

**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**

from **France**

Supervisors of the doctoral thesis:

**Professor Eric Allan**

**Dr. Massimiliano Probo**

**University of Bern and Agroscope**

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The Dean

Prof. Dr.

**Marco Herwegh**

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<b>CURRICULUM VITAE .....</b>	<b>Erreur ! Signet non défini.</b>

## List of Abbreviations

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ADF <sub>OM</sub>	Acid detergent fiber
ADL	Acid detergent lignin
aNDF <sub>OM</sub>	Neutral detergent fiber
Ca	Calcium
CH <sub>4</sub>	Methane
CO <sub>2</sub>	Carbon dioxide
CT	Condensed tannins
Cu	Copper
DM	Dry matter
Fe	Iron
GDD	Growing degree days
HGT	Hohenheim gas test
HNO <sub>3</sub>	Nitric acid
HT	Hydrolysable tannins
IVOMD	<i>In vitro</i> organic matter digestibility
K	Potassium
LDMC	Leaf dry matter content
MB	Mollasses-based blocks
Mg	Magnesium
Mn	Manganese
N	Nitrogen
NH <sub>3</sub>	Ammonia
NH <sub>4</sub> <sup>+</sup>	Ammonium
NO <sub>3</sub> <sup>-</sup>	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

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## 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 20<sup>th</sup> 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; Wiczorkowski 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.

48 ***Alnus viridis* encroachment**

49

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

69



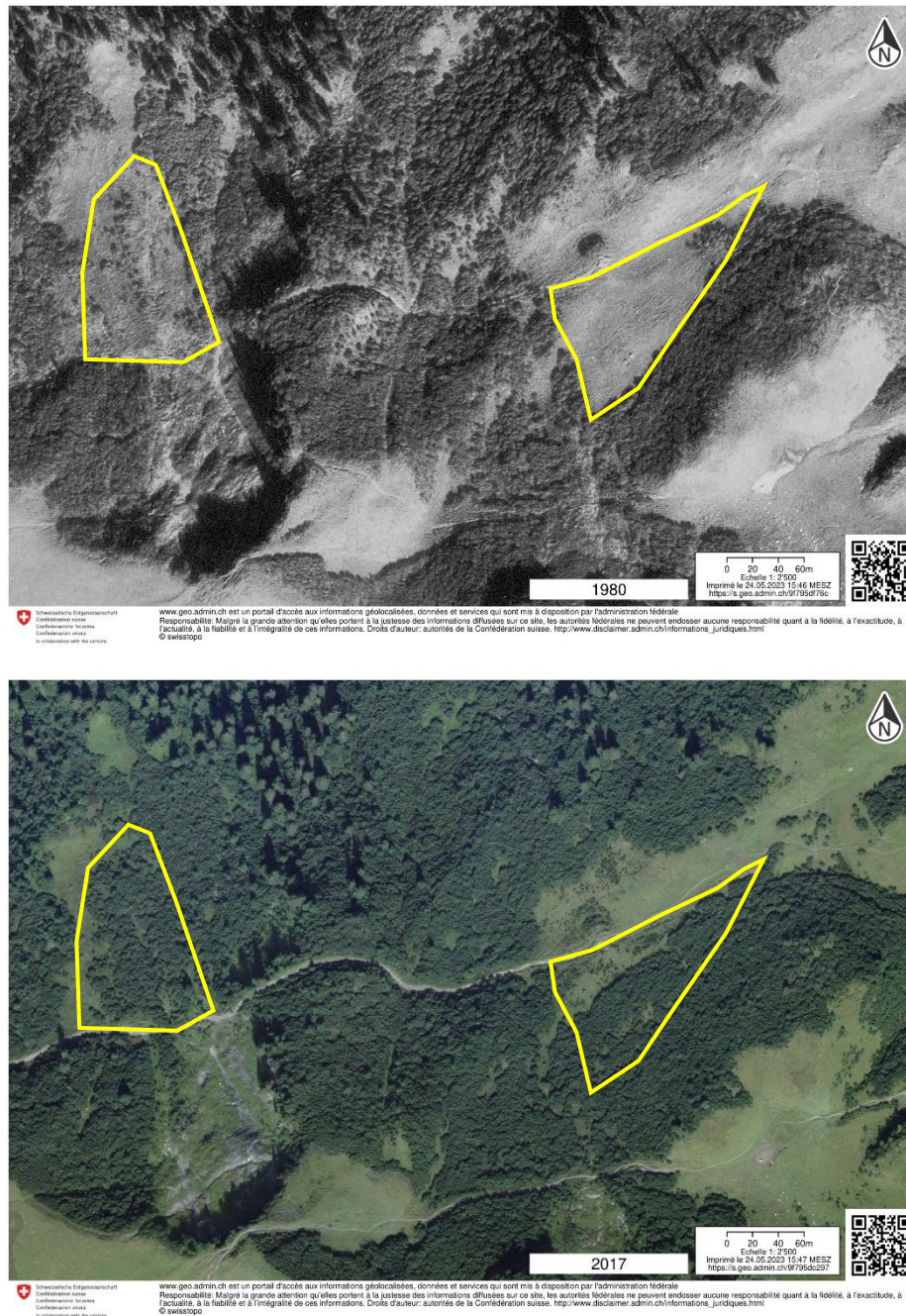
70

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

72

73 In some regions of Switzerland, *A. viridis* was reported to have increased by 50% between 1979  
74 and 2014, and it now represents 70% of the total shrubland cover of the country, with an expansion rate  
75 being two to three times faster than tree forest expansion (Bühlmann et al. 2014; Caviezel et al. 2014;  
76 Abegg et al. 2020). **Figure 2** illustrates an example of the significant expansion of *A. viridis* in a  
77 subalpine pasture used as a study site for this thesis between 1980 and 2017. Nowadays, *A. viridis* is

78 expanding into larger elevation ranges and gentle slopes, as its spreading is not only driven by land use  
79 intensity but also accelerated by climate change at higher elevations (Körner 2012; Caviezel et al. 2017;  
80 Skoczowski et al. 2021).



81  
82 **Figure 2** Changes in *Alnus viridis* cover in a subalpine pasture in Western Switzerland, Bovonne, Bex  
83 (VD), between 1980 and 2017. Source: Swisstopo. Areas with the most visible increase in *A. viridis*  
84 cover are circled in yellow.

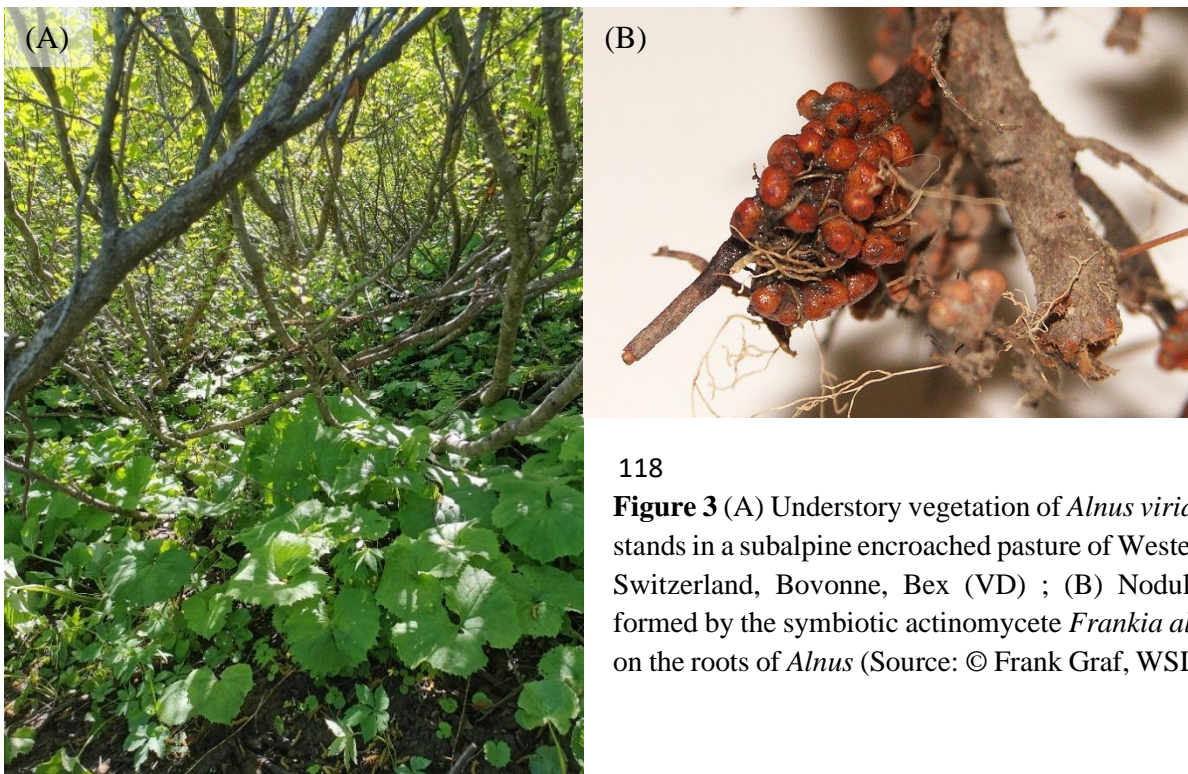
85  
86 *A. viridis* invasion is of major concern as it creates unfavourable agro-environmental conditions.  
87 Indeed, similarly to the case of tree and shrub encroachment in general, *A. viridis* expansion reduces the  
88 available areas for agriculture purposes, as well as prevents the provision of several ecosystem services  
89 from alpine and subalpine pastures. Among other things, *A. viridis* encroachment increases the risks of  
90 natural hazards such as wildfire (Schumacher and Bugmann 2006; Lovreglio et al. 2014) and does not  
91 protect from erosion or landslides (Tasser et al. 2003), or even avalanches, which are facilitated due to

92 the elasticity of their branches that allow the snow to slide (Brüchert et al. 2003; Bühlmann et al. 2014;  
93 Caviezel et al. 2014).

94

95 One of the worrying drawbacks from *A. viridis* encroachment is the reduction of plant and animal  
96 biodiversity (Anthelme et al. 2001; Laiolo et al. 2004; Pornaro et al. 2013; Koch et al. 2015; Zehnder et  
97 al. 2020). Indeed, the shading created by *A. viridis* stands and the vegetation growing under its canopy  
98 reduces the chance of new seedling establishments (Anthelme et al. 2003; Bühlmann et al. 2014). It also  
99 creates cold and humid habitats, where a reduced number of tall shade-tolerant plant species can grow,  
100 and other plant seedling germination is even hampered by its vegetative basal sprouting. Moreover, *A.*  
101 *viridis* lives in symbiosis with the nitrogen-fixing actinomycete *Frankia alni*, which generates high  
102 levels of nitrogen (N) fixation in soils, sometimes leading to eutrophication (Huss-Daniel 1997; Dawson  
103 2008). Indeed, this endosymbiosis is achieved through the formation of N-fixing root nodules (**Figure**  
104 **3**) in which *Frankia alni* converts the atmospheric N ( $N_2$ ) into ammonia ( $NH_3$ ) thanks to nitrogenase  
105 enzyme complexes (Huss-Daniel 1997; Schwob et al. 2017). This N fixation creates a N saturation in  
106 the soils, resulting into soil acidification through the nitrification of ammonium ( $NH_4^+$ ) into nitrate  
107 ( $NO_3^-$ ), during which base cations are released, contributing to soil acidity (Verburg et al. 2001;  
108 Galloway et al. 2003; Thimonier et al. 2010). Therefore, only a few shade- and N-tolerant plants are  
109 able to adapt to these ecological conditions and grow in the understory vegetation of *A. viridis*  
110 shrublands (Zehnder et al. 2020). More specifically, the *A. viridis* understory is dominated by a few fast-  
111 growing and broad-leaved forbs such as *Adenostyles alliariae* (Gouan) A. Kern., *Peucedanum*  
112 *ostruthium* (L.) W. D. J. Koch, and *Cicerbita alpina* (L.) Wallr., and ferns such as *Dryopteris dilatata*  
113 (Hoffm.) A. Gray and *Athyrium filix-femina* (L.) Roth, which take competitive advantage of the high  
114 nutrient availability present in the topsoil (Anthelme et al. 2001; Wiedmer and Senn-Irlet 2006). The N  
115 accumulation also constrains the re-establishment of natural montane forests and prevents forest  
116 succession, by hindering conifers establishment (Hiltbrunner et al. 2014).

117



118

**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).

127

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

151  
152 By decreasing plant and animal diversity, generating soil and water pollution and releasing  
153 greenhouse gases into the atmosphere, *A. viridis* shrubs are direct contributors to the environmental  
154 decline in alpine and subalpine ecosystems. Combined with the agronomical losses related to shrub  
155 encroachment, the “*A. viridis* encroachment problem” has been increasingly reported in literature, and  
156 concerns have risen for grassland conservation and restoration. Therefore, managing the expansion of  
157 *A. viridis* is an important goal for the restoration of the ecosystem services provided by alpine and  
158 subalpine pastures. However, because *A. viridis* stands are located in remote, steep and hard-to-reach  
159 areas, regulating its spread is challenging, and manual removal is often technically difficult, time  
160 consuming and expensive. Until the 1950’s the manual cutting of *A. viridis* shrubs was regularly carried  
161 out for firewood and had an economical purpose (Caviezel et al. 2017). Combined with the high grazing  
162 pressure at that time, *A. viridis* spread was then more controlled. Nowadays, the management of its  
163 expansion is harder and under several constraints that farmers may not be able to overcome. With the  
164 lack of management over several years, some areas have become even harder to reach and the amount  
165 of work needed to counteract *A. viridis* encroachment has become more and more significant.

166  
167 Moreover, *A. viridis* leaves have moderately high contents in cell wall constituents and plant  
168 secondary metabolites, such as tannins. Therefore, they may not be palatable to many production-  
169 oriented dairy livestock breeds (Papanastasis et al. 2008; Waghorn 2008). While the number of dairy  
170 farms have decreased in many alpine regions in the last decades, the majority of alpine farms are still  
171 composed by dairy cows (Mack et al. 2013; Battaglini et al. 2014). For instance, Switzerland hosts 7400  
172 alpine summer farms that are mainly oriented for dairy production (Mack et al. 2013). Because these  
173 high-production breeds are not able to digest highly lignified plants with high tannin contents, *A. viridis*  
174 is not an appreciated feeding resource. Therefore, they are not able to actively reduce shrub stands  
175 through grazing pressure only.

## 176 **Robust livestock**

177

178 Other livestock species and breeds were reported to have the ability to forage on woody species.  
179 Indeed, some robust species and breeds have an adaptative digestive system with bacterial populations  
180 able to deteriorate lignified material and detoxify secondary metabolites, as well as providing high  
181 resistance to tannic compounds (Manousidis et al. 2016). Therefore, they are better suited to digest  
182 lignified plants with low digestibility. For example, goat ability to forage on shrublands is widely  
183 recognized and they are commonly used for shrub encroachment management (Álvarez-Martínez et al.  
184 2016; Marques et al. 2017; Pauler et al. 2022b). This performance is also linked to their ability to browse  
185 in steep slopes and to easily penetrate dense shrub stands due to their small size (Pauler et al. 2022b).  
186 More specifically, the typical alpine breed Camosciata goats have been reported to select several woody  
187 plants in their diet, and even eat potentially toxic plants such as ferns (Iussig et al. 2015a). Other goat  
188 breeds, such as Boer goats also showed high potential for shrub encroachment regulation as they  
189 consumed thorny and spiny invasive shrub species (Elias and Tischew 2016). Some sheep breeds were  
190 also reported to actively graze on shrubs, such as Welsh Mountain or Scottish Blackface sheep, as  
191 reported by Fraser et al. (2009).

192

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

204

205 Highland cattle (**Figure 4**) could be another promising breed to control *A. viridis*-encroachment.  
206 Indeed, this low-productive and rustic breed originating from Scotland displays the ability to forage on  
207 very low forage quality vegetation (Pauler et al. 2020a,b). As other robust species, Highland cattle have  
208 a tannin-tolerant microbial population in their rumen allowing them to digest plants that are unpalatable  
209 for high-production breeds. Feeding in poor nutritional quality pastures does not constrain their weight  
210 gain, as they can maintain sufficient nutrient intake from a vegetation with high neutral detergent fibre  
211 content and low organic matter digestibility, thanks to their low maintenance energy requirement (Berry  
212 et al. 2002). Therefore, this breed may be able to feed on woody species such as *A. viridis*, as they forage  
213 less selectively in comparison to high-yielding breeds. Indeed, Pauler et al. showed in 2019 that tree and  
214 shrub species cover decreased under Highland cattle grazing in comparison to production-oriented  
215 breeds, suggesting their higher feeding preference for these woody species. In 2020, Pauler et al. (2020b)  
216 demonstrated that Highland cattle forage behaviour in semi-natural pastures in Switzerland was less  
217 influenced by some particular plant functional traits in comparison to Angus and Holstein crossbreeds  
218 or Braunvieh breed. More specifically, while high-production oriented breeds avoided plants with higher  
219 C:N ratio and leaf dry matter content, Highland cattle did not withdraw them from their diet (Pauler et  
220 al. 2020b). This suggests that they excluded a smaller range of plant species and they grazed on less  
221 palatable species. Moreover, Highland cattle may be able to break branches through rubbing with their  
222 long horns, as well as to apply wounds in trunks and roots through trampling, which could be additional  
223 ways to weaken shrub species (Della Marianna et al. 2012). Indeed, trunk, branches and root wounds



224 could promote fungal infection, as fungi species have been recorded to effectively affect alder species.  
225 Brasier et al. (2004) described different variants of the pathogen species *Phytophthora alni* that attack  
226 alder species across Europe, and Pisetta et al. (2012) identified the spread of *Cryptodiaporthe (Valsa)*  
227 *oxystoma* (Rehm) Urb in *A. viridis* stands in the Italian Alps, which spreads on necrotic bark along with  
228 other fungi species, such as *Peniophora aurantiaca* (Bres.) Höhn. & Litsch. Most of these pathogens  
229 spread through water (Bjelke et al. 2016), so that a combination of wet weather conditions and lesions  
230 applied to the shrub could help spread the disease to the stands and reduce their expansion. In addition,  
231 Highland cattle are characterized by rather large claw size relatively to their body weight, which reduces  
232 the pressure they exert on the vegetation (Pauler et al. 2020a). Being among the smallest cattle breed,  
233 with 24 kg at birth and generally not exceeding 500 kg at adult age (Pauler et al. 2020a; Radkowski et  
234 al. 2022), their low weight allows them to graze in steep and wet areas. As well as their adaptative  
235 digestive system, this feature was probably gained from the harsh and wet environmental conditions of  
236 the Scottish Highlands (Pauler et al. 2020a). Therefore, this ability could be beneficial in steep slopes  
237 where *A. viridis* is usually found, and where the manual cutting is even more hardly feasible.  
238 Additionally, their robustness drastically reduces any veterinary care compared to other breeds, which  
239 makes them well adapted to remote mountain areas. Finally, their long fur may enable the epizoochoric  
240 transportation of seeds which could positively affect the botanical composition and help restore former  
241 species-rich grasslands (Pauler et al. 2019).

242

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

247



248

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

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## 253 ***Alnus viridis* forage potential**

254

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

260

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

268

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

279

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

284

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

288

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## 290 **Highland cattle foraging behaviour**

291

292 Livestock feeding preferences are widely affected by plant species forage quality and availability.  
293 Indeed, the content in nutrients, fibre, and phenols are essential factors for leaf palatability and  
294 digestibility, and therefore drive herbivore diet selection (Leng 1997; Collins et al. 2017; Ravetto Enri  
295 et al. 2020; Mahieu et al. 2021). While woody species such as *A. viridis* could be a good forage resource  
296 for robust livestock, its voluntary intake by Highland cattle may not be assured under all conditions.  
297 Indeed, the palatability of plant species is relative to their abundance and the abundance of the  
298 surrounding plant species (Pauler et al. 2020b). In pastures, cattle choices are primarily driven by good  
299 quality forage, as it provides the most important resource for daily needs (Pauler et al. 2020b). Moreover,

300 throughout the season, changes in livestock feeding behaviour in grazing systems can also happen,  
301 following the temporal changes in forage yield and quality (Bailey and Brown 2011). Therefore, it is  
302 important to estimate the intake of woody plants by Highland cattle to fully understand to what extent  
303 they can effectively impact shrub encroachment. In addition to the evaluation of their damage on shrub  
304 species through defoliation, the knowledge of their forage preferences under different conditions could  
305 be integrated into management strategies and help provide a good quality nutrition for animals, while  
306 keeping an efficient impact on shrub encroached areas. Even if the understanding of the feeding  
307 preference in woody-dominated areas is rather difficult because of the heterogeneity of the vegetation,  
308 direct observations are a good way to understand feeding preferences and behaviour in heterogeneous  
309 habitats, and they have indeed been commonly used in shrub encroached environments (Elias and  
310 Tischew 2016; Nota et al. 2023).

311

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

319

320

### 321 **Tracking cattle behaviour and distribution using GPS technology**

322

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

346



347  
 348 **Figure 5** (A) GPS Tellus collar for livestock (©Followit); (B) Highland cattle wearing a GPS collar.  
 349

350

### 351 **Targeted grazing**

352

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

376  
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## 378 **Nitrogen translocation in *A. viridis*-encroached pastures**

379

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

406

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

420

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

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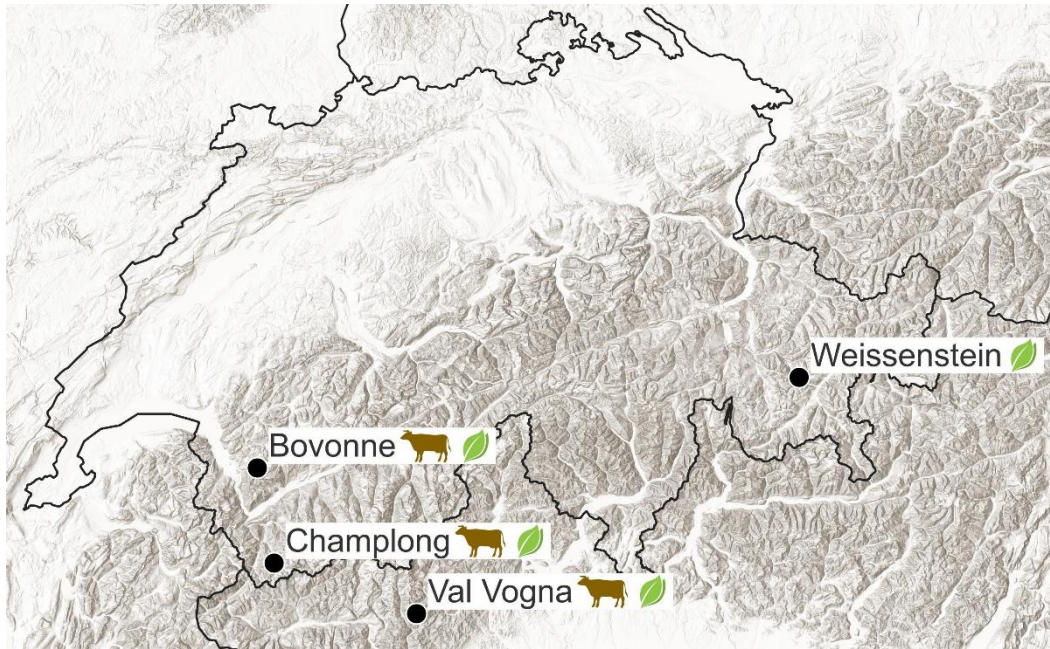
## 426 Study sites

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428 This PhD thesis was carried out in the framework of the RosbustAlps project, between 2019 and  
429 2023, in four study sites across the Swiss and Italian Alps, where *A. viridis* encroached former open  
430 pastures. The sites corresponded to different pedo-climatic conditions with various degrees of *A. viridis*  
431 encroachment. Three of the four sites were grazed by Highland cattle herds, with different paddock sizes  
432 but similar livestock stocking rates (**Figure 6**). Indeed, the first paddock was located in Val Vogna, in  
433 Northern Italy, in the province of Vercelli. This site was the largest of the project and it was grazed by  
434 the largest herd (~ 80 cows) for the whole project length. It was moderately encroached by *A. viridis*  
435 shrubs and had the lowest *A. viridis* cover (20%). The second paddock (summer pasture of “Champlong”)  
436 was located in Western Switzerland, in the canton of Valais, municipality of Bourg-Saint-Pierre. It was  
437 the smallest paddock of the project and was therefore grazed by the smallest herd (12 cows). *A. viridis*  
438 encroachment was of 44%. This paddock was only grazed by Highland cattle during 2019 but served as  
439 a sampling site for *A. viridis* leaves throughout the project. Three other paddocks were placed in the site  
440 of Bovonne, in Western Switzerland, in the canton of Vaud, municipality of Bex. They were grazed by  
441 the same herd of approximately 30 cows every year of the project. Each of these paddocks had a different  
442 degree of *A. viridis* encroachment, from 51 to 71%. Finally, the last site “Weissenstein” was located in  
443 Eastern Switzerland, in the canton of Grisons, municipality of Bergün. This site was not grazed by  
444 Highland cattle and only served as a sampling site for *A. viridis* leaves to provide a larger sample pool  
445 across the Alps.

446

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



456  
 457 **Figure 6** Localisation of the four study sites across Switzerland and Italy. All sites were used for leaf  
 458 sampling in 2020 and 2021. Paddocks with Highland cattle herds were implemented in Bovonne (3  
 459 paddocks, 2019-2023), Val Vogna (1 paddock, 2019-2023) and Champlong (1 paddock, 2019).

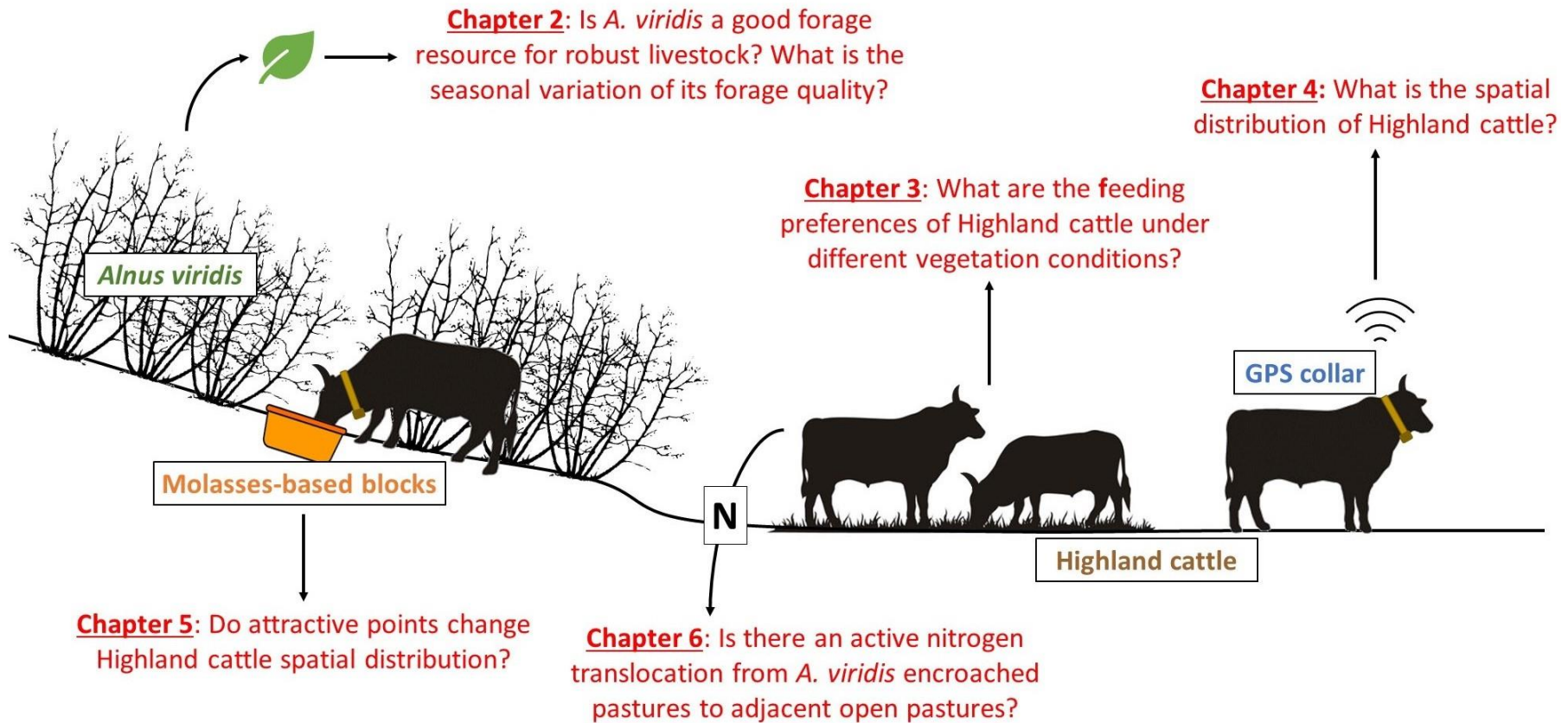
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## 462 Objectives

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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.

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



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487 **Figure 7** Summary of the objectives of the RobustAlps project



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

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

535 **Abstract**

536

537 *Alnus viridis* is a shrub species that has been increasing in many European mountains over the past  
538 decades, leading to a loss of agricultural areas and several negative environmental impacts. Recently,  
539 targeted livestock management systems have been investigated to reduce its encroachment. This study  
540 aims to provide an exhaustive assessment of *A. viridis* leaf composition and its temporal variation across  
541 the grazing season. *A. viridis* leaves were collected throughout the summers of 2020 and 2021 in four  
542 encroached sites across the Swiss and Italian Alps, characterized by different pedo-climatic conditions.  
543 Based on the data collected by meteorological stations, the growing degree days (GDD) were calculated  
544 for each site and year. The leaf functional traits (i.e., Leaf Dry Matter Content – LDMC – and Specific  
545 Leaf Area – SLA), chemical composition (macro- and micro-elements and fibre content), phenolic  
546 content, in vitro organic matter digestibility (IVOMD), and related gas production (CO<sub>2</sub> and CH<sub>4</sub>) were  
547 assessed. The LDMC significantly increased throughout the season, whereas the SLA decreased. All  
548 macro- and micro-elements significantly varied during the season, with leaf nitrogen (N) and phosphorus  
549 (P) decreasing. In contrast, leaf fibre contents significantly increased as the season advanced. There was  
550 a significant decrease in total phenol and total tannin content during the summer season. Finally, adding  
551 *A. viridis* leaves (20% dry matter) to cattle diets significantly reduced IVOMD, methane produced per  
552 digested organic matter (CH<sub>4</sub>/dOM) and CO<sub>2</sub>/dOM, compared to a 100% hay diet. Moreover, CH<sub>4</sub>/dOM  
553 and CH<sub>4</sub>/CO<sub>2</sub> ratio increased during the summer season. These results highlight the potential of *A. viridis*  
554 leaves as a valuable forage resource, especially at the beginning of the summer. Such information could  
555 be used to optimize grazing of robust livestock in *A. viridis*-encroached alpine pastures in order to reduce  
556 its invasion and minimize greenhouse gases production at the same time.

## 557 Introduction

558  
559 Due to agricultural land abandonment in the most marginal areas, tree and shrub-encroachment has  
560 strongly increased in European mountains in the last century (MacDonald et al. 2000; Orlandi et al.  
561 2016). This trend has caused the loss of grassland areas in alpine regions, with a reduction in landscape  
562 diversity and aesthetic value (Strebel and Bühler 2015; Schirpke et al. 2016). *Alnus viridis* (Chaix) DC  
563 is one of the most rapidly spreading shrub species in several European mountain chains (Boscutti et al.  
564 2014; Caviezel et al. 2017; Skoczowski et al. 2021), thanks to high reproduction and growth (Wiedmer  
565 and Senn-Irlet 2006). Its expansion can create unfavourable agro-environmental conditions and the  
566 reduction of key ecosystem services. Indeed, in addition to the reduction of forage yield due to grassland  
567 loss, it also prevents forest succession and does not provide protection from natural hazards such as  
568 avalanches (Caviezel et al. 2014; Faccioni et al. 2019). Moreover, its ability to fix nitrogen (N) due to  
569 the symbiosis with the N<sub>2</sub>-fixing actinomycete *Frankia alni* can lead to N saturation in soils (Bühlmann  
570 et al. 2016). This increases risks of nitrate leaching and emissions of greenhouse gasses, such as nitrous  
571 oxide, and reduces animal and plant biodiversity (Bühlmann et al. 2017; Zehnder et al. 2020). While *A.*  
572 *viridis* is mostly found on northern and west facing slopes, it is predicted to expand to a wider range of  
573 environmental conditions due to its observed occurrence in new habitats (Skoczowski et al. 2021).

