, and 3, as part of another experiment set, to attract Highland herds into these areas. In each herd and during both years, six to ten cows were equipped with GPS collars (Followit AB ©, Tellus GPS System collars, Sweden) that recorded their position every ten minutes during the whole grazing period, with an accuracy of two to five meters. The GPS collars also recorded cattle neck movements through activity sensors of the X and Y axes.

Table 5 Topographical and management characteristics of the four *A. viridis*-encroached paddocks used in Chapter VI.

	Paddock 1	Paddock 2	Paddock 3	Paddock 4
Coordinates	N45°46’18.8" E7°54’9.1"	N46°16'9.8" E7°6'44.2"	N46°16'12.1" E7°6'58.8"	N46°16'15.9" E7°07'02.8"
Elevation (m a.s.l.)	1897 ± 67	1745 ± 46	1789 ± 32	1877 ± 21
Slope (°)	21	23	21	23
Cover of *A. viridis* (%)	26	61	71	51
Grazable area (ha)	30.9	8.26	7.67	7.04
Number of grazing days 2019	44	17	18	-
Number of grazing days 2020	29	17	18	19
Livestock units[a] 2019	45.4	29.8	29.8	-
Livestock units[a] 2020	70.4	29.6	29.6	25.4
Stocking rate[b] 2019	0.177	0.168	0.192	-
Stocking rate[b] 2020	0.181	0.167	0.190	0.187
Number of GPS collars 2019	6	8	8	8
Number of GPS collars 2020	8	10	10	10

2. Dung and vegetation nitrogen measurements

During the grazing seasons of 2019 and 2020, eight to 12 fresh dung pats of different Highland cows were sampled, two to three times (period 1, 2 and 3) in very paddock, every 10 days, around noon. Before analyses, all dung samples were freeze-dried (Christ Delta 2–24, Kühner AG, Birsdelden, Switzerland) and milled through a 1.0 mm sieve (Brabender rotary mill; Brabender GmbH & Co. KG, Duisburg, Germany). Nitrogen (N) content was determined by the Dumas method (ISO 16634-1:2008). To determine residual dry matter content, samples were dried during 3 h at 105 °C.

Three samples of *A. viridis* leaves (1750 g of fresh leaves each) were collected in both sites of Bovonne and Val Vogna, in June, July and August of both years to represent the changes in leaf N content during the summer season. For each sample, *A. viridis* leaves (including petioles) were hand-harvested all around the canopy of five different trees up at a maximum above-ground height of 1.80 m to simulate the potential grazing by Highland cattle (Svensk et al. 2022). In each paddock, 12 to 66 vegetation patches of 0.47 ± 0.05 ha (0.01 ha to 1.7 ha) were defined prior to Highland cattle grazing, representing areas with homogeneous botanical composition and vegetation structure. Herbaceous vegetation samples (250 g of fresh matter) were then sampled before grazing using a handheld grass cutter in each vegetation patch (one vegetation sample per patch) in 2019 for paddocks 1, 2, and 3, and in 2020 for paddock 4. Leaf and herbaceous vegetation samples were dried at 60 °C for 72 h. After being ground to pass a 1-mm sieve (Brabender rotary mill; Brabender GmbH & Co. KG, Duisburg, Germany), leaf and herbaceous vegetation samples were analysed for dry matter content by heating at 105 °C during 3 h. The N content of *A. viridis* leaves and herbaceous vegetation samples was determined by the Dumas method (ISO 16634-1:2008). Moreover, in each vegetation patch the percentage of *A. viridis* cover was also assessed using direct visual observations and satellite pictures, and the slope was calculated through a Digital Terrain Model (90-m resolution) in QGIS 3.6 software.

3. Nitrogen ingested estimation

The weighted mean of N ingested by Highland cattle was estimated during a period of 24 h before dung sampling (N_{24H}), following the methodology on cattle diet timespan estimations (Estermann et al. 2001; Bakker et al. 2004; Browne et al. 2005). For this 24 h timespan, Highland cattle activities (grazing and resting) were discriminated through the analysis of both horizontal distance travelled and activity data from the GPS collars sensors, considering that Highland cattle were ingesting N during the grazing activity phases only. The horizontal distance travelled was calculated from consecutive GPS fixes for each collared cow. The activity from collar sensors was obtained from the mean of X and Y axes activities. Therefore, we identified the phases when grazing occurred most frequently. Grazing phases were identified as times when distance travelled and motion sensor-based activity were higher, and resting phases were assigned times when these values were lower (Probo et al. 2014). The number of GPS fixes within the grazing activity phases was then counted in each vegetation patch. Then, the proportion of GPS fixes in each vegetation patch was related to the measured herbaceous vegetation N content of the patches, to compute the weighted mean of N ingested over the 24 h (N_{24H}) according to the following formula:

$$N_{24H} = \frac{\sum_i^n (NHV_i * GPSga\ fixes_i)}{\sum_i^n GPSga\ fixes_i} \tag{1}$$

where NHV_i and $GPSga\ fixes_i$ represent the N content of the herbaceous vegetation and the proportion of GPS fixes within the grazing activity time in the vegetation patch i, 24 h before dung sampling, respectively.

In addition, in all patches where *A. viridis* was present, the N_{24H} calculated from herbaceous vegetation was corrected based on the N content of *A. viridis* leaves sampled at the closest date to the corresponding dung sample, to account for the N ingested from *A. viridis* leaf consumption. Based on the direct observations of Highland cattle foraging behavior conducted in Bovonne (Nota et al. 2022, 1077 observations), it was estimated that *A. viridis* leaves represented 12% of animal diet on average (although we also incorporated variation in the amount of *A. viridis* leaves eaten, see below). Thus, in the vegetation patches in which *A. viridis* was present, the N_{24H} corresponding to each dung sample was calculated considering a diet characterized by 88% N from herbaceous forage and 12% N from leaves, following this formula:

$$N_{24H} = \frac{\sum_i^n \left[\left[(0.88 * NHV_i) + (0.12 * NAL_i) \right] * GPSga\ fixes_i \right]}{\sum_i^n GPSga\ fixes_i} \tag{2}$$

where NAL_i represents the N content of *A. viridis* leaves sampled at the closest date to the corresponding dung sample.

4. Nitrogen import–export flux estimation

In every paddock and for each vegetation patch, the value of the N flux (N kg ha^{-1} yr^{-1}) was calculated using an import–export model following this formula:

$$Nflux = Nexcreted - Ningested \tag{3}$$

where Nexcreted is the estimated amount of N excreted by livestock through urine and dung in the patch during the whole grazing season, and Ningested is the estimated amount of N eaten by livestock in the same patch and during the same period. For this flux, two assumptions were made: (i) the excretion is proportional to the total time cows spent in the patch, and (ii) the ingestion is proportional to the time cows spent grazing only (White et al. 2001; Koch et al. 2018).

Based on these assumptions, the excreted N was calculated as:

$$Nexcreted = D \times TS \times n \times DM \times (p \times Npaddock) \tag{4}$$

where D is the number of grazing days, TS the percentage of time spent by cows in the vegetation patch, n the number of animals present, DM the dry matter intake (kg $animal^{-1}$ day^{-1}) calculated using previous studies on Highland cattle weight and DM ingestion of both cows and calves (Berry et al. 2002; Pauler et al. 2019), and Npaddock the estimated weighed mean of N eaten by the cows (g kg^{-1} DM) in the whole paddock, using the N content of *A. viridis* leaves and herbaceous vegetation as previously described. The parameter p is the estimated percentage of Ningested that is excreted by grazing beef cattle according to seven trials conducted in comparable conditions and presented in Estermann et al. (2003), Berry et al. (2002) and Estermann et al. (2001). Indeed, those experiments had comparable conditions to our study as they were performed on low growing beef cattle breeds (Highland or Angus cattle with cow and calf pairs), in similar montane environments (1557 ± 237 m) and/or with similar crude protein content diets (14.63 ± 0.75%, compared to 16.05 ± 1.12% from our N ingested estimations).

Ingested N was calculated as:

$$Ningested = D \times TG \times n \times DM \times Npatch \qquad (5)$$

where TG is the percentage of time cows spent grazing in the patch, and Npatch the estimated ingested N content calculated from the N content of *A. viridis* leaves and herbaceous vegetation, at the vegetation patch level.

Since some parameters from the estimation of the N fluxes are subjected to uncertainties, we associated a statistical distribution to each parameter (mean ± SE), following the methodology described in Koch et al. (2018), and performed 500 Monte Carlo simulations, in order to obtain 5%, 50% and 95% quantiles of the N flux for each vegetation patch in every year. We thus included the uncertainty of: (i) p from the seven experiments (92.7 ± 0.7%), (ii) Npaddock, by including the variability of the percentages of *A. viridis* leaves eaten by the cows when in presence of the shrub (11.8 ± 2.8%), at the paddock level, and (iii) Npatch, by including the same variability of the percentages of *A. viridis* leaves eaten by the cows when in presence of the shrub at the patch level.

5. Statistical analysis

All statistical analyses were performed using R version 3.4.4 (R Core Team 2017). The effect of *A. viridis* cover on the N content of the understory herbaceous vegetation was tested by using a Generalized Least Square model (GLS), with *A. viridis* cover as fixed factor and coordinates of vegetation patches centroids nested into “paddock” as random effect, using a Linear Correlation Structure. This random effect structure accounts for the nested structure of the data and for any residual spatial autocorrelation among neighboring vegetation patches.

The relation between dung pats N content (response variable) and N_{24H} (explanatory variable) was tested using a Linear Mixed-effect Model (lme, package “nlme”), with the date of dung sampling as a continuous variable nested into “paddock” as a time correlated covariate (corCAR1), to account for the temporal autocorrelation structure linked to vegetation changes throughout the summer season. The marginal R-squared values were obtained using the “performance” package (function “model_performance”). The effect of *A. viridis* cover (divided into 3 categories: 0–33%, 34–66% and 67–100%), slope (divided into 3 categories: < 10°, 10–20° and > 20°) and their interaction on the N fluxes was tested using a Linear Mixed-effect Model lme, package (“nlme”). Paddock was specified as random factor to account for spatial autocorrelation. Model residuals were affected by heteroscedasticity, therefore a weighting function was used to correct the variances through the argument varIdent in the lme function by setting as grouping variable the *A. viridis* covers. *Post hoc* tests were performed for the models when significant effects between categories were detected (Tukey’s test, $P < 0.05$, emmeans package).

Results

1. Effect of *A. viridis* cover on the nitrogen content of the understory herbaceous vegetation

The herbaceous vegetation had an average N content of 25.9 ± 0.8 g kg^{-1} DM among all paddocks and vegetation patches (the details per paddock can be found in Supplementary materials, **Figures S11–S14**). The N content of the herbaceous vegetation was positively related with *A. viridis* cover percentage (**Figure 28**, $P < 0.001$, $R^2 = 0.36$, $n = 58$). Indeed, the N concentration of the herbaceous vegetation increased with increasing *A. viridis* cover, with averages of 22.9 ± 0.9 g kg^{-1} DM in open areas (0–33% of *A. viridis* cover), 28.2 ± 1.6 g kg^{-1} DM in moderately encroached areas (34–66% of *A. viridis* cover) and 30.5 ± 1.6 g kg^{-1} DM in highly encroached areas (67–100% of *A. viridis* cover).

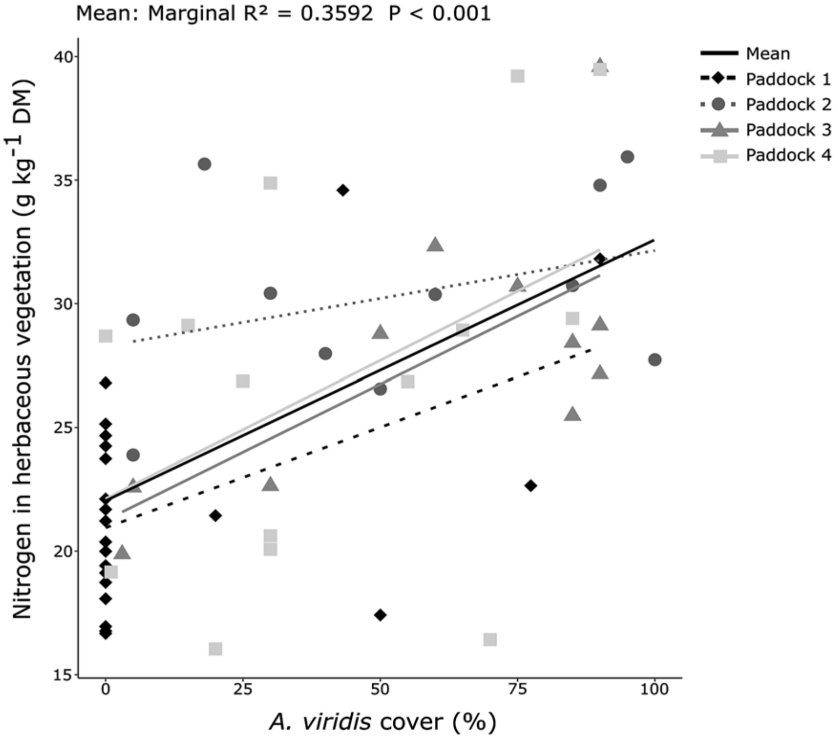


Figure 28 Relationship between the N content (g kg^{-1} DM) of the understory herbaceous vegetation and *A. viridis* cover percentage in the vegetation patches of all paddocks.

2. Effect of animal diet on dung nitrogen content

The N content of Highland cattle dung pats was on average 31.2 ± 0.2 g kg^{-1} DM (**Figure 29**, mean ± SE), and consistent between both years (31.1 ± 0.3 g kg^{-1} DM in 2019, and 31.4 ± 0.4 g kg^{-1} DM in 2020, $P = 0.137$). The N dung content slightly decreased during the grazing season in both years ($P < 0.001$), with averages of 32.8 ± 0.5 g kg^{-1} DM, 31.0 ± 0.3 g kg^{-1} DM and 29.2 ± 0.4 g kg^{-1} DM in dung sample periods 1, 2 and 3 respectively. The N content of *A. viridis* leaves that was used for the estimation of the N_{24H} of the encroached vegetation patches was of 31.8 ± 0.6 g kg^{-1} DM across all sites and periods. The estimated N_{24H} remained stable across dung sampling periods ($P = 0.92$), with 25.8 ± 1.5 g kg^{-1} DM, 26.5 ± 1.6 g kg^{-1} DM and 25.4 ± 2.2 g kg^{-1} DM in period 1, 2 and 3 respectively. The N_{24H} ingested was of 21.4 ± 0.3 g kg^{-1} DM, 30.5 ± 0.2 g kg^{-1} DM, 24.6 ± 1.1 g kg^{-1} DM and 27.6 ± 0.8 g kg^{-1} DM for paddock 1, 2, 3 and 4 respectively, with an overall average of 25.9 ± 1.0 g kg^{-1} DM among all paddocks. There was a marginally significant positive reltionship between dung N content and N_{24H}, (**Figure 29**, P = 0.07, R² = 0.25, n = 17).

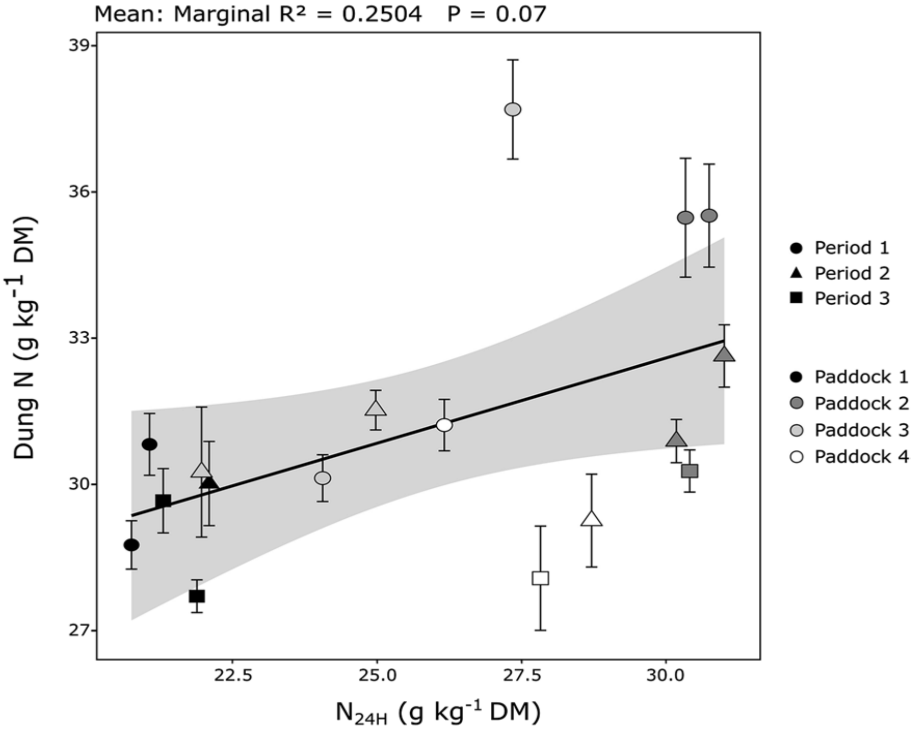


Figure 29 Relationship between the N content of the dung pats (dung N, g kg^{-1} DM) of Highland cattle and the estimated mean content of N ingested 24 h before the dung sampling (N_{24H}, g kg^{-1} DM), with the trend line (grey area) following lm smoothing method, and error bars representing the standard error.

3. N import–export fluxes in vegetation patches

The 5%, 50% and 95% quantiles of the predicted values displayed similar patterns, for N ingested, N excreted and the resulting N fluxes (**Table 6**). The following results are values of the 50% quantile (see Maps of N fluxes per each paddock in Supplementary materials, **Figures S15–S19**).

Based on N fluxes, in all paddocks, Highland cattle visited and actively grazed mostly on the open and moderately flat areas. Indeed, the most open vegetation patches (0–33% of *A. viridis* cover) with medium slope (10–20°) displayed the highest mean N ingestion, i.e. 51.9 ± 8.7 kg ha^{-1} yr^{-1}. On the other hand, the steepest (> 20°) and most encroached areas (67–100%) displayed the lowest average of N ingested, i.e. 11.1 ± 1.3 kg ha^{-1} yr^{-1} (**Table 6**). In parallel, N excretion was the highest in the open areas (0–33%) and in the lowest slopes (< 10°), with a N excreted average of 79.2 ± 16.4 kg ha^{-1} yr^{-1}. Similar to N ingestion, the lowest N excretions were estimated in steepest and most encroached areas, with a N excretion value of 7.0 ± 1.0 kg ha^{-1} yr^{-1}. Consequently, the N fluxes were significantly affected by *A. viridis* cover and slope (**Figure 30**, P < 0.001), with overall positive N fluxes in flat and open areas (i.e. N accumulated), and negative N fluxes in steeper and encroached ones (i.e. N exported; **Figure 30**, **Table 6**). On average, open and flat areas had N fluxes of 29.5 ± 10.3 kg ha^{-1} yr^{-1}, while encroached and steep areas had average N fluxes of -4.1 ± 0.7 kg ha^{-1} yr^{-1}. Indeed, in all paddocks combined, 80.66% of vegetation patches with positive N fluxes were open pastures (0–33% of *A. viridis* cover), while only

2.2% were highly encroached areas (67–100% of *A. viridis* cover), and 17.14% were moderately encroaches areas (34–66% of *A. viridis* cover). Most (44.81%) of these accumulation zones had a moderate slope (17.5 ± 0.6° on average). On the other hand, 37.9% of the vegetation patches that displayed a N depletion were open pastures, 47.08% were highly encroached areas and 15.05% were moderately encroached areas. Most (74.0%) of these depletion zones had high slope (30.5 ± 0.6° on average). Moreover, in terms of spatial distribution, N accumulated in very small areas and most of the grazed land was N depleted. Indeed, paddocks 1, 2, 3 and 4 displayed N accumulation in only 11.8%, 15.1%, 12.1% and 19.2% of their areas, respectively.

N fluxes were very similar among years. Indeed, vegetation patches with negative N fluxes had on average −6.9 ± 0.8 kg ha^{-1} yr^{-1} in 2019, and −5.6 ± 0.7 kg ha^{-1} yr^{-1} in 2020, while vegetation patches with positive N fluxes had an average of 26.0 ± 10.3 kg ha^{-1} yr^{-1} in 2019, and 19.3 ± 5.7 kg ha^{-1} yr^{-1} in 2020. In 2019, four vegetation patches were not visited by Highland cattle in paddock 1, corresponding to 0.98% of the paddock size (0.30 ha). In 2020, three of these patches remained unvisited with an addition of two others, corresponding to 1.34% of the paddock size (0.41 ha). As a result, estimations of N ingested, N excreted and N fluxes were equal to zero for those unvisited areas. Most of these vegetation patches were covered by Rhododendron ferrugineum or were *A. viridis*-encroached areas, and one was an open pasture with steep slope (34.8°). All vegetation patches of paddocks 2, 3 and 4 were visited in both years.

Table 6 Mean ± SE of the N fluxes, and detailed N ingestion and N excretion (kg ha^{-1} yr^{-1}) values for 5%, 50% and 95% quantiles, estimated on vegetation patches of all paddocks on both years, depending on slope and *A. viridis*-cover categories

	5% quantile			50% quantile			95% quantile		
Slope / Cover	< 10°	10 - 20°	> 20°	< 10°	10 - 20°	> 20°	< 10°	10 - 20°	> 20°
	N fluxes								
0 - 33%	20.86 ± 9.67	-3.64 ± 4.21	-3.99 ± 1.13	29.46 ± 10.32	6.82 ± 5.35	-1.14 ± 1.42	37.99 ± 11.19	17.32 ± 6.94	1.78 ± 2.04
34 - 66%	-20.61 ± 0.18	-14.07 ± 2.79	-6.55 ± 1.24	-16.52 ± 0.38	-11.07 ± 2.48	-4.98 ± 1.29	-11.88 ± 0.17	-7.93 ± 2.26	-3.39 ± 1.49
67 - 100%	-8.76 ± 1.42	-15.73 ± 3.47	-5.31 ± 0.76	-7.73 ± 1.31	-13.18 ± 2.83	-4.09 ± 0.65	-6.7 ± 1.14	-10.75 ± 2.33	-2.81 ± 0.6
	N ingestion								
0 - 33%	43.01 ± 6.9	44.35 ± 7.41	12.81 ± 2.44	49.68 ± 7.97	51.85 ± 8.67	15.07 ± 2.98	56.63 ± 9.18	59.27 ± 9.93	17.33 ± 3.52
33 - 67%	27.12 ± 0.25	27.77 ± 2.56	14.05 ± 1.95	31.13 ± 0.49	30.24 ± 2.77	15.24 ± 2.13	34.65 ± 0.48	32.59 ± 3.01	16.37 ± 2.35
67 - 100%	17.54 ± 3.25	27.33 ± 4.15	10.03 ± 1.2	18.34 ± 3.42	28.91 ± 4.54	11.08 ± 1.34	19.13 ± 3.57	30.53 ± 5.01	12.11 ± 1.49
	N excretion								
0 - 33%	74.27 ± 15.9	51.89 ± 10.58	12.28 ± 3.27	79.15 ± 16.41	58.62 ± 12.23	13.91 ± 3.77	84.46 ± 17.08	65.41 ± 13.83	15.6 ± 4.27
33 - 67%	12.72 ± 0.19	17.33 ± 1.61	9.22 ± 1.6	14.54 ± 0.18	19.17 ± 1.69	10.23 ± 1.82	16.59 ± 0.16	21.09 ± 1.8	11.27 ± 2.05
67 - 100%	9.94 ± 2.04	14.09 ± 1.76	6.41 ± 0.88	10.61 ± 2.12	15.71 ± 2.06	6.99 ± 1	11.28 ± 2.21	17.4 ± 2.37	7.62 ± 1.13

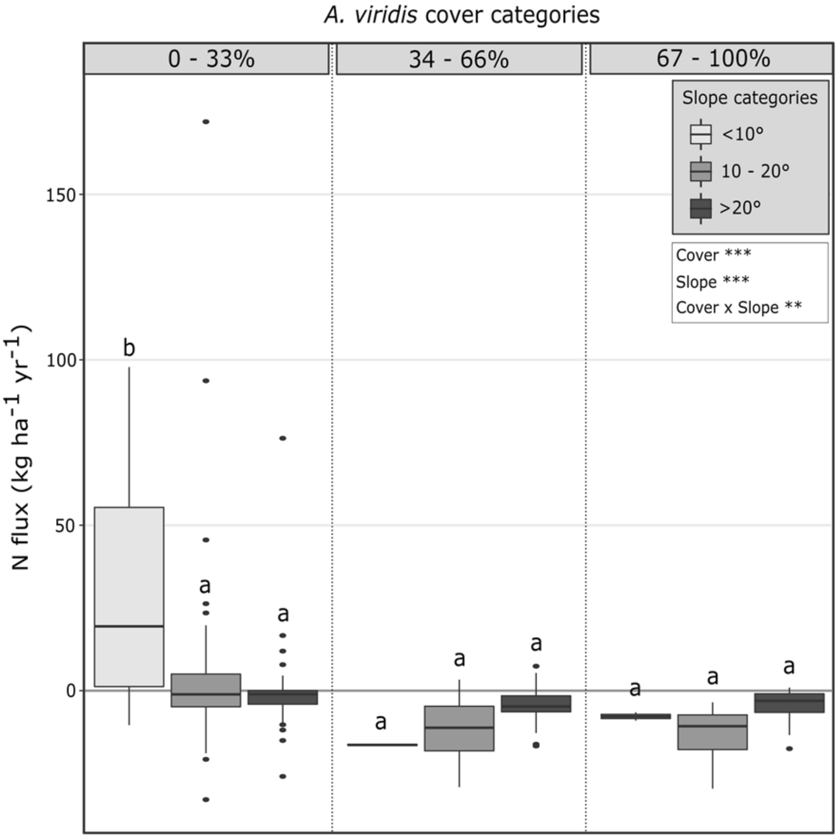


Figure 30 N fluxes (kg ha^{-1} yr^{-1}) of all paddocks and both years for the different categories of *A. viridis* cover (0–33%; 34–66%; 67–100%) and slope (< 10°; 10–20°; > 20°). Different letters indicate significant differences between categories.

Discussion

1. *A. viridis* cover increases the nitrogen content of the understory herbaceous vegetation

In all paddocks of the study, the N content of herbaceous vegetation samples was significantly higher in the understory of *A. viridis* stands than in adjacent open pastures, as previously observed by Bühlmann et al. (2016), Zehnder et al. (2016b, 2020), and Svensk et al. (2021). Bühlmann et al. (2016) also demonstrated that the N content in herbaceous plant leaves under *A. viridis* canopy was as high as the N content in *A. viridis* leaves. Indeed, they measured similar concentrations in plant leaves growing in the understory of *A. viridis* shrublands, with values between 27.3 ± 4.5 g kg^{-1} DM and 30.7 ± 3.3 g kg^{-1} (at 1650 and 1950 m elevation respectively), similar to our mean concentration of 30.5 ± 1.6 g kg^{-1} DM g kg^{-1} measured in highly encroached areas. Therefore, *A. viridis* shrublands accumulate N in the ground soil and provide an ideal habitat for nitrophilous plants with high N concentrations in their leaves (Anthelme et al. 2001; Bühlmann et al. 2016; Zehnder et al. 2020). Moreover, because *Alnus* species do not need to mobilize N from their leaves in autumn (Rodríguez-Barrueco et al. 1984), they resorb very little of their leaf N content. Combined with the late shading of their leaves, this leads to high N contents in the litter and to increased soil N saturation over time (Bühlmann et al. 2016).

2. Animal diet affects dung nitrogen content

The N content measured in Highland cattle dung pats was high, with an average of 31.2 ± 0.2 g kg^{-1} DM, if compared to values found for other cattle breeds with high crude protein rich diets, thus confirming our first hypothesis. For instance, two studies conducted by Koenig and Beauchemin (2013b, a) found that Angus beef cattle fed with silage of similar crude protein content (14–14.5%) excreted a fecal N of about 21.2 g kg^{-1} DM to 23.2 g kg^{-1} DM on average. Haynes and Williams (1993) determined that the average N content in the dung of dairy cattle grazing in open pastures varied between 20 and 28 g kg^{-1} DM, whereas the N dung concentration was about 27 g kg^{-1} DM in nutrient-rich pastures Williams and Haynes (1995). Similarly, Lançon (1978) found a dung N content of 20.6 g kg^{-1} DM for non-fertilized grasslands and 28.7 g kg^{-1} DM for fertilized grasslands. Other studies have also assessed dung N contents around 20 g kg^{-1} DM for dairy cows grazing on open pastures (Yokoyama et al. 1991; Bakker et al. 2004).