574  
575 Since it is costly and difficult to control its spread through manual cuts, recent studies have  
576 investigated the potential of robust livestock to forage on *A. viridis* and reduce its encroachment. For  
577 example, (Pauler et al. 2022b) showed that Dexter cattle, Pfauen goats and Engadine sheep can cause  
578 significant damage to *A. viridis* shrubs. Indeed, cattle were able to break branches and trample on young  
579 shrubs, while goats and sheep foraged on and debarked the plants, with Engadine sheep being the most  
580 effective livestock in clearing encroached areas. Other robust breeds, such as Highland cattle, were also  
581 evaluated and shown to be efficient in opening dense *A. viridis* stands (Svensk et al. 2022). While it was  
582 observed that some species and breeds are thus able to feed on *A. viridis* shrubs, it is essential to assess  
583 the full potential of this plant species as a forage resource for livestock. Tree and shrub species can have  
584 high potential for ruminant nutrition (Luske and van Eekeren 2017), as they maintain higher nutrient  
585 content during the late summer season compared to herbaceous species (Papachristou and Papanastasis  
586 1994; Ravetto Enri et al. 2020). Therefore, shrubs such as *A. viridis* could become an interesting forage  
587 supplement and help compensate for animal feeding during the summer and periods with forage  
588 shortage. Bühlmann et al. (2016) measured the N concentration of *A. viridis* leaves at different elevations  
589 (1650 m and 1950 m) and found slightly higher N concentration at higher elevations. Another study  
590 assessed the biomass production of *A. viridis* (Wiedmer and Senn-Irlet 2006), and Stević et al. (2010)  
591 measured the total tannin content of *A. viridis* leaves ( $4.4 \pm 0.4\%$ ), which is an important factor in leaf  
592 digestibility assessment. However, to the best of our knowledge, no study has ever conducted an  
593 exhaustive analysis of *A. viridis* leaf functional traits, chemical composition, and nutritive value across  
594 the whole summer season. This aspect is crucial to fully understand the forage potential of *A. viridis* and  
595 to identify the ideal period for it to be grazed. Several studies have highlighted the importance of  
596 assessing leaf characteristics at different development stages to better estimate the nutritive value for  
597 livestock. For example, Pauler et al. (2020b) pointed out the significant impact of leaf functional traits,  
598 such as specific leaf area (SLA) and leaf dry matter content (LDMC), on cattle foraging behaviour,  
599 where larger leaves (i.e., with higher SLA) were preferred for grazing, while leaves with higher LDMC  
600 were avoided. Other studies found that these plant traits impacted digestibility, as smaller and denser  
601 leaves reduced ruminal degradation (Pontes et al. 2007). Pauler et al. (2020b) also found that leaves with  
602 higher N and phosphorus (P) contents were more often selected by cattle, and other research highlighted  
603 a significant impact of primary and secondary leaf compounds on forage selection, digestibility, and  
604 animal health (Leng 1997; Collins et al. 2017; Ravetto Enri et al. 2020; Mahieu et al. 2021). The impact

605 of phenolic compounds on forage quality is also well known, as high tannin and non-tannin phenolic  
606 concentrations tend to reduce palatability and digestibility (Sunnerheim et al. 1988; Kumar and  
607 Vaithiyathan 1990). Finally, leaf neutral detergent fibre (NDF) content is an essential factor for  
608 livestock digestibility as well, and has previously been found to increase throughout the growing season  
609 for woody fodder species (Papachristou and Papanastasis 1994). Moreover, since livestock production  
610 is one of the most important contributors to direct greenhouse gas emissions, especially methane (CH<sub>4</sub>)  
611 production from ruminants (Slade et al. 2016), it is relevant to investigate the potential mitigating effect  
612 of *A. viridis* on cattle digestion and CH<sub>4</sub> emissions. Previous studies investigated the impact of the  
613 inclusion of woody plants on forage intake, nutrient digestibility, and gas production (Terranova et al.  
614 2018, 2019), but the impact of *A. viridis* leaves in livestock diet has not been assessed yet, hampering  
615 the implementation of livestock management systems to control *A. viridis* expansion.

616  
617 To fill these knowledge gaps, this study aimed to assess the temporal variation in *A. viridis* leaf  
618 functional traits, chemical composition and *in vitro* digestibility and the impact on gas production when  
619 they are added to cattle diet. Specifically, the objectives of this study were to assess: (1) the functional  
620 traits (LDMC and SLA), (2) the chemical composition (macro/micro- elements and fibre fractions), (3)  
621 the phenol composition, (4) the *in vitro* organic matter digestibility (IVOMD) and gas production of *A.*  
622 *viridis* leaves, as well as (5) the relationships among all measured leaf characteristics. We expected that  
623 *A. viridis* leaf functional traits, chemical composition, IVOMD, and gas production varied across the  
624 season, with a loss of leaf forage quality as the season advanced. We also expected the IVOMD and  
625 related gas production of a livestock diet partially composed by *A. viridis* leaves to be lower than that  
626 of a control diet purely composed of hay.

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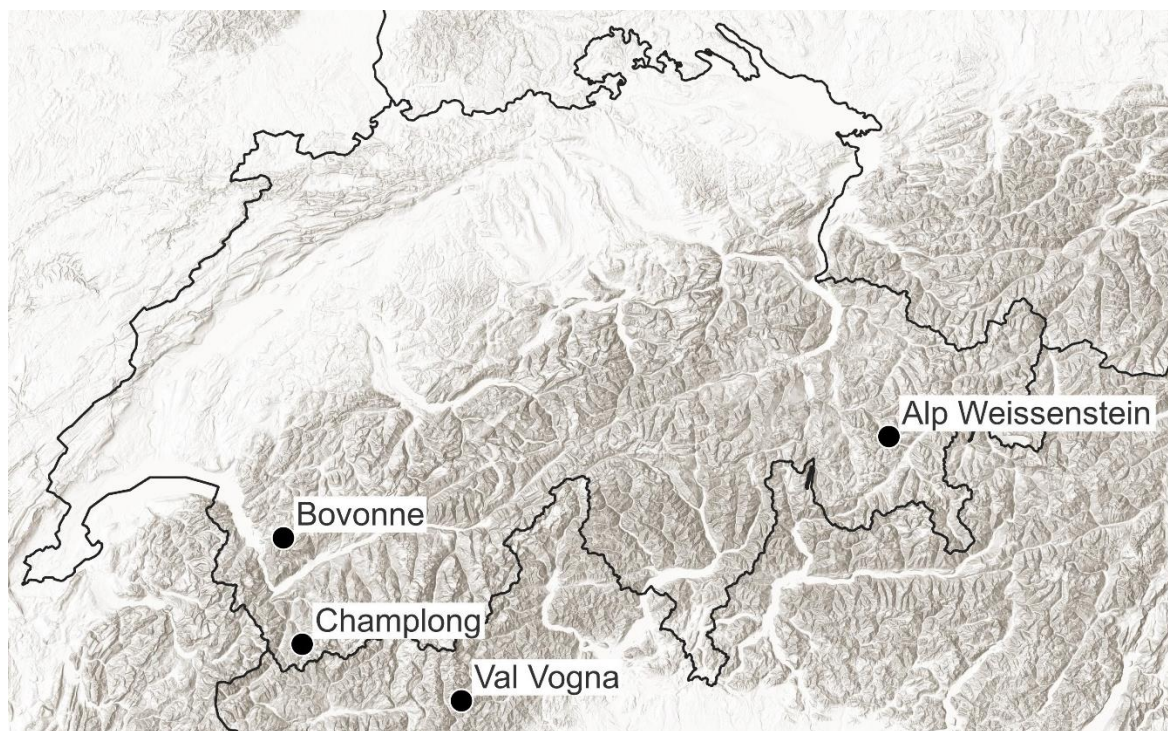
## 629 **Methods**

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### 631 **1. Study sites**

632

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



642  
643 **Figure 8** Location of the four sites of the study in the Swiss and Italian Alps. Basemap: ESRI Terrain.  
644

645  
646 **Table 1** Characteristics of the four experimental sites. Soil data refer to the laboratory analyses of the  
647 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 <sup>a</sup>	1674 <sup>b</sup>	1023 <sup>b</sup>	1200 <sup>b</sup>
Mean annual temperature (°C)	7.46 <sup>c</sup>	4.51 <sup>b</sup>	4.21 <sup>b</sup>	1.79 <sup>b</sup>
Soil total organic carbon (TOC mg kg <sup>-1</sup> )	548.53 ± 37.48	282.04 ± 42.45	483.89 ± 70.93	370 ± 33.37
Soil total nitrogen (TN mg kg <sup>-1</sup> )	56.33 ± 4.61	19.48 ± 1.21	49.02 ± 9.41	29.22 ± 3.04
Soil dissolved inorganic nitrogen (DIN mg kg <sup>-1</sup> )	16.74 ± 5.1	12.36 ± 1.1	19.35 ± 4.49	10.44 ± 1.86
Soil available phosphorus (mg kg <sup>-1</sup> )	11.27 ± 5.32	0.56 ± 0.13	9.36 ± 4.47	5.23 ± 1.77
pH (H <sub>2</sub> O)	3.84 ± 0.03	6.27 ± 0.28	5.44 ± 0.18	4.71 ± 0.22

648 <sup>a</sup>Mean annual precipitation (mm) for the period from 1977 to 2007.

649 <sup>b</sup>Mean annual precipitation (mm) or mean annual temperature (°C) for the period from 1991 to 2021.

650 <sup>c</sup>Mean annual temperature (°C) for the period from 1977 to 2007, calculated on a 10 km<sup>2</sup> grid.

651

652

653 **2. *Alnus viridis* leaf sampling**

654

655 During the summers of 2020 and 2021, 1.75 kg of *A. viridis* fresh leaves were collected three times  
656 (i.e. in June, July and August) at each site to assess the seasonal changes in leaf functional traits,  
657 chemical composition, phenolic contents, and IVOMD. For each sample, *A. viridis* leaves and petioles  
658 were hand-harvested from different points in the canopy of five different trees, at a maximum height of  
659 1.80 m to simulate the potential browsing of cattle (Svensk et al. 2022). New trees were selected for  
660 each harvest at all four sites to analyze seasonal changes without repeated harvesting, which would  
661 imply feeding pressure. Moreover, sampled *A. viridis* shrubs were chosen outside of grazed areas and  
662 they were comparable in terms of height (three to five meters high).

663

664

665 **3. Leaf functional traits**

666

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

671

$$672 \text{LDMC (mg g}^{-1}\text{)} = \text{DW(mg)} / \text{FW(g)}$$

673

674 The SLA was measured according to Cornelissen et al. (2003) using the leaves collected for LDMC  
675 described above. Before drying the leaves, we measured the surface of all 10 leaves of each sample by  
676 photocopying them and calculating their area with an image analysis software (ImageJ, Schneider et al.  
677 2012). This allowed us to calculate SLA as followed:

678

$$679 \text{SLA (cm}^2 \text{g}^{-1}\text{)} = \text{leaf surface (cm}^2\text{)} / \text{DW (g)}$$

680

681

682 **4. Leaf chemical composition**

683

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

696

697 Fibre fractions were analysed according to Van Soest et al. (1991). The neutral detergent fiber  
698 (aNDF<sub>OM</sub>) and acid detergent fiber (ADF<sub>OM</sub>) contents (ISO 16472:2006 for NDF and ISO 13906:2008  
699 for ADF) were determined gravimetrically (ISO 6865:2000) after acid and alkaline digestions using a  
700 fibre analyser (Fibretherm Gerhardt FT-12, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) and

701 were expressed without residual ash after incineration at 600°C for 3 h. The aNDF<sub>OM</sub> was evaluated with  
702 heat stable  $\alpha$ -amylase and sodium sulfite. Acid detergent lignin (ADL) was analysed according to ISO  
703 13906:2008. Hemicellulose and cellulose concentrations were estimated as aNDF<sub>OM</sub> minus ADF<sub>OM</sub> and  
704 ADF<sub>OM</sub> minus ADL, respectively.

705  
706

## 707 **5. Leaf phenolic compounds**

708

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

715  
716

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

718

719 The assessment of IVOMD and gas production was made using the *in vitro* incubation with the  
720 Hohenheim gas test (HGT) method, which was performed as outlined by Menke and Steingass (1988).  
721 For each sampling date, the remaining leaves from the five trees were pooled in one sample, ending up  
722 in 12 samples per year. *A. viridis* leaves were freeze dried and ground with a centrifugal mill (Model  
723 ZM1, Retsch GmbH, Haan, Germany) to pass through a 1 mm sieve. The incubation of *A. viridis* leaves  
724 was performed by combining the leaves with hay (ryegrass dominated sward, crude protein = 11.63%;  
725 NDF = 48.35%), in a ratio of 1:4 on a DM basis. This proportion was chosen to simulate the diet of  
726 Highland cattle in *A. viridis*-encroached pastures, as described in Svensk et al. (2023). A diet of hay  
727 (100%) served as the control. For each year, rumen fluid was collected from three ruminally-cannulated  
728 multiparous late lactating Original Braunvieh cows, and was collected before morning feeding three  
729 times, across three weeks in both 2020 and 2021. It was then transported in a pre-heated thermos flask  
730 to the laboratory. Within one hour after collection, rumen fluid was strained through four layers of gauze  
731 and added to a buffer solution in a 1:2 ratio according to the protocol of Menke and Steingass (1988).  
732 For incubation, modified 100-ml glass syringes with two outlets, one for fluid and one for gas sampling,  
733 were used as described in Soliva and Hess (2007). The incubation lasted for 24 h at 39 °C in an incubator  
734 with an integrated rotor. After 24 h, the fermentation gas volume was recorded from the calibrated scale  
735 printed onto the syringes, and the fermentation was terminated by removing the incubation fluid from  
736 the syringes while the gas phase remained inside. Fermentation gas samples of 150  $\mu$ l were taken from  
737 the incubation syringes and injected using a gas-tight Hamilton syringe (Hamilton AG, Bonaduz,  
738 Switzerland) into a gas chromatograph (6890N, Agilent Technologies, Wilmington, DE, USA) equipped  
739 with a thermal conductivity detector. Concentrations of CH<sub>4</sub> and CO<sub>2</sub> were analysed with this detector,  
740 and expressed per digested organic matter (dOM). Each pooled sample and the control were incubated  
741 in duplicates per run resulting in a total number of n = 6 per each year. Two syringes filled with only  
742 rumen fluid buffer mixture were incubated as blank.

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## 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):

$$GDD = \sum_{i=1}^n \left[ \left( \frac{T_{max} + T_{min}}{2} \right) - T_{base} \right]$$

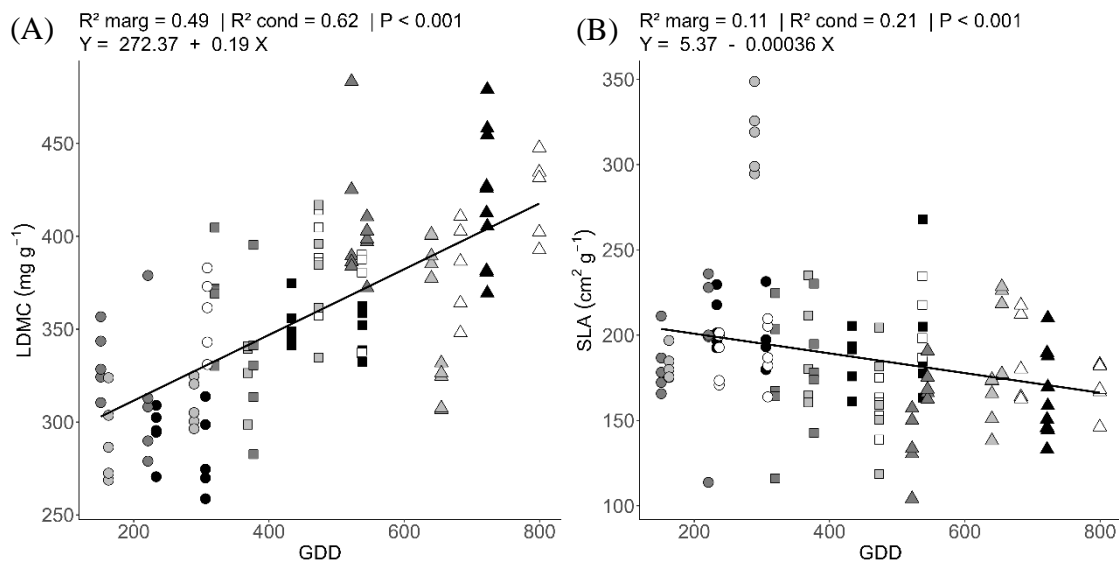
where  $i = n$  are the days with a temperature above 0°C,  $T_{max}$  and  $T_{min}$  are the daily maximum and minimum temperature respectively, and  $T_{base}$  is a threshold temperature defined here at 5°C following the description for pasture grasses from Grigorieva et al. (2010). The GDD was then 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<sup>-1</sup> (**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<sup>2</sup> g<sup>-1</sup>.



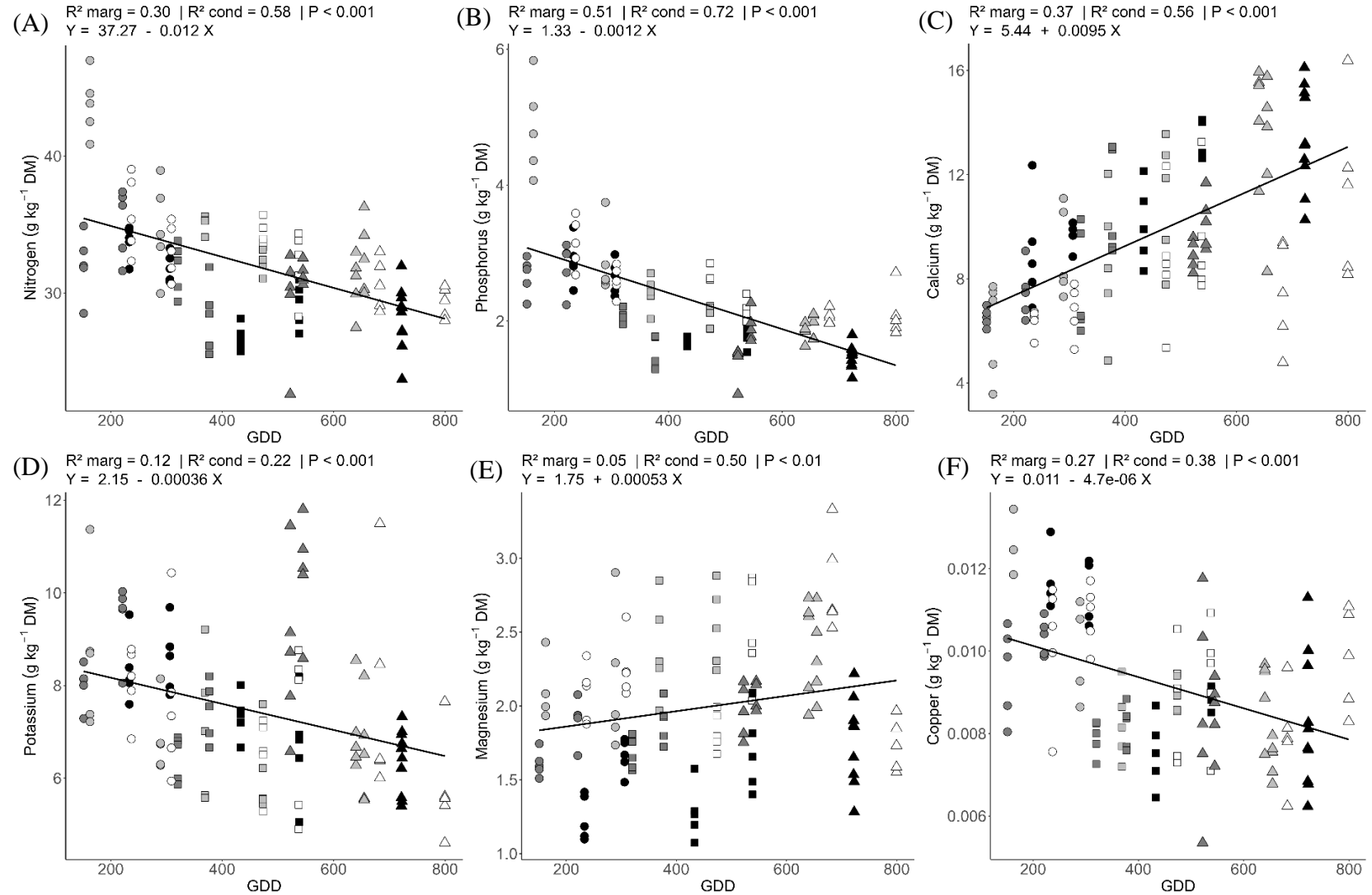


786  
 787 **Figure 9** Variation of (A) Leaf Dry Matter Content (LDMC) and (B) Specific Leaf Area (SLA) in  
 788 relation to growing degree days (GDD) in Val Vogna (white), Bovonne (black), Champlong (dark grey)  
 789 and Weissenstein (light grey). Sampling periods are represented with different shapes as circles (June),  
 790 squares (July) and triangles (August).

791  
 792  
 793 **2. Leaf chemical composition**

794  
 795 All macro- and micro-elements of *A. viridis* leaves were significantly affected by seasonal  
 796 temperature changes. Indeed, N, P, K, Cu and Zn all significantly decreased with increasing GDD  
 797 (**Figure 10A, B, D, F, I**,  $P < 0.001$ ). In contrast, Ca, Mn, Fe and Mg significantly increased with GDD  
 798 (**Figure 10C, E, G, H**). The N and P contents ranged from 46.98 to 22.62 g kg<sup>-1</sup> DM and from 5.84 to  
 799 0.92 g kg<sup>-1</sup> DM, respectively. Therefore, leaf crude protein content had minimum and maximum values  
 800 of 141.37 g kg<sup>-1</sup> DM and 293.63 g kg<sup>-1</sup> DM, respectively. Residual ash ranged from 32.07 g kg<sup>-1</sup> DM to  
 801 62.79 g kg<sup>-1</sup> DM, and increased significantly with GDD ( $P < 0.001$ ).

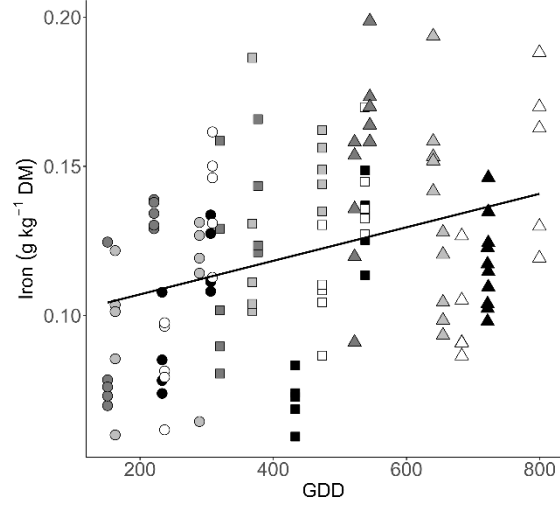
802  
 803 The fibre content of *A. viridis* leaves, aNDF<sub>OM</sub>, aADF<sub>OM</sub>, and ADL concentrations significantly  
 804 increased with increasing GDD (**Figure 11**,  $P < 0.01$ ), ranging from 202.77 to 454.59 g kg<sup>-1</sup> DM, from  
 805 116.14 to 353.46 g kg<sup>-1</sup> DM and from 41.61 to 257.28 g kg<sup>-1</sup> DM, respectively. *A. viridis* leaves had a  
 806 hemicellulose content of 124.8 g kg<sup>-1</sup> DM, and a cellulose content of 97.4 g kg<sup>-1</sup> DM on average.



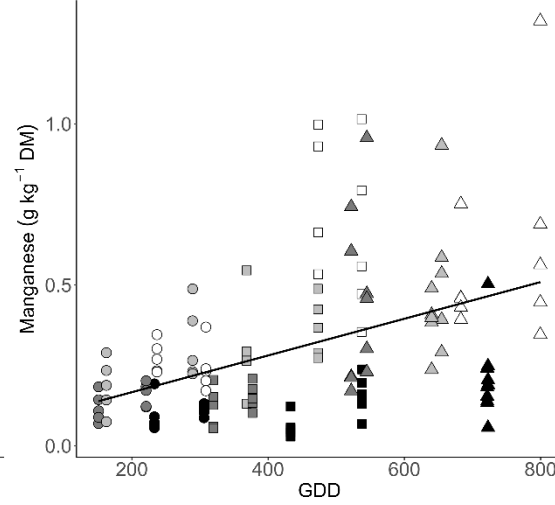
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 810

**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).

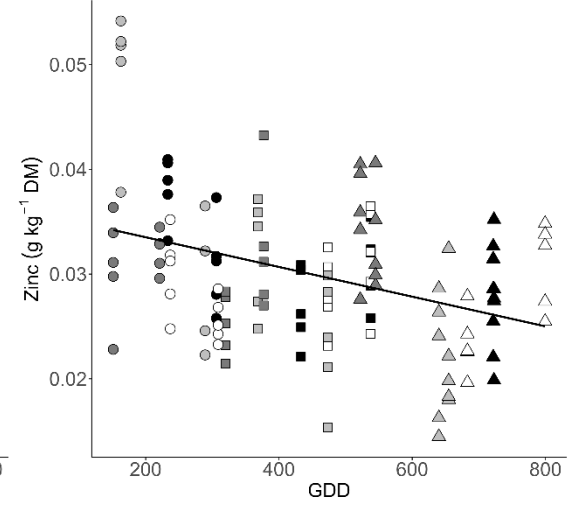
(G)  $R^2 \text{ marg} = 0.20$  |  $R^2 \text{ cond} = \text{NA}$  |  $P < 0.001$   
 $Y = 0.095 + 5.7\text{e-}05 X$



(H)  $R^2 \text{ marg} = 0.20$  |  $R^2 \text{ cond} = 0.62$  |  $P < 0.001$   
 $Y = -2.29 + 0.0019 X$

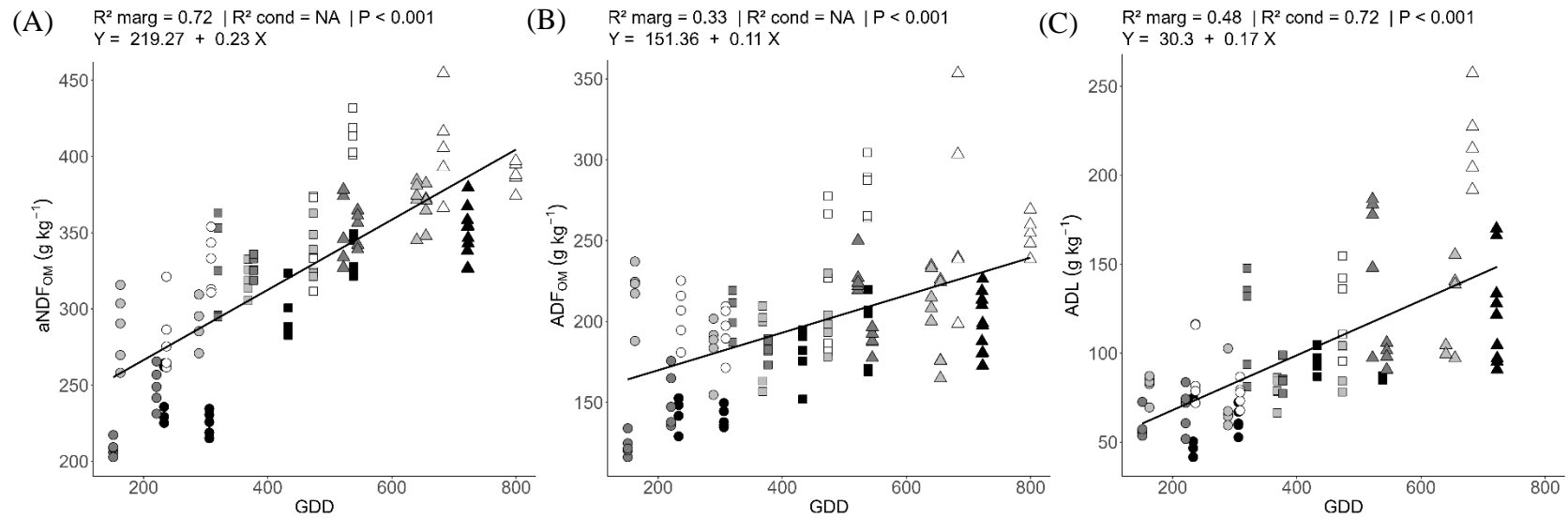


(I)  $R^2 \text{ marg} = 0.14$  |  $R^2 \text{ cond} = \text{NA}$  |  $P < 0.001$   
 $Y = 0.036 - 1.4\text{e-}05 X$



811

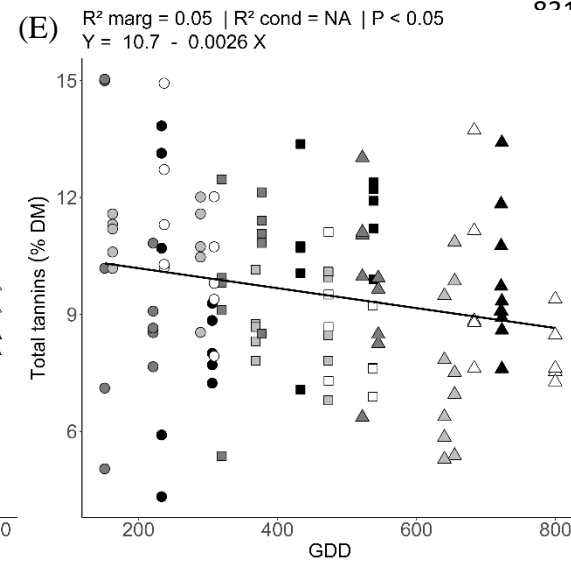
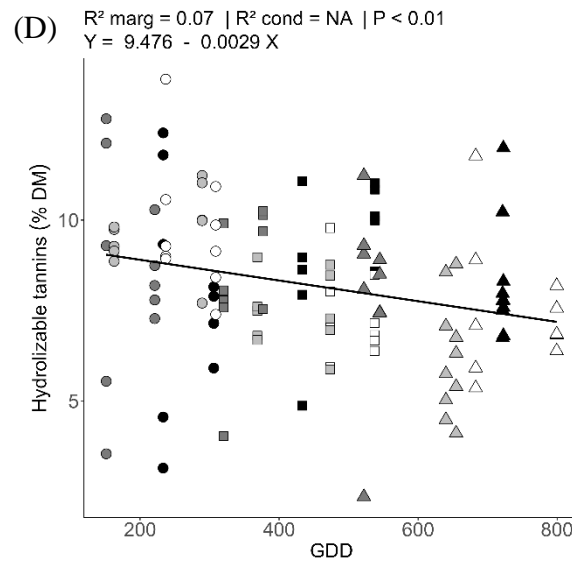
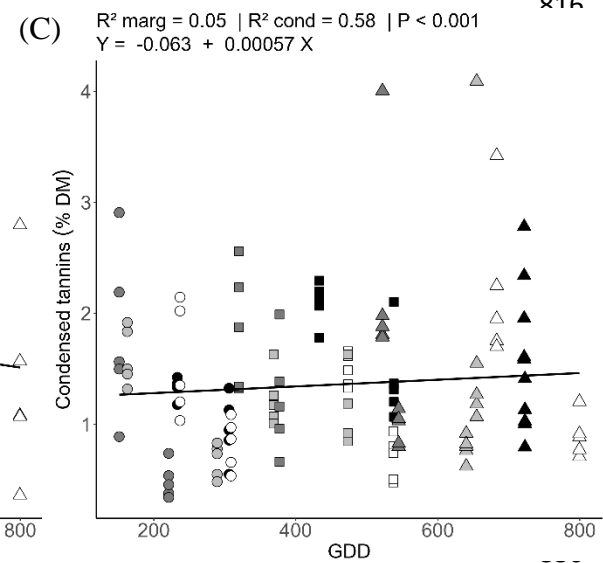
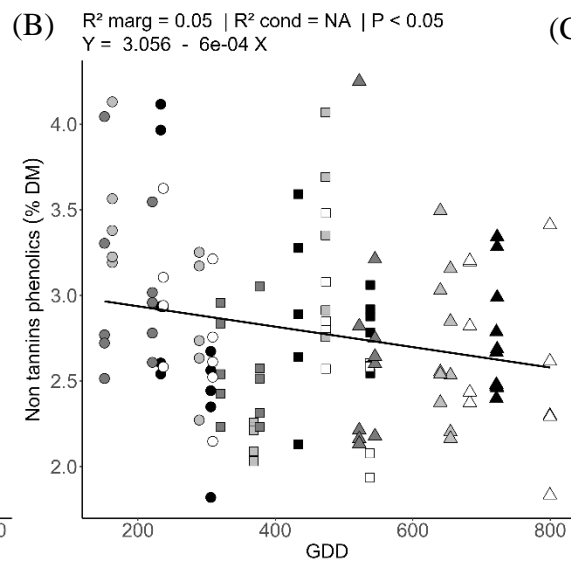
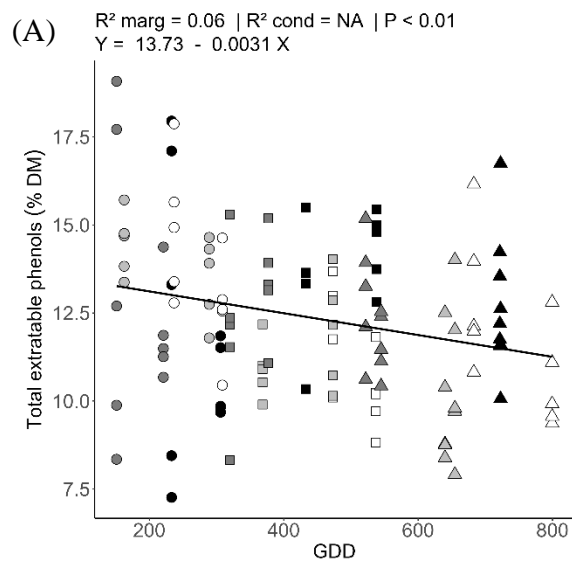
812 **Figure 10** continued



813

814 **Figure 11** Variation of aNDF<sub>OM</sub> (A), ADF<sub>OM</sub> (B) and ADL (C) in relation to growing degree days (GDD) in Val Vogna (white), Bovonne (black), Champlong

815 (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) hydrolyzable 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).

841 **3. Leaf phenolic compound**

842

843 While having the weakest relationships with GDD compared to other leaf compounds, all phenolic  
844 compounds measured in *A. viridis* leaves significantly changed over the season (**Figure 12**). The TEP  
845 significantly decreased with GDD (**Figure 12A**), as did the non-tannin phenolics (NTP, **Figure 12B**).  
846 Leaf TEP ranged from 19.08% DM to 7.26% DM, and NTP ranged from 4.25% DM to 1.82% DM.  
847 Condensed tannins (CT) slightly increased with GDD (**Figure 12C**), from 0.34% DM to 4.09% DM. In  
848 contrast, HT and TT concentrations in the leaves decreased (**Figure 12D, E**), with values ranging from  
849 13.90% DM to 2.36% DM and 15.03% DM to 4.32% DM, respectively.

850

851

852 **4. Leaf in vitro organic matter digestibility and gas production**

853

854 Including 20% of *A. viridis* leaves in the hay diet for in vitro incubation, reduced the IVOMD by  
855 5.80% on average (**Figure 13A**), with an average digestibility of  $63.30 \pm 0.19\%$  compared to  $69.10 \pm$   
856  $0.46\%$  for the control. The IVOMD of the *A. viridis*-composed diet also significantly decreased over the  
857 season (**Figure 13A**), from a maximum value of 66.80% to a minimum value of 60.11%.

858

859 The amount of CH<sub>4</sub> produced per unit of digestible OM (CH<sub>4</sub>/DOM) was reduced on average by  
860 17.73% when adding *A. viridis* leaves (**Figure 13B**, Student's *t*-test  $P < 0.01$ ), with a mean value of  
861  $43.83 \pm 0.48$  ml g<sup>-1</sup>, compared to  $53.27 \pm 0.46$  ml g<sup>-1</sup> of the control. It significantly increased with GDD  
862 (**Figure 13B**), ranging from 34.54 ml g<sup>-1</sup> to 49.86 ml g<sup>-1</sup>.

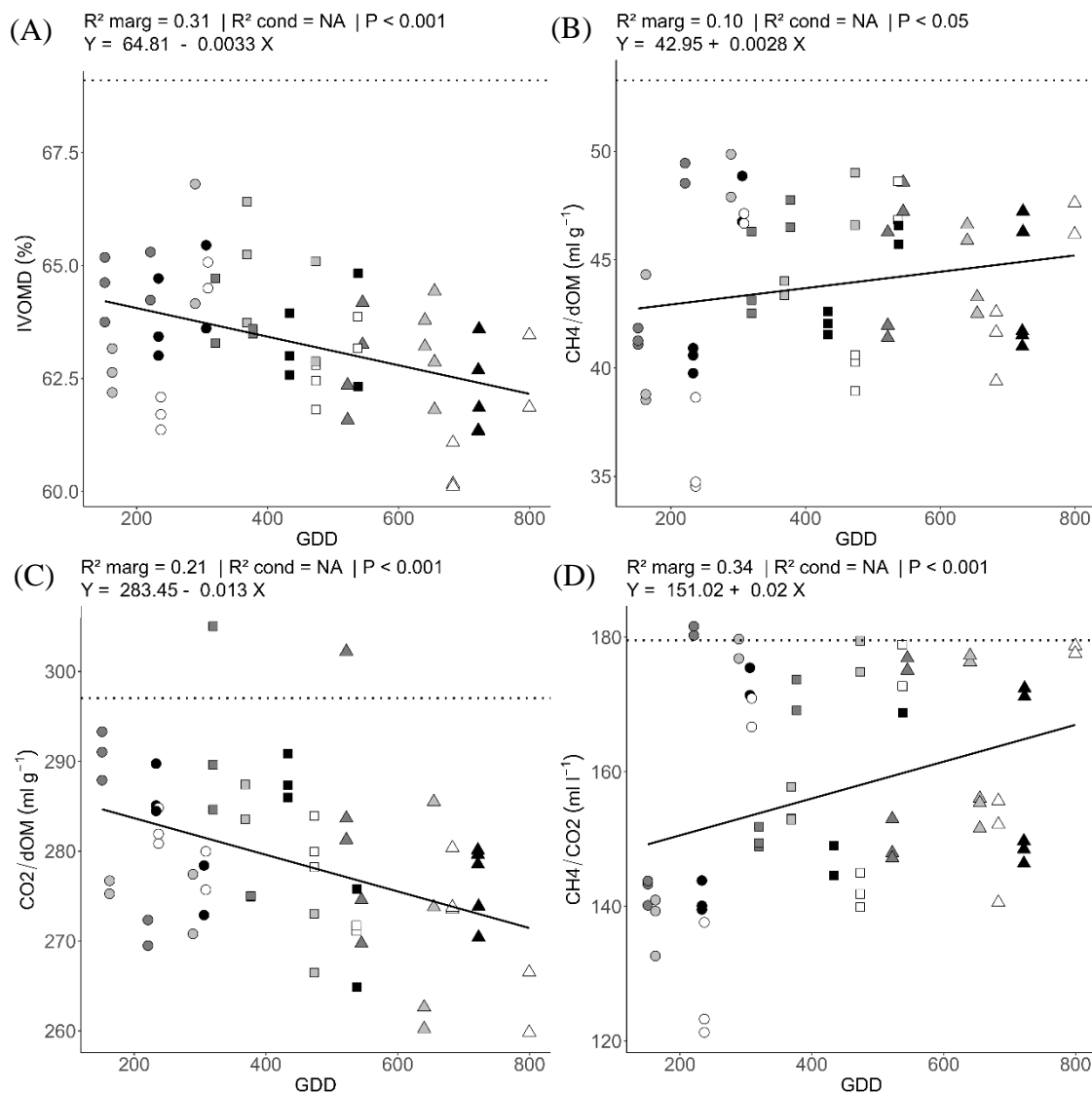
863

864 The amount of CO<sub>2</sub> produced per unit of digestible OM (CO<sub>2</sub>/DOM) followed an opposite trend,  
865 with a significant reduction with the seasonal increase in GDD (**Figure 13C**). It ranged from 305.03 to  
866 259.84 ml g<sup>-1</sup>, and was also significantly reduced (by 5.84%) compared to the control (**Figure 13C**,  
867 Student's *t*-test  $P < 0.01$ ), with average values of  $278.74 \pm 0.22$  ml g<sup>-1</sup> and  $297.03 \pm 2.09$  ml g<sup>-1</sup>,  
868 respectively.

869

870 Therefore, the CH<sub>4</sub>/CO<sub>2</sub> ratio obtained from the *A. viridis* diet was also significantly reduced  
871 compared to the control (**Figure 13D**, Student's *t*-test  $P < 0.01$ ), and increased significantly with GDD,  
872 ranging from 121.28 to 181.62 ml l<sup>-1</sup> (**Figure 13D**).

873

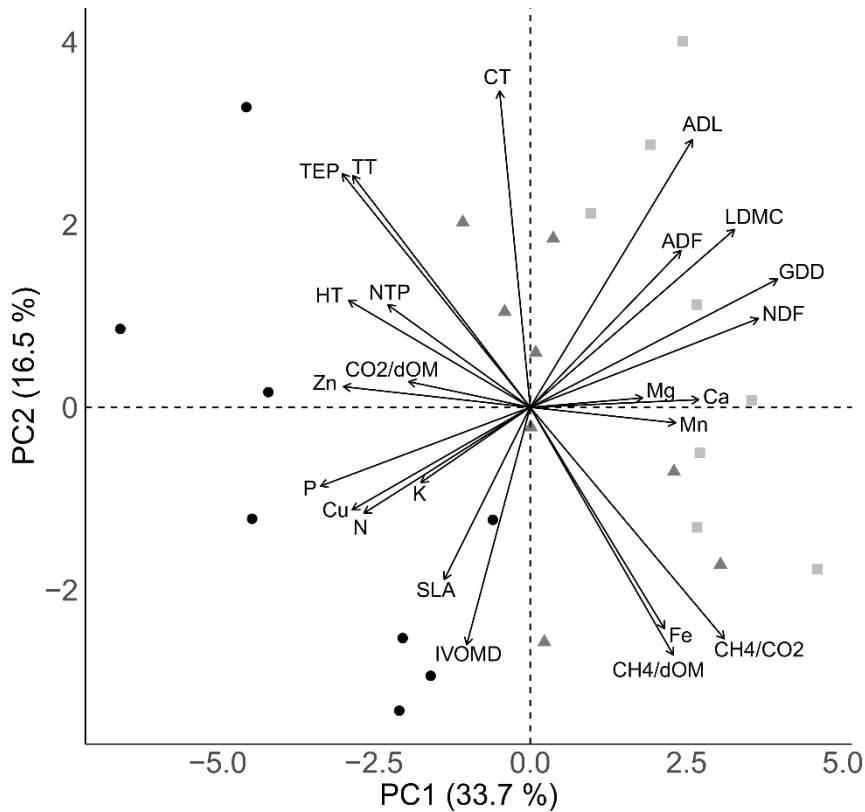


874  
 875 **Figure 13** Variation of (A) in vitro organic matter digestibility (IVOMD), (B) methane per digested  
 876 organic matter ( $\text{CH}_4/\text{dOM}$ ), (C) carbon dioxide per digested organic matter ( $\text{CO}_2/\text{dOM}$ ) and (D) the ratio  
 877 between methane and carbon dioxide ( $\text{CH}_4/\text{CO}_2$ ) in relation to growing degree days (GDD) in Val Vogna  
 878 (white), Bovonne (black), Champlong (dark grey) and Weissenstein (light grey). Sampling periods are  
 879 represented with different shapes as circles (June), squares (July) and triangles (August). The dotted line  
 880 represents the control for comparison, i.e. the diet composed by 100% hay.

881  
 882

### 883 5. Principal component analysis

884  
 885 The results of the PCA are presented in **Figure 14**. The first PCA axis (PC1, accounting for 33.7%  
 886 of the variance) reflected the seasonal changes in *A. viridis* leaves, with increasing GDD, NDF, LDMC,  
 887 and Ca, and decreasing P, HT, Zn, Cu and N. The second axis (PC2, accounting for 16.5% of the  
 888 variance) reflected a gradient of increasing CT, ADL, TEP and TT, and decreasing  $\text{CH}_4/\text{dOM}$ , IVOMD,  
 889  $\text{CH}_4/\text{CO}_2$  and Fe. The PCA grouped the different phenolic compounds together, as well as the fibre  
 890 fractions.  
 891



892  
 893 **Figure 14** Principal component analysis (PCA) for *A. viridis* leaf functional traits, macro- and micro-  
 894 elements, fibre fractions, phenolic compounds, gases per digested organic matter, in vitro organic matter  
 895 digestibility, and the GDD. The samples are represented in black dots (June), dark grey triangles (July)  
 896 or light grey squares (August).

897  
 898

## 899 Discussion

900

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

904  
 905

### 906 1. Leaf functional traits

907

908 Throughout the season, we found similar SLA and LDMC values for *A. viridis* compared to  
 909 previous studies (Skoczowski et al. 2021) and compared to other alder species (Pierce et al. 1994). As  
 910 we expected, *A. viridis* leaf functional traits varied during the season, and leaf SLA and LDMC changed  
 911 in opposite directions, leading to denser leaves with higher dry matter content as the season advanced.  
 912 This trend was supported by the PCA that showed these two functional traits following opposite patterns,  
 913 with LDMC positively correlated to GDD. A study on *A. glutinosa* made by De Kort et al. in 2014  
 914 showed similar trends, as the alder had smaller leaves with increasing temperature. Skoczowski et al.  
 915 (2021) also found variation in *A. viridis* leaf morphology according to elevation, linked to the different  
 916 climatic conditions and stressful environments. Previous studies on other woody shrubs and trees have  
 917 also provided similar results (Wilson et al. 1999; Qin et al. 2018). This pattern is explained by tree  
 918 strategies to acquire or retain resources depending on the environment. SLA and LDMC are directly  
 919 linked with leaf biomass and nutrient assimilation, and lower SLA and higher LDMC values at the end



920 of the season occur because the plant focuses on conservation of acquired resources (Qin et al. 2018;  
921 Ravetto Enri et al. 2020). Consequently, as for other forage plants, *A. viridis* leaves may be less selected  
922 by cows at the end of the summer Pauler et al. (2020b).

923  
924

## 925 2. Leaf chemical composition

926

927 The chemical composition of *A. viridis* leaves varied significantly as the season advanced. Indeed,  
928 we found similar decreasing patterns for many important leaf nutrients (N, P, K, Cu, and Zn) during the  
929 summer season. This decrease is in line with the findings on *A. glutinosa* by Rodríguez-Barrueco et al.  
930 (1984), who showed that leaf N, P, and K decreased during the vegetative season as well. Other studies  
931 on alder species (Dawson and Funk 1981; Chapin and Kedrowski 1983; Luske and van Eekeren 2017)  
932 or on other woody plants (James and Smith 1978; Niinemets and Tamm 2005; Gowda et al. 2019)  
933 detected similar trends, with the lowest values for these leaf nutrients in autumn. Our results from the  
934 PCA also highlighted tight correlations between some of these elements (i.e., positive correlations  
935 between N-P and N-K), as also shown by Rodríguez-Barrueco et al. (1984) for *A. glutinosa* leaves. The  
936 relationship between leaf N, P and K is well documented, and plays an essential role in the eco-  
937 physiological processes of the plant, contributing to photosynthesis, growth and reproduction (Tian et  
938 al. 2019). Moreover, in the case of N-fixing trees such as *A. viridis*, the N fixation leads to a higher P  
939 demand compared to non-N-fixing plants, thus creating a positive relationship between N and P  
940 (Dawson 2008). On the other hand, other *A. viridis* leaf nutrients displayed an increase with leaf  
941 senescence, such as leaf Ca, Fe, and Mn, which were also shown to increase in *A. glutinosa* leaves  
942 (Rodríguez-Barrueco et al. 1984; Luske and van Eekeren 2017). However, the increase in *A. viridis* leaf  
943 Mg was not found in *A. glutinosa* but was found in other woody plants (James and Smith 1978). The  
944 increase in *A. viridis* leaf Ca contrasts with herbaceous species, where Ca is generally reported to be  
945 stable across the growing season by Schlegel et al. (2016). This increase, as well as the increase in leaf  
946 Mn, could be explained by their low mobility in the phloem and the lack of remobilization of these  
947 elements from leaves with senescence (White 2012; Maillard et al. 2015).

948

949 Despite this significant variation throughout the season, *A. viridis* N leaf concentrations (22.62 to  
950 46.98 g kg<sup>-1</sup> DM) were similar to previous measurements made on this species, e.g. Bühlmann et al.  
951 (2016) measured in late July and early August 28.2 ± 0.2 g kg<sup>-1</sup> DM and 29.4 ± 0.4 g kg<sup>-1</sup> DM at low  
952 (1650 m) and high (1950 m) elevations, respectively. These results are relatively high in comparison to  
953 other woody species, as Tian et al. (2018) found a global mean of 21.13 g kg<sup>-1</sup> DM when analysing a  
954 compiled dataset of several deciduous woody species across continents. This high N concentration in *A.*  
955 *viridis* leaves was expected due to its symbiosis with *F. alni*, which leads to an accumulation of N in the  
956 roots and therefore higher N uptake by the plant (Dawson 2008; Luske and van Eekeren 2017). In  
957 addition, as they are not N limited, *Alnus* species do not need to resorb leaf N before winter, leading to  
958 potentially higher leaf N concentrations at the end of the season (Dawson and Funk 1981; Rodríguez-  
959 Barrueco et al. 1984; Han et al. 2013; Maillard et al. 2015). Consequently, as previously found for other  
960 alder species (Luske and van Eekeren 2017; Mahieu et al. 2021), *A. viridis* leaf crude protein  
961 concentrations, which always exceeded 140 g kg<sup>-1</sup> DM, were higher than those usually found in  
962 temperate green fodder or in permanent grassland at the beginning of the season (Agabriel 2010;  
963 Schlegel 2021). Indeed, if compared to typical leguminous forage species found in nutrient-rich  
964 grasslands, such as *Trifolium pratense* L. or *Trifolium repens* L., the crude protein content is similar and  
965 sometimes higher for *A. viridis* leaves, with values ranging from 141.37 to 293.63 g kg<sup>-1</sup> DM compared  
966 to 161 to 261 g kg<sup>-1</sup> DM for the two *Trifolium* species (Daccord and Arrigo 2001). Additionally, *A.*  
967 *viridis* leaf N content also mostly exceeded some nutrient optimum requirements for sheep and cattle,

968 as described by Hejcman et al. (2016). Since leaf crude protein concentrations lower than 7% have been  
969 shown to decrease forage intake by livestock (Minson and Milford 1967), *A. viridis* could remain a good  
970 source of crude protein for livestock throughout the season, despite its seasonal decrease (González-  
971 Hernández et al. 2000).

972  
973 Similarly, high concentrations were found for other nutrients measured in *A. viridis* leaves. For  
974 instance, leaf Ca, Mg and P concentrations were similar or higher than that found in typical leguminous  
975 forage species. Indeed, *A. viridis* leaf P content ranged between 0.92 to 5.84 g kg<sup>-1</sup> DM compared to 4.0-  
976 4.1 g kg<sup>-1</sup> DM usually found in *T. pratense* and *T. repens* (Daccord and Arrigo 2001). They were also  
977 comparable to the optimum nutritive range, as described by (Hejcman et al. 2016). *A. viridis* leaf P was  
978 also similar to that of *A. glutinosa* measured by Luske and van Eekeren (2017), and to other deciduous  
979 woody plants (Tian et al. 2018). On the other hand, *A. viridis* leaf K was lower than that of leguminous  
980 species but within the range defined by Hejcman et al. (2016). A few out-of-range values for P were  
981 found in the samples from Weissenstein at the beginning of the 2020 season, for which the highest N,  
982 Cu and Zn concentrations were also recorded. Because macro- and micro- elements are essential for  
983 animal health, in terms of growth and reproduction (Mahieu et al. 2021), *A. viridis* leaves could become  
984 a significant resource for livestock.

985  
986 The range of fibre and lignin concentrations of *A. viridis* leaves were very similar to those found  
987 for other alder species in France by Mahieu et al. (2021), and were higher than contents found for  
988 herbaceous vegetation used as a comparison in the same study. As the season advanced, leaves became  
989 more fibrous, as all fibre fractions showed an increase from June to August. This is in line with previous  
990 studies on other alder species such as *A. rubra* (González-Hernández et al. 2000) and other deciduous  
991 shrubs and trees (Happe et al. 1990; Papachristou and Papanastasis 1994), which showed that NDF,  
992 ADF, and lignin leaf content increased from spring to autumn, leading to a lower forage quality at the  
993 end of the summer season. Indeed, forage with high fibre contents leads to lower nutrient digestibility  
994 and assimilation with plant senescence, as lignin has been described as one of the most important factors  
995 limiting the degradation of cell walls in the rumen (Van Soest et al. 1991; Cherney et al. 1993; Mahieu  
996 et al. 2021). Moreover, high fibre content tends to reduce voluntary intake from livestock (Allison 1985),  
997 which would lead to less selection of the shrub at the end of the summer.

998  
999 These results show that *A. viridis* leaves decrease in overall nutritive value across the grazing  
1000 season, which is similar to previous studies on fodder shrubs and trees. Indeed, in 2022 Navale et al.  
1001 studied the variation in the leaf composition of several fodder trees throughout the season and found that  
1002 most of the nutritive and mineral content decreased as leaves matured.

### 1003 1004 1005 **3. Leaf phenolic compounds**

1006  
1007 Leaf total tannin concentrations from our study were higher than what has been previously found  
1008 for *A. viridis* (as well as for *A. incana*), with values up to 3.5 times higher than the values measured by  
1009 Stević et al. (2010). Because their results focused on lower elevations, we could hypothesize that *A.*  
1010 *viridis* could display higher phenol concentrations at higher elevations, which has been described for  
1011 other plant species due to their tendency to synthesise phenols to protect against UV radiations that  
1012 increase with altitude (Alonso-Amelot et al. 2007; Bernal et al. 2013; Zargoosh et al. 2019). Except for  
1013 leaf CT, the phenolic compounds of *A. viridis* leaves significantly decreased as the season advanced.  
1014 Previous studies on alder species and on other woody plants have shown a similar phenol decrease  
1015 during the season, with a higher phenolic allocation to young leaves, while the variation in leaf CT

1016 seems to be highly species-dependent (González-Hernández et al. 2000; Gowda et al. 2019). While leaf  
1017 phenol content has been primarily linked to leaf ontogeny and air temperature, the high phenol content  
1018 of *A. viridis* leaves in June could also be linked to the seasonal variations of UV radiations that tend to  
1019 peak in June in Europe (Bernal et al. 2013). The variation in phenols in *A. viridis* leaves during the  
1020 season may have a significant impact on livestock feeding behaviour, as they are responsible for  
1021 astringency and affect digestibility. For instance, if consumed in large quantities, HT can be toxic, while  
1022 CT can negatively impact ruminant digestion rate, leading to lower voluntary feed intake (Waghorn  
1023 2008; Piñeiro-Vázquez et al. 2015; Gowda et al. 2019). Indeed, CT create binding complexes with  
1024 protein that protect them from ruminal degradation and intestinal absorption, leading to a reduced  
1025 apparent total tract N digestibility (Waghorn 2008; Woodfield et al. 2019). This is supported by the  
1026 results of our PCA, which showed a negative relationship between CT and IVOMD. On the other hand,  
1027 this phenomenon may lead to a higher proportion of N excreted through dung instead of urine, which is  
1028 less subjected to ammonia volatilization and nitrate leaching (Woodmansee et al. 1981; Tamminga 2006;  
1029 Angelidis et al. 2019). Woodfield et al. (2019) recommended a CT concentration to range between 20  
1030 and 40% DM, which aligns well with the concentrations we measured in a diet composed by 20% of *A.*  
1031 *viridis* leaves. Including phenols in that proportion in the diet of grazing ruminants might increase energy  
1032 and N utilisation (Gowda et al. 2019).

1033  
1034

#### 1035 **4. Leaf *in vitro* OM digestibility and gas production**

1036

1037 The IVOMD of *A. viridis* leaves was very similar to the OM digestibility found for *A. glutinosa*  
1038 leaves by Luske and van Eekeren (2017). As expected, including 20% *A. viridis* leaves in a hay-based  
1039 diet led to a lower IVOMD when compared to the control. This is most probably due to the higher  
1040 content of phenols in *A. viridis* leaves compared to hay, which can highly affect digestibility. On the  
1041 other hand, the IVOMD of the mixed diet, including *A. viridis* leaves, seemed to be at its maximum at  
1042 the beginning of the season, which is not in line with our results on phenols, except for CT. This is  
1043 however consistent with previous studies on fodder tree species (Papachristou and Papanastasis 1994)  
1044 and with our results on functional traits and nutrient variation during the season. Indeed, SLA and  
1045 LDMC have a positive and negative effect on digestibility respectively (Pontes et al. 2007), while leaf  
1046 N content may have a positive effect on digestibility (Bumb et al. 2018), and the highest values of N in  
1047 *A. viridis* leaves at the beginning of the season might thus be one factor for the highest measured  
1048 digestibility. In addition, the lower fibre fraction found at the beginning of the summer might  
1049 compensate for the decreased digestibility generated by the phenols.

1050

1051 Interestingly, a diet including 20% of *A. viridis* leaves in the DM significantly decreased *in vitro*  
1052 total gas production when compared to the control diet consisting of hay only. This is in line with the  
1053 findings of Terranova et al. (2018), where most of the woody plant leaves tested led to a decrease in  
1054 CH<sub>4</sub> production in comparison to the control diet. However, CH<sub>4</sub>/dOM production from *A. viridis* leaves  
1055 diet significantly increased as the season advanced, highlighting that leaves have a better CH<sub>4</sub>/dOM  
1056 mitigation effect in the early season. These CH<sub>4</sub>/dOM emission values are in line with our results on  
1057 phenols as high leaf tannin content seems to reduce methanogenesis (Patra and Saxena 2010; Jayanegara  
1058 et al. 2011; Piñeiro-Vázquez et al. 2015). Therefore, the CH<sub>4</sub>/dOM increase during the season is related  
1059 to decrease in measured total leaf extractable phenols, which is confirmed by the PCA results displaying  
1060 CH<sub>4</sub>/dOM and phenols in opposite positions. Similarly to CH<sub>4</sub> production, CO<sub>2</sub> production from  
1061 *A. viridis* leaves diet significantly decreased in comparison to the control, showing that the fermentation  
1062 was generally affected. However, contrary to CH<sub>4</sub>, CO<sub>2</sub>/dOM decreased with an increasing GDD, which

1063 is the consequence of the reduced degradation of the leaves that is in line with the changing chemical  
1064 composition. Consequently, the CH<sub>4</sub>/CO<sub>2</sub> ratio increased with increasing GDD.

1065  
1066 Therefore, *A. viridis* leaves tend to have a better mitigation effect on gas emissions at the beginning  
1067 of the season. According to Tavendale et al. (2005) and Terranova et al. (2018), a CT concentration  
1068 below 8% in the DM as found in our study could reduce CH<sub>4</sub>/dOM production without restraining  
1069 fermentation rate. Because CH<sub>4</sub> is the most important greenhouse gas emitted in animal production  
1070 (Slade et al. 2016), making robust livestock graze in *A. viridis*-encroached pastures at the beginning of  
1071 the season could thus help mitigate greenhouse gas emissions in these mountain livestock production  
1072 systems, while keeping an appropriate ruminal fermentation and therefore a high animal performance.

1073  
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## 1075 **Conclusion**

1076  
1077 This study described the composition of *A. viridis* leaves and their OM digestibility and related gas  
1078 production throughout two summer seasons at four different subalpine sites. The high amounts of crude  
1079 protein and macro- and micro-elements found in *A. viridis* leaves highlighted its value as fodder for  
1080 robust livestock, offering a complementary forage resource to grass that could be well integrated in an  
1081 adapted management of encroached mountain pastures. High seasonal variation in leaf chemical  
1082 composition was found, and because fibre, lignin, crude protein and nutrient contents can impact  
1083 voluntary intake in foraging behaviour, these results could help defining targeted management strategies  
1084 for alpine pastures to optimize robust livestock grazing in *A. viridis* encroached areas while increasing  
1085 beef cattle productivity and reducing the greenhouse gas emissions of CH<sub>4</sub>. In particular, the beginning  
1086 of the summer (June and July) seems to be the best period to graze *A. viridis* leaves, as in this period of  
1087 the year they have the highest nutritional value and potential to reduce greenhouse gas emissions.

1088 **CHAPTER III: Foraging behavior of Highland cattle in silvopastoral**  
1089 **systems in the Alps**

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1132 Based on: Nota G, Svensk M, Barberis D, Frund D, Pagani R, Pittarello M, Probo M, Ravetto Enri S,  
1133 Lonati M, Lombardi G. Foraging behavior of Highland cattle in silvopastoral systems in the Alps.  
1134 (under review in Agroforestry Systems).

1135 **Abstract**

1136

1137         Trees and shrubs expanded in the last decades in European mountains due to land abandonment  
1138 and the decrease in grazing pressure, and are expected to further spread also due to climate change. As  
1139 a consequence of low forage quality and topographic constraints, the management of mountain  
1140 environments dominated by woody vegetation with livestock is often challenging. Silvopastoral systems  
1141 based on cattle hardy breeds able to forage on woody plants, such as Highland cattle, could be a suitable  
1142 option for the management and restoration of such environments. In this study, we used direct  
1143 observations to explore the foraging behavior of Highland cattle in four study areas across the western  
1144 Alps. In particular, we assessed 1) cattle diet composition, 2) the selection for more than 30 tree and  
1145 shrub species, and 3) the relationships between species consumption and their abundance in the  
1146 environment. Highland cattle fed on a mixture of both woody and herbaceous species, including between  
1147 15 and 46% of woody plants in the diet. Some trees (e.g., *Celtis australis*, *Fraxinus* spp., and *Populus*  
1148 *tremula*) and shrubs (e.g., *Frangula Alnus*, *Rhamnus* spp., and *Rubus idaeus*) were positively selected  
1149 by cattle, thus could be an important forage supplement to their diet. Moreover, the results highlighted  
1150 that relative species consumption increased with increasing species abundance in the environment,  
1151 suggesting that this cattle breed could be suitable to control shrub expansion in highly encroached areas.  
1152 The outcomes of this study can support the development of targeted silvopastoral systems in the Alps.

1153 **Introduction**

1154

1155 In European mountains, trees and shrubs expanded in the last decades to the detriment of open  
1156 habitats (mainly pasturelands) due to land abandonment and grazing pressure decrease, and are expected  
1157 to further spread due to the additional effect of climate change (Espunyes et al. 2019). Such changes  
1158 resulted in a general reduction of the ecosystem services associated to mountain agroecosystems (e.g.,  
1159 forage production, protection from natural hazards, and landscape aesthetic value) (Schirpke et al. 2016;  
1160 Faccioni et al. 2019) and of plant diversity (Maurer et al. 2006; Koch et al. 2015; Orlandi et al. 2016).  
1161 Mountain environments dominated by tree and shrub species are scarcely exploited by production-  
1162 oriented livestock, as they provide lower quality forage than open pastures and are usually located in  
1163 marginal areas with rough topography. Indeed, woody plants are generally characterized by a higher  
1164 content of lignin and secondary compounds (e.g., tannins, saponins, alkaloids) than the herbaceous  
1165 forage (Seidavi et al. 2020; Mahieu et al. 2021). Because of these constraints, many mountain shrub-  
1166 encroached grasslands, shrublands, and forests are left unmanaged and become dense stands  
1167 characterized by lower biodiversity (Laiolo et al. 2004; Zehnder et al. 2020).