The high dung N content found in our study was marginally related to the amount of N ingested during 24 h before excretion, showing a trend of increasing N in the dung with increasing ingested N, which is in line with our second hypothesis. Despite the non-significance of this effect, the trend shown in this relation suggests that the more Highland cattle grazed in highly encroached areas, where N content in vegetation was high, the richer their dung became in N. This result is in line with previous studies, such as Kebreab et al. (2001) and Angelidis et al. (2019). For example, by using data of 69 different studies, Angelidis et al. (2019) showed that the N intake had a significant positive effect on the excreted dung N. While dung N seemed to increase with increasing N_{24H}, even the lowest dung N concentrations recorded in this study remained rather high compared to other experiments in similar conditions. This might be mainly explained by the overall high N content in the diet of Highland cattle grazing in these *A. viridis*-encroached areas. The high N content in dung pats may also be explained by the high condensed tannin concentration of *A. viridis* leaves. Indeed, Stević et al. (2010) demonstrated that *A. viridis* leaves can have a tannin concentration of 44 ± 4 g kg^{-1} DM, which can form tannin-protein complexes by binding with plant proteins when consumed by livestock (Harris et al. 1998; Waghorn 2008; Woodfield et al. 2019). These complexes are better protected from rumen microbial degradation and they can thus reduce the degradation of N during digestion (Waghorn et al. 1987; Burggraaf and Snow 2010; Piñeiro-Vázquez et al. 2017). Therefore, the passage of undegraded N could be favored through the intestine and through dung, and a smaller proportion of N might be excreted in urine (Burggraaf and Snow 2010; Theodoridou et al. 2011). Further studies on Highland cattle digestion are needed to confirm whereas the proportion of N excreted in urine will be reduced through the consumption of *A. viridis*-encroached vegetation with high tannins concentrations, as this could be beneficial. Indeed, a reduction of urine N may decrease N pollution, as urea N is subjected to ammonia (NH_3) volatilization and nitrate leaching (Woodmansee et al. 1981; Jarvis 1994; Whitehead 1995; Tamminga 2006; Angelidis et al. 2019). On the other hand, NH_3 volatilization through the dungs is restrained by the crust formation of dung pats, even though heavier rainfall might alter this protection (Longhini et al. 2020). Fecal N is in a less mobile form that needs mineralization before N can be lost through leaching, and NH_3 volatilization through urine is 5–6 times higher than through dung pats (Lockyer and Whitehead 1990; Kebreab et al. 2001; Berry et al. 2002; Woodfield et al. 2019). Furthermore, the slow process of N release through dung pats can allow a better use by the surrounding vegetation and for the soil on the long term (Woodmansee et al. 1981; Lantinga et al. 1987; Berry et al. 2001; Burggraaf and Snow 2010). Moreover, the excretion of condensed tannins through dung may also inhibit nitrification and slow down the microbial activity of the soil, preventing nitrate leaching and consequently soil acidification (Burggraaf and Snow 2010). Finally, requirements for cattle diet show that a minimum of 20 to 40 g kg^{-1} DM of condensed tannin concentration is needed to improve animal performance (Woodfield et al. 2019), which, combined with the N supply *A. viridis* leaves provide, highlight their forage potential for robust breeds, such as Highland cattle. However, further research is needed to assess the *A. viridis* leaf ingestion by grazing cattle on a higher range of environmental conditions.

3. An active nitrogen translocation occurred from shrub-encroached areas to pastures

Our study demonstrated that the excretion of N after its uptake from *A. viridis* leaves and the understory herbaceous vegetation allowed its redistribution across the pastures. Overall, the N transfer was significantly dependent on *A. viridis* cover as well as on topographic features (i.e. slope), confirming our third hypothesis. Indeed, our estimations of the N fluxes in the paddocks showed that there was an active N translocation from the steep, shrub-encroached, and N saturated areas to the adjacent flat and open pastures that had comparatively lower herbaceous N content. This is consistent with the findings of Schnyder et al. (2010), who showed a significant N accumulation in the flattest zones and N removal from the steepest areas in hilly pastures. In the same study, the authors found similar results for phosphorus (P), which is a good estimate of other nutrients such as N, with the difference that this element is much less present in urine and thus easier to evaluate on field and less subjected to transformations. Similar results on P were found in previous studies, such as Koch et al. (2018), where P was significantly translocated from feeding areas to the flattest resting areas, and Jewell et al. (2007), who also found a significant redistribution and fewer dung deposition in the steepest areas of subalpine pastures. As in previous studies, our flat and open areas with high N deposition often correspond to livestock resting areas, where there is generally a high excretion deposition, especially during the night. Costa et al. (1990) found that 90% of cattle excretion was deposited in resting-adapted areas, with slopes lower than 40%. In the present study, while the open areas displayed the highest N ingestion as well as the highest N excretion, the latter often exceeded N ingestion, making the final N fluxes positive. In the same way, the highly encroached areas displayed the lowest N ingestion, but displayed an even lower N excretion, highlighting the final depletion of N induced by Highland cattle grazing. On the other hand, the depletion areas of our paddocks were larger than the accumulation zones, meaning that cattle actively removed N from a larger area and concentrated it in relatively small patches. This is in line with previous findings, which showed that nutrients are often returned into small spots, with most of the area displaying a negative balance and net nutrient loss (Bakker et al. 2004; Jewell et al. 2007; Koch et al. 2018).

The assessment of the N fluxes over two years allows us to suggest that Highland cattle grazing could moderately reduce N accumulation under *A. viridis* encroached stands on the long-term, while simultaneously providing it in targeted areas. Particularly, the proper management of resting areas could help fertilize specific nutrient-poor pastures which are subjected to early encroachment processes (Probo et al. 2016), such as pastures dominated by *Nardus stricta* L., *Rhododendron ferrugineum* L. or *Vaccinium myrtillus* L. The translocation of N towards these areas could improve their forage yield and quality, potentially transforming the N saturation issue into an environmental and agronomical resource. Schellberg et al. (1999) demonstrated in a long-term study that N and P inputs on nutrient-poor grassland drastically increased DM production and forage quality and changed floristic composition. In this study, oligotrophic species decreased with nutrient fertilization, and created ecological favorable conditions for other grasses and legumes. At a moderate level, fertilization can improve botanical diversity (Vintu et al. 2011) and, with N and P enrichment by Highland cattle grazing, we could expect similar results on the long-term whether such management should continue. However, further studies, not only on N import–export fluxes related to grazing, but also on N pool changes are needed to better understand the potential of nutrients translocation, and the benefits for the vegetation and soil characteristics. Indeed, N inputs from atmospheric deposition and N fixation can be quite elevated in these environments (50 to 100 kg ha^{-1} yr^{-1} according to Binkley et al. (1994). Despite our results showing moderate N translocation towards open and flat areas (29.52 kg DM ha^{-1} yr^{-1} on average), attention should be paid to possible over-fertilization issues resulting from high levels of N accumulation in the resting areas. Indeed, the distribution patterns of grazing cattle can be stable across years (Koch et al. 2018), consequently leading to a repeated distribution of cattle dung and thus potential over-fertilization of the resting areas. To avoid such negative impacts and preserve nutrient capital, it is advised to adopt a rotational management, as it allows more homogeneous utilization of the pasture compared to free-grazing systems. Previous studies have shown that the presence of fences, shaded zones, attractive points (such as salty or molasses blocks), and a water trough may also impact the spatial distribution of dung and urine pats (Jewell et al. 2007; Buttler et al. 2008; Auerswald et al. 2010; Pittarello et al. 2016a; Carnevalli et al. 2019; Svensk et al. 2022). Thus, these features should be included in the management of cattle grazing to better redistribute nutrients across the pastures. The results of this study highlight the potential of Highland cattle grazing to become an efficient tool to effectively translocate part of the ingested N, which could be accounted for in livestock management, and help in the restoration of former open pastures in the long-term. However additional research is needed to assess the effect of such grazing management to counteract N deposition in the shrublands, including long term effects on nutrients pools.

CHAPTER VII: General discussion

The goal of this thesis was to evaluate wether Highland cattle can become a management tool to reduce *A. viridis* encroachment in subalpine pastures. This was achieved through an experiment conducted for four years in the Swiss and Italian Alps, during which Highland cattle herds grazed in highly *A.viridis*-encroached pastures. During this experiment, we assessed the forage potential of *A. viridis* leaves (Chapter II) as well as Highland cattle feeding behavior (Chapter III) in order to understand to what extend Higland cattle could forage on *A.viridis* and actively damage it. We monitored Highland cattle using GPS collars to understand their grazing and movement behavior in the harsh conditions of highly encroached pastures (Chapter IV), and we tested management strategies with the implementation of attractive points to increase their impact on *A. viridis* stands (Chapter V). Finally, we estimated the N translocation made by Highland cattle grazing to understand the potential impacts produced by this livestock management on the long-term (Chapter VI).

The forage potential of *A. viridis* leaves

Chapter II investigated the forage nutritional value of *A. viridis* leaves, through the description of the seasonal variation of their chemical composition. The leaves displayed high crude protein and macro- and micro- elements concentrations that are essential for livestock health (Collins et al. 2017; Mahieu et al. 2021), and could therefore be integrated as complementary forage resource in addition to a grass-based diet. Thanks to measurements conducted throughout the summer season, *A. viridis* leaves were identified to be most interesting for nutritive purposes for livestock in June and July, with decreasing digestibility over the season. In addition, *A. viridis* leaves showed a mitigation effect on CH_4 emission by cattle, with a maximized effect at the beginning of the season. Therefore, this study emphasized the potential to integrate *A. viridis* leaves for robust livestock foraging on encroached vegetation conditions, as it had been previously described for other woody species (Terranova et al. 2018). Indeed, these results could be applied not only to Highland cattle but also to other robust species and breeds which were previously shown to be able to forage on *A. viridis* (Pauler et al. 2022b). Other measurements on the chemical composition of the buds and the bark of *A. viridis* could be carried out in order to deepen the knowledge on *A. viridis* forage potential. Indeed, Dexter cattle and Engadine sheep have been reported to feed on the buds, with sheep actively debarking the shrub as well (Zehnder et al. 2017). The phenolic compounds of these plant parts have already been investigated by Stević et al. (2010), but no full description on their forage quality was made yet.

While this chapter allowed a comprehensive assessment of the feeding potential of *A. viridis*, providing a direct link with livestock nutritional requirements is complex. Indeed, nutritive needs of livestock highly depends on animal condition, i.e. its maintenance, age, growth, reproduction or lactation. Therefore, it is crucial to include other source of feed intake with complementary forage quality in order to prevent potential loss in weight leading to health problems and a reduction of productivity, above all at the end of the summer grazing season, when *A.viridis* leaves were shown to decrease their overall good nutritional quality.

Feeding behaviour of Highland cattle

Chapter III disentangled the diet composition of Highland cattle through the direct observation of their feeding behaviour under different vegetation conditions. This cattle breed selected a mix of different plant species, with herbaceous plants of high forage quality as well as woody plants, which composed up to 46% of their diet. The *A. viridis* accounted for 12% of Highland cattle diet on average, and its consumption was dependent to its abundance. Therefore, in highly encroached pastures, Highland cattle consume more of *A. viridis* leaves, depending on the availability of other forage resources. While some species composing *A. viridis* understory vegetation were avoided by Highland cattle, such as *Adenostyles alliareae* (Gouan), some ferns such as *Dryopteris dilatata* (Hoffm.) were recorded to be positively selected despite being known to be potentially toxic (Iussig et al. 2015a, **Figure 31**). This behaviour is particularly interesting, as Highland cattle were able to intensively defoliate plants growing under *A. viridis* stands in addition to directly grazing the shrub, which could facilitate the process of subalpine grassland restoration on the long term.

Figure 31 Highland eating ferns under *Alnus viridis*-encroached stands in Western Switzerland, Bovonne, Bex (VD).

Because Highland cattle diet is composed by a mix of various species, providing a mosaic of high quality vegetation and woody plants is essential to preserve their health and avoid reducing meat production, as mentionned in the previous chapter. In 2022, livestock weight measurements were performed on all Highland cows, heifers, and calves before and after the grazing season (72 days) in the site of Bovonne, Switzerland (VD). The results (unpublished) showed an average weight gain of 21.75 kg for adult cows ($\geq$ 2.5 years) and 44.7 kg (i.e. 0.62 kg day^{-1}) for heifers and calves (< 2.5 year) during the grazing season. Interestingly, these results are very much in line with those by Berry et al. (2002), who measured an average weigh gain of 0.69 kg day^{-1} for Highland calves after 77 grazing days but in un-encroached subalpine pastures. In the same study, the comparison with dairy cattle highlighted the ability of Highland cattle to utilize poor nutritive quality pastures without losing productivity, while more productive breeds tend to lose weight under those difficult conditions. Therefore, *A. viridis* encroached pastures provided sufficient forage resources for Highland cattle growth, without negatively affecting their productivity.

Highland cattle grazing patterns and their effect on *A. viridis* encroached pastures

Chapter IV described the distribution patterns of Highland cattle in *A. viridis* encroached pastures, underlying their ability to exploit every areas of the paddocks. Indeed, while Highland cattle preferred to graze in open areas with higher pastoral value vegetation, they also grazed in areas with high *A. viridis* cover composed by poorer forage quality vegetation. In accordance with the findings of Pauler et al. (2020a), they were able to travel and graze in the steepest slopes and in areas far from any water source, which is of high interest as *A. viridis* shrubs mainly thrive in steep slopes and remote areas. Therefore, Highland cattle were able to effectively penetrate the denser *A. viridis* stands, as it has been observed for smaller species such as goats or sheep (Pauler et al. 2022b).

While these findings described the influence of topographical, vegetation and management factors determining grazing behaviour, they did not include the possible effects related to weather conditions during the summer season. In dry and warm conditions, cattle may be subjected to heat stress and will tend to look for shaded locations that could increase the time they spent in highly encroached areas (Dash et al. 2016). Therefore, weather could impact their spatial distribution during the day as well as their activity (grazing or resting). In addition, heavy rainfalls might highly impact the use of steeper slopes, where it might be more difficult to move. While the following years did not depitcted any drastic change in Highland cattle spatial distribution patterns, the limitation of this study to the summer season of 2019 should also be considered.

Highland cattle showed a high potential to impact *A. viridis* stands as they grazed in highly-encroached areas. Their impact on the shrubs and the understory vegetation was further investigated in Chapter V, where Highland cattle were lured into the denser parts of the paddocks. In this chapter, we demonstrated the attractiveness of molasses-based blocks as they increased the time cattle spent in *A.viridis* stands. While previous studies have shown that molasses-based blocks could attract cattle in underused areas (Bailey and Welling 1999), this study demonstrated that they could attract Highland cattle despite being placed in areas with steep slopes and high shrub density. Their increased grazing and trampling around attractive points generated considerable impacts on both *A. viridis* shrubs and the surrounding vegetation (57.3% decrease), increasing the amount of bare soil (59.1% increase). Indeed, the understory vegetation was mainly grazed or trampled and several branches of *A. viridis* shrubs were found broken after grazing. By placing molasses-based blocks at the base of the shrubs, the mechanical effects produced by livestock were thus enhanced. In addition, because the attraction had an effect up to 50 m around the molasses-based blocks, these impacts on vegetation were carried out on a rather large scale instead of being restricted to small areas.