1168

1169 Silvopastoral systems with hardy cattle breeds could be a suitable option for the management of  
1170 these environments. Indeed, while production-oriented cattle behave as typical grazers, with grass as  
1171 their main forage source, hardy cattle breeds can include a greater proportion of woody plants in their  
1172 diet. For instance, Alberes cattle can feed year-round with a predominantly woody diet in Mediterranean  
1173 forests of the Pyrenees (Bartolomé et al. 2011). In the Alps, Dexter (Pauler et al. 2022b) and Highland  
1174 (Pauler et al. 2020a; Svensk et al. 2022) cattle are acknowledged to feed on woody species as well. The  
1175 ability of certain livestock species and breeds to consume woody plants is linked with microbial  
1176 populations in their rumen able to detoxify secondary metabolites and degrade lignin. This characteristic  
1177 is well documented in goats (Silanikove 2000; Giger-Reverdin et al. 2020), which are considered as  
1178 mixed feeders (i.e., they feed on a mixture of both herbaceous and woody species) (Hofmann 1989). In  
1179 addition to the exploitation of otherwise unused forage resources, livestock grazing can be a valuable  
1180 ecological restoration tool to contribute in counteracting shrub expansion (Casasús et al. 2007; Öllerer  
1181 et al. 2019). Moreover, silvopastoral systems can favor a better adaptation of mountain farms to climate  
1182 change, as woody plants can be an important alternative forage during critical grass shortages (e.g., in  
1183 summer droughts) and provide shade relief to animals during hot periods (Vandermeulen et al. 2018a,  
1184 b; Sales-Baptista and Ferraz-de-Oliveira 2021).

1185

1186 Due to their robustness and ability to consume woody plants, Highland cattle were proposed as a  
1187 tool to control shrub encroachment in different silvopastoral systems and geographic regions, from  
1188 heterogeneous grass-shrub-woodland communities in The Netherlands (Cromsigt et al. 2018) to coastal  
1189 dunes in Belgium (Lamoot et al. 2005), North American oak savannas (Harrington and Kathol 2009;  
1190 Hedtcke et al. 2009), and, more recently, shrub-encroached pastures in the Alps (Pauler et al. 2019;  
1191 Svensk et al. 2021, 2022). Particularly, in the Alps, Svensk et al. (2022) observed that they could damage  
1192 *Alnus viridis* (Chaix) DC., which is among the most rapidly expanding shrub species in Central Europe  
1193 (Anthelme et al. 2007), by the combination of foliage direct consumption, trampling, and mechanical  
1194 damage to branches. Pauler et al. (2019) observed that grazing by Highland cattle can improve plant  
1195 diversity in Swiss subalpine pastures and reduce shrub cover more efficiently compared to other cattle  
1196 breeds.

1197

1198 Despite the fact that the ability of Highland cattle to forage on woody plants in the Alps, as  
1199 documented by Pauler et al. (2020a) and Svensk et al. (2022), no studies explored their foraging behavior  
1200 across different mountain silvopastoral systems and assessed their feeding preferences for different tree

1201 and shrub species. This information would be essential to support the possible development of  
1202 silvopastoral systems based on the Highland cattle breed in the Alps. To fill this knowledge gap, we  
1203 used direct observations to study the foraging behavior of Highland cattle in four study areas in the  
1204 western Alps characterized by contrasting woody vegetation. Specifically, the aim of this study was to  
1205 assess the foraging behavior of Highland cattle in the Alps based on their diet composition, feeding  
1206 preferences, and the influence of species abundance on plant consumption and selection. We  
1207 hypothesized that: 1) Highland cattle fed on a mixture of both herbaceous and woody plant species, like  
1208 in a mixed feeder strategy; 2) some woody plants were positively selected, i.e., they were palatable to  
1209 Highland cattle; and 3) the relative consumption of plant species was influenced by their abundance in  
1210 the environment.

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## 1213 **Methodology**

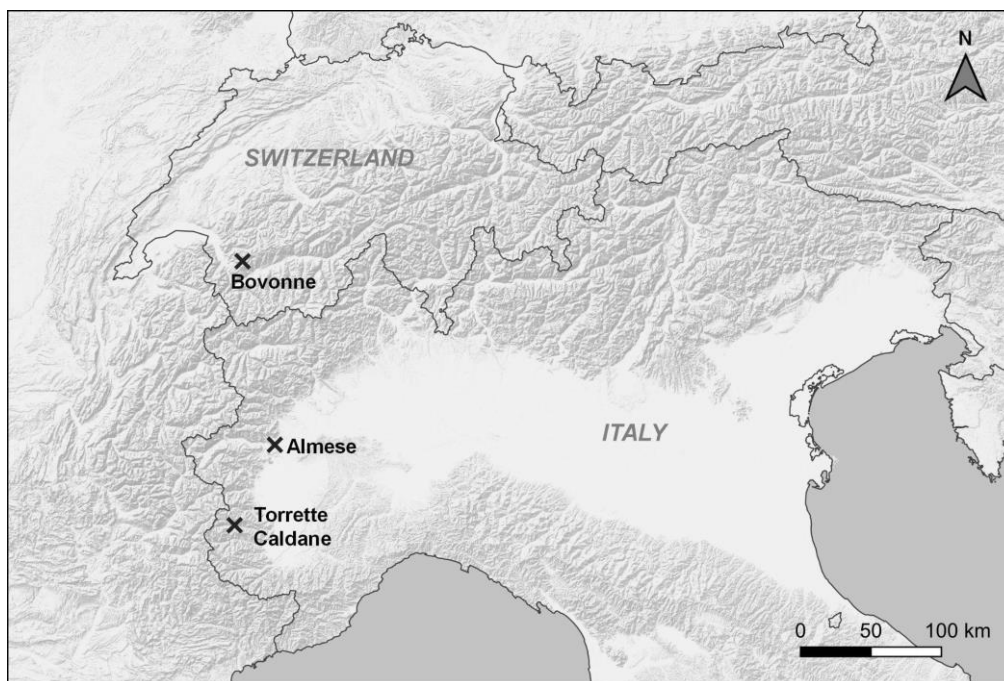
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### 1215 **1. Study areas and grazing management**

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1217 The study was carried out in four paddocks located along an elevation gradient (480-1745 m a.s.l.;  
1218 Tab. 1) and extensively grazed by Highland cattle herds in the western Alps, i.e, Almese (Piedmont  
1219 Region, Italy), Torrette (Piedmont Region, Italy), Caldane (Piedmont Region, Italy), and Bovonne  
1220 (Canton of Vaud, Switzerland) (**Figure 15**). The paddocks were representative of the Alpine encroached  
1221 pastures, being dominated between 50% and 100% by woody plant communities (shrubs and trees) (Tab.  
1222 1). In Almese, the vegetation was a mosaic of small meso-xerophile forests, shrublands, and dry-  
1223 grasslands. Dominant trees were *Fraxinus ornus* L., *Populus tremula* L., and the alien species *Quercus*  
1224 *rubra* L.; dominant shrubs were *Prunus spinosa* L. and *Rubus ulmifolius* aggr., while *Bromus erectus*  
1225 Huds., *Carex caryophylla* Latourr., and *Chrysopogon gryllus* (L.) Trin. were the most abundant species  
1226 in the open grassland patches. In Torrette, the vegetation was a deciduous mesophile forest dominated  
1227 by *Acer pseudoplatanus* L., *Fraxinus excelsior* L., and *Larix decidua* Mill. The shrub *Rubus idaeus* L.  
1228 was abundant in the understory and dominated the open clearings, while *Festuca flavescens* Bellardi  
1229 was the most frequent grass. In Caldane, the vegetation was a mosaic of *F. excelsior* and *Sorbus aria*  
1230 (L.) Crantz-dominated forests, *P. spinosa* and *Rosa canina* aggr. shrublands, and dry-grasslands  
1231 dominated by *B. erectus* and *Festuca ovina* aggr. Bovonne was characterized by meso-hygrophilous  
1232 communities dominated by *A. viridis* and by open mesophilous grasslands. In the grassland patches,  
1233 *Alchemilla xanthochlora* Rothm and *Calamagrostis villosa* (Chaix) J.F.Gmel. were the dominant  
1234 species, whereas in the *A. viridis* understory, *Adenostyles alliariae* (Gouan) A. Kern. and *Dryopteris*  
1235 *dilatata* (Hoffm.) A. Gray were the most abundant species. Aerial photographs (AGEA 2018;  
1236 SWISSIMAGE 2020) and vegetation maps of the four paddocks are available in Supplementary  
1237 Materials (**Figures S1-S4**).





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1239 **Figure 15** Location of the four study areas (Almese, Torrette, Caldane, and Bovonne) in the western  
1240 Alps. Basemap: ESRI Terrain.

1241  
1242 The study was carried out in the grazing season 2021. The paddocks Almese, Torrette, and Caldane  
1243 were managed by the same farm. From late April to mid-June, the herd grazed within the Almese  
1244 paddock. The animals were then moved to summer pastures in the municipality of Casteldelfino, where  
1245 the herd was divided into two groups: a larger group grazed within the Caldane paddock from late June  
1246 to the end of August while a smaller group grazed within the Torrette paddock from mid-August to mid-  
1247 September. Bovonne paddock was grazed for three weeks in July by a different herd. Paddock size,  
1248 livestock units, herd composition, and grazing season are reported in **Table 2**.

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1250 **Table 2** Characteristics of the four paddocks used in Chapter III (Almese, Torrette, Caldane, and  
1251 Bovonne).

	<b>Almese</b>	<b>Torrette</b>	<b>Caldane</b>	<b>Bovonne</b>
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

1252 \* According to EU Regulation 2018/1091 of the European Parliament and of the Council, Annex 1  
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1256 **2. Direct observations on livestock foraging behavior**

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1258 To study the foraging behavior, we used direct observations adapting the methodology proposed  
1259 by Nota et al. (2023) to Highland cattle. Each observer randomly chose an adult cow (focal animal) and  
1260 recorded its foraging behavior during 15 sec. observation sessions followed by 20 sec. breaks between  
1261 each observation. Each cow was continuously observed for two hours, on average, before the observer  
1262 moved to another focal cow. The identification number of the cow was recorded. We used the feeding  
1263 station as the spatial scale where decisions on plant selection were made by the cow. According to  
1264 Bailey et al. (1996) classification, the feeding station is the front feet placement explored by grazing  
1265 animals during a 5-100 second temporal period. Specifically, we spatially delimited the feeding station  
1266 as a buffer area having a height of 1.5-m from the ground level and a 50-cm radius around the head of  
1267 the cow. We assumed that all the plants available in this buffer layer were detectable by the cow and  
1268 that 1.5-m above ground corresponded to the maximum height that animals could exploit (threshold set  
1269 according to our field observations). For each observation session, two variables were recorded: (1) the  
1270 plant species relative abundance (SA) and (2) the plant species relative consumption (SC). The SA  
1271 represented the proportion of biomass (ranging from 10 to 100% and visually estimated with a pace of  
1272 10%) of each species available in the feeding station. The SC represented the proportion of biomass  
1273 (ranging from 0 to 100% and recorded with a pace of 10%) of each species consumed during the 15 sec.  
1274 session. All woody plant species, tall herbs and ferns were identified at the species level. All other  
1275 herbaceous species were grouped in a broad category ‘herbage’, as the identification at the species level  
1276 through direct observations could be extremely difficult for such plants, especially in grassland patches.  
1277 The nomenclature of plant species followed Landolt et al. (2010).

1278 The direct observations were performed during four days in Almese, Torrette, and Caldane, and  
1279 three days in Bovonne, for a total of 11’286 observation sessions, corresponding to 153 monitoring  
1280 hours. The monitoring days were performed approximately once every week to be representative of  
1281 cattle foraging behavior during the whole grazing period in the paddocks.

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1284 **3. Data and statistical analysis**

1285 **a. Diet composition and Jacobs’ Selection Index**

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1287 All analyses were performed separately for the four study areas.

1288 The proportion in the feeding stations (%FS<sub>*i*</sub>) of each woody, tall herb and fern species, and of the  
1289 ‘herbage’ category was calculated as follows

1290

1291 
$$\%FS_i = \frac{\sum SA_i}{\sum_{i=1}^n SA_i} \times 100 \quad (1)$$

1292

1293 where SA<sub>*i*</sub> is the abundance of the species *i* or of the ‘herbage’ category at each observation session.

1294

1295 Then, the overall proportion of woody species in the feeding stations was obtained by summing the  
1296 proportions of all woody plant species. Likewise, the overall proportion of herbaceous species in the  
1297 feeding stations was obtained by summing the proportions of all tall herbs and ferns, and the ‘herbage’  
1298 category.

1299

1300 The proportion in the diet (%DIET<sub>*i*</sub>) of each woody, tall herb and fern species, and of the ‘herbage’  
1301 category was calculated as follows:

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

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:

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

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  $\geq 80\%$ ; 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.

1346 The analyses were performed with R Software (R Core Team, 2018). The ‘mgcv’ package (Wood  
1347 2011) was used to fit the GAMs and the ‘vegan’ package (Oksanen et al., 2020) was used to perform  
1348 the cluster analyses.

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## 1351 Results

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### 1353 1. Diet composition and plant species selection

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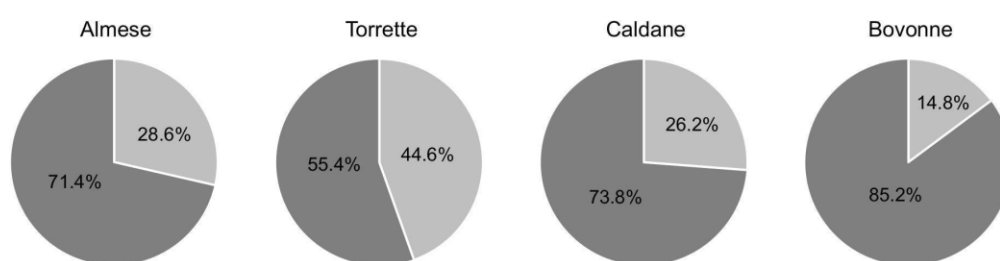
1355 Thirty different woody plant species were recorded in Almese, 24 in Torrette, 21 in Caldane, and  
1356 six in Bovonne. About tall herbs and ferns, one species was recorded in Almese, six in Torrette, none in  
1357 Caldane, and 18 in Bovonne.

1358 The proportion of woody species in the feeding stations used by Highland cattle ranged from 14.8%  
1359 in Bovonne to 44.6% in Torrette (**Figure 16a**). The proportions in the diet showed similar patterns to  
1360 those in the feeding stations, with the lowest proportion of woody species in Bovonne (15.1%),  
1361 intermediate proportions in Almese and Caldane (28.6% and 26.2%, respectively), and the highest in  
1362 Torrette (45.8%).

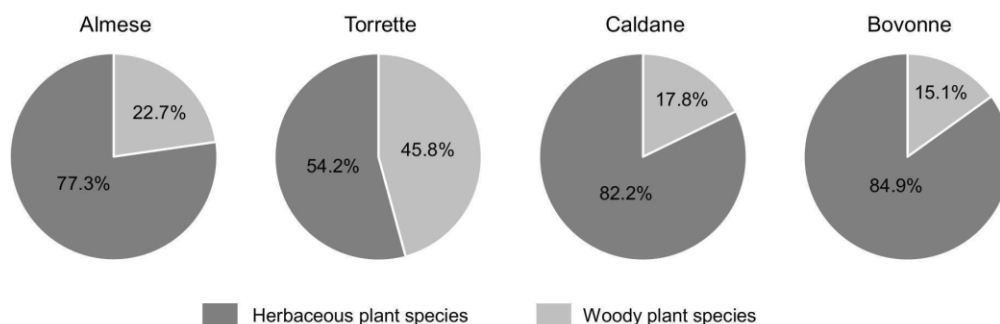
1363 In Almese, the most consumed woody species were *Frangula Alnus* Mill. (3.3% of the diet), *P.*  
1364 *spinosa* (2.9%), and *F. ornus* (2.9%). *Rubus idaeus* accounted for about 40% of the diet in Torrette,  
1365 while *P. spinosa* (4.8%) and *R. ulmifolius* aggr. (2.7%) were the main woody plants browsed in Caldane.  
1366 In Bovonne, *A. viridis* accounted for about 12% of cattle diet. The complete list with all recorded woody  
1367 and tall herb and fern species, the corresponding number of observations, number of cows that  
1368 encountered the species, and the proportions in the feeding stations and in cattle diet is reported in the  
1369 Supplementary Materials (**Table S1**).

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a) Proportions in the feeding stations



b) Proportions in the diet

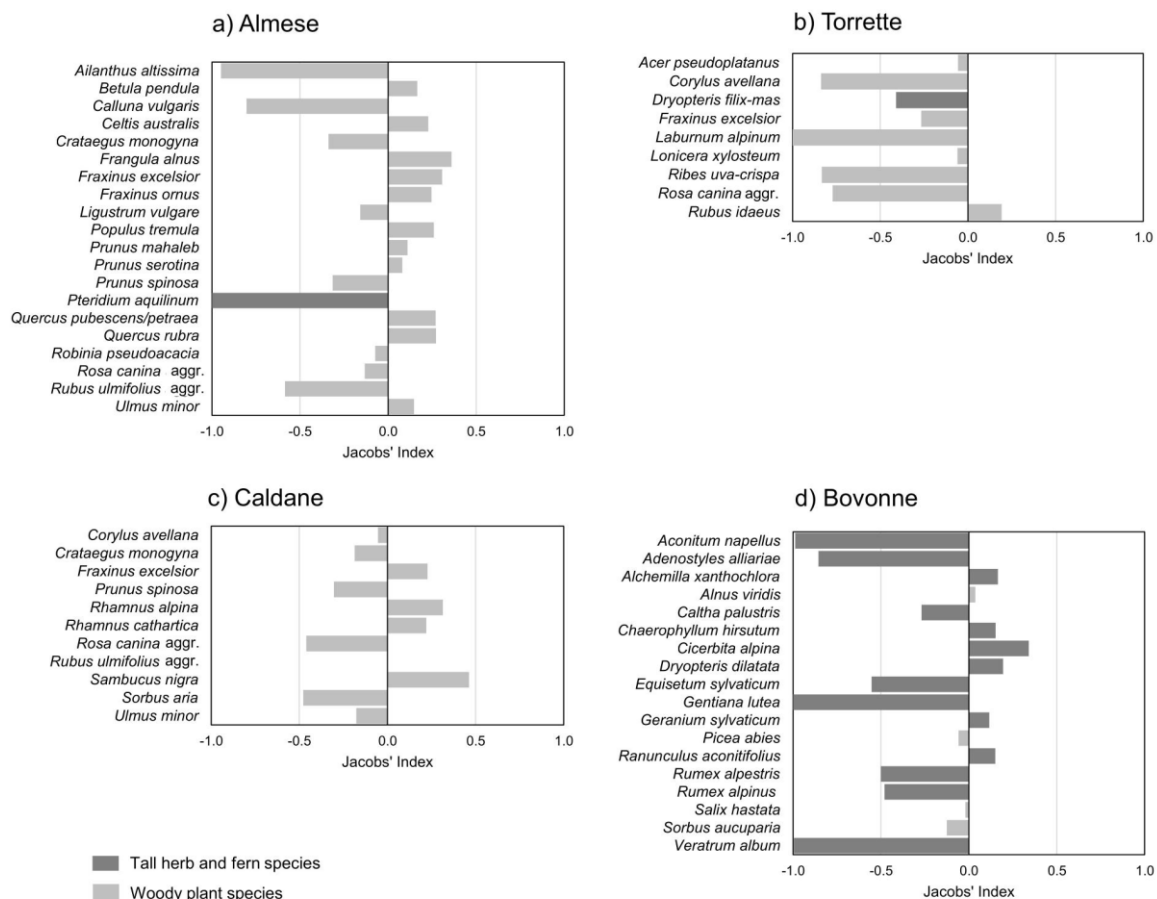


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1372 **Figure 16** Proportion of woody and herbaceous plant species in a) the feeding stations and b) in  
1373 Highland cattle diet in the four study areas.

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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.



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**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.

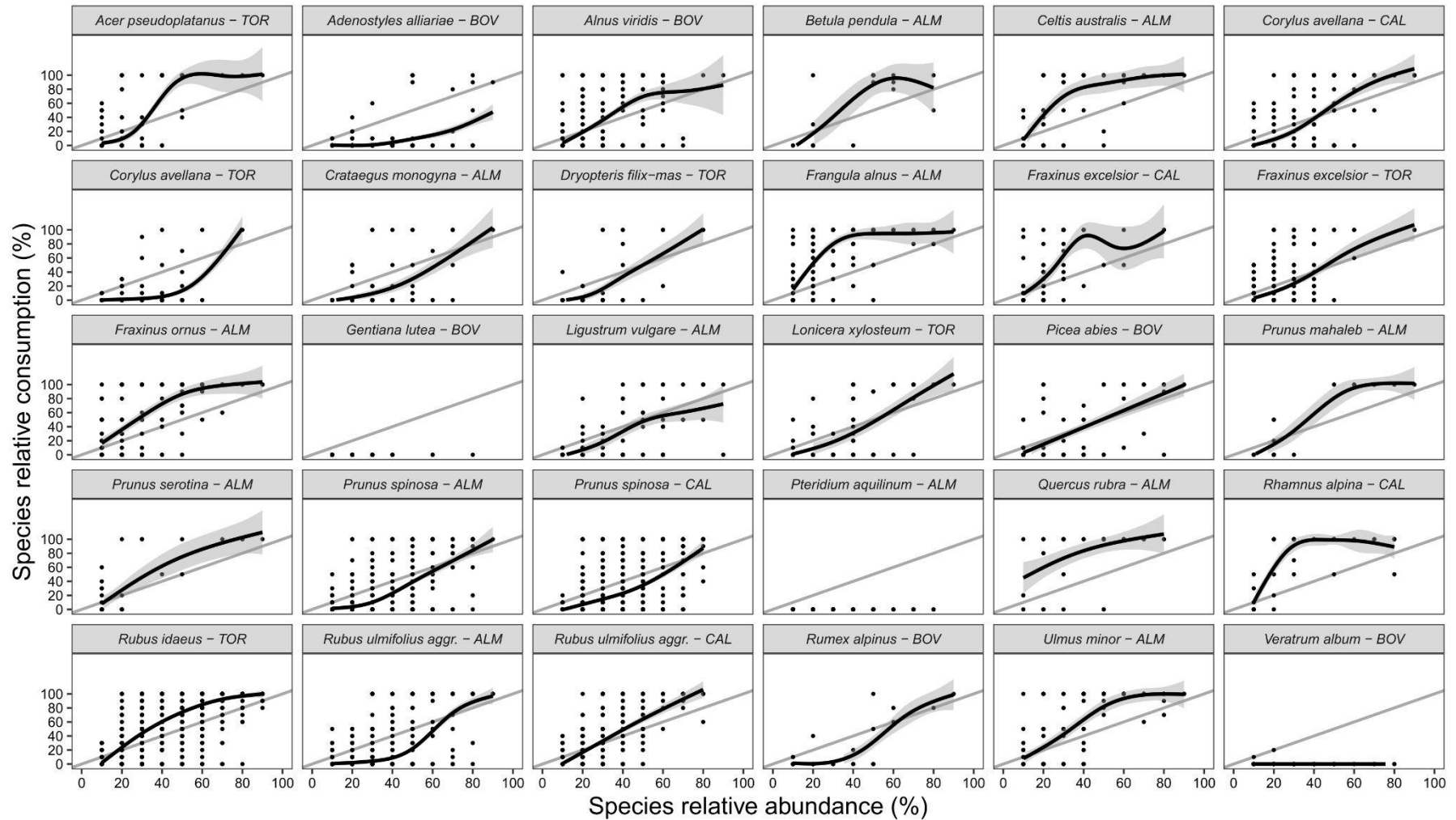
## 2. Relationships between species consumption and abundance

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  $\geq 20$ -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 ( $\geq 70\%$ ). 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.

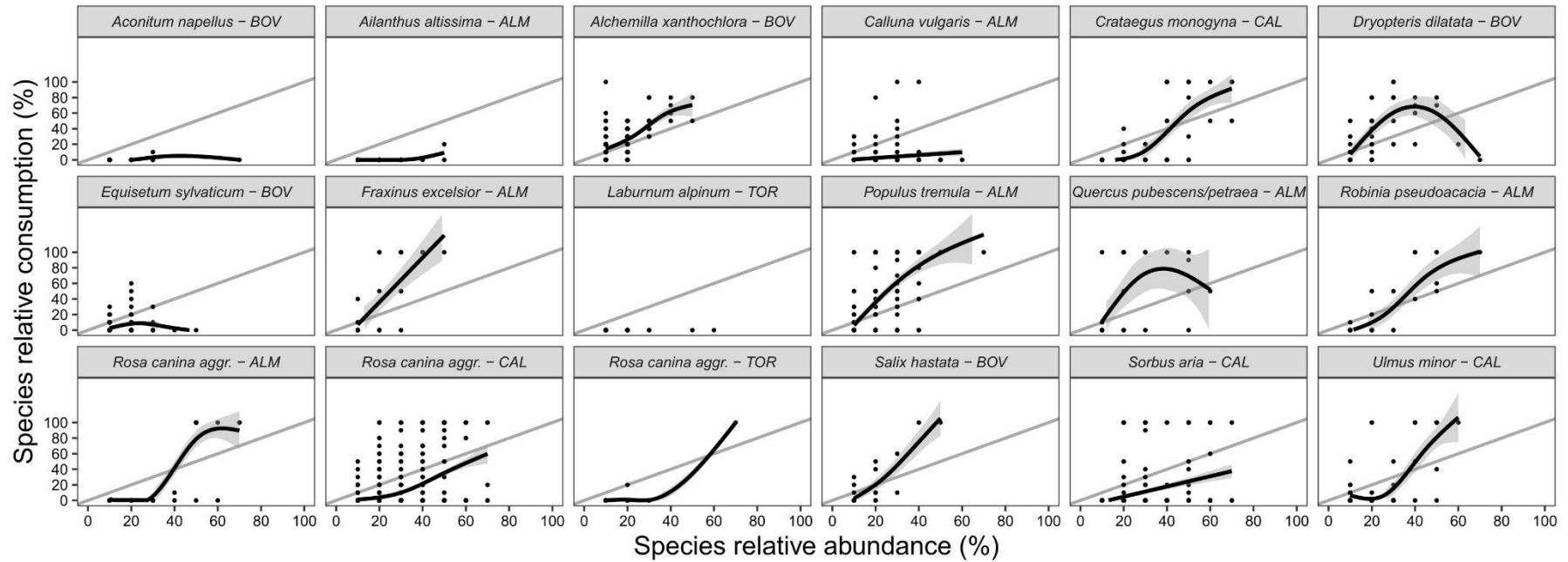
## Discussion

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).



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**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  $\geq 80\%$ ) 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.



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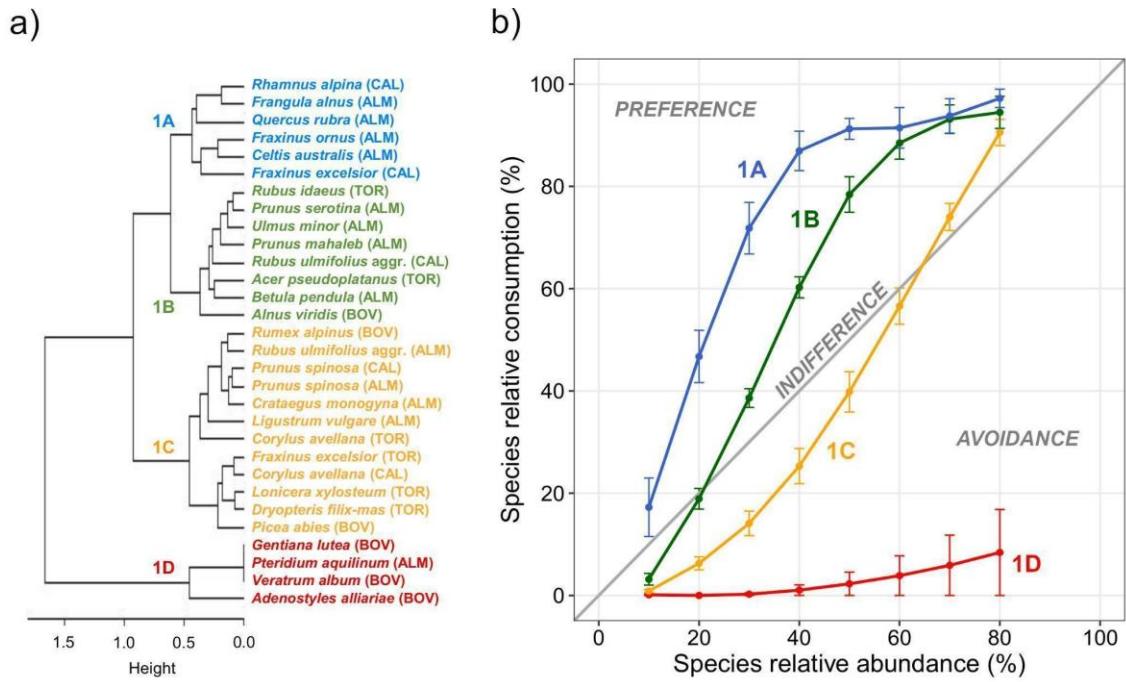
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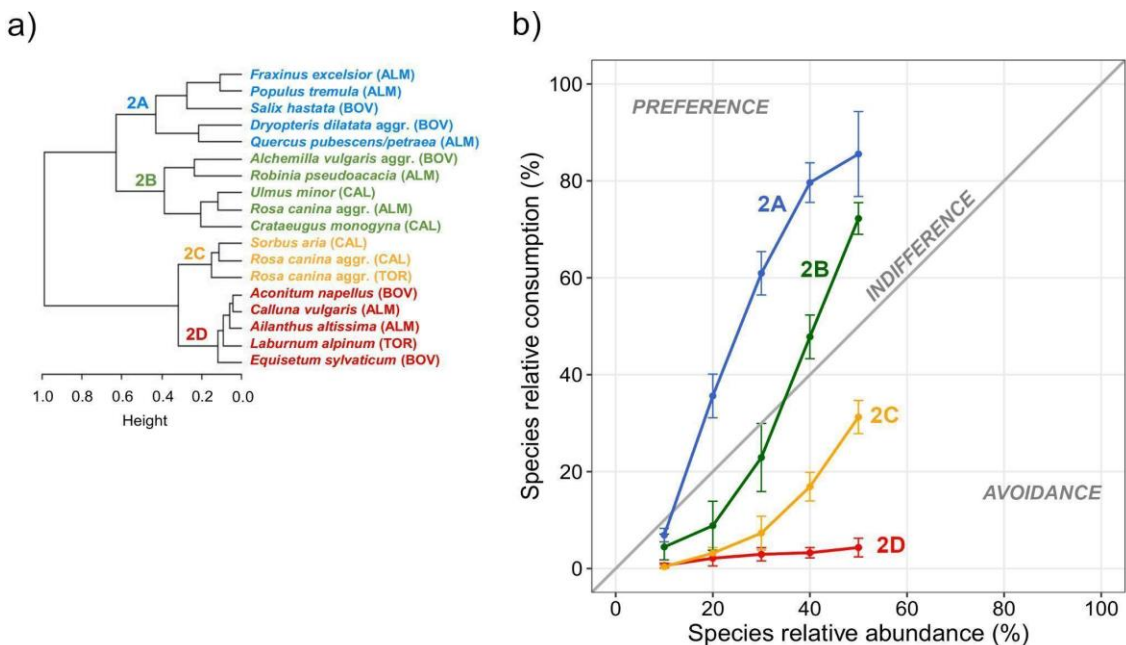
**Figure 19** Relationships between species relative consumption and species relative abundance modeled with Generalized Additive Models for group 2 (upper range of species abundance values between 50 and 70%) of plant species. *Laburnum alpinum* showed no consumption and thus was not modeled due to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





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**Figure 20** a) Dendrogram and b) relationships between species relative consumption and abundance for group 1 (upper range of species abundance values  $\geq 80\%$ ) 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.



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**Figure 21** a) Dendrogram and b) relations between species relative consumption and abundance for group 2 (upper range of SA values  $\geq 50\%$  and  $\leq 70\%$ ) 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.