On the long term, such management could allow effective grassland restoration effects on the denser parts of the encroached-pastures, with a slow opening of the canopy that could allow light to reach the herbaceous levels and new light-demanding plants to settle in the gaps created by livestock grazing and trampling. However, implementing attractive points over several years requires adapted strategies, and rotational management, as well as a regular relocation of attractive points is needed to avoid the over-utilization of targeted areas. Indeed, an excessive exploitation of certain areas could lead to excessive trampling with possible localized erosion on steep slopes. Pietola et al. (2005) demonstrated that highly visited sites (such as water sources) with high trampling led to a reduced porosity in the soil and consequently higher risks of erosion, even at low grazing intensity. This risk could also be increased during particularly rainy years, and special care should be given during those events. Moreover, while livestock supplements are widely used by farmers, they are usually placed in accessible zones in order to improve animal diet or to facilitate regular inventories or health care (Pittarello et al. 2016a). With the additionnal aim to reduce *A. viridis* cover, the set up of molasses-based blocks is more complicated. Indeed, it requires the transportation of heavy blocks in steep and dense areas that are hardly accessible, and needs a sustainable installation that can last the whole grazing period without being moved by the herd. It is therefore important to take into account these practical challenges when discussing possible management strategies, which need to be adapted to site-specific conditions.

Nitrogen translocation

Chapter VI described the N content of herbaceous vegetation, *A. viridis* leaves and Highland cattle dung pats, and used these data to make an estimation of the N translocation fluxes in the studied areas. In this chapter, we confirmed that the vegetation under *A. viridis* stands displayed higher N content in comparison to adjacent open areas. Moreover, the N content of Highland cattle dung pats was higher in comparison to the content measured for other full-grazing beef cattle breeds in previous studies, probably due to the high N and tannin content of *A. viridis* leaves they were ingesting. Indeed, Angus cattle have shown lower N content in their dung despite having similar crude protein content in their diet (Koenig and Beauchemin 2013a). With increasing grazing time spent in highly encroached areas, where the N content in the vegetation is high, Highland cattle excreted higher N concentrations. Finally, the estimations on the N fluxes confirmed that Highland cattle actively transported N from steeper and denser areas to open and flat adjacent pastures. Indeed, as previously assessed by other studies with different breeds, dung pats (and therefore N) tend to accumulate in flattest areas that are mainly used as resting spots for cattle (Jewell et al. 2007; Schnyder et al. 2010). Moreover, while Highland cattle grazed primarily high-quality forage vegetation found in open and flat areas, which is in accordance with the findings of Chapter IV, the N ingestion in these areas was lower than the N deposition. In contrast, *A. viridis* encroached and steep areas displayed the lowest N ingestion, yet it exceeded N deposition, leading to an overall N depletion. Nutrient availability is particularly important for the restoration of former open pastures, and Highland cattle grazing could thus help fertilizing nutrient-poor areas, while contributing to the reduction of N accumulation under *A. viridis* stands. However, while this study focused on N translocation due to the N accumulation generated by *A. viridis* shrubs, similar estimations could be done for other nutrients to provide detailed nutrient traslocation related to Highland cattle grazing. For example, Koch et al. (2018) focused on the translocation of P, as this nutrient has great effect on plant species composition, and is a good indicator of other nutrient levels. In addition, particular care should be given to Highland cattle ability to modify plant available nutrient, as an over-utilization of some areas could lead to excessive nutrient accumulation on the long term. In that regard, the effectiveness of molasses-based blocks in attracting cows farther from resting areas could be used to avoid this possible negative impact. In addition, management strategies such as rotational grazing, or fencing and water trough placement could help preventing the overuse of specific resting areas (Jewell et al. 2007).

While the calculations used in Chapter VI are a common method for nutrient estimations and are increasingly precise thanks to the use of GPS-tracking (Koch et al. 2018), they are limited to the data gathered from literature when direct field measurements were not possible. For example, cattle urine collection is particularly challenging with grazing animals, and could not be carried out in remote mountain areas. In this study, a maximum of comparable studies with similar grazing conditions were gathered to provide the most precise missing information for the calculation of N fluxes. However, the possibility of a bias remains, either linked to specific field conditions or breed-dependant traits. In addition, while grazing and resting activity periods were discriminated through the use of two different variables (i.e., activity calculated by activity sensors and distance travelled by the cow), these estimations could be lightly biased as well. Indeed, periods mainly attributed to grazing periods may see a few animals resting, and inversely, as not all cows were equipped with GPS collars. Finally, there is room for improvement as well in the estimation scales, as vegetation intake and excretions were determined at the vegetation patch level. While vegetation is relatively homogeneous in these patches, variations in livestock distribution patterns may occur at a finer scale due to the local presence of water troughs, or due to localized slope shifts.

Long-term restoration objective

This project was carried out for four years, and allowed us to make a comprehensive analysis of the potential for Highland cattle to graze in *A.viridis* encroached pastures. However, the restoration of plant and animal biodiversity is a long-term process, and several years may be needed to assess the effect of management practices on vegetation composition changes. In high elevations specifically, ecological responses of plant species are slower due to the short growing season (Körner 2003). The restoration of herbaceous grassland species is also highly dependent on the presence of seed sources, as the range for seed dispersal is spatially limited (Barbaro et al. 2001). Therefore, Highland cattle grazing in subalpine pastures may take several years to efficiently restore former open grasslands with light-demanding, slow growing and less competitive species. Elias et al. (2018) have demonstrated the recovery of formerly shrub-encroached areas in Germany after they were grazed by goats over seven years, with improved light conditions that allowed light-demanding species to settle, thanks to the reduced shrub cover. Similar results were found by Silva et al. (2019), who measured an increased species richness after several years of goat grazing in shrub-grassland habitats. Consequently, Highland cattle grazing could also show similar positive effects on plant composition over the next years, through the slow opening of the canopy of *A. viridis* stands, the seed dispersal through dungs and fur transportation, and the effective redistribution of nutrients within grazed paddocks. This medium to long-term process may be particularly true for the most encroached areas, where a strong grazing impact is needed to allow the opening of the canopy and the installation of new grassland species. If repeated over the long term, such management could thus lead to a more heterogeneous vegetation composition, with pastures progressively composed by a mosaic of open pastures with higher forage quality and smaller stands of *A. viridis*. In one of our experiment study (Val Vogna, Italy), typical species of nutrient-rich pastures (e.g. *Phleum rhaeticum*) have already been observed to grow in the newly opened areas, where attractive points were placed, only two years after their implementation (**Figure 32**). In addition, an un-published work was carried out in 2019 to evaluate the seed germination in Highland cattle dung pats. This was done by sampling several Highland cattle dung pats and placing them in a greenhouse after vernalization. While very few seeds had grown, probably due to vernalization protocol issues, a majority of them were graminoid plants (77%) such as *Poa chaixi*, *Carex sp.*, *Avenella flexuosa*, and the rest were legumes (23%, mainly *Lotus alpinus* and *Trifolium pratense*). Therefore, there is a potential for Highland cattle to transport seeds of valuable grass and legume species through their dung excretions. An in-depth study on the seeds carried by Highland cattle, both through the dungs and through their furs, could help emphasize their ability to bring them in newly opened areas. In the future, the monitoring of the plant communities of the paddocks used in this project could be done using the permanent linear transects placed in the vegetation patches in 2019. This prospect has already been planned in the near future (2024, 5 years after the first grazing season), and could be continued over the following years.

Figure 32 Picture of the vegetation of newly open *A. viridis* stands, two years after the placement of molasses-based blocks. Val Vogna, Italy, 2022. Several individuals of *Phleum rhaeticum* are present, where very few were recorded in the botanical surverys of 2020. In the picture it is also possible to see several broken branches of *A. viridis* on the ground, thanks to Highland cattle grazing impacts.

Result communication

To successfully restore *A. viridis*-encroached pastures, it is however essential for Highland cattle herds to continue grazing in the pastures on the long term, in order to keep constraining *A. viridis* expansion and apply sufficient damages to the shrubs. With the end of the scientific project, this is achieved through the persuasion of farmers that such management is worth mainting on the long-term. Indeed, the implementation of paddocks in remote areas is difficult and time-consuming. It involves the set up of fences and water troughs in steep slopes, the monitoring of the herd during the summer season, in addition to potential unforeseen events that could request immediate care (e.g., animal heath issues, water shortage, etc.). Therefore, farmers with pre-existing activities, such as dairy farms, may not have the resources to maintain this type of management without the help of a funded project. Personal goals and perspectives of the farmers, as well as a change in personnel on farm may also intervene in the management decisions. In the paddock of Champlong, Highland cattle grazing stopped after the first year due to the farmers' will to reduce the herd size, which was not compatible with the project objectives. Yet, farmers are aware of forage losses due to shrub-encroachement, and management attemps such as mechanical cuts are regularly set up to try reducing the shrub cover. Moreover, the gain weight witnessed in one of the study area (Bovonne, VD) is an important argument to convince Highland cattle owners that cows can graze in highly encroached areas without losing productivity. Therefore, communicating the potential of Highland cattle management to farmers is essential to ensure the continuity and spread of these restoration projects. Regular presentations of the results during the experiment to key stakeholders is needed as they do not take part to the scientific assessments of the management practices, and long-term prospects may be difficult to grasp. During our project, this was achieved through public agricultural events (Salon des alpages 2019 and 2022), during more targeted events with professional interventions to field days (Journée herbagère AGRIDEA-ADCF, cours montagne AGRIDEA), or through the publication of the results in local agricultural journals (AgriHebdo and Gest'Alpe). By reaching a wide range of stakeholders (farmers, agricultural advisors, agricultural schools, park and forest managers, local associations, etc.), the transmission of information goes beyond the boundaries of the scientific community, and is delivered to actors that play a fundamental role in pasture management. This could also help unlocking the implementation of political measures to finance service herds and compensate the costs linked to such management systems. Direct payments for particular agricultural practices allow farmers to be compensated for their contribution to the provision of ecosystem services to society (El Benni et al. 2012). Future policies supporting service herds could thus be set up to help reducing *A. viridis* encroachement in alpine and subalpine pastures through diverse management techniques, including robust livestock cattle grazing.

Perspectives

While this project included GPS technology, which allowed a better monitoring of Highland cattle behaviour in such environments, additional technological devices could help the real-time monitoring of the herds and reduce the workload needed for livestock management. For example, the implementation of virtual fencing technologies could relieve farmers from fencing installation in remote areas. Indeed, virtual fencing is of increasing interest in agriculture, as it has the potential to improve farmers work by increasing flexibility and reducing the labour and costs of livestock management (Umstatter 2011). This method is particularly interesting for an adapted management along the grazing season and to provide faster reaction to changing circumstances, in comparison to physical fences. Several trials have been conducted on cattle herds, and positive results show their ability to rapidly respond to virtual fencing (Campbell et al. 2020). However, this technology is not widely spread among farmers and a few products are available on the market, mostly due to regulations in many european countries, where these devices are not yet authorized, such as in Switzerland (Aaser et al. 2022). Additional trials are necessary to test virtual fencing in mountain areas and potentially help farmers for livestock control in remote areas to preserve these open habitats of great nature conservation value.

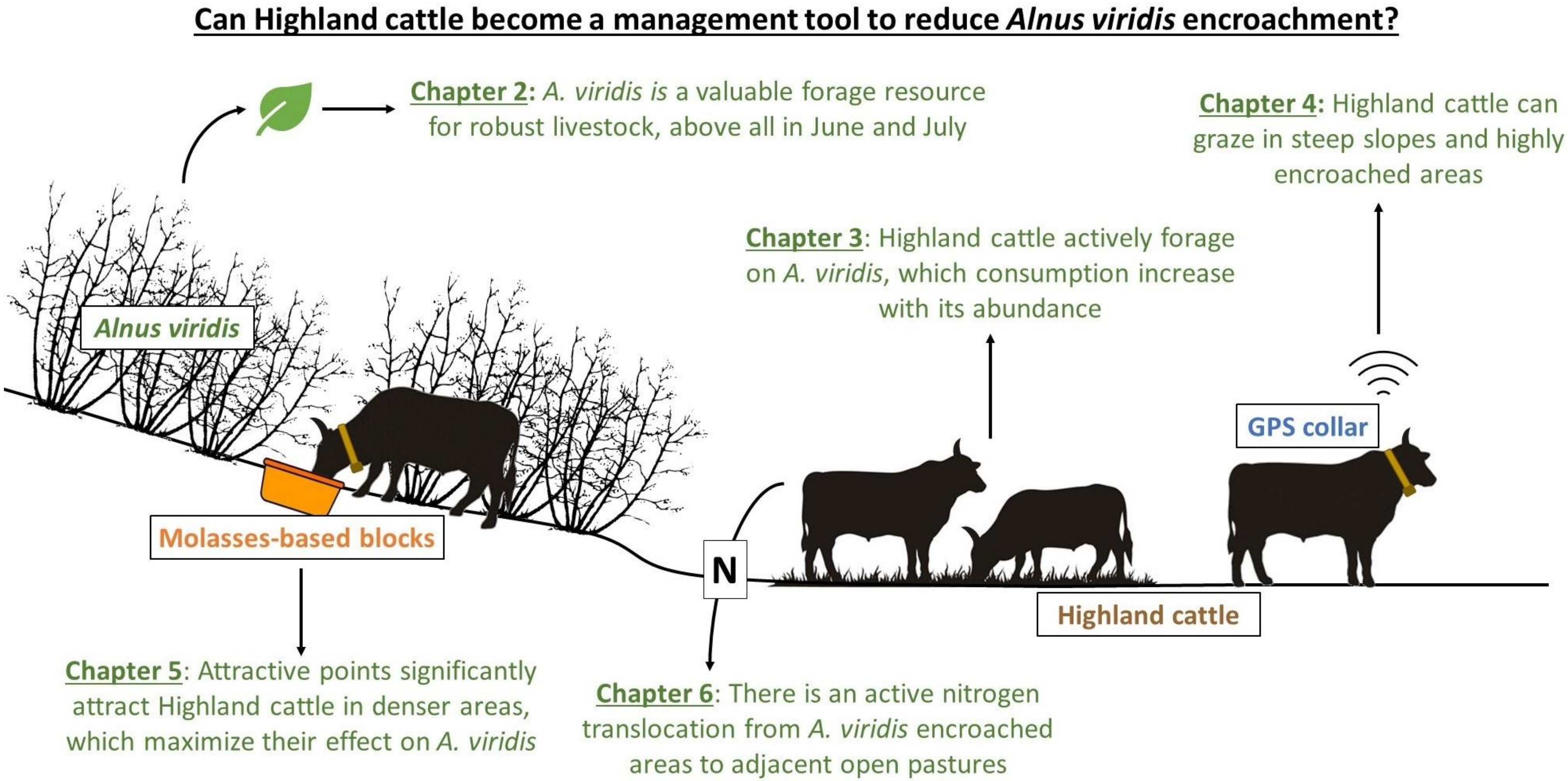
In addition, the utilisation of satellite and drone images could help monitor *A. viridis* cover and keep track of Highland cattle effect on the canopy. Indeed, satellite and drone technology is increasingly used for vegetation mapping and monitoring as it provides aerial images that can be analyzed to detect different plant communities (James and Bradshaw 2020). It has been used in several contexts, such as the detection of invasive plants, or in agriculture for the estimation of crop coverage and health (Pajares 2015). For example, Olariu et al. (2022) have used very high resolution drone imagery to map woody plant encroachment in grasslands in Texas (USA). They were able to accurately classify different woody plant species and provide precise vegetation mapping using machine learning algorithms. Drone technology has also been used to monitor leaf phenology and ontogeny at the tree-crown level (Wu et al. 2021). Therefore, this technology could be applied in *A. viridis*-encroached pastures to follow leaf seasonal changes and also potentially track Highland cattle grazing impacts that can generate the drying out of impacted branches.