1468 Results based on Jacobs' Selection Index and consumption-abundance relationships were coherent  
1469 and confirmed our second hypothesis that some woody plants were palatable to Highland cattle and  
1470 could represent an important forage resource in silvopastoral systems. For instance, leaves of *C.*  
1471 *australis*, *P. tremula*, and *F. ornus* were positively selected by cows. *Celtis australis* is considered a  
1472 nutritious and high palatable forage species (Singh et al. 2010), while *P. tremula* is of intermediate  
1473 quality (Hejcmanová et al. 2014). *Fraxinus ornus* is acknowledged as an important browse species for  
1474 goats in Mediterranean environments (Papachristou and Papanastasis 1994; Papachristou et al. 1999).  
1475 *Fraxinus excelsior*, which has high forage quality (Hejcmanová et al. 2014; Ravetto Enri et al. 2020),  
1476 was positively selected by cows too, except in Torrette study area. The avoidance for this species  
1477 observed in Torrette may be explained by the age of the plants, as in this paddock there were abundant  
1478 *F. excelsior* seedlings about 20-cm tall, whereas in the other sites the trees were mainly adult and the  
1479 cows fed on their lowest branches. The reduction in plant chemical defenses and increase in herbivory  
1480 with increasing plant age has been documented for some trees (Boege and Marquis 2005). Other plants  
1481 largely appreciated by cows as browse species were the shrubs *F. Alnus*, *S. nigra*, and *R. idaeus*, in line  
1482 with their high nutritional quality (Mahieu et al. 2021). The leaves of *Rhamnus* species (*R. alpina* and  
1483 *R. cathartica*) were positively selected too, despite their bark and berries are acknowledged for the  
1484 presence of toxic compounds (e.g., anthraquinones; Wink 2010). Interestingly, the alien invasive tree  
1485 *Q. rubra* was palatable to Highland cattle, whereas cows expressed indifference for the alien invasive  
1486 tree *R. pseudoacacia*, and totally refused the alien invasive tree *A. altissima*, which is rich in secondary  
1487 compounds (Kowarik and Säumel 2007). Selection of *A. pseudoplatanus* and *C. avellana*, typical  
1488 species of European temperate forests, ranged from indifference to avoidance. This result agrees with  
1489 the low forage quality of their leaves (i.e., low digestibility and high phenols concentration) as  
1490 documented in literature (Papachristou and Papanastasis 1994; Ravetto Enri et al. 2020; Mahieu et al.  
1491 2021). As recently observed by Svensk et al. (2022), Highland cattle fed on *A. viridis* leaves, which were  
1492 consumed according to their abundance in the environment. Despite its moderately high tannin  
1493 concentration (Stević et al. 2010), this shrub's foliage is rich in protein (Bühlmann et al. 2016; Pauler et  
1494 al. 2022a) and could represent an important constituent of cattle diet in subalpine pastures. When  
1495 foraging in *A. viridis* shrublands, cows expressed a strong selection towards understory species, as they  
1496 preferred to graze *C. alpina* and the fern *D. dilatata* while completely avoiding other frequent tall herbs  
1497 such as *A. alliariae* and the toxic *A. napellus* and *V. album*. Additionally, according to its well-known  
1498 poisonous effects for livestock species (Marrs and Watt 2006), the fern *P. aquilinum* was totally refused  
1499 in Almese. Finally, cows expressed neutral to negative selection for spiny shrubs such as *C. monogyna*,  
1500 *P. spinosa*, *R. canina* aggr., and *R. ulmifolius* aggr. Despite the leaf quality of such shrubs is rather high  
1501 (excellent in *P. spinosa*; Mahieu et al. 2021), spines represented an important deterrent to browsing.

1502 The information about cattle feeding preferences obtained with this study could contribute to a  
1503 better definition of the forage provision and the vegetation carrying capacity in silvopastoral systems  
1504 managed with Highland cattle. Indeed, in the Alps, the computation of the vegetation carrying capacity  
1505 has been developed for pastures and is based on the quality indices of herbaceous plants, which represent  
1506 the main forage source for conventional cattle breeds, whereas trees and shrubs are generally considered  
1507 with a low nutritional value for domestic ruminants. For instance, several indices of specific quality  
1508 targeted for herbaceous plants (Roggero et al. 2002; Cavallero et al. 2007) have been defined for the  
1509 calculation of the pastoral value sensu Daget and Poissonet (1971). The inclusion of woody plants in the  
1510 estimation of the vegetation carrying capacity, such as through the definition of their specific quality  
1511 indices (see for example the study by Gusmeroli et al. 2007), could help a better and sustainable  
1512 management of these mountain environments.

1513

1514 Finally, based on consumption-abundance relationships, we accept our third hypothesis that species  
1515 consumption was influenced by their abundance in the feeding station. Particularly, most of the species  
1516 were increasingly consumed when they were increasingly available to cows. These results agree with  
1517 other studies on grass (Agnusdei and Mazzanti 2001; Chen et al. 2015) and woody (Elias and Tischew  
1518 2016) species performed at the pasture scale. Exceptions to this trend were the strongly avoided species,  
1519 i.e., those of clusters 1D and 2D, which were seldom or never eaten regardless of their abundance,  
1520 probably because of high unpalatability and toxicity issues. Interestingly, consumption-abundance  
1521 relationships showed that the selection by cattle for some species (i.e., clusters 1B, 2B, and 1C) varied  
1522 from avoidance to preference along the gradient of increasing species abundance. This trend may be  
1523 explained in the context of momentary maximization theories, which assume that animals select the best  
1524 available alternative at any given time (Senft et al. 1987; Bailey et al. 1996). Particularly, the most  
1525 palatable plants in the feeding station are selected until palatability of remaining forage decreases to a  
1526 threshold value, then cows move to another feeding station. The threshold value is not fixed but increases  
1527 when animals encounter high quality plants and decreases with lower quality plants (Senft et al. 1987;  
1528 Bailey et al. 1996). According to this interpretation, when cows are foraging in a feeding station highly  
1529 encroached by tree and shrub species of medium-low palatability (i.e., those of clusters 1B, 2B, and 1C),  
1530 the threshold decreases and the consumption and selection on these plants increase. Conversely, when  
1531 these species are occasionally present in the feeding station, they are avoided as cows prefer to forage  
1532 on more palatable species. In this regard, we highlight that Jacobs' Selection Index was ineffective to  
1533 detect changes of species selection along the gradient of increasing abundance as it only provides an  
1534 overall selection value. Consumption-abundance relationships, instead, allowed to scrutinize more  
1535 thoroughly feeding preferences, highlighting how some generally avoided plants can be positively  
1536 selected when their abundance is high. This behavior has implications especially for species like *A.*  
1537 *viridis*, *P. spinosa*, *R. canina* aggr., and *R. ulmifolius* aggr., whose encroachment into open grasslands  
1538 represents a crucial issue in European mountains due to land abandonment (Casasús et al. 2007; Svensk  
1539 et al. 2021; Verdinelli et al. 2022). Our results highlighted that browsing on these species is more  
1540 effective when cows are foraging in highly encroached patches rather than when these species are  
1541 sparsely distributed within a matrix of more palatable species. Silvopastoral systems with the Highland  
1542 breed may thus represent a management tool to control undesirable woody plant species, particularly  
1543 when cows exploit highly encroached areas. Increases in the animal stocking rates (Pauler et al. 2022b)  
1544 and the use of attractive points (Pittarello et al. 2016b; Svensk et al. 2022) could further contribute to  
1545 intensify the impact of cattle on target trees and shrubs.

1546

1547

## 1548 **Conclusion**

1549

1550 This study showed that Highland cattle had a mixed diet consisting of both woody and herbaceous  
1551 plants, suggesting that silvopastoral systems based on this breed could be a valuable option for the  
1552 management and restoration of abandoned environments in the Alps. Some trees (e.g., *Celtis australis*,  
1553 *Fraxinus* spp., and *Populus tremula*) and shrubs (e.g., *Frangula Alnus*, *Rhamnus* spp., and *Rubus idaeus*)  
1554 were very palatable to Highland cattle, thus can be an important forage resource and a supplement to  
1555 cattle diet. In addition, our results highlighted that cows increased the consumption of plant species with  
1556 their increasing abundance in the feeding station, suggesting that this breed may be suitable to control  
1557 shrub expansion in highly encroached areas. Further research should integrate foraging behavior  
1558 evaluations with tree and shrub forage quality and animal performance analyses. Moreover, the effects  
1559 of the grazing management with Highland cattle on the restoration of shrub-encroached grasslands,  
1560 shrublands, and forests should be assessed in the long term.

1561 **CHAPTER IV: Spatial distribution of Highland cattle in *Alnus viridis*-**  
1562 **encroached subalpine pastures**

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1602 Based on: Svensk, M., Pittarello, M., Nota, G., Schneider, M. K., Allan, E., Mariotte, P., Probo, M.  
1603 (2021). Spatial Distribution of Highland Cattle in *Alnus viridis* Encroached Subalpine Pastures.  
1604 *Frontiers in Ecology and Evolution*. 9:626599. doi: 10.3389/fevo.2021.626599

1605 **Abstract**

1606

1607 Green alder (*Alnus viridis*) is a shrub species that has expanded over former pastures in Central  
1608 Europe due to land abandonment, leading to negative agri-environmental impacts, such as a reduction  
1609 in forage yield and quality and an increase in nitrate leaching. Robust livestock breeds such as Highland  
1610 cattle could be used to control *A. viridis* encroachment. The objectives of this study were to investigate  
1611 the impact of *A. viridis* encroachment on plant community composition and diversity and to map the  
1612 spatial distribution of Highland cattle in *A. viridis*-encroached pastures. During the summer of 2019,  
1613 three different Highland cattle herds were placed along an *A. viridis* encroachment gradient. A total of  
1614 58 botanical surveys were carried out before grazing to assess plant community composition, pastoral  
1615 value, and ecological indicator values. The spatial distribution of cattle was studied during the whole  
1616 grazing period by monitoring six to eight cows equipped with GPS collars in each herd. Plant species  
1617 associated with higher pastoral values of the vegetation were found in areas with lower *A. viridis* cover,  
1618 while highly encroached areas were dominated by a few nitrophilus and shade-tolerant broad-leaved  
1619 species and by ferns. Cattle spent more time in areas with higher pastoral value but did not avoid areas  
1620 with high cover of *A. viridis*, on steep slopes or far from water. These results show that Highland cattle  
1621 are able to tolerate harsh environmental conditions and that they can exploit *A. viridis*-encroached  
1622 pastures. This suggests that they have a high potential to reduce *A. viridis* encroachment in the long-  
1623 term.

1624 **Introduction**

1625

1626 Throughout the last decades, socioeconomic changes have led to a reduction in agropastoral  
1627 activities in European mountain areas, triggering profound landscape modifications, and widespread  
1628 natural reforestation and shrub encroachment in former pastures (Estel et al. 2015). For instance, from  
1629 1985 to 2013, shrublands and forests have increased by 10.6% across Switzerland, with the largest  
1630 increases in the Alps (Abegg et al. 2020). Shrublands now cover an area of 679 km<sup>2</sup> in Switzerland, with  
1631 about 70% of them dominated by pure stands of green alder [*Alnus viridis* (Chaix.) D. C.], which is the  
1632 most rapidly expanding shrub species in Central Europe (Anthelme et al. 2007). *A. viridis* is a pioneer  
1633 species, which lives in symbiosis with the N<sub>2</sub>-fixing actinomycete *Frankia alni* (Huss-Daniel 1997), and  
1634 its presence is determined by land use intensity (Caviezel et al. 2017). Encroachment by *A. viridis* can  
1635 have several negative agri-environmental impacts: in particular, *A. viridis*-dominated shrublands have  
1636 lower plant and animal diversity and forage quality than open grazed areas (Anthelme et al. 2001). A  
1637 recent study showed that *A. viridis* encroachment resulted in a rapid decline in plant species richness,  
1638 with dense *A. viridis* patches hosting 62% less species than adjacent open pastures (Zehnder et al. 2020).  
1639 Moreover, there is high nitrate and dissolved organic carbon leaching from *A. viridis* shrublands, which  
1640 results in water pollution, soil acidification, hydrological drawbacks, and gaseous nitrogen losses  
1641 (Bühlmann et al. 2016; van den Bergh et al. 2018). It is therefore important to find methods that control  
1642 *A. viridis* encroachment in order to reverse biodiversity losses and to protect key ecosystem services.

1643

1644 Controlling shrub encroachment is challenging because mechanical removal is extremely difficult  
1645 and expensive in harsh and marginal mountain conditions due to steep slopes and a lower density of  
1646 road network. Moreover, many shrub species are not palatable to grazing animals, since their foliage  
1647 has low digestibility, mostly due to high tannin concentrations (Waghorn 2008). However, some robust  
1648 livestock species and breeds can forage on shrubs and digest their leaves, thanks to ruminal bacterial  
1649 populations, which can degrade lignified material and which have high resistance to tannins (Manousidis  
1650 et al. 2016). Highland cattle are a robust breed that are increasingly being reared in different European  
1651 alpine countries due to their ability to forage on poor quality and shrub-encroached pastures (Pauler et  
1652 al. 2020b, a). As a consequence, the particular grazing behavior of Highland cattle can also result in  
1653 distinct pasture botanical composition, with reduced woody plant species cover in the long term (Pauler  
1654 et al. 2019). However, the impact of robust livestock on shrub encroachment may depend on  
1655 environmental and management conditions, such as slope, pastoral value of the vegetation, and distance  
1656 to water sources and attractive supplements for livestock (Probo et al. 2014; Homburger et al. 2015;  
1657 Pittarello et al. 2016a). It is therefore important to evaluate the conditions under which robust livestock  
1658 breeds are able to effectively reduce shrub encroachment in alpine pasture.

1659

1660 However, to the best of our knowledge, no study has assessed in detail the drivers related to plant  
1661 community composition and diversity in *A. viridis*-encroached pastures nor the potential of Highland  
1662 cattle to reduce *A. viridis* encroachment. For this reason, in the present study, we aimed (i) to disentangle  
1663 the topographic and ecological drivers of plant community composition and diversity in *A. viridis*-  
1664 encroached pastures, from Northern to Southern Alps and (ii) to measure the spatial distribution of  
1665 Highland cattle in *A. viridis*-encroached pastures to determine whether they are attracted to or avoid *A.*  
1666 *viridis* patches. Here, we hypothesized that spatial distribution of Highland cows would vary depending  
1667 on *A. viridis* cover and other factors such as distance to water, slope, and the pastoral value of the  
1668 vegetation. More specifically, we expected cows to spend more time in areas around water sources and  
1669 in more valuable pasture and to avoid steep slopes.

1670 **Methods**

1671

1672 To investigate vegetation features and cattle spatial distribution, three Highland cattle herds were  
 1673 placed in four different *A. viridis*-encroached paddocks in the Swiss and Italian Alps in the summer of  
 1674 2019. Three paddocks were located in Switzerland: Bovonne 1 and Bovonne 2 (in the canton of Vaud)  
 1675 and Champlong (in the canton of Valais). Bovonne 1 and Bovonne 2 were adjacent paddocks, grazed  
 1676 one after the other by the same herd. The fourth paddock was located in Val Vogna (in the province of  
 1677 Vercelli) in the Italian Alps (**Table 3**). Highland cattle grazed each paddock for approximately 1 month:  
 1678 in July (Bovonne 1), from mid-July to late-August (Champlong), and in August (Bovonne 2 and Val  
 1679 Vogna). All the herds included suckler cows, heifers, and calves, varying in age from 6 months to 17  
 1680 years. The paddocks had similar environmental conditions in terms of elevation and slope, were grazed  
 1681 at comparable livestock stocking rates, and were representative of a gradient of *A. viridis* encroachment,  
 1682 i.e., with *A. viridis* cover values ranging from 20 to 70% (**Table 3**). A water trough was installed in each  
 1683 of the two paddocks in Bovonne, while many natural streams were present both in Val Vogna and  
 1684 Champlong paddocks.

1685

1686 **Table 3** Topographical, management, and vegetation characteristics of the four *A. viridis*-encroached  
 1687 paddocks used in Chapter IV.

	<b>Bovonne 1</b>	<b>Bovonne 2</b>	<b>Champlong</b>	<b>Val Vogna</b>
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 <sup>-1</sup> year <sup>-1</sup> )	0.22	0.20	0.21	0.30
Number of GPS-equipped cows	8	8	7	6
Cover of <i>Alnus viridis</i> (%)	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

1688 Cover of *Alnus viridis*, distance to water, elevation, slope, and pastoral value of the vegetation are mean values  
 1689 (± SD) measured at a 10-m × 10-m cell scale over the whole paddock area. One livestock unit = 1 animal of  
 1690 500 kg.

1691

1692 In order to characterize the drivers of plant community composition and diversity within each  
 1693 paddock, the botanical composition was determined along 12.5-m transects. Transects were placed in  
 1694 patches (1.08 ± 0.22 ha, **Table 3**) with homogeneous botanical composition and vegetation structure,  
 1695 and the vertical point-quadrat method was used to record plant species (Daget and Poissonet 1971). At  
 1696 50-cm intervals along each transect, the plant species touching a steel needle were identified and  
 1697 recorded (i.e., 25 points per transect). Since rare species are often missed by this method, all other  
 1698 species within a 1-m buffer area around the transect were also recorded (Kohler et al. 2004). A total of  
 1699 58 vegetation transects were surveyed in the four paddocks (**Table 3**). Plant species nomenclature

1700 followed Aeschimann et al. (2004). The relative abundance of each plant species was computed by  
1701 dividing species frequency of occurrence by the sum of frequency of occurrence values for all species  
1702 in the transect and multiplying it by 100. A species relative abundance value of 0.3% was assigned to  
1703 all the species found only in the buffer zone and not in the main transect, following (Pittarello et al.  
1704 2016a). We then calculated the pastoral value (PV) of the vegetation, based on the species composition.  
1705 The PV is a synthetic index summarizing forage yield, quality, and palatability for livestock (Pittarello  
1706 et al. 2018). To estimate PV, we attributed each species an index of specific quality (Cavallero et al.  
1707 2007). The index of specific quality depends on the preference, morphology, structure, and productivity  
1708 of the plant species, and it ranges from 0 (low) to 5 (high). The PV, which ranges from 0 to 100, was  
1709 calculated as follows (Daget and Poissonet 1971):  
1710

$$1711 \quad PV = \sum_{i=1}^n (SRA_i \times ISQ_i) \times 0.2$$

1712  
1713 where  $SRA_i$  is the species relative abundance, and  $ISQ_i$  is the index of specific quality value of the  
1714 species  $i$ .  
1715

1716 Moreover, we used the indicator values of Landolt et al. (2010) for each plant species. These  
1717 indicate the environmental conditions in the habitats in which the given species occurs and are a measure  
1718 of its environmental preferences. We used the light (L), soil moisture (F), and nutrient value (N)  
1719 (nitrogen) indicators. We then determined the mean ecological conditions of each vegetation patch, as  
1720 the mean Landolt value for L, F, and N by multiplying each species indicator value by its relative  
1721 abundance. For each vegetation transect, two plant diversity indexes were computed: species richness  
1722 and Shannon diversity index  $H_r$  (Magurran 1998). *A. viridis* canopy cover (%) was visually estimated  
1723 within each vegetation patch.  
1724

1725 Each paddock was subdivided into 10 × 10-m grid cells, and the distance to water sources (i.e.,  
1726 streams and water troughs), the aspect, elevation, and slope were calculated for the centroid of each cell  
1727 using (QGIS/ArcView/SAGA/R). Moreover, *A. viridis* cover and PV were calculated for each grid cell  
1728 using the values of the corresponding vegetation patch. If a cell covered more than one vegetation patch,  
1729 the patch accounting for most of the cell area was considered. To avoid issues with circular variables  
1730 (where high and low values are close together), aspect was transformed into southness ( $180 - |\text{aspect}$   
1731  $- 180|$ ) (Chang et al. 2004). In each herd, six to eight cows were equipped with GPS collars (Followit  
1732 Tellus GPS collars) that recorded their position every 10 min during the whole grazing period, with an  
1733 average accuracy of 2–5 m (**Table 3**). The number of GPS fixes was calculated for each grid cell.  
1734

1735 All statistical analyses were performed using R version 3.4.4. A partial canonical correspondence  
1736 analysis (CCA, vegan package), taking into account the spatial distance between botanical surveys, was  
1737 performed to describe how PV, biodiversity indices, ecological indicators (i.e., Landolt indexes), and  
1738 topography (i.e., slope and aspect) were related to plant community composition (after Hellinger  
1739 transformation). Moreover, relationships between *A. viridis* cover and diversity indexes and PV were  
1740 analyzed with generalized linear mixed models with Poisson (species richness and PV) and quasi-  
1741 Poisson (Shannon diversity index  $H_r$ ) distributions, with paddock considered as a random factor (lme4  
1742 package). The spatial distribution of cattle calculated from GPS data (i.e., the number of GPS fixes  
1743 counted in each grid cell) was investigated separately for three distinct periods within each paddock,  
1744 i.e., the beginning (P1), middle (P2), and end (P3) of the grazing period. These periods had equal lengths  
1745 within each paddock and varied between 1 and 2 weeks depending on the length of the grazing period



1746 (Table 3). The impacts of slope, *A. viridis* cover, distance to water, and PV on the number of GPS fixes  
1747 were assessed using linear regression models with zero-inflated negative binomial likelihood, as this  
1748 error distribution accounted for overdispersion in the response variable (tested using the AER package).  
1749 Homburger et al. (2015) found this likelihood structure to be appropriate for GPS counts in zones  
1750 incompletely exploited by cattle. In order to account for the spatial autocorrelation of data, a spatial  
1751 structure error term (using a two-dimensional random walk of second order) was included, and the model  
1752 was fitted by Integrated Nested Laplace Approximation (INLA).

1753  
1754

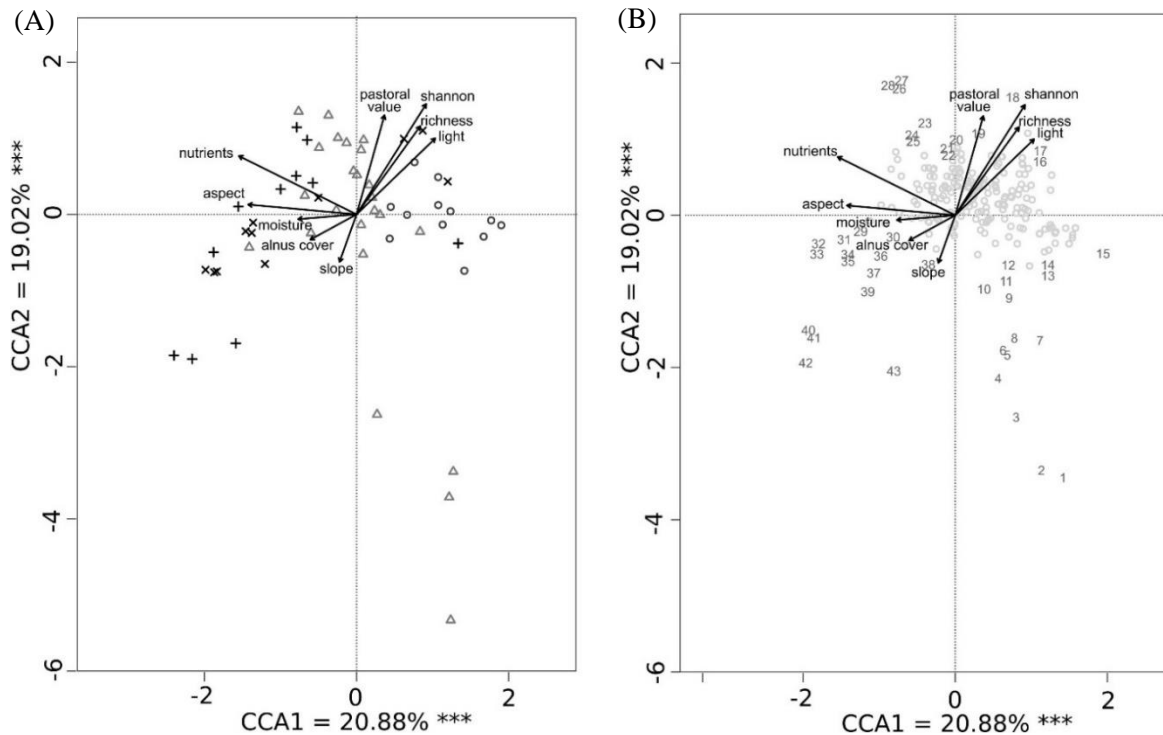
## 1755 Results

1756

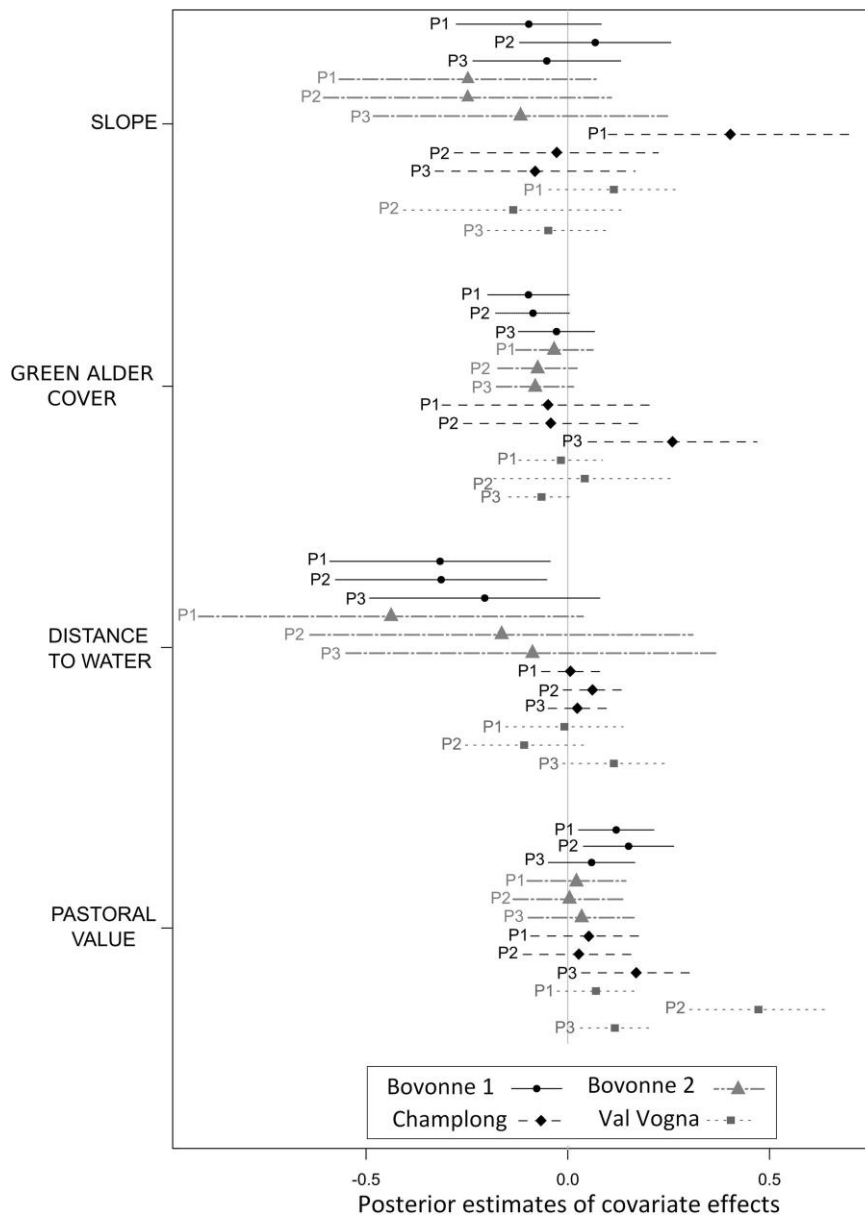
1757 We found a total of 252 plant species in all the paddocks, with the highest species richness detected  
1758 in Val Vogna (157 species, Table 3). The results of the CCA analysis highlighted different ecological,  
1759 topographical, and vegetation impacts on plant community composition (Figure 22), which significantly  
1760 explained the variation in community composition across the paddocks (CCA 1 = 20.9%, CCA2 =  
1761 19.0%, CCA3 = 12.9%, CCA 4 = 10.9%, and total = 84.6%). Vegetation patches within the two Bovonne  
1762 paddocks were characterized by higher *A. viridis* cover and nitrogen enrichment (i.e., higher values for  
1763 N indicator). In contrast, the Champlong paddock had many open pasture patches (with higher light  
1764 availability), while Val Vogna patches were more heterogeneous. Four patches in Val Vogna were well  
1765 distinguished and corresponded to vegetation dominated by dwarf shrubs, such as *Vaccinium vitis-idaea*,  
1766 *Juniperus nana*, and *Rhododendron ferrugineum*, with low PV (Figure 22B). *A. viridis* cover had a  
1767 significant negative effect on plant species richness, Shannon diversity index  $H_r$ , and PV ( $p < 0.001$ ).  
1768 Indeed, plant species associated with higher PV (e.g., *Festuca pratensis* and *Trifolium pratense*) were  
1769 often found in the most open areas, characterized by a lower *A. viridis* cover, as well as typical small  
1770 sized pasture species (e.g., *Medicago lupulina* and *Polygala alpestris*), which were associated with the  
1771 highest light availability and biodiversity (both in terms of species richness and Shannon diversity index  
1772  $H_r$ ). Conversely, areas with high levels of *A. viridis* encroachment and with high indicator values for N  
1773 were dominated by a few tall broad-leaved species, such as *Adenostyles alliariae* and *Cicerbita alpina*,  
1774 together with ferns (mainly *Athyrium filix-femina* and *Dryopteris dilatata*).

1775

1776 Estimates of linear regression models showed that livestock spatial distribution was often positively  
1777 influenced by PV. Cattle spent more time in high PV areas in Bovonne 1 (during periods 1 and 2),  
1778 Champlong (period 3), and Val Vogna (periods 2 and 3) (Figure 23). In Bovonne 2, the PV did not  
1779 influence cattle spatial distribution. The cover of *A. viridis*, slope, and distance to water sources did not  
1780 generally affect livestock spatial distribution. *A. viridis* cover and slope only had significant effects in  
1781 the Champlong paddock during the third and the first grazing period, respectively; in both cases, cattle  
1782 spent more time in areas with high *A. viridis* cover and on steeper slopes. Distance to water had a  
1783 negative effect only in periods 1 and 2 in Bovonne 1 paddock, where water sources were less abundant,  
1784 meaning cattle spent more time close to water.



1785  
 1786 **Figure 22** Partial canonical correspondence analysis (CCA) showing plant community composition  
 1787 explained by *A. viridis* cover, slope, aspect, pastoral value, species richness, Shannon index, and  
 1788 ecological indicators for nutrients, light, and moisture (Landolt indexes). Both dimensions presented are  
 1789 significant ( $***p < 0.001$ ). (A) Botanical surveys are represented for Bovonne 1 (+), Bovonne 2 (x),  
 1790 Champlong (o), and Val Vogna ( $\Delta$ ). (B) Plant species are represented by numbers (detailed below) for  
 1791 the most important species and by dots for the remaining species. (1) *Vaccinium vitis-idaea*, (2)  
 1792 *Juniperus nana*, (3) *Cryptogramma crista*, (4) *Gymnocarpium Dryopteris*, (5) *Astrantia minor*, (6)  
 1793 *Rhododendron ferrugineum*, (7) *Arnica montana*, (8) *Avenella flexuosa*, (9) *Festuca scabriculmis*, (10)  
 1794 *Rosa pendulina*, (11) *Vaccinium myrtillus*, (12) *Calamagrostis villosa*, (13) *Melampyrum sylvaticum*,  
 1795 (14) *Plantago lanceolata*, (15) *Epilobium montanum*, (16) *Medicago lupulina*, (17) *Thlaspi montanum*,  
 1796 (18) *Polygala alpestris*, (19) *Molinia arundinacea*, (20) *Hypericum richeri*, (21) *Festuca pratensis*, (22)  
 1797 *Trifolium pratense*, (23) *Arabis ciliata*, (24) *Rumex alpestris*, (25) *Campanula latifolia*, (26) *Capsella*  
 1798 *bursa-pastoris*, (27) *Chenopodium bonus-henricus*, (28) *Trifolium badium*, (29) *Cardamine resedifolia*,  
 1799 (30) *Adenostyles alliariae*, (31) *Acer pseudoplatanus*, (32) *Rubus saxatilis*, (33) *Thalictrum*  
 1800 *aquilegifolium*, (34) *Saxifraga rotundifolia*, (35) *Cicerbita alpina*, (36) *Urtica dioica*, (37) *Dryopteris*  
 1801 *dilatata*, (38) *Rubus idaeus*, (39) *Athyrium filix-femina*, (40) *Prenanthes purpurea*, (41) *Sorbus*  
 1802 *aucuparia*, (42) *Picea abies*, and (43) *Oxalis acetosella*.



1803  
 1804 **Figure 23** Effects of four covariates on livestock spatial distribution. The dots show the mean estimated  
 1805 effects and the line the 95% confidence interval, in the paddocks of Bovonne 1 (black line), Bovonne 2  
 1806 (gray dashed line), Champlong (black dashed line), and Val Vogna (gray dotted line) for the three  
 1807 grazing periods (P1, P2, and P3).