Robust breed grazing in *A. viridis*-encroached areas in subalpine pastures could also be complemented by regular manual/mechanical cutting of the woody shrubs. Mechanical cuts alone may not be efficient to effectively stop the invasion of shrubs, because of the difficulty to access the areas and because it may not always help restore grass resources (Camacho et al. 2008). However, a combination of cutting and grazing could be efficient on the long term. Indeed, in a six year study conducted in the French Pre-Alps by Barbaro et al. (2001), the combination of manual cuttings and livestock grazing was efficient in reducing shrub invasion and restoring grasslands, wether it was grazed by goats, sheep, or cattle. In their study, the number of species was doubled in four years after shrub cleaning by cuts and grazing, which led to a restoration of species-rich grasslands. While it is an additional labour for farmers to perform these cuttings at a regular basis, associations that aim to help protecting the environment could help by providing workforce. In the canton of Vaud in Switzerland, the association “Alpes Vivantes” has previously organized cuttings of *A. viridis* in subalpine pastures, and discussions have been initiated with them to perform cutting interventions in the encroached areas in Bovonne as a follow up of the current project.

Finally, the results of this project could be useful for other grazing breeds and species. Indeed, several studies have highlighted the efficiency of goats and sheep to apply damages on *A. viridis* shrubs and other invasive woody plants (Elias and Tischew 2016; Elias et al. 2018; Pauler et al. 2022b). They are used by farmers as service herds to counteract the propagation of undesirable species. The knowledge gained on the forage potential of *A. viridis* leaves, the effect of molasses-based blocks, as well as the N translocation evaluated in this project, could be used to implement other management strategies with different livestock categories, and provide future alternatives for farmers.

Conclusion

The present thesis showed how Highland cattle can become an efficient management tool to counteract *A.viridis* encroachment (**Figure 33**). Indeed, they could effectively damage the shrubs through grazing, in addition to appling severe mechanical damages through trampling and the breaking of branches. Thanks to their low weight and their adaptation to low forage quality vegetation, Highland cattle were able to graze in highly encroached and steep areas. These effects can be enhanced by the placement of attractive points in the form of molasses-based blocks. These impacts, combined with their effective rebalance of N between encroached areas and open pastures could lead to a progressive restoration of former grassland vegetation in the long-term. Nowadays, Highland cattle are being increasely reared in many alpine countries, and could therefore become a trending management tool to protect grasslands against *A. viridis* encroachment.


Can Highland cattle become a management tool to reduce *Alnus viridis* encroachment?

Chapter 2: *A. viridis is* a valuable forage resource for robust livestock, above all in June and July

Chapter 3: Highland cattle actively forage on *A. viridis*, which consumption increase with its abundance

Chapter 4: Highland cattle can graze in steep slopes and highly encroached areas

Chapter 5: Attractive points significantly attract Highland cattle in denser areas, which maximize their effect on *A. viridis*

Chapter 6: There is an active nitrogen translocation from *A. viridis* encroached areas to adjacent open pastures