1808  
 1809  
 1810 **Discussion**

1811  
 1812 Overall, plant communities with higher *A. viridis* cover had lower plant diversity and were  
 1813 dominated by a few broad-leaved species, like *A. alliariae* and *C. alpina*, which prefer N-enriched  
 1814 conditions. These results support the findings of Anthelme et al. (2001) and Zehnder et al. (2020), who  
 1815 also showed a decline in alpine plant diversity with *A. viridis* encroachment. The few species that survive  
 1816 in *A. viridis*-dominated areas are able to take advantage of the N-enrichment and increased shading, and  
 1817 they replace many typical pasture species that are highly light demanding. Interestingly, other than  
 1818 broad-leaved species, the understory vegetation of many *A. viridis* patches was dominated by ferns (in  
 1819 particular, *A. filix-femina* and *D. dilatata*). These ferns are also highly shade tolerant, but they typically

1820 prefer areas with lower N levels, compared to the broad-leaved species (Landolt et al. 2010). They may  
1821 therefore be more abundant in areas recently invaded by *A. viridis* where N levels have not increased so  
1822 much. These results show that encroachment by *A. viridis* threatens the diversity of Alpine pastures and  
1823 leads to a loss of typical pasture species.

1824  
1825 The Highland cattle did not avoid patches with a high density of *A. viridis*. In fact, they were able  
1826 to graze in most *A. viridis*-covered areas, therefore indicating that they could be valuable in grazing and  
1827 reducing *A. viridis* patches. In contrast to many other breeds (e.g., Homburger et al. 2015), Highland  
1828 cattle were also capable of grazing on the steepest slopes and were able to spend time in areas further  
1829 from water sources, as they were not significantly influenced by these harsh conditions. This is in line  
1830 with the observations of Pauler et al. (2020a) who found that Highland cattle used space more evenly  
1831 and avoid steep slopes less than other breeds. Moreover, cattle even preferred *A. viridis* patches and  
1832 steep slopes in Champlong, during certain grazing periods, which shows that cattle are able to move to  
1833 areas with the most unfavorable conditions and can stay there for relatively long periods (approximately  
1834 2 weeks). Cattle also responded differently to the various factors during the time they were on the  
1835 paddocks. This indicates that the herds may explore the paddock intensively at the beginning of the  
1836 grazing period before selecting particular areas later on. However, despite the fact that Highland cattle  
1837 graze less selectively on plants compared to other breeds (Pauler et al. 2020b), they were still mostly  
1838 attracted to valuable pastures. Thus, providing a mosaic of open pasture areas with higher quality forage  
1839 in *A. viridis*-encroached paddocks may be a good solution to respect grazing preferences while  
1840 increasing grazing pressure on *A. viridis*-encroached patches. Moreover, this type of setting would  
1841 enhance the potential for seed translocation through endo- and epi-zoochory by Highland cattle  
1842 (Mouissie 2004; Cosyns et al. 2005). Indeed, in the study of Pauler et al. (2019), plant species relying  
1843 on epizoochory were significantly more frequent in pastures grazed by Highland cattle than in paddocks  
1844 grazed by other breeds, probably because the long fur of this breed is ideal for transporting seeds. Animal  
1845 movements might thus provide additional seed translocation fluxes from pastures to encroached areas  
1846 to facilitate the restoration of typical pasture vegetation in the long-term (Pittarello et al. 2016a).  
1847 However, future research is needed to assess the simultaneous effects of cattle grazing, trampling, and  
1848 seed transportation on the restoration of pasture vegetation in *A. viridis*-encroached areas. We observed  
1849 considerable impact of the cattle on vegetation within the encroached areas (data not shown), including  
1850 intense defoliation of *A. viridis* shrubs and a reduction of their canopy cover, due to browsing, as well  
1851 as damage to branches due to cattle movement and scratching. Moreover, an increase in bare soil due to  
1852 livestock trampling and a considerable number of dung pats was observed within *A. viridis*-encroached  
1853 areas. If repeated for several years, these effects of the cattle could provide favorable conditions for the  
1854 recolonization of typical pasture vegetation in formerly encroached areas. The repetition of the botanical  
1855 surveys along the fixed vegetation transects installed during this study could allow us to assess the  
1856 medium- and long-term impacts produced by Highland cattle on vegetation. In addition, testing  
1857 improved techniques to attract livestock to the most encroached areas, such as the placement of specific  
1858 attractive points (Pittarello et al. 2016a; Bailey et al. 2019), might be relevant to further increase the  
1859 grazing pressure and thus livestock impacts on *A. viridis*-encroached subalpine pastures. In conclusion,  
1860 our results show that Highland cattle can graze in harsh environmental conditions and exploit *A. viridis*-  
1861 encroached pastures. This suggests that they have a high potential to reduce *A. viridis* encroachment in  
1862 the long-term.

1863 **CHAPTER V: Use of molasses-based blocks to modify grazing patterns and**  
1864 **increase Highland cattle impacts on *Alnus viridis*-encroached pastures**

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1906 Based on: Svensk M, Nota G, Mariotte P, Pittarello M, Barberis D, Lonati M, Allan E, Perotti E, Probo  
1907 M (2022) Use of molasses-based blocks to modify grazing patterns and increase Highland cattle impacts  
1908 on *Alnus viridis*-encroached pastures. *Frontiers in Ecology and Evolution*. [https://doi.org/10.3389/fevo.](https://doi.org/10.3389/fevo.2022.849809)  
1909 2022.849809

1910 **Abstract**

1911

1912 *Alnus viridis* is a pioneer species that has expanded in Central Europe in the last decades, causing  
1913 a series of negative agro-environmental impacts. Robust livestock grazing could be used as a targeted  
1914 tool to reduce its encroachment, but more information is needed to find the best approach to achieve  
1915 this goal. In this study, we assessed the potential of molasses-based blocks (MB) to lure Highland cattle  
1916 into *A. viridis*-encroached areas and monitored impacts on the vegetation after grazing. In 2019 and  
1917 2020, two Highland cattle herds equipped with GPS collars were placed in three paddocks in the Swiss  
1918 and Italian Alps, differing in the degree of *A. viridis* encroachment. In 2020, MB were added to highly  
1919 encroached areas within each paddock to attract the herds to feed on *A. viridis*. Botanical surveys  
1920 were carried out before and after grazing, around MB and control areas. Highland cattle grazed  
1921 significantly more around MB (up to 50 m from the MB) compared to the previous year (i.e., same area  
1922 without MB) and compared to control areas. The increased targeted grazing around MB led to a  
1923 significant decrease in herbaceous cover and an increase in bare soil compared to control areas. Livestock  
1924 grazing and trampling significantly reduced the cover of ferns, tall herbs, medium and small herbs, and  
1925 woody species around MB compared to control areas. *A. viridis* leaves and branches were significantly  
1926 removed and damaged up to 10 m from the MB, due to the more intense livestock grazing. Such results  
1927 highlight the potential of this management regime to effectively reduce *A. viridis* encroachment in  
1928 montane grasslands.

1929 **Introduction**

1930

1931 During the last century, socio-economic transformations have led to a large-scale decrease in agro-  
1932 pastoral activities across the mountain areas of Europe, resulting in many challenges for grassland  
1933 management and biodiversity conservation (MacDonald et al. 2000; Valkó et al. 2018). For instance, in  
1934 Switzerland, the last 30 years have witnessed a significant decrease in grassland area in alpine regions  
1935 (Strebel and Bühler 2015; Zehnder et al. 2016a). The reduction of livestock farming has caused profound  
1936 landscape modification and widespread shrub and tree encroachment in former meadows and pastures  
1937 (Estel et al. 2015). For example, from 1985 to 2013, shrublands and forests increased by 10.6% across  
1938 Switzerland, with the largest increases in the Alps (Abegg et al. 2020). Encroachment of montane  
1939 grasslands by woody species has been even more pronounced in the Italian Alps (Orlandi et al. 2016).  
1940 Shrublands now cover an area of 679 km<sup>2</sup> in Switzerland (Abegg et al. 2020), with about 70% of them  
1941 composed by green alder [*Alnus viridis* (Chaix.) D. C.], which is among the most rapidly expanding  
1942 shrub species in Central Europe (Anthelme et al. 2007). *Alnus viridis* is a pioneer shrub species that lives  
1943 in symbiosis with the N<sub>2</sub>-fixing actinomycete *Frankia alni* (Huss-Daniel 1997). It is found mostly in  
1944 steep, north and west-facing slopes, but it can easily expand into other habitats in montane environments,  
1945 thanks to its efficient colonization ability and substantial seed production (Farmer et al. 1985; Caviezel  
1946 et al. 2017). Its presence is strongly affected by land-use intensity, as land-abandonment is a key driver  
1947 of its spread (Caviezel et al. 2017).

1948

1949 Encroachment by *A. viridis* can have several negative agro-environmental impacts on montane  
1950 grasslands, which in turn reduce the provision of key ecosystem services for society. For instance, *A.*  
1951 *viridis* encroachment prevents forest succession, and causes nitrogen enrichment in soils leading to  
1952 increased nitrate leaching and higher risk of dissolved organic carbon leaching (Bühlmann et al. 2016;  
1953 Hunziker et al. 2017). This can result in soil acidification, water pollution and gaseous nitrogen losses  
1954 (Caviezel et al. 2014; Bühlmann et al. 2017). van den Bergh et al. (2018) also showed that *A. viridis*-  
1955 encroachment increases the evaporative water loss. Moreover, *A. viridis*-dominated shrublands are  
1956 characterized by a lower forage quality and host lower animal and plant diversity than adjacent  
1957 grasslands (Anthelme et al. 2001; Bühlmann et al. 2014; Svensk et al. 2021). A recent study by Zehnder  
1958 et al. (2020) showed that *A. viridis* encroachment resulted in a rapid reduction in plant species richness  
1959 and grassland specialist species, with dense *A. viridis* patches hosting 62% less species than nearby open  
1960 pastures. This decrease in biodiversity is linked to reduced light levels in highly encroached areas, as  
1961 well as to soil nitrogen enrichment. Indeed, only a few tall and shade-tolerant plants with broad leaves,  
1962 such as *Adenostyles alliariae* (Gouan) A. Kern and *Cicerbita alpina* L. (Wallr.), together with a few fern  
1963 species, are able to adapt to these ecological conditions and dominate the understory vegetation of *A.*  
1964 *viridis* shrublands (Svensk et al. 2021). Furthermore, in contrast to coniferous forests, *A. viridis* stands  
1965 do not provide protection against erosion and avalanches on steep slopes, mostly due to the elasticity of  
1966 their branches that easily bend under snow pressure (Caviezel et al. 2014). Their resistance to this  
1967 environmental pressure provides an advantage for *A. viridis* in these areas, compared to other shrubs or  
1968 trees that can easily break under such disturbance. Finally, the encroachment by *A. viridis* can also  
1969 adversely impact landscape quality in montane areas, resulting in reduced attractiveness for tourists.

1970

1971 Because *A. viridis* predominantly establishes on steep slopes and marginal locations, with few  
1972 roads, the mechanical removal of this shrub species can be technically difficult, expensive and time  
1973 consuming. One alternative and sustainable nature-based solution to counteract shrub encroachment  
1974 could be the use of targeted grazing (Elias and Tischew 2016; Elias et al. 2018; Pauler et al. 2022b).  
1975 Meisser et al. (2014) monitored the grazing behavior of Hérens cows in *A. viridis* dominated pastures  
1976 and showed that they did spend time in half or more encroached areas, despite their usual preference for

1977 open areas. However, many shrub species like *A. viridis* are not palatable for production-oriented  
1978 livestock due to their low foliage digestibility. Stević et al. (2010) found that *A. viridis* leaves  
1979 contained an average of 4.4% of tannins, which could eventually cause an astringent taste and reduce  
1980 palatability (Kumar and Vaithyanathan 1990). Nevertheless, some robust livestock species and breeds  
1981 have higher resistance to tannins, with ruminal bacterial populations that can better degrade lignified  
1982 material, allowing them to feed on shrubs and digest their leaves (Berry et al. 2002; Marques et al. 2017).  
1983 For instance, previous studies have shown that sheep and goats can efficiently feed on woody plants and  
1984 significantly reduce their cover (Iussig et al. 2015a; Álvarez-Martínez et al. 2016; Pauler et al. 2022b).  
1985 Some cattle breeds can also feed on shrub species (Zehnder et al. 2016a; Pauler et al. 2019). For example,  
1986 Highland cattle, a robust breed originating from Scotland, are able to graze on low quality shrub foliage  
1987 (Pauler et al. 2020a) due to their low maintenance energy requirements and their more efficient use of  
1988 nutrients from the vegetation (Berry et al. 2002). This has recently led, together with the low costs of  
1989 their maintenance and care, to an increased rearing of this livestock breed in alpine regions (Pauler et  
1990 al. 2020b). Previous studies have demonstrated their capacity to reduce woody plant species cover over  
1991 time, with a turnover in plant diversity (Pauler et al. 2019, 2020a). In addition to the direct grazing of  
1992 the leaves, Highland cattle are also able to damage shrub branches and trunks thanks to their long horns  
1993 (Svensk et al. 2021), even if they do not directly debark trees as some robust goat or sheep breeds do  
1994 (Pauler et al. 2022b). Recently, Svensk et al. (2021) showed that Highland cattle can graze on steep  
1995 montane pastures characterized by high *A. viridis* cover and associated low forage quality vegetation in  
1996 the understory. Therefore, Highland cattle grazing could be an efficient and sustainable tool to reduce  
1997 *A. viridis* encroachment and restore encroached pastures in the long-term.

1998  
1999 Together with cattle feeding behavior, livestock management techniques are also key elements to  
2000 increase livestock impacts on targeted shrub-encroached vegetation. For instance, livestock supplements  
2001 could be used to attract them to underused and shrub-encroached locations (Probo et al. 2013, 2016).  
2002 Different types of dietary supplementation exist, mainly composed of salt (i.e., mineral mix  
2003 supplements) or sugar (i.e., molasses-based blocks, MB). Previous studies have already demonstrated  
2004 the efficacy of mineral supplements in attracting beef cattle herds within steep montane shrub-  
2005 encroached areas (Probo et al. 2013; Pittarello et al. 2016a). For example, in a 5-year study, Probo et al.  
2006 (2016) highlighted a significant reduction in shrub cover, together with the establishment of typical  
2007 pasture species with higher forage quality, thanks to the effects produced by grazing, trampling, seed  
2008 and dung translocation at mineral supplement locations. Dehydrated MB were also proven to be efficient  
2009 in influencing cattle grazing patterns in unfavorable field conditions (i.e., steep slopes, far from water  
2010 sources and usually undergrazed zones) and across large areas in the North American steppes (Bailey  
2011 and Welling 1999; Bailey et al. 2001). However, they have never been tested in montane shrub-  
2012 encroached areas, despite their lower cost and labor compared to fencing and herding. The MB for cattle  
2013 grazing management provides different advantages, such as an enrichment of cattle diets through  
2014 mineral supplementation, that can also reduce the risks of foot pathology and mycotoxicosis, and an  
2015 enhancement of the intake of forage with low palatability, thanks to their appetizing role (Mordenti et  
2016 al. 2021). However, more information is needed on the efficiency of molasses-based supplements in  
2017 changing livestock spatial distribution and increasing the cover of target plant species of functional  
2018 groups, in montane environments. Indeed, previous studies (e.g., Tocco et al. 2013), showed that the  
2019 analysis of functional group cover in the short-term can be essential to assess the effectiveness of  
2020 targeted grazing techniques in a longer term.

2021  
2022 In the present study, we placed MB on *A. viridis*-encroached pastures to attract Highland cattle  
2023 herds and increase their effects on shrub vegetation. Specifically, we aimed to investigate: (i) the effect  
2024 of strategically placed MB on Highland cattle spatial distribution and (ii) the impacts of targeted grazing



2025 by livestock around MB on *A. viridis* shrubs, understory plant functional groups and soil cover. We  
2026 hypothesized that (i) Highland cattle would significantly use more the areas around MB, and (ii) their  
2027 targeted grazing and trampling pressure would have a higher impact on *A. viridis* shrubs around MB,  
2028 with an increased removal of leaves and damage on trunks and branches compared to control areas.  
2029 Simultaneously, we expected (iii) the understory vegetation to be more affected around MB, with an  
2030 increase in bare soil due to livestock grazing and trampling, which could lead to a potential re-  
2031 colonization of those areas by typical pasture species in the long-term.

2032  
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## 2034 **Methods**

2035

### 2036 **1. Study areas and grazing management**

2037

2038 During the summer seasons of 2019 and 2020, two Highland cattle herds were placed in three *A.*  
2039 *viridis*-encroached paddocks in the Swiss and Italian Alps. The first one (paddock 1, 17.88 ha) was  
2040 located in Val Vogna, in the province of Vercelli (Italy). The other two paddocks (paddock 2, 8.26 ha  
2041 and paddock 3, 7.67 ha) were located in Bovonne, in the canton of Vaud (Switzerland) and were grazed  
2042 by the same herd. All paddocks were grazed at a comparable stocking rate between years (**Table 4**) and  
2043 had similar topographical conditions, with an elevation of 1,861.45 m a.s.l (mean  $\pm$  s.e.) and slope of  $23$   
2044  $\pm 8^\circ$  (mean  $\pm$  s.e.). The three paddocks were representative of an *A. viridis* cover gradient, with an  
2045 average cover of 20, 61, and 71%, respectively, in paddock 1, 2, and 3. The herds grazed in the summer  
2046 pastures from the middle of June to the beginning of September (**Table 4**). All the herds included  
2047 cow/calf pairs and heifers, varying in age from 6 months to 17 years (with an average of 5 years for  
2048 paddock 1 and 4 years for paddock 2 and 3) and about 70% of the animals were present in both years at  
2049 the same site. A water trough was installed in paddocks 2 and 3, while natural streams were present in  
2050 paddock 1. In each herd and during both years, six to ten cows (**Table 4**) were equipped with GPS collars  
2051 (Followit AB, Tellus GPS System collars, Sweden) that recorded their position every 10 min during the  
2052 whole grazing period, with an accuracy of 2–5 m.

2053 **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 <sup>a</sup> )	Grazable area (ha)	Stocking rate (LU/ha × year)	Number of GPS collars
2019	Paddock 1	July 19 <sup>th</sup> to September 3 <sup>rd</sup>	44	45.4	17.88	0.31	6
	Paddock 2	July 2 <sup>nd</sup> to 19 <sup>th</sup>	17	29.8	8.26	0.17	8
	Paddock 3	July 30 <sup>th</sup> to August 17 <sup>th</sup>	19	29.8	7.67	0.19	8
2020	Paddock 1	July 20 <sup>th</sup> to August 18 <sup>th</sup>	29	70.4	17.88	0.31	8
	Paddock 2	June 15 <sup>th</sup> to July 2 <sup>nd</sup>	17	29.6	8.26	0.17	10
	Paddock 3	July 2 <sup>nd</sup> to 20 <sup>th</sup>	18	29.6	7.67	0.19	10

2054 <sup>a</sup>LU, Livestock Unit. One livestock unit = 1 animal of 500 kg.

2055 In 2020, five dehydrated MB (with 2–3% of residual moisture) of 22.5 kg each were added to each  
2056 paddock as attractive points. They were provided within small boxes of 40 × 28 × 20 cm. They were  
2057 mostly composed of sugar (40%), which has an appetizing effect and fosters the intake of low forage  
2058 quality vegetation (Mordenti et al. 2021), and contained mineral supplements which are often lacking  
2059 in natural montane environments (Schlegel and Kessler 2001), thus complementing cattle feeding  
2060 (detailed composition available in **Table S2**). The number of blocks was defined based on the average  
2061 consumption by cattle given by the producer (i.e., 35–50 g/calf × day, 100 g/heifer day, and 150–  
2062 200 g/cow × day). The consumption of MB was monitored in each paddock every 2 days to check that  
2063 enough molasses was available during the grazing period. At the end of the grazing period, the MB were  
2064 weighed and the average consumption per animal was estimated by dividing the total amount consumed  
2065 by the number of animals and grazing days. The MB were placed at five points along a 40-m line, lying  
2066 along a contour line, and separated by 10 m from each other (**Figure S5**), in highly *A. viridis*-encroached  
2067 areas (i.e., areas with more than 2/3 of *A. viridis* cover). A control line was also established in each  
2068 paddock in areas with comparable *A. viridis* cover, slope, botanical composition and distance to water  
2069 sources. These two similar zones (190 ± 72 m apart) were identified in each paddock and assigned  
2070 randomly to either control or MB areas before the grazing season of 2020. Moreover, to avoid any pre-  
2071 existing effect of the area on cattle distribution, we also checked that the MB and control locations were  
2072 similarly exploited by livestock in 2019, before the MB were established in 2020. Around control and  
2073 MB lines, buffer areas with a radius of 10 and 50 m were created in a GIS environment, and the number  
2074 of GPS positions was calculated for each cow within each buffer (using QGIS 3.6 software). As one  
2075 GPS location was recorded every 10 min, we calculated the average number of minutes a cow spent per  
2076 day in every buffer area. The 10-m buffer was used to assess the attractive effect in a small area, in  
2077 which livestock were likely present to actively consume MB, while the 50-m buffer was used to estimate  
2078 the attractive effect on a larger scale, i.e., including areas where livestock grazed and walked around  
2079 MB locations.

2080  
2081

## 2082 2. Vegetation surveys

2083

2084 Around both MB and control points, botanical surveys were carried out in 2020 both before grazing  
2085 (June-July) and after grazing (August), in cross-shaped vegetation transects of 10 meters (see **Figure**  
2086 **S5**), using the vertical point-quadrat method (Daget and Poissonet 1971). At 50 cm intervals along each  
2087 transect, the vascular plant species touching a steel needle up to a grazable height (i.e., 1.8 m) were  
2088 identified and recorded. To account for rare species around the transect, all other species within a one  
2089 meter buffer around the transect were also recorded (Kohler et al. 2004). Each line of MB and control  
2090 points in all three paddocks was made up of 16 transects (i.e., 48 vegetation transects in control areas  
2091 and 48 in MB areas in total). The percentages of herbaceous cover and bare soil were visually assessed  
2092 before and after grazing within a one-meter buffer around each transect, to assess the changes in  
2093 vegetation cover produced by livestock targeted grazing and trampling. Plant species nomenclature  
2094 followed Aeschimann et al. (2004).

2095

2096 Along the vegetation transect, the percentage species cover (%SC) was calculated by converting  
2097 the recorded frequency of occurrence of each plant species to 100 measurements. A %SC of 0.3% was  
2098 assigned to the species found only in the 1-m buffer zone and not along the transect, following Pittarello  
2099 et al. (2016a). Species were grouped in three main functional groups (see **Table S3**) that were common  
2100 among all paddocks and typical of the understory of *A. viridis*-encroached pastures (Svensk et al. 2021):  
2101 (1) Ferns and tall herbs, i.e., all ferns and tall broad-leaved forbs having a leaf diameter greater than 10  
2102 cm and taller than 40 cm, following Pignatti (1982); (2) medium and small herbs, i.e., other non-

2103 graminoid species that were not classified as tall herbs; and 3) woody, i.e., all woody species (including  
2104 *A. viridis*). Graminoids were not taken into account as they were a minor component of the understory  
2105 vegetation, representing only  $5.9 \pm 3.1\%$  on average (mean  $\pm$  SE) of the vegetation cover in each  
2106 paddock (with a maximum of 11.5% in paddock 1).

2107  
2108 Along each transect three *A. viridis* shrubs were selected every 3–4 m and marked. We did not  
2109 include the central transect in this selection as the central transects had two MBs close to them. One  
2110 branch from each tree was selected to assess the number of living leaves before and after grazing, and  
2111 thus estimate the removal of *A. viridis* leaves by Highland cattle, and the damage done to the observed  
2112 branch. Whenever the branch was totally broken and untraceable after grazing, the number of remaining  
2113 living leaves was considered equal to zero. The damage made on *A. viridis* branches through grazing,  
2114 scratching or trampling was also visually assessed with the following scale: 0 (no damage), 1 (moderate  
2115 damage), 2 (intense damage) and 3 (branch totally damaged or broken). The maximum height at which  
2116 damage was observed on each branch was also recorded.

2117  
2118 For the analyses of soil and functional group cover as well as leaf removal, a response ratio (RR)  
2119 was computed for all the assessed variables according to the following formula:

2120  
2121 
$$RR = \frac{Value_{Post} - Value_{Pre}}{Value_{Pre}}$$

2122  
2123 where “ValuePost” is the value measured after grazing and “ValuePre” the value measured before  
2124 grazing. This formula provides an indication of the effect size, which is not biased by the initial (before  
2125 grazing) differences among replicates. A negative RR indicates that the value decreased after grazing,  
2126 while a positive RR indicates that the value increased after grazing.

2127  
2128  
2129 **3. Statistical analysis**

2130  
2131 All statistical analyses were performed using R version 3.4.4 (R Core Team, 2017). The effects of  
2132 treatment (control vs. MB), the year (2019, 2020) and their interactions on the spatial distribution of  
2133 cows were tested separately for the 10- and 50-m buffers by using a linear mixed-effect model (lme,  
2134 package “nlme”), with “paddock” as a random factor. Spatial distribution of cows was measured as the  
2135 average time spent by a cow at the herd level within the buffer (minutes  $\text{day}^{-1} \text{cow}^{-1}$ , obtained from GPS  
2136 location counts), i.e., the sum of the minutes spent by all GPS-equipped cows in the considered buffer,  
2137 divided by the number of GPS-equipped cows. The effects of treatment (control vs. MB) on 2020 soil  
2138 cover (RRs of herbaceous cover and bare soil) and on functional group cover (RRs of ferns and tall  
2139 herbs, medium and small herbs, and woody species cover) were tested using generalized least square  
2140 models (GLS), with treatment (control vs. MB) as fixed factors and coordinates of the transects, nested  
2141 in paddock, as random effects. This random effect structure takes into account the nested structure of  
2142 the data and accounts for any residual spatial autocorrelation among neighboring transects. The effects  
2143 of MB, distance of shrubs along the transect, and their interactions, on the RR of *A. viridis* leaves were  
2144 tested using the same type of GLS model, again with the coordinates of the transects nested in paddock.  
2145 Although the GLS analysis takes into account the potential spatial autocorrelation and  
2146 pseudoreplication, we carried out an additional, conservative analysis in which we analyzed only the  
2147 average RR in each area of the paddocks (i.e., 3 control points vs. 3 MB, one control and one MB from  
2148 each paddock: 6 data points in total), using ANOVA. The ANOVA has much lower power than the GLS  
2149 model but is completely free from any spatial autocorrelation or pseudoreplication issues. *Post hoc* tests

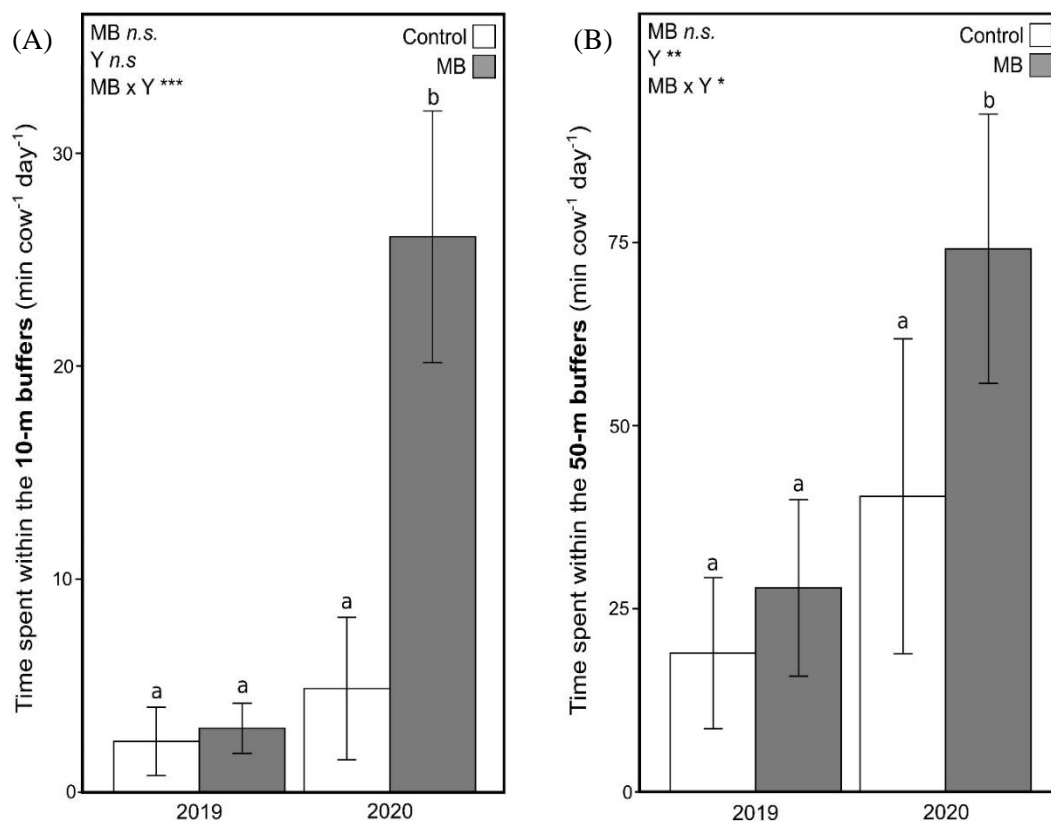
2150 were performed for the models when significant effects were detected (Tukey’s test,  $P < 0.05$ ), and  
 2151 additional Student’s test ( $t$ -test) were performed to assess the difference of RRs from zero.  
 2152  
 2153

## 2154 Results

### 2155 1. Effects of molasses-based blocks placement on livestock spatial distribution

2156 Based on the remaining molasses after grazing, we calculated that each animal consumed 134 g of  
 2157 molasses per day on average. All MB (except two placed in paddock 3) were completely consumed at  
 2158 the end of each grazing period.  
 2159  
 2160

2161 There were no significant differences in the average number of minutes spent by cows between  
 2162 control and MB areas in 2019, in both 10 and 50-m buffers ( $P = 0.99$  and  $P = 0.78$ , respectively; **Figure**  
 2163 **24** and **Table S4**), indicating that the locations chosen were equally attractive to livestock, before the  
 2164 MB were placed in 2020. There was a significant increase of livestock use in MB areas in 2020 compared  
 2165 to 2019 for both 10-m (MB Y,  $P < 0.001$ , + 771% on average; **Figure 24A**) and 50-m (MB Y,  $P < 0.05$ ,  
 2166 + 166% on average; **Figure 24B**) buffers.  
 2167  
 2168



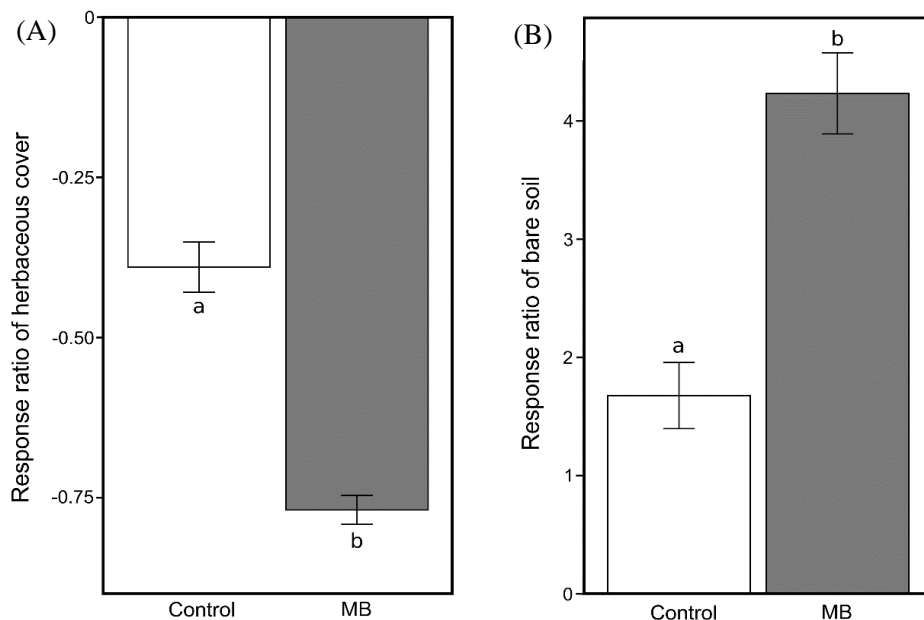
2169 **Figure 24** Number of minutes spent (mean  $\pm$  SE) per day per cow within the herd in 10-m buffers (A)  
 2170 and 50-m buffers (B) in 2019 and 2020 for both molasses-based blocks (MB, gray) and control (white)  
 2171 areas. Significance of treatment (MB), year (Y) and their interaction (MB  $\times$  Y) is reported on the graph  
 2172 (“n.s” as non significant, “\*” for  $p < 0.05$ , “\*\*” for  $p < 0.01$ , “\*\*\*” for  $p < 0.001$ ). Different letters  
 2173 indicate significant differences (Tukey’s test,  $P < 0.05$ ) between areas for both years.  
 2174  
 2175  
 2176

2177 **2. Effects of livestock on soil cover**

2178

2179 The percentage of bare soil was highly impacted by livestock targeted grazing and trampling. There  
2180 was a significant decrease in herbaceous cover and a significant increase in bare soil after grazing, in  
2181 both control and MB areas, with response ratios always significantly different from zero ( $P < 0.001$ ,  
2182 **Figure 25** and **Table S4**). The impacts of livestock around the MB always had a significant effect on  
2183 both herbaceous cover and bare soil ( $P < 0.001$ , **Figure 25**), with a higher impact on bare soil around  
2184 MB compared to control areas. The herbaceous cover had an average RR of  $-0.77$  around MBs, and  
2185 decreased from 73.5% before grazing to 16.2% after grazing. In control areas, herbaceous cover had an  
2186 average RR of  $-0.39$  and decreased from 61.9% before grazing to 35.5% after grazing. On the other  
2187 hand, the percentage of bare soil had an average RR of 4.23 around MB, and represented 17.1% before  
2188 grazing and 76.2% after grazing. In control areas, bare soil had an average RR of 1.68, and represented  
2189 29.2% before grazing and 56.7% after grazing. The ANOVA analysis performed on the average RR  
2190 cover per paddock provided the same results, except for percentage of bare soil for which the effect of  
2191 MB was marginally significant (see **Table S5**).

2192



2193

2194 **Figure 25** Response ratio (mean  $\pm$  SE) of herbaceous cover (A) and bare soil (B) in both molasses-based  
2195 blocks (MB, gray) and control (white) areas. Different letters indicate significant differences (Tukey's  
2196 test,  $P < 0.05$ ) between areas. Response ratios from both control and MB areas were significantly  
2197 different from zero for both herbaceous cover and bare soil ( $t$ -test,  $P < 0.001$ ).

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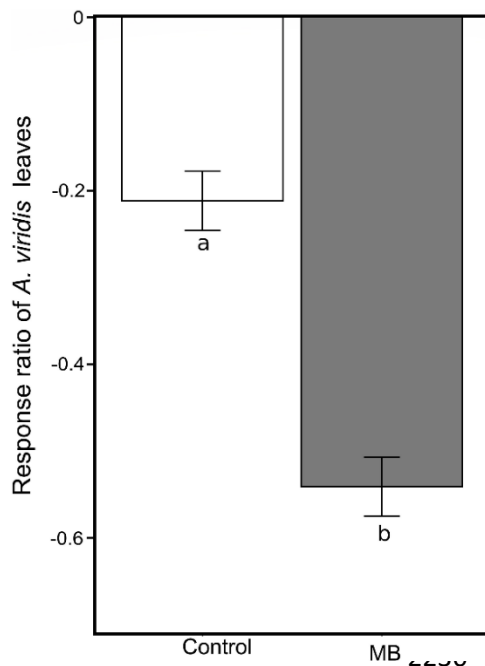
2199

2200 **3. Effects of livestock on *Alnus viridis* shrubs**

2201

2202 The *A. viridis* shrubs were highly damaged by Highland cattle targeted grazing. There was a  
2203 significant decrease in the number of *A. viridis* leaves after grazing in both control and MB areas, with  
2204 a higher decrease around MB and negative response ratios significantly different from zero ( $P < 0.001$ ,  
2205 **Figure 26** and **Table S4**). The interaction between MB and distance was significant ( $P < 0.05$ ), with the  
2206 removal being higher on shrubs closer to the MB compared to further shrubs, while in control areas the  
2207 effect was the same independent of the distance. In addition to leaf removal, damage on *A. viridis*  
2208 branches was assessed at MB locations at an average height of  $1.48 \pm 0.53$  m (mean SE), with 68% of  
2209 branches having damage scores between 1 and 2 (moderate to intense damages) and 10% with damage

2210 scores of 3 (very severe damage or broken branches). Reducing our dataset to 6 data points and analyzing  
2211 the average RR of number of *A. viridis* leaves showed that the effect of MB was also significant (see  
2212 **Table S5**).  
2213



**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,  $P < 0.001$ ).

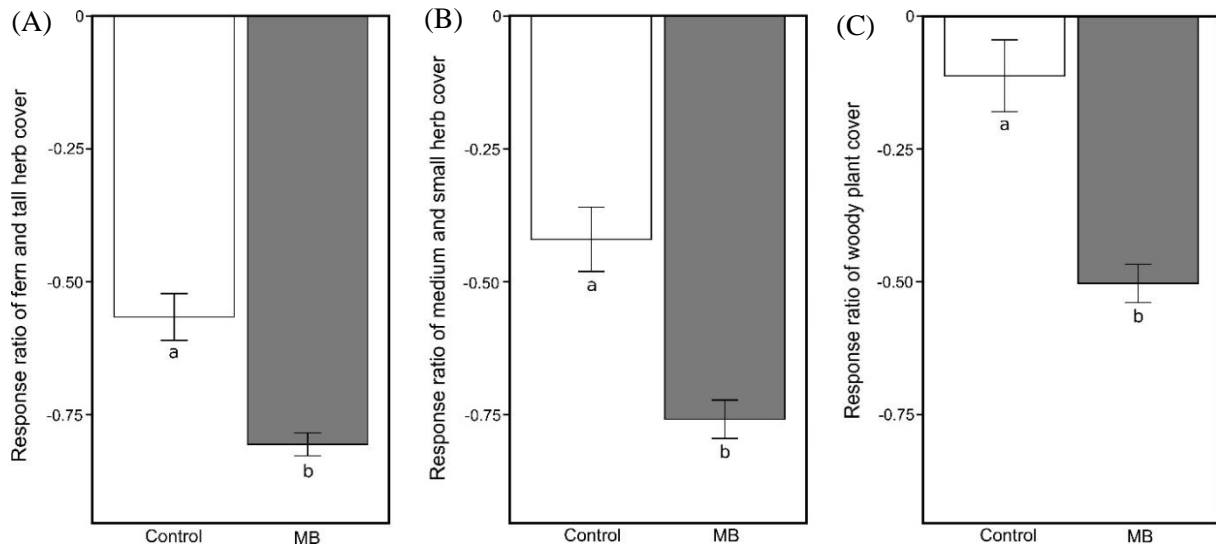
2231  
2232  
2233

#### 4. Effects of livestock on understory plant functional group cover

2234 A total of 149 plant species were recorded during the botanical surveys in all paddocks (the species  
2235 list and their corresponding functional group are available in **Table S3**). Ferns and tall herbs included  
2236 37 species and had the highest % SC (44.4%), with *Adenostyles alliariae* (14.1%), *Athyrium filix-femina*  
2237 (L.) Roth (10.4%) and *Dryopteris dilatata* (Hoffm.) A. Gray (4.3%) being the most dominant species.  
2238 Most species (78) were medium and small herbs (23.5%), with *Ranunculus aconitifolius* L. (8.7%)  
2239 *Stellaria nemorum* L. (2.7%) and *Viola biflora* L. (2.2%) being the most dominant. Woody species  
2240 (26.1%) comprised 14 species, including *Alnus viridis* (18.5%), *Vaccinium myrtillus* L. (2.0%) and  
2241 *Rhododendron ferrugineum* L. (1.5%). Graminoids were not abundant in the botanical surveys (20  
2242 species and 5.9%).

2243

2244 The increased targeted grazing and trampling around MB had a significant impact on understory  
2245 plant functional groups. There was a significant decrease in fern and tall herb cover and in medium and  
2246 small herb cover after grazing in both control and MB areas, with negative response ratios always  
2247 significantly different from zero ( $P < 0.001$ , **Figure 27A, B** and **Table S4**). The presence of MB always  
2248 had a significant effect on both groups, leading to a higher decrease compared to control areas ( $P <$   
2249  $0.001$ , **Figure 27A, B**). Woody species decreased significantly more around MB than in control areas  
2250 ( $P < 0.001$ ; **Figure 27C**), in which they were not significantly damaged, with response ratios not  
2251 different from zero ( $-0.11 \pm 0.07$ ,  $P = 0.10$ ). Reducing our dataset to only 6 data points and analyzing  
2252 the average RR of functional groups showed the same results, except for fern and tall herb cover for  
2253 which the effect of MB was not significant (see **Table S5**).  
2254



2255  
 2256 **Figure 27** Response ratio (mean ± SE) of the cover of ferns and tall herbs (A), medium and small herbs  
 2257 (B) and woody species (C) for both molasses-based blocks (MB, gray) and control (white) areas.  
 2258 Different letters indicate significant differences (Tukey's test,  $P < 0.05$ ) among areas. Response ratios  
 2259 from both control and MB areas were significantly different from zero for all functional groups ( $t$ -test,  
 2260  $P < 0.001$ ), except for woody plants where the control area showed an average response ratio not  
 2261 different from zero ( $t$ -test,  $P = 0.104$ ).

2262  
 2263  
 2264 **Discussion**

2265  
 2266 Overall, the strategic placement of MB significantly altered Highland cattle spatial distribution, and  
 2267 increased livestock use of areas around MB locations, compared to the previous year and to control  
 2268 areas. This result confirmed our first hypothesis that Highland cattle were attracted to MB and that they  
 2269 were able to use steep and highly encroached *A. viridis* areas, as recently pointed out by Svensk et al.  
 2270 (2021). The results on the effectiveness of MB are thus consistent with those found by Bailey and  
 2271 Welling (1999), who showed that they could attract herds into underused rangelands with poor quality  
 2272 forage. Those authors assessed the efficiency of dehydrated blocks in un-encroached zones with gentle  
 2273 to moderate slopes, while our study showed that such strategic placements can attract herds even in areas  
 2274 with steeper slopes and high levels of woody species encroachment, where grazing conditions could be  
 2275 more challenging. When analyzing the attractive effect at different spatial scales, a significant increase  
 2276 in the use of areas up to 50-m from MB locations, compared to control areas, was detected. This 50-m  
 2277 scale effect of MB underlines the potential of such management techniques to increase livestock use  
 2278 over large *A. viridis* encroached patches. The effect of MB within 10-m buffers was even more  
 2279 significant, with substantially increased livestock use at a close range near the molasses-based blocks.  
 2280 Such measurements at different spatial scales highlight the influence of MB on Highland cattle spatial  
 2281 distribution, with this type of management enabling cattle to increase their visits to the surrounding  
 2282 encroached zones once they are attracted by the molasses. Other studies have also shown high potential  
 2283 for different strategic placements to reduce shrub-encroachment at different scales, and to attract herds  
 2284 into under-grazed areas (Bailey and Welling 2007; Bailey and Jensen 2008; Pittarello et al. 2016a). In  
 2285 particular, Pittarello et al. (2016a) found a significant attractive effect at 10- and 50-m around  
 2286 supplement blocks in dwarf-shrub encroached montane pastures, using a different supplement type  
 2287 (mineral mix supplements) for another cattle breed (Piedmontese breed). In our study, under comparable



2288 mountain topographic conditions, livestock use and supplement consumption were much higher, with  
2289 134 g of molasses consumed per animal per day, corresponding to ca. 80 g of minerals without taking  
2290 into account the 40% of sugar from molasses content (see **Table S2**) compared to 13.7 g of mineral mix  
2291 supplement in Pittarello et al. (2016a), suggesting a higher attractiveness and efficiency of molasses-  
2292 based than mineral mix blocks in modifying livestock spatial distribution. Indeed, the average time spent  
2293 around MB (i.e., 74 min cow<sup>-1</sup> day<sup>-1</sup>) was much higher than the 18 min cow<sup>-1</sup> day<sup>-1</sup> around mineral mix  
2294 supplements in a 50-m buffer found by Pittarello et al. (2016a). Furthermore, the familiarity of the cattle  
2295 with the paddock characteristics might be an important factor to consider when implementing such  
2296 management in the long term, as the exploration behavior could increase over time and have an enhanced  
2297 effect on vegetation.

2298

2299 Overall, the increased use by Highland cattle of areas with MB led to a significant removal of leaves  
2300 and damage to the branches of *A. viridis* shrubs. Moreover, it resulted in a decrease in herbaceous cover  
2301 and an increase in bare soil after grazing, compared to control areas. Further, the cover of medium and  
2302 small herbs and woody plants was significantly reduced by the increased livestock use of MB areas.  
2303 These impacts were related both to targeted grazing and to the mechanical damage caused by Highland  
2304 cattle once attracted to the MB areas. Such damage may result from trampling, scratching and breaking  
2305 branches while walking (see **Figure S6-10**), as recently observed by Svensk et al. (2021). The significant  
2306 effect of the interaction between MB and the distance of shrubs suggests a different effect of the distance  
2307 between control and MB areas. Indeed, large paths created by the animals were observed between MB  
2308 and the surrounding area, due to grazing activity and movement from MB to water sources. While we  
2309 could not differentiate between effects of grazing and trampling on the vegetation, Highland cattle were  
2310 observed to graze on species with particularly low forage quality, such as ferns (*A. filix-femina*, *D.*  
2311 *dilatata*) and tall herbs (*A. alliariae*). This demonstrates the ability of this breed to feed not only on *A.*  
2312 *viridis* shrubs but also on other unpalatable understory species, which is consistent with our second and  
2313 third hypotheses. Previous studies have also shown that Highland cattle tend to be less selective with  
2314 regard to forage quality compared to other breeds, and are able to feed on woody plants (Pauler et al.  
2315 2020b, a). Moreover, they tend to be more adapted to low quality vegetation and lower nutrient intake,  
2316 as they continue to gain weight under such constraining conditions (Berry et al. 2002). *A. viridis* leaves  
2317 and the understory vegetation have also proven to be a valuable fodder resource, but only for certain  
2318 robust cattle breeds, such as Dexter cattle or Hérens cattle (Meisser et al. 2014; Zehnder et al. 2016a).  
2319 Even if only measured in the short-term, the intense impacts exerted on *A. viridis* shrubs and other  
2320 woody species indicate the potential for Highland cattle to reduce woody species encroachment in the  
2321 medium- and long-term.

2322

2323 Maintaining Highland cattle grazing and the strategic placement of MB over the long term could  
2324 enable typical pasture species to colonize areas where vegetation cover was highly impacted and new  
2325 gaps were created. Indeed, several studies have shown that long-term targeted grazing by goats led to  
2326 increased light conditions in formerly highly shrub-encroached pastures, with the establishment of light-  
2327 demanding, low-growing and less competitive grassland species (Elias et al. 2018; Silva et al. 2019;  
2328 Köhler et al. 2020). Similarly, we can expect cows to alleviate light competition for the understory  
2329 vegetation cover (thanks to the combination of increased bare soil and removed of *A. viridis* leaves)  
2330 likely producing a shift in plant functional groups in the long term, and an increase in plant diversity  
2331 (Hautier et al. 2009). Moreover, to avoid possible risks of localized erosion linked to excessive livestock  
2332 trampling, it would be advisable to regularly move MB to different shrub-encroached areas during the  
2333 grazing season and throughout the years. This would also allow the expansion of the livestock impacts  
2334 on vegetation to a wider area (Probo et al. 2013). While our paddocks always had comparable stocking  
2335 rates between years, they did not always have exactly the same stocking densities (e.g., paddock 1, in

2336 which in the second year a bigger herd grazed for a shorter period compared to the first year). Therefore,  
2337 future studies with similar stocking densities among years would be useful to confirm the present  
2338 findings. The assessment of the potential effect of social interactions within the herd (Stephenson et al.  
2339 2016) could also be useful to refine the effect of MB in the herd at an individual level. Moreover, as  
2340 Highland cattle periodically traveled from open pastures to highly encroached zones (Svensk et al.  
2341 2021), the restoration of former pastures could be enhanced by seed translocation through endo- and  
2342 epi-zoochory. Indeed, a recent study (Pauler et al. 2019) demonstrated that a higher number of  
2343 epizoochoric plant species were found in pastures grazed by Highland cattle compared to pastures grazed  
2344 by production-oriented cattle breeds, since the long fur of the Highland cattle resulted in more efficient  
2345 seed dispersal. The same study also found that the less selective grazing behavior of this breed led to  
2346 higher plant species richness in pastures, as Highland cattle consumed a larger range of species  
2347 compared to other breeds. They were thus able to graze on less palatable dominant species and reduce  
2348 their abundance for the benefit of other herbaceous species. Moreover, a high number of dung droppings  
2349 was already observed around MB after grazing, which could further promote seed dispersal from  
2350 adjacent pastures through endozoochory (Cosyns et al. 2005). Indeed, even if other management  
2351 strategies such as controlled fires or clear-cutting have proven effective at reducing shrub encroachment  
2352 in montane environments, targeted grazing seems to be best suited to restore montane pastures,  
2353 especially considering the enhanced plant dispersion by livestock (Alados et al. 2019). In addition, the  
2354 mechanical damage caused to *A. viridis* branches by livestock might increase pathogen attacks on this  
2355 shrub species and therefore reduce its spread. For example, fungi species of the genus *Phytophthora* can  
2356 spread through water and have the potential to infect bark lesions of a plant, and have been reported to  
2357 have severe impacts on alder shrubs including *A. viridis*, sometimes leading to mortality (Pisetta et al.  
2358 2012; Bregant et al. 2020).

2359

2360

## 2361 **Conclusion**

2362

2363 In conclusion, our findings demonstrate that the strategic placement of molasses-based blocks was  
2364 efficient in attracting Highland cattle toward areas highly invaded by *A. viridis*, despite the harsh terrain  
2365 (steep slopes). Highland cattle successfully fed on, and damaged, *A. viridis* shrubs around the MB, thus  
2366 significantly altering the understory vegetation through grazing and trampling, in just one grazing  
2367 season. Highland cattle could thus have the potential to effectively reduce *A. viridis*-encroachment in  
2368 the long term, and, by increasing light conditions for understory species and translocating seeds, they  
2369 could promote montane pasture restoration.

2370 **CHAPTER VI: Nitrogen translocation by Highland cattle grazing in *Alnus***  
2371 ***viridis*-encroached pastures**

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2411 Based on: Svensk M, Pittarello M, Mariotte P, Nota G, Schneider M K, Frund D, Dubois S, Allan E,  
2412 Probo M (2023) Nitrogen translocation by Highland cattle grazing in *Alnus viridis*-encroached  
2413 pastures. Nutrient Cycling in Agroecosystems. <https://doi.org/10.1007/s10705-023-10282-0>

2414 **Abstract**

2415

2416 During the last decades, *Alnus viridis* has expanded over former montane pastures and meadows,  
2417 due to land use and abandonment. This nitrogen-fixing woody species has triggered negative agro-  
2418 environmental impacts, such as nitrogen (N) leaching, soil acidification and a reduced biodiversity. The  
2419 aim of this study was to estimate the N translocation from *A. viridis*-encroached areas to adjacent open  
2420 pastures by Highland cattle. In 2019 and 2020, Highland cattle herds equipped with GPS collars were  
2421 placed in four *A. viridis*-encroached paddocks across Italy and Switzerland. The N content was measured  
2422 in *A. viridis* leaves, herbaceous vegetation, and cattle dung pats, which were collected throughout the  
2423 grazing season. Using GPS locations and collar activity sensors, livestock activity phases were  
2424 discriminated. The N ingested by cattle was estimated through the N content of herbaceous vegetation  
2425 and *A. viridis* leaves of vegetation patches visited by cattle during 24 h before dung sampling ( $N_{24H}$ ).  
2426 The N content of herbaceous vegetation significantly increased with increasing *A. viridis* cover. The  
2427 average N content in dung pats ( $31.2 \pm 3.4 \text{ g kg}^{-1} \text{ DM}$ ) was higher than average values from literature  
2428 on grazing cattle. Moreover, it was positively related to the  $N_{24H}$ . Most of this N ( $29.5 \pm 10.3 \text{ kg ha}^{-1}$   
2429  $\text{yr}^{-1}$ ) was translocated towards resting areas, which generally occurred on flat open pastures. Our results  
2430 highlight the potential of Highland cattle to effectively translocate part of the ingested N from *A. viridis*-  
2431 encroached towards targeted open areas, thus bringing new perspective for forage yield and quality  
2432 improvement in the long-term.

## 2433 Introduction

2434

2435 Encroachment of grasslands by woody species is a worldwide phenomenon (Eldridge et al. 2011;  
2436 Wieczorkowski and Lehmann 2022). During the last decades, European mountain chains have faced  
2437 major socio-economic transformations, above all on marginal areas, such as a decrease in agro-pastoral  
2438 activities and land abandonment, with a consequent increase in shrub encroachment (MacDonald et al.  
2439 2000; Strebel and Bühler 2015; Orlandi et al. 2016). For instance, in Switzerland, shrub forests have  
2440 increased by 22% between 1983 and 2017, with the greatest expansion recorded in the Alps (Abegg et  
2441 al. 2020). In Central Europe, *Alnus viridis* (Chaix., D.C.) is the most rapidly expanding shrub species,  
2442 as it is a pioneer species that has efficient sexual and vegetative reproduction traits (Farmer et al. 1985;  
2443 Mallik et al. 1997; Caviezel et al. 2017). In Switzerland, its expansion rate is two to three times faster  
2444 than the forest, and nowadays it represents 70% of the Swiss shrubland cover (Anthelme et al. 2007;  
2445 Bühlmann et al. 2014; Abegg et al. 2020). This shrub can be found mostly in north and west facing steep  
2446 slopes, but has recently started to expand in more gentle slopes (Caviezel et al. 2017). Its presence has  
2447 led to many agro-ecological issues, since it can reduce some of the ecosystem services provided by  
2448 montane grasslands. Indeed, its encroachment into open grasslands has led to a loss of potential  
2449 agricultural production and has reduced landscape attractiveness, which has an important economic  
2450 value for tourism (Ewald 2001). *A. viridis* encroachment also prevents forest succession by hindering  
2451 montane conifers' establishment (Hiltbrunner et al. 2014).

2452

2453 Because of its symbiosis with the N<sub>2</sub>-fixing actinomycete *Frankia alni* (Huss-Daniel 1997; Dawson  
2454 2008), the presence of *A. viridis* is leading to nitrogen enrichment in soils (Bühlmann et al. 2016). This  
2455 creates a nitrogen saturated habitat which, combined with the reduction of light, temperature and the  
2456 increased humidity under *A. viridis* canopy, facilitates the growth and dominance of a few shade-tolerant  
2457 and nitrophilous species, such as *Adenostyles alliariae* (Gouan) A. Kern, *Cicerbita alpina* L. (Wallr.)  
2458 and ferns (e.g. *Athyrium filix-femina* (L.) Roth and *Dryopteris dilatata* (Hoffm.), having low forage  
2459 quality (Svensk et al. 2021). Therefore, encroached areas have lower plant and animal species richness  
2460 if compared to adjacent open grasslands (Anthelme et al. 2001; Susan and Ziliotto 2004; Bühlmann et  
2461 al. 2014; Cislighi et al. 2019; Zehnder et al. 2020; Svensk et al. 2021).

2462

2463 Nitrogen fixation by *A. viridis* increases nitrification and thus enhances possibilities of nitrate and  
2464 dissolved organic carbon leaching, leading to the pollution of streamlets and groundwater (Bühlmann et  
2465 al. 2014, 2016; Hunziker et al. 2017). Consequently, *A. viridis*-encroached habitats display higher risk  
2466 of soil acidification, through the loss of base cations leached along with nitrates (Galloway et al. 2003;  
2467 Bühlmann et al. 2016). Moreover, high nitrogen availability increases gaseous nitrogen loss such as the  
2468 greenhouse gases NO and N<sub>2</sub>O (Galloway et al. 2003; Caviezel et al. 2014; Hiltbrunner et al. 2014).

2469

2470 Managing the expansion of *A. viridis* is thus an important goal for the restoration of the ecosystem  
2471 services provided by montane pastures. Historically, until the 1950's, the mechanical cutting of *A. viridis*  
2472 for firewood combined with the higher grazing pressure at the time helped in controlling *A. viridis* spread  
2473 (Caviezel et al. 2017). Nowadays, because of the loss of its economic benefit and the reduced workforce  
2474 available in mountain areas, other more economically sustainable solutions have to be found to restore  
2475 open pastures. Moreover, single cuts of *A. viridis* shrublands may lead to sprouting and thus denser  
2476 stands, and may not be efficient to restore the below-ground conditions, as they are highly altered by the  
2477 shrub encroachment (Schwob et al. 2017).

2478

2479 The use of targeted grazing to reduce tree and shrub-encroachment has already been recognized as  
2480 being efficient and promising in the long-term (Mittlacher et al. 2002; Meisser et al. 2014; Elias et al.

2481 2018). Because *A. viridis* leaves contain moderately high tannins levels (Stević et al. 2010), they can  
2482 have low digestibility and palatability, preventing their use as a forage resource for production-oriented  
2483 livestock (Kumar and Vaithiyathan 1990; Besharati and Taghizadeh 2011). However, robust livestock  
2484 breeds have the ability to digest lignified and tannin-rich vegetation through their tannin-tolerant rumen  
2485 bacteria (Manousidis et al. 2016). Previous studies have shown that robust cattle breeds, such as  
2486 Highland cattle, are able to feed on shrub species foliage with low forage quality (Pauler et al. 2020b;  
2487 Svensk et al. 2021). The exploitation of woody species-encroached areas by Highland cattle can help  
2488 controlling shrub and tree cover over time, by combining the effect of direct foliage consumption,  
2489 trampling, and the mechanical breaking of branches, thanks to their long horns, and could potentially  
2490 lead to the slow opening of the canopy, allowing the recolonization of typical pasture species in the  
2491 long-term (Probo et al. 2016; Pauler et al. 2019, 2020a; Svensk et al. 2022).

2492  
2493 In addition to their use to limit *A. viridis* encroachment, robust livestock could be used to balance  
2494 the level of nitrogen in shrub-encroached areas and adjacent pastures. Indeed, nutrients can be  
2495 transported through animal excretions, by taking in nutrients while foraging, and returning them through  
2496 urine and dung excretions (Haynes and Williams 1993; Schnyder et al. 2010). Up to 95% of the nitrogen  
2497 ingested by grazing animals can be excreted, mostly in urine (Whitehead 1970; Burggraaf and Snow  
2498 2010). Cattle activity (e.g., grazing, resting) is usually affected by topographic, vegetation and  
2499 management factors (Probo et al. 2014; Homburger et al. 2015). Thus, the spatial distribution of dung  
2500 pats and urine is not uniform and excretions are mainly deposited in resting areas, which are usually flat  
2501 areas with low shrub cover (Costa et al. 1990; White et al. 2001; Kohler et al. 2006; Buttler et al. 2008;  
2502 Koch et al. 2018). As a consequence, nutrients can be spatially redistributed from grazing areas, where  
2503 they are taken in, to resting areas, where they are deposited (Kohler et al. 2006). Therefore, in *A. viridis*  
2504 encroached pastures subjected to targeted grazing with Highland cattle, we expect an active N  
2505 translocation from shrub-encroached to open and flat areas. *A. viridis*-encroached areas can have a high  
2506 N level in the vegetation, due to the high N content of *A. viridis* leaves (Bühlmann et al. 2016) and that  
2507 of the understory herbaceous vegetation. Indeed, the understory herbaceous vegetation is characterized  
2508 by a significantly higher N content than that of both nutrient-rich and nutrient-poor pastures in the  
2509 surroundings (Zehnder et al. 2017). Moreover, condensed tannins rich-species, such as *A. viridis*, may  
2510 induce proportionally higher nitrogen excreted through dung than through urine (Burggraaf and Snow  
2511 2010), thus limiting ammonia volatilization from urea, and making nitrogen more available for plant  
2512 utilization on the long-term (Lantinga et al. 1987; Berry et al. 2001).

2513  
2514 The aim of this study was to evaluate whether Highland cattle grazing in *A. viridis*-encroached  
2515 pastures can become a management tool to translocate N from shrublands to adjacent open pastures, and  
2516 thus help reduce the negative environmental impacts of *A. viridis* expansion. Therefore, we studied  
2517 Highland cattle herds grazing in Swiss and Italian *A. viridis*-encroached pastures and we measured the  
2518 N content of the herbaceous vegetation, green alder leaves and cattle dung pats during two grazing  
2519 seasons. Specifically, we aimed to: (i) measure the N content in Highland cattle dung pats and compare  
2520 with literature data on grazing cattle dung pats; (ii) assess its relationship with the ingested N content  
2521 during 24 h prior dung deposition; and (iii) estimate the N import–export fluxes within *A. viridis*-  
2522 encroached areas and adjacent open pastures. We hypothesized that: (i) the N content in Highland cattle  
2523 dung pats is higher than in dung pats from cattle grazing on open pastures and/or with similar crude  
2524 protein-rich diets; (ii) the more often cattle have grazed in *A. viridis* encroached areas during 24 h before  
2525 dunging, the more nitrogen-rich the dung pats are; and (iii) *A. viridis*-encroached areas have negative N  
2526 fluxes (i.e., N is exported from these areas), while adjacent open pastures have positive N fluxes (i.e., N  
2527 accumulates).

2528

2529 **Methods**

2530

2531 **1. Study areas and grazing management**

2532

2533 The study was conducted in 2019 and 2020 on four *A. viridis*-encroached paddocks grazed by two  
 2534 Highland cattle herds in the Swiss and Italian Alps (**Table 5**). The first paddock (paddock 1, 30.86 ha)  
 2535 was located in Val Vogna, Italy (province of Vercelli). The other three paddocks (paddock 2, 8.26 ha;  
 2536 paddock 3, 7.67 ha; paddock 4, 7.04 ha) were located in Bovonne, Switzerland (canton of Vaud) and  
 2537 were grazed in rotation by the same herd. All paddocks were grazed at a comparable stocking rate and  
 2538 had similar topographical conditions (**Table 5**). The four paddocks were representative of an *A. viridis*  
 2539 cover gradient, with an average cover of 26%, 51%, 61% and 71% respectively in paddock 1, 4, 2 and  
 2540 3. Paddock 1, 2 and 3 were grazed during two summer seasons (2019 and 2020), while paddock 4 was  
 2541 grazed during one summer season (2020). More detailed information on the vegetation characteristics  
 2542 of paddocks 1, 2 and 3 can be found in Svensk et al. (2021). Each year, the herds grazed from the middle  
 2543 of June to the beginning of September. All the herds included cow/ calf pairs and heifers, varying in age  
 2544 from 6 months to 17 years (with an average of five years for paddock 1, and four years for paddock 2,  
 2545 3 and 4) and about 70% of the animals were present in both years at the same site. A water trough was  
 2546 installed in paddocks 2, 3 and 4, while natural streams were present in paddock 1 for the entire grazing  
 2547 period. As described in Svensk et al. (2022), in the second year of the project (2020), five molasses-  
 2548 based blocks were installed in small highly encroached areas of paddocks 1, 2, and 3, as part of another  
 2549 experiment set, to attract Highland herds into these areas. In each herd and during both years, six to ten  
 2550 cows were equipped with GPS collars (Followit AB ©, Tellus GPS System collars, Sweden) that  
 2551 recorded their position every ten minutes during the whole grazing period, with an accuracy of two to  
 2552 five meters. The GPS collars also recorded cattle neck movements through activity sensors of the X and  
 2553 Y axes.

2554

2555 **Table 5** Topographical and management characteristics of the four *A. viridis*-encroached paddocks  
 2556 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 <i>A. viridis</i> (%)	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 <sup>a</sup> 2019	45.4	29.8	29.8	-
Livestock units <sup>a</sup> 2020	70.4	29.6	29.6	25.4
Stocking rate <sup>b</sup> 2019	0.177	0.168	0.192	-
Stocking rate <sup>b</sup> 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

2557

2558

2559

2560

## 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, Birseldlen, 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  $0.47 \pm 0.05$  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:

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



2606 where  $NHV_i$  and  $GPSga\ fixes_i$  represent the N content of the herbaceous vegetation and the proportion  
 2607 of GPS fixes within the grazing activity time in the vegetation patch  $i$ , 24 h before dung sampling,  
 2608 respectively.