Figure 33 Summary of the main findings of the RobustAlps project

SUPPLEMENTARY MATERIAL

Supplementary materials of CHAPTER III

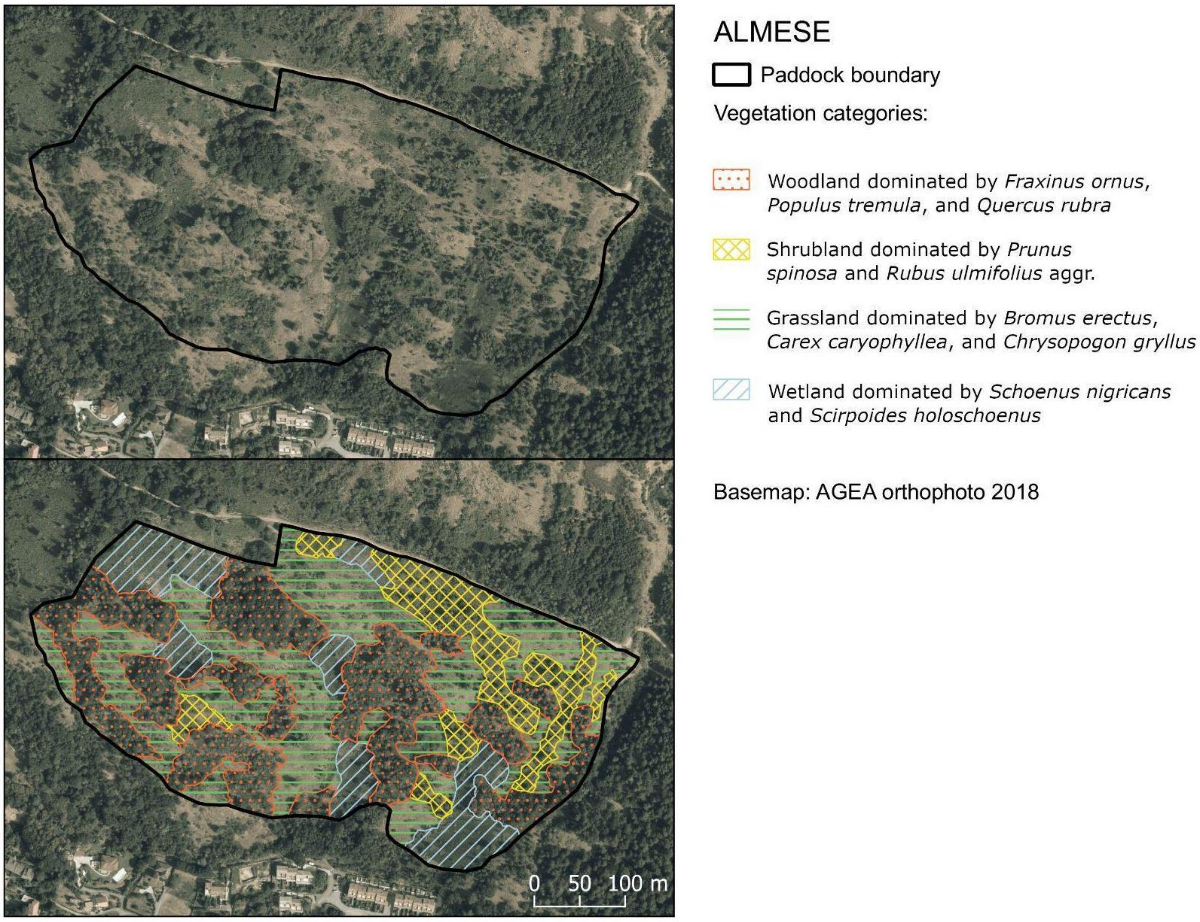


Figure S1 Aerial photograph and vegetation map of Almese study area

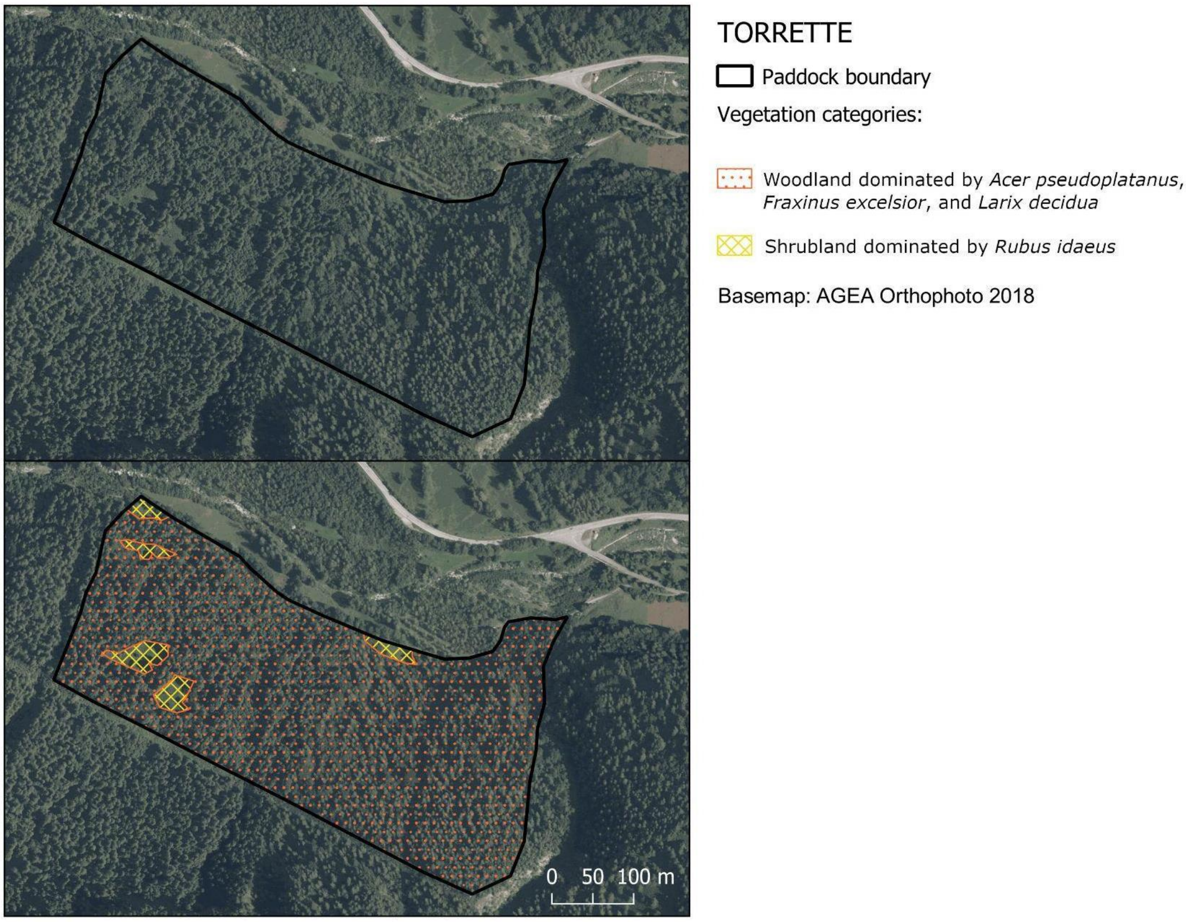


Figure S2 Aerial photograph and vegetation map of Torrette study area

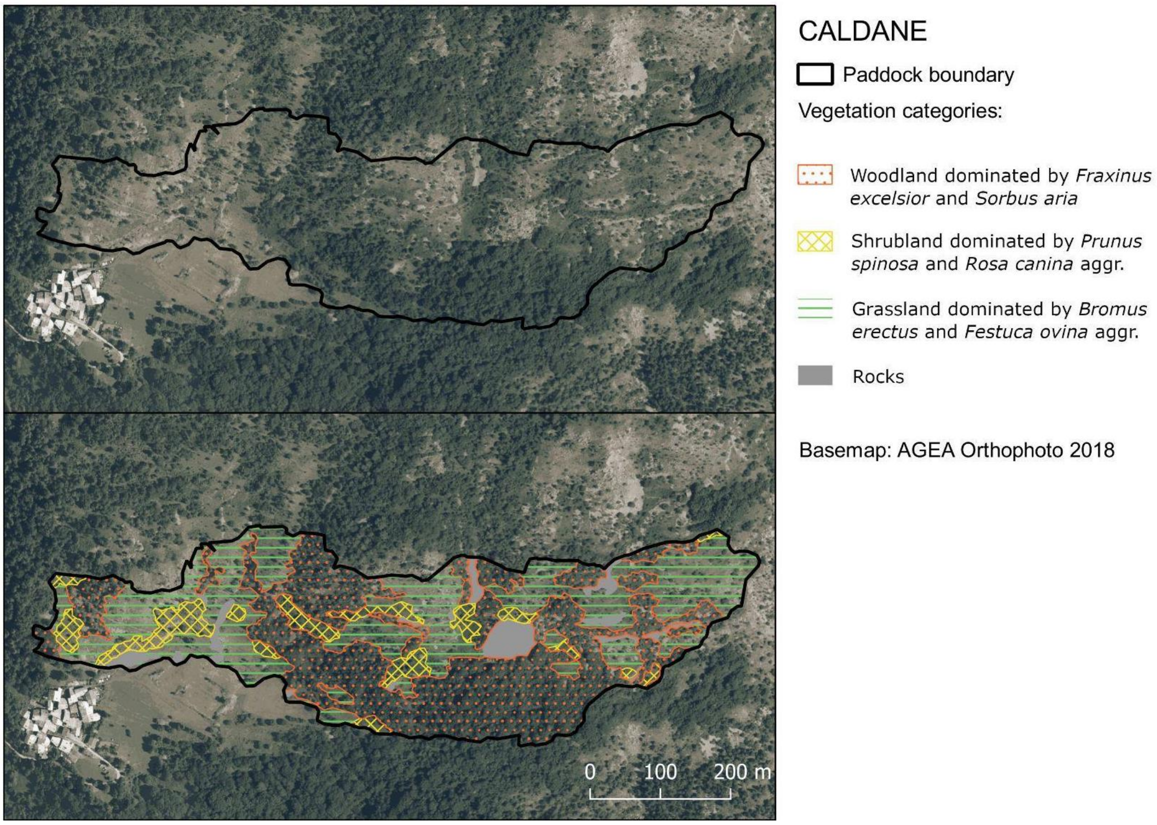


Figure S3 Aerial photograph and vegetation map of Caldane study area

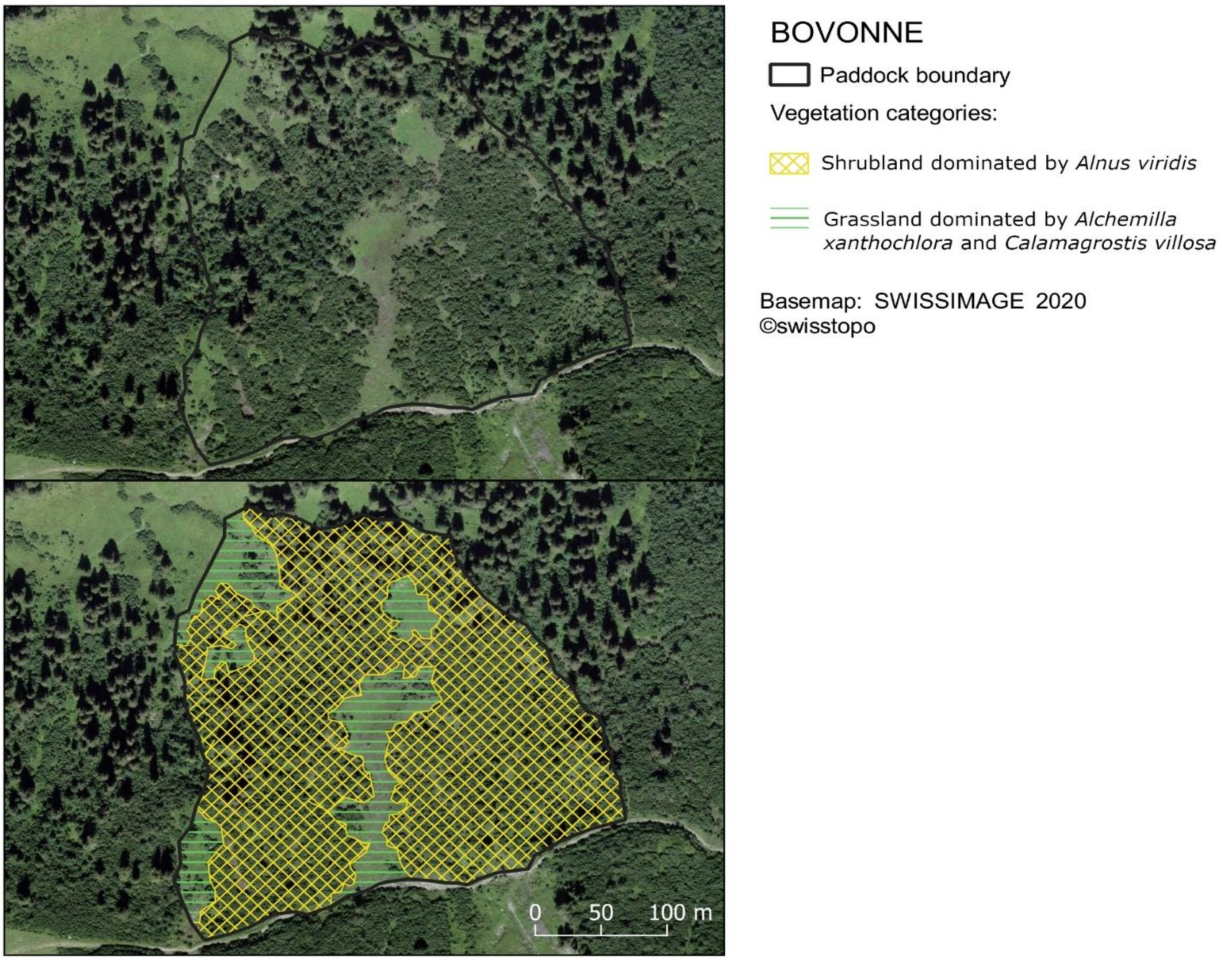


Figure S4 Aerial photograph and vegetation map of Bovonne study area

Table S1 List of the woody (W) and herbaceous (H) plant species recorded in the paddocks of the four study areas (i.e., Almese, Torrette, Caldane, and Bovonne) during the direct observations, with the corresponding number of observation sessions, number of cows that met the species, and the proportion in the feeding stations and in cattle diet.

Study area	Plant species	Life form	Observation sessions (n)	Cows that met the species during grazing (n)	Proportion in the feeding stations (%)	Proportion in the diet (%)
Almese	*Ailanthus altissima*	W	42	5	0.19	0.00
Almese	*Alnus glutinosa*	W	3	1	0.02	0.02
Almese	*Betula pendula*	W	24	7	0.24	0.33
Almese	*Calluna vulgaris*	W	342	10	1.47	0.16
Almese	*Cedrus atlantica*	W	15	3	0.09	0.00
Almese	*Celtis australis*	W	85	6	0.88	1.39
Almese	*Clematis recta*	W	19	3	0.16	0.38
Almese	*Crataegus monogyna*	W	151	11	1.16	0.58
Almese	*Euonymus europaeus*	W	15	3	0.07	0.23
Almese	*Ficus carica*	W	6	1	0.02	0.00
Almese	*Frangula Alnus*	W	212	9	1.61	3.35
Almese	*Fraxinus excelsior*	W	27	5	0.15	0.27
Almese	*Fraxinus ornus*	W	211	10	1.76	2.87
Almese	*Hedera helix*	W	7	2	0.02	0.00
Almese	*Juniperus communis*	W	18	4	0.14	0.00
Almese	*Ligustrum vulgare*	W	121	8	1.07	0.78
Almese	*Malus* sp.	W	12	2	0.10	0.19
Almese	*Populus tremula*	W	211	9	1.06	1.79
Almese	*Prunus avium*	W	6	4	0.03	0.10
Almese	*Prunus mahaleb*	W	25	3	0.34	0.42
Almese	*Prunus serotina*	W	39	7	0.29	0.35
Almese	*Prunus spinosa*	W	764	11	5.38	2.87
Almese	*Pteridium aquilinum*	H	161	8	1.07	0.00
Almese	*Pyrus pyraster*	W	11	2	0.08	0.18
Almese	*Quercus pubescens/petraea*	W	30	8	0.18	0.31
Almese	*Quercus rubra*	W	49	7	0.65	1.12
Almese	*Robinia pseudoacacia*	W	36	4	0.20	0.17
Almese	*Rosa canina* aggr.	W	63	9	0.45	0.35
Almese	*Rubus ulmifolius* aggr.	W	1415	12	9.22	2.59
Almese	*Sorbus aria*	W	3	1	0.04	0.05
Almese	*Ulmus minor*	W	138	10	1.39	1.86
Torrette	*Acer pseudoplatanus*	W	244	3	2.05	1.83
Torrette	*Alnus incana*	W	4	2	0.06	0.00
Torrette	*Athyrium filix-femina*	H	27	2	0.26	0.00
Torrette	*Betula pendula*	W	3	1	0.02	0.00
Torrette	*Corylus avellana*	W	489	3	4.37	0.40
Torrette	*Dryopteris affinis*	H	6	1	0.03	0.00
Torrette	*Dryopteris dilatata*	H	3	1	0.01	0.00
Torrette	*Dryopteris filix-mas*	H	131	3	1.09	0.46
Torrette	*Fagus sylvatica*	W	26	2	0.24	0.00
Torrette	*Fraxinus excelsior*	W	466	3	3.49	2.05
Torrette	*Gymnocarpium dryopteris*	H	2	1	0.02	0.00
Torrette	*Laburnum alpinum*	W	55	3	0.35	0.00
Torrette	*Larix decidua*	W	22	2	0.15	0.00
Torrette	*Lonicera alpigena*	W	1	1	0.04	0.04
Torrette	*Lonicera xylosteum*	W	95	3	1.64	1.46
Torrette	*Polystichum aculeatum*	H	2	2	0.01	0.00
Torrette	*Populus alba*	W	12	2	0.08	0.06
Torrette	*Prunus avium*	W	21	2	0.10	0.03
Torrette	*Quercus petraea*	W	12	1	0.08	0.00
Torrette	*Ribes uva-crispa*	W	26	3	0.15	0.01
Torrette	*Rosa canina* aggr.	W	63	3	0.41	0.05
Torrette	*Rubus idaeus*	W	1354	3	30.70	39.54
Torrette	*Rubus ulmifolius* aggr.	W	1	1	0.01	0.01
Torrette	*Salix alba*	W	4	2	0.04	0.04

Torrette	*Salix caprea*	W	8	2	0.05	0.00
Torrette	*Salix purpurea*	W	1	1	0.00	0.00
Torrette	*Sorbus aria*	W	59	2	0.40	0.07
Torrette	*Sorbus aucuparia*	W	15	2	0.08	0.02
Torrette	*Tilia platyphyllos*	W	5	2	0.06	0.15
Torrette	*Ulmus minor*	W	2	2	0.01	0.00
Caldane	*Acer pseudoplatanus*	W	19	3	0.63	0.72
Caldane	*Berberis vulgaris*	W	2	2	0.02	0.00
Caldane	*Corylus avellana*	W	308	8	2.63	2.37
Caldane	*Crataegus monogyna*	W	88	9	0.64	0.44
Caldane	*Fagus sylvatica*	W	1	1	0.00	0.00
Caldane	*Fraxinus excelsior*	W	90	7	0.53	0.85
Caldane	*Juniperus communis*	W	11	7	0.06	0.03
Caldane	*Laburnum alpinum*	W	3	1	0.02	0.00
Caldane	*Malus domestica*	W	1	1	0.01	0.03
Caldane	*Prunus avium*	W	13	3	0.05	0.05
Caldane	*Prunus spinosa*	W	1096	9	8.57	4.77
Caldane	*Quercus pubescens/petraea*	W	15	5	0.05	0.00
Caldane	*Rhamnus alpina*	W	65	8	0.73	1.40
Caldane	*Rhamnus cathartica*	W	29	4	0.16	0.25
Caldane	*Ribes uva-crispa*	W	4	3	0.02	0.00
Caldane	*Rosa canina* aggr.	W	965	9	5.98	2.29
Caldane	*Rubus ulmifolius* aggr.	W	406	9	2.72	2.72
Caldane	*Salix caprea*	W	3	1	0.03	0.05
Caldane	*Sambucus nigra*	W	28	5	0.17	0.46
Caldane	*Sorbus aria*	W	360	9	2.62	0.94
Caldane	*Ulmus minor*	W	92	6	0.55	0.39
Bovonne	*Acer pseudoplatanus*	W	18	9	0.18	0.14
Bovonne	*Aconitum napellus*	H	126	11	1.52	0.01
Bovonne	*Adenostyles alliariae*	H	515	13	11.42	0.99
Bovonne	*Alchemilla xanthochlora*	H	286	10	4.42	6.06
Bovonne	*Alnus viridis*	W	490	14	11.10	11.85
Bovonne	*Caltha palustris*	H	40	8	0.48	0.28
Bovonne	*Chaerophyllum hirsutum*	H	120	11	1.29	1.75
Bovonne	*Cicerbita alpina*	H	28	4	0.34	0.70
Bovonne	*Cirsium oleraceum*	H	10	4	0.09	0.16
Bovonne	*Dryopteris dilatata*	H	52	3	1.15	1.70
Bovonne	*Equisetum sylvaticum*	H	195	12	2.35	0.69
Bovonne	*Gentiana lutea*	H	61	8	1.13	0.00
Bovonne	*Geranium sylvaticum*	H	126	11	1.48	1.86
Bovonne	*Heracleum sphondylium*	H	10	2	0.12	0.22
Bovonne	*Petasites albus*	H	1	1	0.02	0.00
Bovonne	*Peucedanum ostruthium*	H	3	2	0.04	0.03
Bovonne	*Picea abies*	W	94	8	2.57	2.29
Bovonne	*Ranunculus aconitifolius*	H	148	10	1.72	2.31
Bovonne	*Rubus idaeus*	W	6	4	0.06	0.00
Bovonne	*Rumex alpestris*	H	33	6	0.33	0.11
Bovonne	*Rumex alpinus*	H	65	8	1.44	0.51
Bovonne	*Salix hastata*	W	32	6	0.48	0.46
Bovonne	*Sorbus aucuparia*	W	37	9	0.42	0.32
Bovonne	*Veratrum album*	H	570	14	10.16	0.03

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Supplementary materials of CHAPTER V

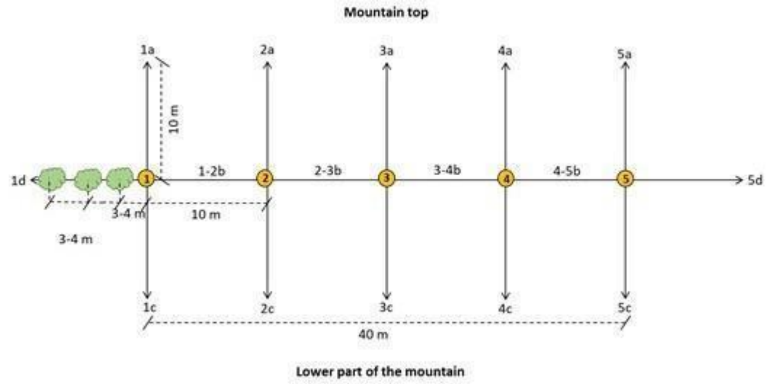

Figure S5 Scheme of the 16 cross-shaped vegetation transects performed in attractive points and control areas. Yellow numbers represent the attractive points (molasses-based blocks) or the control points, depending on the area. The green figures represent the *Alnus viridis* shrubs selected in the external transects which are only linked to one molasses-based block (i.e. not the central transects labeled as 1-2b, 2-3b, 3-4b, and 4-5b).

Figure S6: Intense defoliation on small *Alnus viridis* shrubs having 1-2 meters of height.

Figure S7 Impacts on a tall *Alnus viridis* shrub, 3-4 meters in height. Branches were intensively defoliated and some of them broken by cattle scratching and herd movement.

Figure S8 Impacts on a tall *Alnus viridis* shrub, 3-4 meters high. Branches were intensively defoliated and some of them broken by cattle scratching and herd movement. In the red circle, it is possible to see some debarking effects produced by horn scratching, which led to subsequent fungi infections and drying off of the whole branch.

Figure S9 Impacts on *Alnus viridis* shrubs, 3-4 meters high. Branches were intensively damaged by Highland cattle scratching, trampling and herd movement.

Figure S10 Impacts on *Alnus viridis* shrubs, 3-4 meters high. Branches were intensively grazed and damaged by Highland cattle scratching, trampling and herd movement.

Table S2 Composition of molasses-based blocks. The blocks contained 2-3% of residual moisture.

Components (%)		Additives per kg (mg)	
Raw protein	3.0%	Inorganic zinc	900
Raw fat	2.0%	Inorganic manganese	900
Raw ash	24.0%	Inorganic iodine	60
Sugar	40.0%	Inorganic cobalt	12
Calcium	4.0%	Inorganic selenium	9
Phosphorus	2.0%		
Magnesium	0.2%		
Sodium	2.5%		

Table S3 List of all plant species found in the botanical surveys made in both 2019 and 2020 in all paddocks combined, sorted by their corresponding functional groups. Plant species nomenclature follows Aeschimann et al. (2004).

Ferns and tall herbs		Medium and small herbs			Woody	Graminoids
Achillea macrophylla	*Gymnocarpium dryopteris*	*Achillea millefolium*	*Geum montanum*	*Ranunculus aconitifolius*	*Acer pseudoplatanus*	*Agrostis capillaris*
Aconitum lamarckii	*Heracleum sphondylium*	*Ajuga reptans*	*Geum rivale*	*Ranunculus montanus aggr.*	*Alnus viridis*	*Agrostis schraderiana*
Aconitum napellus	*Hieracium prenanthoides*	*Alchemilla alpina aggr.*	*Hieracium intybaceum*	*Rhodiola rosea*	*Larix decidua*	*Anthoxanthum alpinum*
Adenostyles alliariae	*Huperzia selago*	*Alchemilla pentaphyllea*	*Hieracium murorum aggr.*	*Rumex scutatus*	*Lonicera caerulea*	*Avenella flexuosa*
Alchemilla xanthochlora	*Lamium galeobdolon aggr.*	*Anthyllis vulneraria*	*Homogyne alpina*	*Saxifraga rotundifolia*	*Lonicera nigra*	*Calamagrostis villosa*
Asplenium viride	*Oreopteris limbosperma*	*Aposeris foetida*	*Hypericum maculatum*	*Saxifraga stellaris*	*Picea abies*	*Carex flacca*
Astrantia major	*Petasites albus*	*Aster bellidiastrum*	*Hypericum richeri*	*Sedum acre*	*Rhododendron ferrugineum*	*Carex leporina*
Athyrium filix-femina	*Peucedanum austriacum*	*Astrantia minor*	*Knautia dipsacifolia*	*Sempervivum montanum*	*Rosa pendulina*	*Carex sp.*
Campanula latifolia	*Peucedanum ostruthium*	*Caltha palustris*	*Leontodon helveticus*	*Silene dioica*	*Rubus idaeus*	*Dactylis glomerata*
Carduus defloratus	*Phegopteris connectilis*	*Campanula scheuchzeri*	*Leucanthemum vulgare aggr.*	*Silene nutans*	*Rubus saxatilis*	*Deschampsia cespitosa*
Chaerophyllum hirsutum	*Polystichum lonchitis*	*Campanula trachelium*	*Lotus alpinus*	*Silene rupestris*	*Salix hastata*	*Festuca rubra aggr.*
Cicerbita alpina	*Prenanthes purpurea*	*Cardamine amara*	*Orchis sp.*	*Silene vulgaris*	*Sorbus aucuparia*	*Festuca scabriculmis*
Cirsium oleraceum	*Rumex alpestris*	*Cardamine resedifolia*	*Oxalis acetosella*	*Soldanella alpina*	*Sorbus chamaemespilus*	*Luzula alpinopilosa*
Cryptogramma crispa	*Rumex alpinus*	*Cerastium vulgare*	*Paris quadrifolia*	*Solidago virgaurea*	*Vaccinium myrtillus*	*Luzula campestris aggr.*
Dryopteris dilatata aggr.	*Seseli libanotis*	*Crocus albiflorus*	*Pedicularis tuberosa*	*Streptopus amplexifolius*		*Luzula sylvatica*
Dryopteris filix-mas	*Urtica dioica*	*Cuscuta europaea*	*Phyteuma betonicifolium*	*Stellaria nemorum*		*Phleum rhaeticum*
Epilobium angustifolium	*Veratrum album*	*Dactylorhiza fuchsii*	*Phyteuma hemisphaericum*	*Taraxacum officinale aggr.*		*Poa chaixii*
Equisetum sylvaticum	*Veronica urticifolia*	*Dactylorhiza maculata*	*Phyteuma spicatum*	*Thalictrum aquilegiifolium*		*Poa nemoralis*
Gentiana lutea		*Daucus carota*	*Pimpinella saxifraga*	*Trifolium badium*		*Poa pratensis*
		Epilobium alpestre	*Plantago atrata*	*Trifolium pratense*		*Trisetum flavescens*
		Epilobium obscurum	*Polygonum viviparum*	*Trifolium repens*		
		Euphorbia cyparissias	*Potentilla crantzii*	*Trollius europaeus*		
		Galeopsis tetrahit	*Potentilla aurea*	*Valeriana tripteris*		
		Galium pusillum aggr.	*Potentilla erecta*	*Veronica chamaedrys*		
		Gentiana purpurea	*Primula veris*	*Veronica serpyllifolia*		
		Geranium sylvaticum	*Pulsatilla montana*	*Viola biflora*		

Table S4 Statistical results of the analyses made on spatial distribution (generalized linear model), soil cover (herbaceous cover and fraction of bare soil), *Alnus viridis* leaves and understory plant functional groups (generalized least square model).

Spatial distribution			
Variables		**10 m**	**50m**
	Df	*p*	*p*
Molasses blocks (MB)	1	0.8589	0.2513
Year (Y)	1	0.4713	0.0058
MB x Y	1	2.26E-05	0.0237

Vegetation cover			
Variables		**Herbaceous cover**	**Bare soil**
	Df	*p*	*p*
Molasses blocks (MB)	1	3.65E-05	2.41E-03

***A. viridis* leaves**		
Variables		
	Df	*p*
Molasses blocks (MB)	1	2.718E-10
Distance (D)	1	0.4238
MB x D	1	0.01019

Functional groups				
Variables		**Tall herbs**	**Forbs**	**Woody plants**
	Df	*p*	*p*	*p*
Molasses blocks (MB)	1	0.01051	0.007926	2.88E-07

Table S5 Statistical results of the additional analyses (Anovas) made on the average RR in each area of the paddocks (i.e. 3 control vs. 3 molasses-based blocks areas, one control and one MB area from each paddock: 6 data points in total).

Vegetation cover			
Variables		**Herbaceous cover**	**Bare soil**
	Df	*p*	*p*
Molasses blocks (MB)	1	0.0313	0.05649

***A. viridis* leaves**		
Variables		
	Df	*p*
Molasses blocks (MB)	1	0.0479

Functional groups				
Variables		**Ferns and tall herbs**	**Medium and small herbs**	**Woody plants**
	Df	*p*	*p*	*p*
Molasses blocks (MB)	1	0.126	0.024	0.006349

Supplementary materials of CHAPTER VI

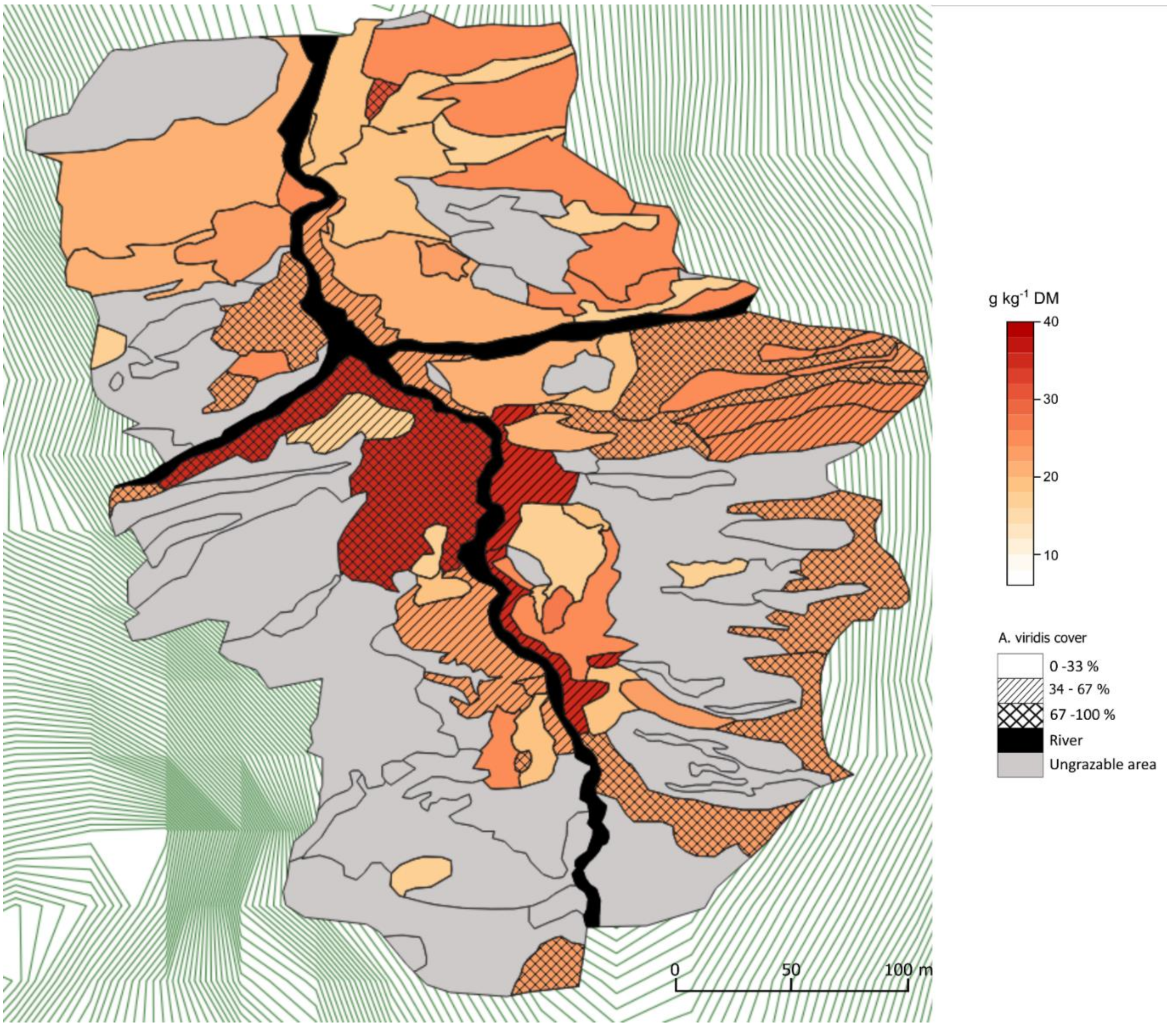

Figure S11 Nitrogen content (g kg^{-1} DM) of the herbaceous vegetation and *Alnus viridis* cover (%) of all vegetation patches of paddock 1.

Figure S12 Nitrogen content (g kg^{-1}) of the herbaceous vegetation and *Alnus viridis* cover (%) of all vegetation patches of paddock 2

Figure S13 Nitrogen content (g kg^{-1}) of the herbaceous vegetation and *Alnus viridis* cover (%) of all vegetation patches of paddock 3.

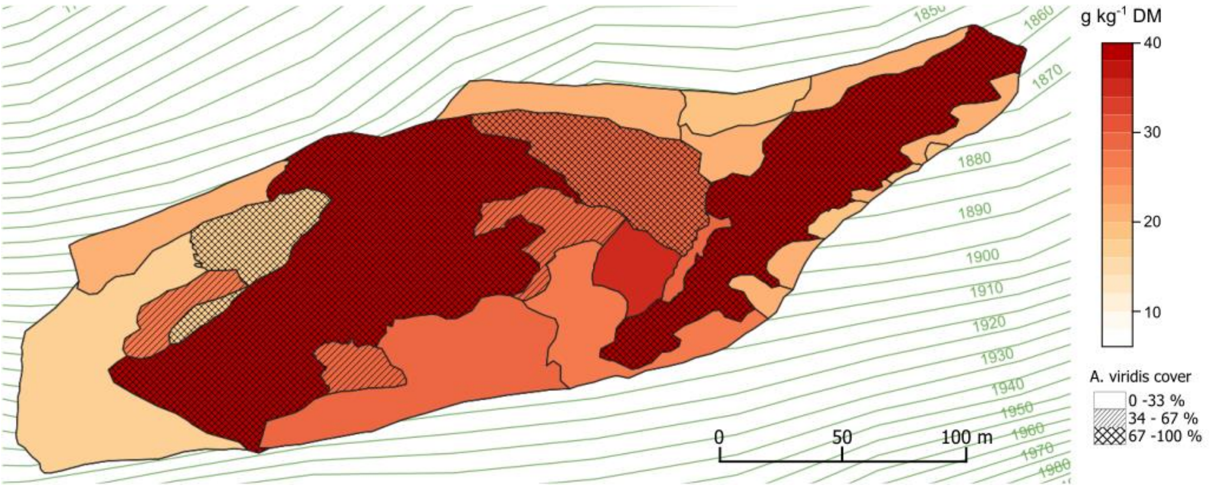

Figure S14 Nitrogen content (g kg^{-1}) of the herbaceous vegetation and *Alnus viridis* cover (%) of all vegetation patches of paddock 4.

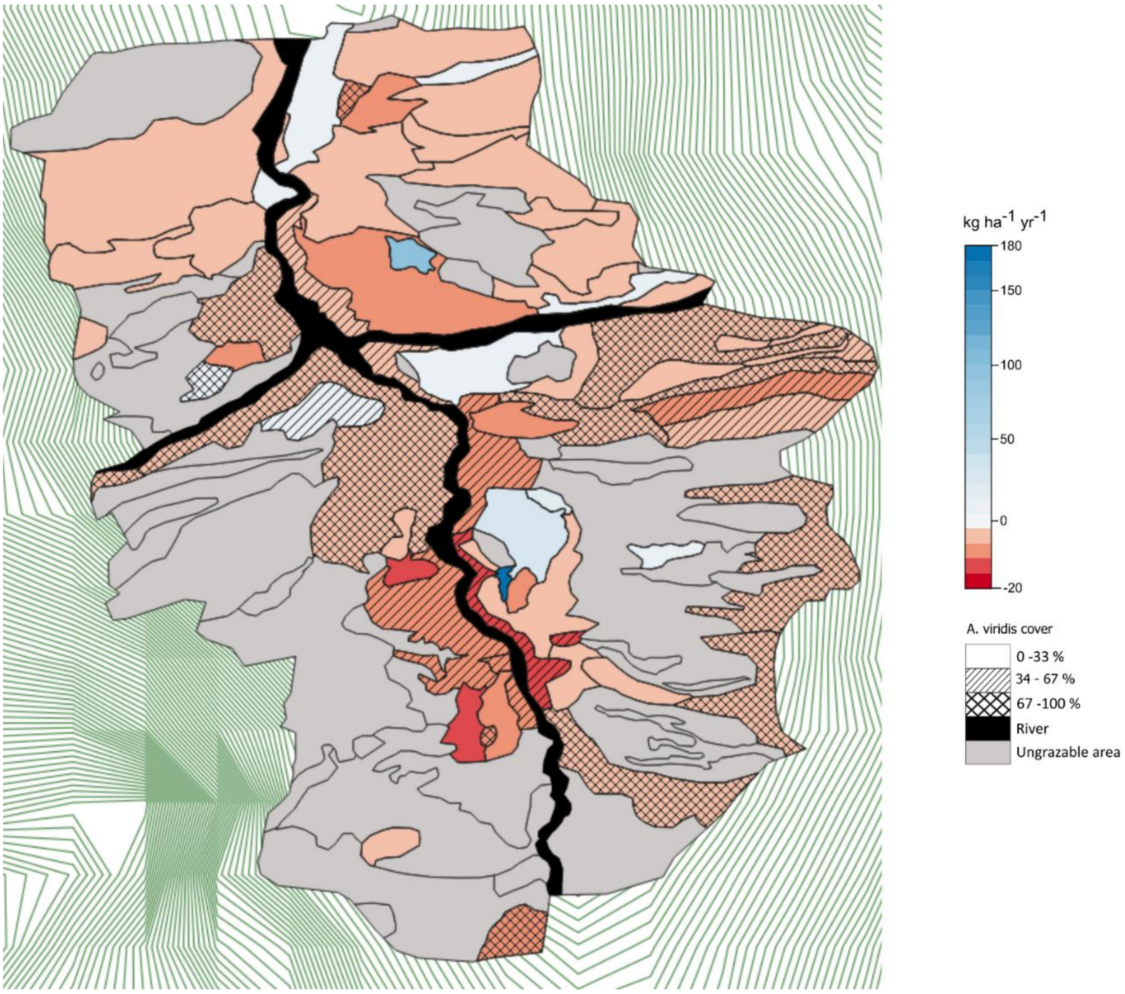

Figure S15 Nitrogen fluxes (50% quantile, kg ha^{-1} yr^{-1}) of the vegetation patches of paddock 1 in 2019.

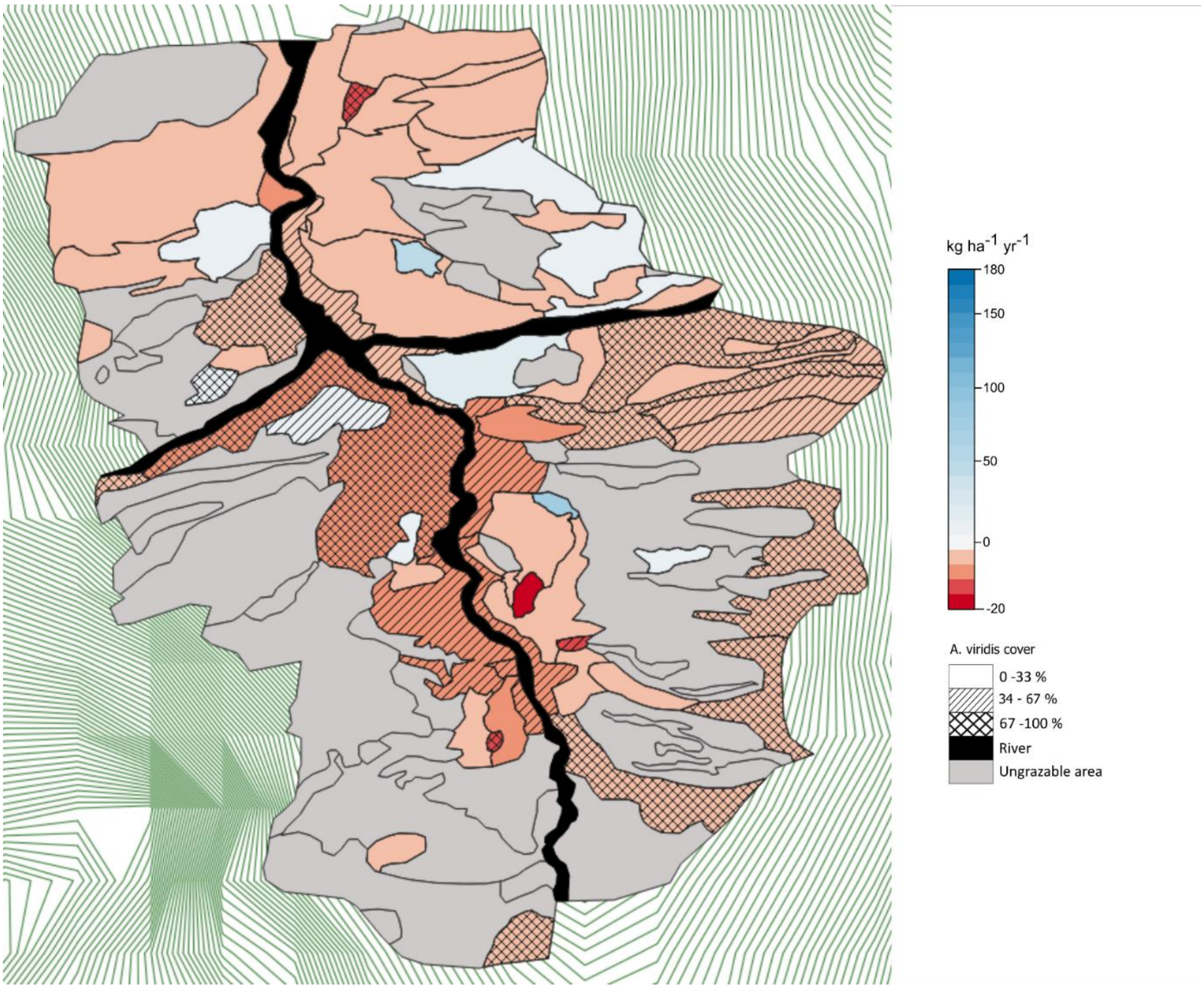

Figure S16 Nitrogen fluxes (50% quantile, kg ha^{-1} yr^{-1}) of the vegetation patches of paddock 1 in 2020.

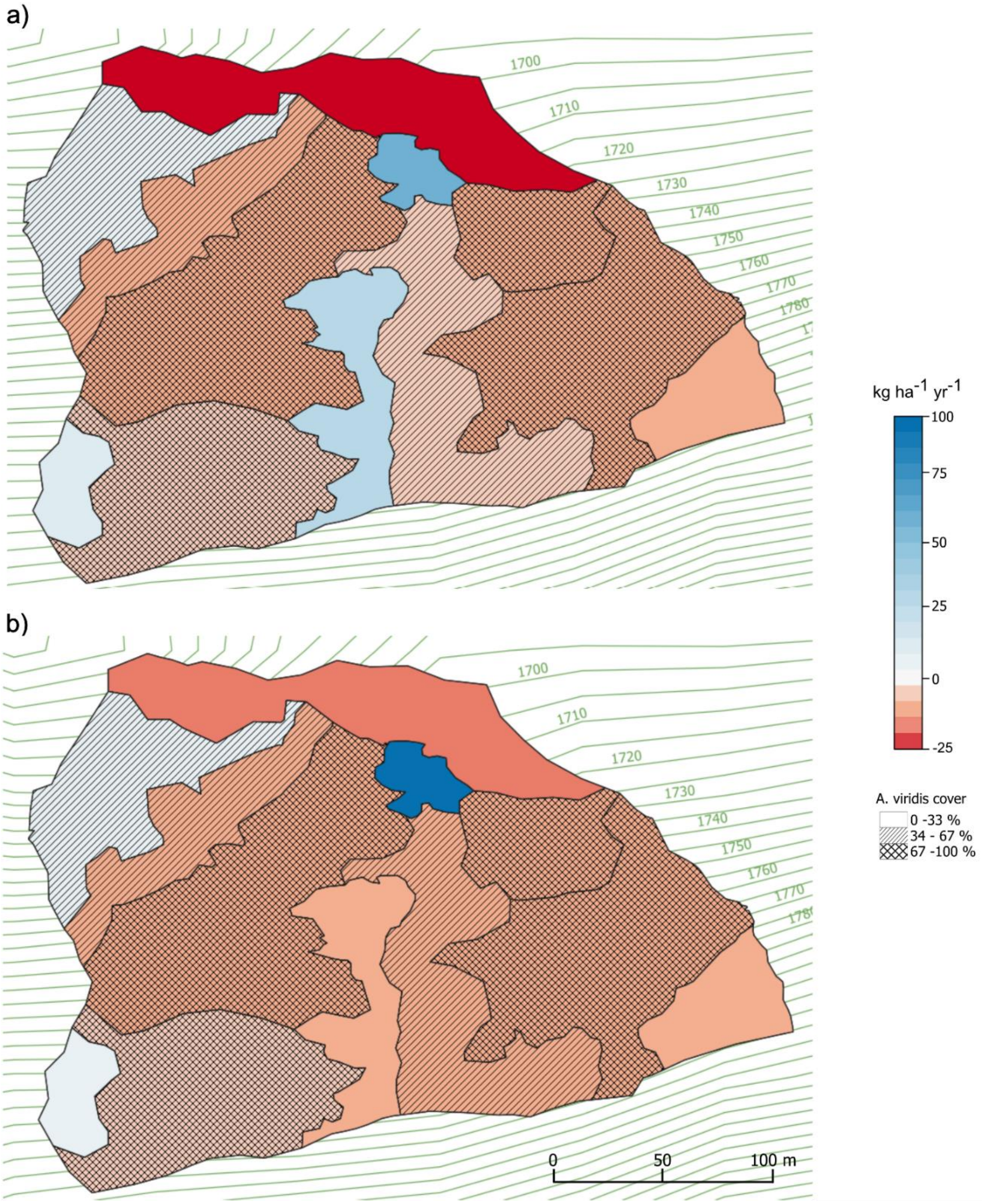

Figure S17 Nitrogen fluxes (50% quantile, kg ha^{-1} yr^{-1}) of the vegetation patches of paddock 2 in: a) 2019, and b) 2020.

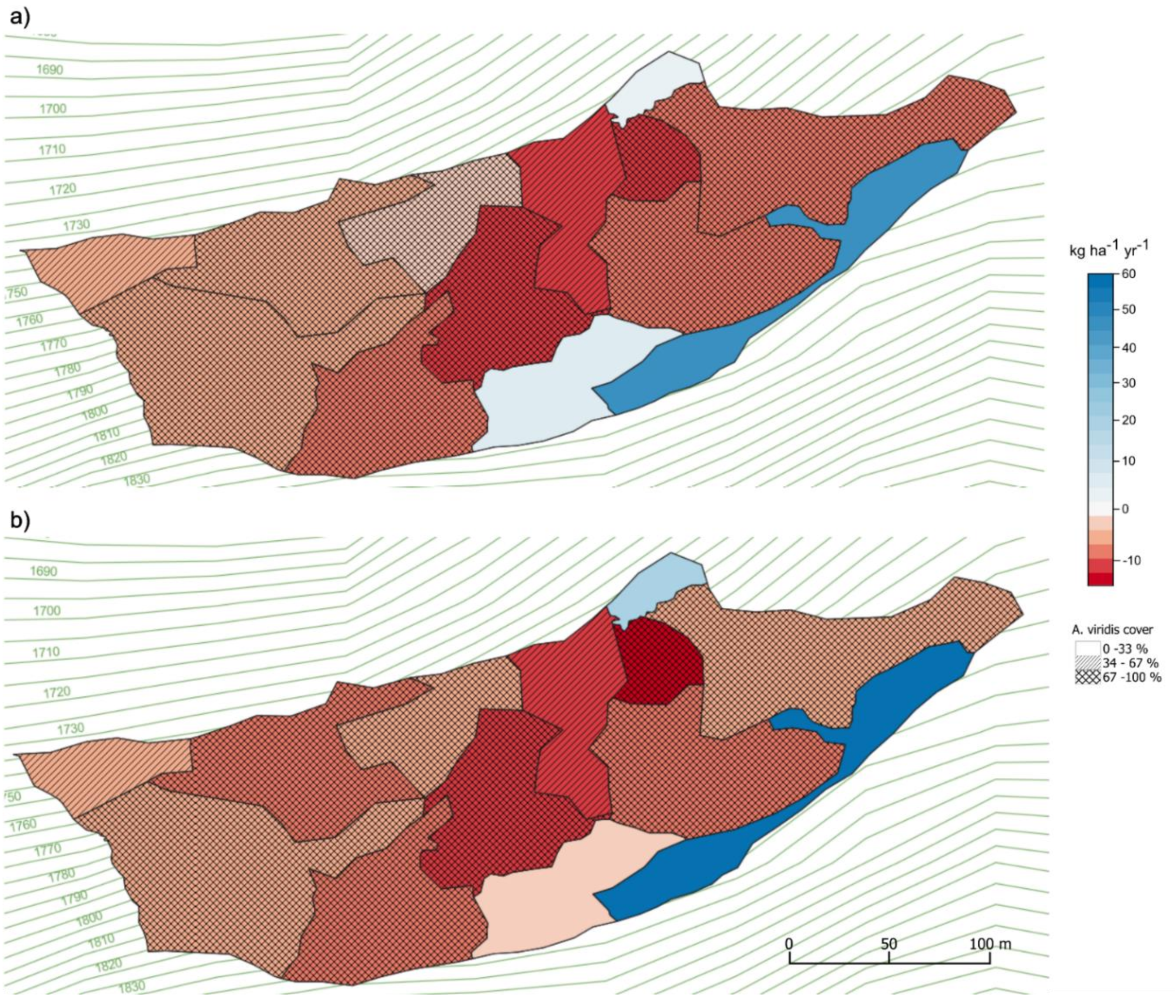

Figure S18 Nitrogen fluxes (50% quantile, kg ha^{-1} yr^{-1}) of the vegetation patches of paddock 3in: a) 2019, and b) 2020.

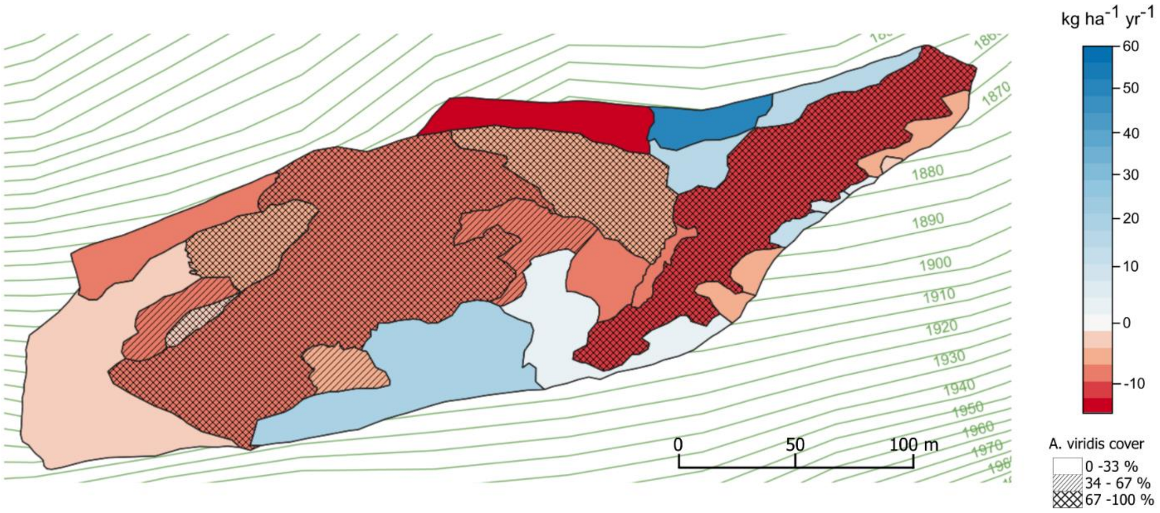

Figure S19 Nitrogen fluxes (50% quantile, kg ha^{-1} yr^{-1}) of the vegetation patches of paddock 4 in 2020.

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Title of the thesis: Targeted grazing of robust livestock for the restoration of greenalder-encroached pastures (RobustAlps Project)

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