2609  
 2610 In addition, in all patches where *A. viridis* was present, the  $N_{24H}$  calculated from herbaceous  
 2611 vegetation was corrected based on the N content of *A. viridis* leaves sampled at the closest date to the  
 2612 corresponding dung sample, to account for the N ingested from *A. viridis* leaf consumption. Based on  
 2613 the direct observations of Highland cattle foraging behavior conducted in Bovonne (Nota et al. 2022,  
 2614 1077 observations), it was estimated that *A. viridis* leaves represented 12% of animal diet on average  
 2615 (although we also incorporated variation in the amount of *A. viridis* leaves eaten, see below). Thus, in  
 2616 the vegetation patches in which *A. viridis* was present, the  $N_{24H}$  corresponding to each dung sample was  
 2617 calculated considering a diet characterized by 88% N from herbaceous forage and 12% N from leaves,  
 2618 following this formula:

$$2620 \quad N_{24H} = \frac{\sum_i^n [(0.88 * NHV_i) + (0.12 * NAL_i)] * GPSga\ fixes_i}{\sum_i^n GPSga\ fixes_i} \quad (2)$$

2621  
 2622 where  $NAL_i$  represents the N content of *A. viridis* leaves sampled at the closest date to the corresponding  
 2623 dung sample.

2624  
 2625

#### 2626 4. Nitrogen import–export flux estimation

2627  
 2628 In every paddock and for each vegetation patch, the value of the N flux ( $N\ kg\ ha^{-1}\ yr^{-1}$ ) was  
 2629 calculated using an import–export model following this formula:

$$2631 \quad Nflux = Nexcreted - Ningested \quad (3)$$

2632  
 2633 where  $Nexcreted$  is the estimated amount of N excreted by livestock through urine and dung in the patch  
 2634 during the whole grazing season, and  $Ningested$  is the estimated amount of N eaten by livestock in the  
 2635 same patch and during the same period. For this flux, two assumptions were made: (i) the excretion is  
 2636 proportional to the total time cows spent in the patch, and (ii) the ingestion is proportional to the time  
 2637 cows spent grazing only (White et al. 2001; Koch et al. 2018).

2638  
 2639 Based on these assumptions, the excreted N was calculated as:

$$2641 \quad Nexcreted = D \times TS \times n \times DM \times (p \times Npaddock) \quad (4)$$

2642  
 2643 where  $D$  is the number of grazing days,  $TS$  the percentage of time spent by cows in the vegetation patch,  
 2644  $n$  the number of animals present,  $DM$  the dry matter intake ( $kg\ animal^{-1}\ day^{-1}$ ) calculated using previous  
 2645 studies on Highland cattle weight and DM ingestion of both cows and calves (Berry et al. 2002; Pauler  
 2646 et al. 2019), and  $Npaddock$  the estimated weighed mean of N eaten by the cows ( $g\ kg^{-1}\ DM$ ) in the  
 2647 whole paddock, using the N content of *A. viridis* leaves and herbaceous vegetation as previously  
 2648 described. The parameter  $p$  is the estimated percentage of  $Ningested$  that is excreted by grazing beef  
 2649 cattle according to seven trials conducted in comparable conditions and presented in Estermann et al.  
 2650 (2003), Berry et al. (2002) and Estermann et al. (2001). Indeed, those experiments had comparable  
 2651 conditions to our study as they were performed on low growing beef cattle breeds (Highland or Angus  
 2652 cattle with cow and calf pairs), in similar montane environments ( $1557 \pm 237\ m$ ) and/or with similar

2653 crude protein content diets ( $14.63 \pm 0.75\%$ , compared to  $16.05 \pm 1.12\%$  from our N ingested  
2654 estimations).

2655  
2656 Ingested N was calculated as:

$$2657 \quad \quad \quad N_{ingested} = D \times TG \times n \times DM \times N_{patch} \quad (5)$$

2659  
2660 where TG is the percentage of time cows spent grazing in the patch, and N<sub>patch</sub> the estimated ingested  
2661 N content calculated from the N content of *A. viridis* leaves and herbaceous vegetation, at the vegetation  
2662 patch level.

2663  
2664 Since some parameters from the estimation of the N fluxes are subjected to uncertainties, we  
2665 associated a statistical distribution to each parameter (mean  $\pm$  SE), following the methodology described  
2666 in Koch et al. (2018), and performed 500 Monte Carlo simulations, in order to obtain 5%, 50% and 95%  
2667 quantiles of the N flux for each vegetation patch in every year. We thus included the uncertainty of: (i)  
2668 p from the seven experiments ( $92.7 \pm 0.7\%$ ), (ii) N<sub>paddock</sub>, by including the variability of the  
2669 percentages of *A. viridis* leaves eaten by the cows when in presence of the shrub ( $11.8 \pm 2.8\%$ ), at the  
2670 paddock level, and (iii) N<sub>patch</sub>, by including the same variability of the percentages of *A. viridis* leaves  
2671 eaten by the cows when in presence of the shrub at the patch level.

2672  
2673

## 2674 **5. Statistical analysis**

2675  
2676 All statistical analyses were performed using R version 3.4.4 (R Core Team 2017). The effect of *A.*  
2677 *viridis* cover on the N content of the understory herbaceous vegetation was tested by using a Generalized  
2678 Least Square model (GLS), with *A. viridis* cover as fixed factor and coordinates of vegetation patches  
2679 centroids nested into “paddock” as random effect, using a Linear Correlation Structure. This random  
2680 effect structure accounts for the nested structure of the data and for any residual spatial autocorrelation  
2681 among neighboring vegetation patches.

2682  
2683 The relation between dung pats N content (response variable) and N<sub>24H</sub> (explanatory variable) was  
2684 tested using a Linear Mixed-effect Model (lme, package “nlme”), with the date of dung sampling as a  
2685 continuous variable nested into “paddock” as a time correlated covariate (corCAR1), to account for the  
2686 temporal autocorrelation structure linked to vegetation changes throughout the summer season. The  
2687 marginal R-squared values were obtained using the “performance” package (function  
2688 “model\_performance”). The effect of *A. viridis* cover (divided into 3 categories: 0–33%, 34–66% and  
2689 67–100%), slope (divided into 3 categories:  $< 10^\circ$ ,  $10\text{--}20^\circ$  and  $> 20^\circ$ ) and their interaction on the N  
2690 fluxes was tested using a Linear Mixed-effect Model lme, package (“nlme”). Paddock was specified as  
2691 random factor to account for spatial autocorrelation. Model residuals were affected by  
2692 heteroscedasticity, therefore a weighting function was used to correct the variances through the  
2693 argument varIdent in the lme function by setting as grouping variable the *A. viridis* covers. *Post hoc*  
2694 tests were performed for the models when significant effects between categories were detected (Tukey’s  
2695 test,  $P < 0.05$ , emmeans package).

2696  
2697  
2698  
2699

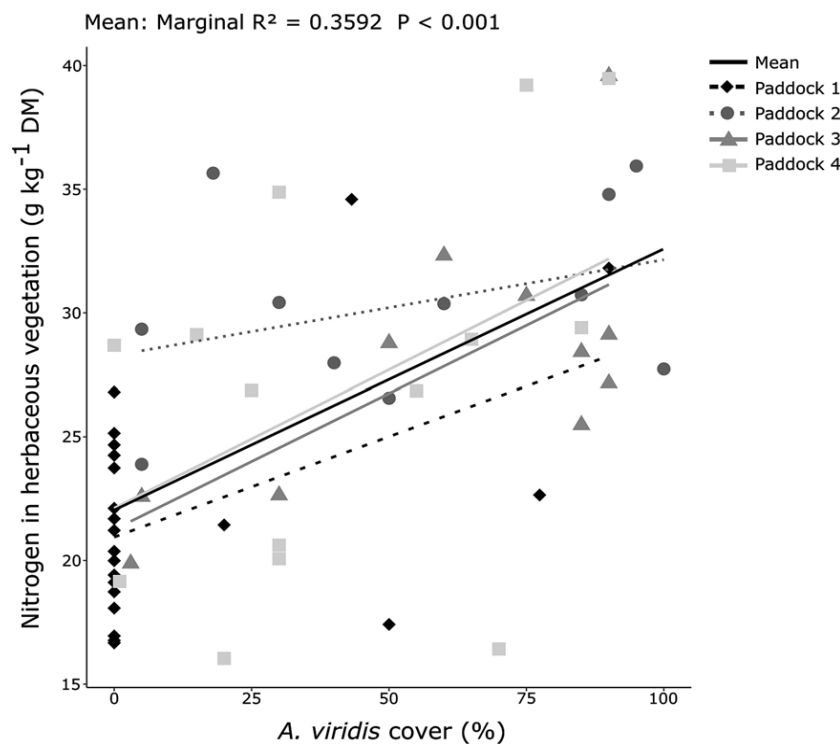
2700 **Results**

2701

2702 **1. Effect of *A. viridis* cover on the nitrogen content of the understory herbaceous vegetation**

2703

2704 The herbaceous vegetation had an average N content of  $25.9 \pm 0.8 \text{ g kg}^{-1} \text{ DM}$  among all paddocks  
2705 and vegetation patches (the details per paddock can be found in Supplementary materials, **Figures S11–**  
2706 **S14**). The N content of the herbaceous vegetation was positively related with *A. viridis* cover percentage  
2707 (**Figure 28**,  $P < 0.001$ ,  $R^2 = 0.36$ ,  $n = 58$ ). Indeed, the N concentration of the herbaceous vegetation  
2708 increased with increasing *A. viridis* cover, with averages of  $22.9 \pm 0.9 \text{ g kg}^{-1} \text{ DM}$  in open areas (0–33%  
2709 of *A. viridis* cover),  $28.2 \pm 1.6 \text{ g kg}^{-1} \text{ DM}$  in moderately encroached areas (34–66% of *A. viridis* cover)  
2710 and  $30.5 \pm 1.6 \text{ g kg}^{-1} \text{ DM}$  in highly encroached areas (67–100% of *A. viridis* cover).  
2711



2712

2713 **Figure 28** Relationship between the N content ( $\text{g kg}^{-1} \text{ DM}$ ) of the understory herbaceous vegetation and  
2714 *A. viridis* cover percentage in the vegetation patches of all paddocks.

2715

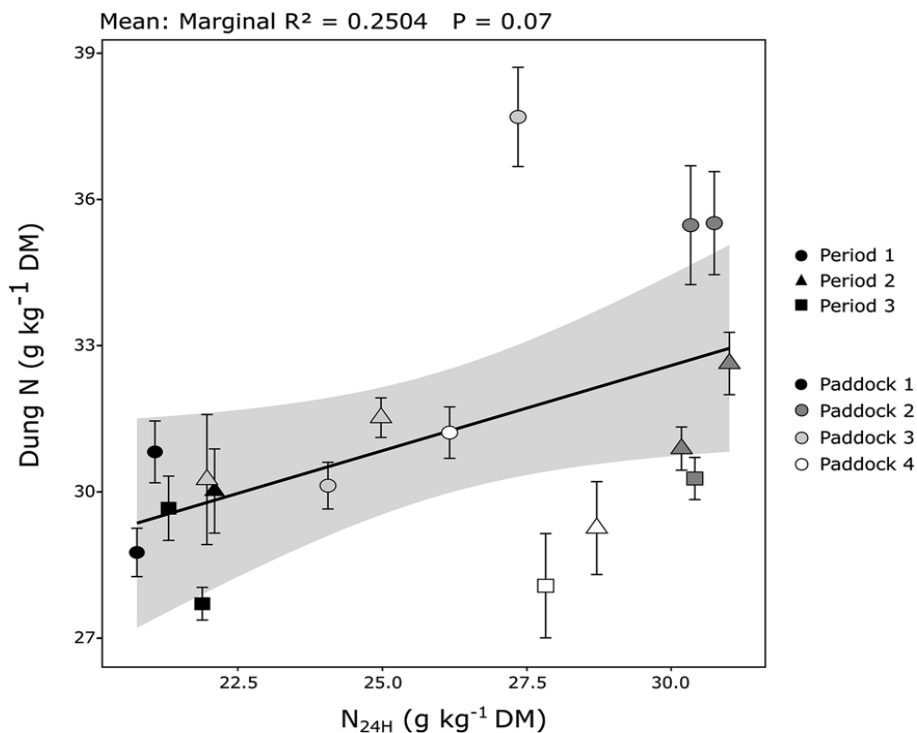
2716

2717 **2. Effect of animal diet on dung nitrogen content**

2718

2719 The N content of Highland cattle dung pats was on average  $31.2 \pm 0.2 \text{ g kg}^{-1} \text{ DM}$  (**Figure 29**, mean  
2720  $\pm \text{SE}$ ), and consistent between both years ( $31.1 \pm 0.3 \text{ g kg}^{-1} \text{ DM}$  in 2019, and  $31.4 \pm 0.4 \text{ g kg}^{-1} \text{ DM}$  in  
2721 2020,  $P = 0.137$ ). The N dung content slightly decreased during the grazing season in both years  
2722 ( $P < 0.001$ ), with averages of  $32.8 \pm 0.5 \text{ g kg}^{-1} \text{ DM}$ ,  $31.0 \pm 0.3 \text{ g kg}^{-1} \text{ DM}$  and  $29.2 \pm 0.4 \text{ g kg}^{-1} \text{ DM}$  in  
2723 dung sample periods 1, 2 and 3 respectively. The N content of *A. viridis* leaves that was used for the  
2724 estimation of the  $N_{24\text{H}}$  of the encroached vegetation patches was of  $31.8 \pm 0.6 \text{ g kg}^{-1} \text{ DM}$  across all sites  
2725 and periods. The estimated  $N_{24\text{H}}$  remained stable across dung sampling periods ( $P = 0.92$ ), with  
2726  $25.8 \pm 1.5 \text{ g kg}^{-1} \text{ DM}$ ,  $26.5 \pm 1.6 \text{ g kg}^{-1} \text{ DM}$  and  $25.4 \pm 2.2 \text{ g kg}^{-1} \text{ DM}$  in period 1, 2 and 3 respectively. The  
2727  $N_{24\text{H}}$  ingested was of  $21.4 \pm 0.3 \text{ g kg}^{-1} \text{ DM}$ ,  $30.5 \pm 0.2 \text{ g kg}^{-1} \text{ DM}$ ,  $24.6 \pm 1.1 \text{ g kg}^{-1} \text{ DM}$  and  $27.6 \pm 0.8$   
2728  $\text{g kg}^{-1} \text{ DM}$  for paddock 1, 2, 3 and 4 respectively, with an overall average of  $25.9 \pm 1.0 \text{ g kg}^{-1} \text{ DM}$  among

2729 all paddocks. There was a marginally significant positive relationship between dung N content and  $N_{24H}$ ,  
 2730 (Figure 29,  $P = 0.07$ ,  $R^2 = 0.25$ ,  $n = 17$ ).  
 2731



2732  
 2733 **Figure 29** Relationship between the N content of the dung pats (dung N,  $g\ kg^{-1}\ DM$ ) of Highland  
 2734 cattle and the estimated mean content of N ingested 24 h before the dung sampling ( $N_{24H}$ ,  $g\ kg^{-1}\ DM$ ),  
 2735 with the trend line (grey area) following lm smoothing method, and error bars representing the standard  
 2736 error.

2737  
 2738

### 2739 3. N import–export fluxes in vegetation patches

2740  
 2741 The 5%, 50% and 95% quantiles of the predicted values displayed similar patterns, for N ingested,  
 2742 N excreted and the resulting N fluxes (Table 6). The following results are values of the 50% quantile  
 2743 (see Maps of N fluxes per each paddock in Supplementary materials, Figures S15–S19).  
 2744

2745 Based on N fluxes, in all paddocks, Highland cattle visited and actively grazed mostly on the open  
 2746 and moderately flat areas. Indeed, the most open vegetation patches (0–33% of *A. viridis* cover) with  
 2747 medium slope (10–20°) displayed the highest mean N ingestion, i.e.  $51.9 \pm 8.7\ kg\ ha^{-1}\ yr^{-1}$ . On the other  
 2748 hand, the steepest (> 20°) and most encroached areas (67–100%) displayed the lowest average of N  
 2749 ingested, i.e.  $11.1 \pm 1.3\ kg\ ha^{-1}\ yr^{-1}$  (Table 6). In parallel, N excretion was the highest in the open areas  
 2750 (0–33%) and in the lowest slopes (< 10°), with a N excreted average of  $79.2 \pm 16.4\ kg\ ha^{-1}\ yr^{-1}$ . Similar  
 2751 to N ingestion, the lowest N excretions were estimated in steepest and most encroached areas, with a N  
 2752 excretion value of  $7.0 \pm 1.0\ kg\ ha^{-1}\ yr^{-1}$ . Consequently, the N fluxes were significantly affected by *A.*  
 2753 *viridis* cover and slope (Figure 30,  $P < 0.001$ ), with overall positive N fluxes in flat and open areas (i.e.  
 2754 N accumulated), and negative N fluxes in steeper and encroached ones (i.e. N exported; Figure 30,  
 2755 Table 6). On average, open and flat areas had N fluxes of  $29.5 \pm 10.3\ kg\ ha^{-1}\ yr^{-1}$ , while encroached and  
 2756 steep areas had average N fluxes of  $-4.1 \pm 0.7\ kg\ ha^{-1}\ yr^{-1}$ . Indeed, in all paddocks combined, 80.66% of  
 2757 vegetation patches with positive N fluxes were open pastures (0–33% of *A. viridis* cover), while only

2758 2.2% were highly encroached areas (67–100% of *A. viridis* cover), and 17.14% were moderately  
2759 encroached areas (34–66% of *A. viridis* cover). Most (44.81%) of these accumulation zones had a  
2760 moderate slope ( $17.5 \pm 0.6^\circ$  on average). On the other hand, 37.9% of the vegetation patches that  
2761 displayed a N depletion were open pastures, 47.08% were highly encroached areas and 15.05% were  
2762 moderately encroached areas. Most (74.0%) of these depletion zones had high slope ( $30.5 \pm 0.6^\circ$  on  
2763 average). Moreover, in terms of spatial distribution, N accumulated in very small areas and most of the  
2764 grazed land was N depleted. Indeed, paddocks 1, 2, 3 and 4 displayed N accumulation in only 11.8%,  
2765 15.1%, 12.1% and 19.2% of their areas, respectively.

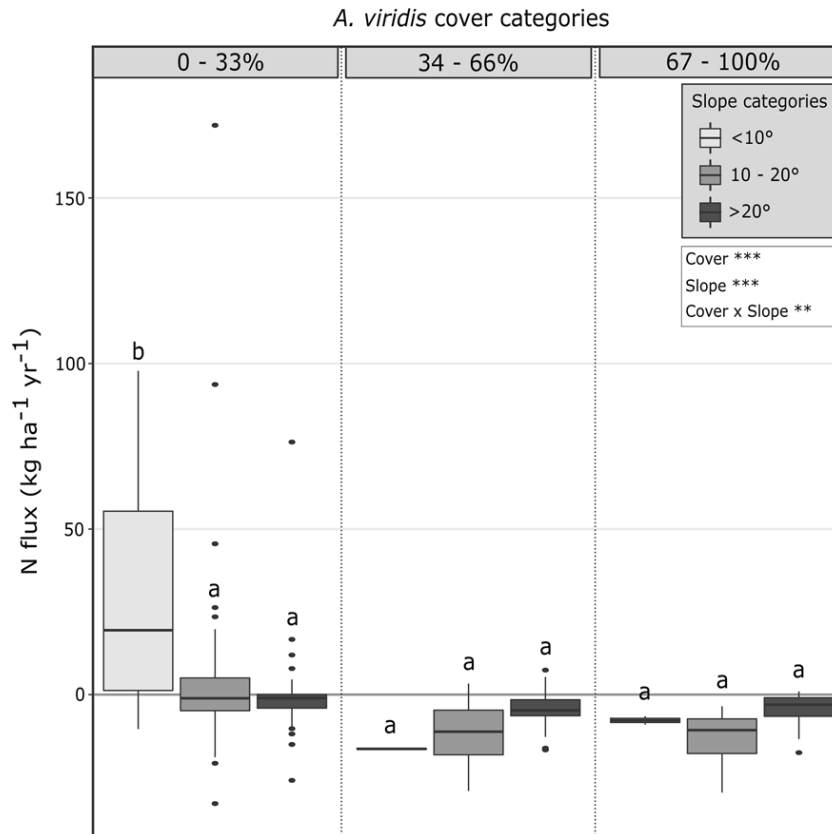
2766

2767 N fluxes were very similar among years. Indeed, vegetation patches with negative N fluxes had on  
2768 average  $-6.9 \pm 0.8 \text{ kg ha}^{-1} \text{ yr}^{-1}$  in 2019, and  $-5.6 \pm 0.7 \text{ kg ha}^{-1} \text{ yr}^{-1}$  in 2020, while vegetation patches with  
2769 positive N fluxes had an average of  $26.0 \pm 10.3 \text{ kg ha}^{-1} \text{ yr}^{-1}$  in 2019, and  $19.3 \pm 5.7 \text{ kg ha}^{-1} \text{ yr}^{-1}$  in 2020.  
2770 In 2019, four vegetation patches were not visited by Highland cattle in paddock 1, corresponding to  
2771 0.98% of the paddock size (0.30 ha). In 2020, three of these patches remained unvisited with an addition  
2772 of two others, corresponding to 1.34% of the paddock size (0.41 ha). As a result, estimations of N  
2773 ingested, N excreted and N fluxes were equal to zero for those unvisited areas. Most of these vegetation  
2774 patches were covered by *Rhododendron ferrugineum* or were *A. viridis*-encroached areas, and one was  
2775 an open pasture with steep slope ( $34.8^\circ$ ). All vegetation patches of paddocks 2, 3 and 4 were visited in  
2776 both years.

2777 **Table 6** Mean  $\pm$  SE of the N fluxes, and detailed N ingestion and N excretion ( $\text{kg ha}^{-1} \text{yr}^{-1}$ ) values for 5%, 50% and 95% quantiles, estimated on vegetation  
 2778 patches of all paddocks on both years, depending on slope and *A. viridis*-cover categories

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

2779



**Figure 30** N fluxes (kg ha<sup>-1</sup> yr<sup>-1</sup>) 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  $27.3 \pm 4.5$  g kg<sup>-1</sup> DM and  $30.7 \pm 3.3$  g kg<sup>-1</sup> (at 1650 and 1950 m elevation respectively), similar to our mean concentration of  $30.5 \pm 1.6$  g kg<sup>-1</sup> DM g kg<sup>-1</sup> 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).

## 2807 2. Animal diet affects dung nitrogen content

2808  
2809 The N content measured in Highland cattle dung pats was high, with an average of  $31.2 \pm 0.2 \text{ g kg}^{-1}$   
2810 DM, if compared to values found for other cattle breeds with high crude protein rich diets, thus  
2811 confirming our first hypothesis. For instance, two studies conducted by Koenig and Beauchemin (2013b,  
2812 a) found that Angus beef cattle fed with silage of similar crude protein content (14–14.5%) excreted a  
2813 fecal N of about  $21.2 \text{ g kg}^{-1}$  DM to  $23.2 \text{ g kg}^{-1}$  DM on average. Haynes and Williams (1993) determined  
2814 that the average N content in the dung of dairy cattle grazing in open pastures varied between 20 and 28  
2815  $\text{g kg}^{-1}$  DM, whereas the N dung concentration was about  $27 \text{ g kg}^{-1}$  DM in nutrient-rich pastures Williams  
2816 and Haynes (1995). Similarly, Lançon (1978) found a dung N content of  $20.6 \text{ g kg}^{-1}$  DM for non-  
2817 fertilized grasslands and  $28.7 \text{ g kg}^{-1}$  DM for fertilized grasslands. Other studies have also assessed dung  
2818 N contents around  $20 \text{ g kg}^{-1}$  DM for dairy cows grazing on open pastures (Yokoyama et al. 1991; Bakker  
2819 et al. 2004).

2820  
2821 The high dung N content found in our study was marginally related to the amount of N ingested  
2822 during 24 h before excretion, showing a trend of increasing N in the dung with increasing ingested N,  
2823 which is in line with our second hypothesis. Despite the non-significance of this effect, the trend shown  
2824 in this relation suggests that the more Highland cattle grazed in highly encroached areas, where N  
2825 content in vegetation was high, the richer their dung became in N. This result is in line with previous  
2826 studies, such as Kebreab et al. (2001) and Angelidis et al. (2019). For example, by using data of 69  
2827 different studies, Angelidis et al. (2019) showed that the N intake had a significant positive effect on the  
2828 excreted dung N. While dung N seemed to increase with increasing  $N_{24\text{H}}$ , even the lowest dung N  
2829 concentrations recorded in this study remained rather high compared to other experiments in similar  
2830 conditions. This might be mainly explained by the overall high N content in the diet of Highland cattle  
2831 grazing in these *A. viridis*-encroached areas. The high N content in dung pats may also be explained by  
2832 the high condensed tannin concentration of *A. viridis* leaves. Indeed, Stević et al. (2010) demonstrated  
2833 that *A. viridis* leaves can have a tannin concentration of  $44 \pm 4 \text{ g kg}^{-1}$  DM, which can form tannin-protein  
2834 complexes by binding with plant proteins when consumed by livestock (Harris et al. 1998; Waghorn  
2835 2008; Woodfield et al. 2019). These complexes are better protected from rumen microbial degradation  
2836 and they can thus reduce the degradation of N during digestion (Waghorn et al. 1987; Burggraaf and  
2837 Snow 2010; Piñeiro-Vázquez et al. 2017). Therefore, the passage of undegraded N could be favored  
2838 through the intestine and through dung, and a smaller proportion of N might be excreted in urine  
2839 (Burggraaf and Snow 2010; Theodoridou et al. 2011). Further studies on Highland cattle digestion are  
2840 needed to confirm whereas the proportion of N excreted in urine will be reduced through the  
2841 consumption of *A. viridis*-encroached vegetation with high tannins concentrations, as this could be  
2842 beneficial. Indeed, a reduction of urine N may decrease N pollution, as urea N is subjected to ammonia  
2843 ( $\text{NH}_3$ ) volatilization and nitrate leaching (Woodmansee et al. 1981; Jarvis 1994; Whitehead 1995;  
2844 Tamminga 2006; Angelidis et al. 2019). On the other hand,  $\text{NH}_3$  volatilization through the dungs is  
2845 restrained by the crust formation of dung pats, even though heavier rainfall might alter this protection  
2846 (Longhini et al. 2020). Fecal N is in a less mobile form that needs mineralization before N can be lost  
2847 through leaching, and  $\text{NH}_3$  volatilization through urine is 5–6 times higher than through dung pats  
2848 (Lockyer and Whitehead 1990; Kebreab et al. 2001; Berry et al. 2002; Woodfield et al. 2019).  
2849 Furthermore, the slow process of N release through dung pats can allow a better use by the surrounding  
2850 vegetation and for the soil on the long term (Woodmansee et al. 1981; Lantinga et al. 1987; Berry et al.  
2851 2001; Burggraaf and Snow 2010). Moreover, the excretion of condensed tannins through dung may also  
2852 inhibit nitrification and slow down the microbial activity of the soil, preventing nitrate leaching and  
2853 consequently soil acidification (Burggraaf and Snow 2010). Finally, requirements for cattle diet show  
2854 that a minimum of 20 to  $40 \text{ g kg}^{-1}$  DM of condensed tannin concentration is needed to improve animal



2855 performance (Woodfield et al. 2019), which, combined with the N supply *A. viridis* leaves provide,  
2856 highlight their forage potential for robust breeds, such as Highland cattle. However, further research is  
2857 needed to assess the *A. viridis* leaf ingestion by grazing cattle on a higher range of environmental  
2858 conditions.

2859  
2860

### 2861 **3. An active nitrogen translocation occurred from shrub-encroached areas to pastures**

2862

2863 Our study demonstrated that the excretion of N after its uptake from *A. viridis* leaves and the  
2864 understory herbaceous vegetation allowed its redistribution across the pastures. Overall, the N transfer  
2865 was significantly dependent on *A. viridis* cover as well as on topographic features (i.e. slope), confirming  
2866 our third hypothesis. Indeed, our estimations of the N fluxes in the paddocks showed that there was an  
2867 active N translocation from the steep, shrub-encroached, and N saturated areas to the adjacent flat and  
2868 open pastures that had comparatively lower herbaceous N content. This is consistent with the findings  
2869 of Schnyder et al. (2010), who showed a significant N accumulation in the flattest zones and N removal  
2870 from the steepest areas in hilly pastures. In the same study, the authors found similar results for  
2871 phosphorus (P), which is a good estimate of other nutrients such as N, with the difference that this  
2872 element is much less present in urine and thus easier to evaluate on field and less subjected to  
2873 transformations. Similar results on P were found in previous studies, such as Koch et al. (2018), where  
2874 P was significantly translocated from feeding areas to the flattest resting areas, and Jewell et al. (2007),  
2875 who also found a significant redistribution and fewer dung deposition in the steepest areas of subalpine  
2876 pastures. As in previous studies, our flat and open areas with high N deposition often correspond to  
2877 livestock resting areas, where there is generally a high excretion deposition, especially during the night.  
2878 Costa et al. (1990) found that 90% of cattle excretion was deposited in resting-adapted areas, with slopes  
2879 lower than 40%. In the present study, while the open areas displayed the highest N ingestion as well as  
2880 the highest N excretion, the latter often exceeded N ingestion, making the final N fluxes positive. In the  
2881 same way, the highly encroached areas displayed the lowest N ingestion, but displayed an even lower  
2882 N excretion, highlighting the final depletion of N induced by Highland cattle grazing. On the other hand,  
2883 the depletion areas of our paddocks were larger than the accumulation zones, meaning that cattle actively  
2884 removed N from a larger area and concentrated it in relatively small patches. This is in line with previous  
2885 findings, which showed that nutrients are often returned into small spots, with most of the area  
2886 displaying a negative balance and net nutrient loss (Bakker et al. 2004; Jewell et al. 2007; Koch et al.  
2887 2018).

2888

2889 The assessment of the N fluxes over two years allows us to suggest that Highland cattle grazing  
2890 could moderately reduce N accumulation under *A. viridis* encroached stands on the long-term, while  
2891 simultaneously providing it in targeted areas. Particularly, the proper management of resting areas could  
2892 help fertilize specific nutrient-poor pastures which are subjected to early encroachment processes (Probo  
2893 et al. 2016), such as pastures dominated by *Nardus stricta* L., *Rhododendron ferrugineum* L. or  
2894 *Vaccinium myrtillus* L. The translocation of N towards these areas could improve their forage yield and  
2895 quality, potentially transforming the N saturation issue into an environmental and agronomical resource.  
2896 Schellberg et al. (1999) demonstrated in a long-term study that N and P inputs on nutrient-poor grassland  
2897 drastically increased DM production and forage quality and changed floristic composition. In this study,  
2898 oligotrophic species decreased with nutrient fertilization, and created ecological favorable conditions  
2899 for other grasses and legumes. At a moderate level, fertilization can improve botanical diversity (Vintu  
2900 et al. 2011) and, with N and P enrichment by Highland cattle grazing, we could expect similar results  
2901 on the long-term whether such management should continue. However, further studies, not only on N  
2902 import–export fluxes related to grazing, but also on N pool changes are needed to better understand the

2903 potential of nutrients translocation, and the benefits for the vegetation and soil characteristics. Indeed,  
2904 N inputs from atmospheric deposition and N fixation can be quite elevated in these environments (50 to  
2905 100 kg ha<sup>-1</sup> yr<sup>-1</sup> according to Binkley et al. (1994). Despite our results showing moderate N translocation  
2906 towards open and flat areas (29.52 kg DM ha<sup>-1</sup> yr<sup>-1</sup> on average), attention should be paid to possible  
2907 over-fertilization issues resulting from high levels of N accumulation in the resting areas. Indeed, the  
2908 distribution patterns of grazing cattle can be stable across years (Koch et al. 2018), consequently leading  
2909 to a repeated distribution of cattle dung and thus potential over-fertilization of the resting areas. To avoid  
2910 such negative impacts and preserve nutrient capital, it is advised to adopt a rotational management, as it  
2911 allows more homogeneous utilization of the pasture compared to free-grazing systems. Previous studies  
2912 have shown that the presence of fences, shaded zones, attractive points (such as salty or molasses  
2913 blocks), and a water trough may also impact the spatial distribution of dung and urine pats (Jewell et al.  
2914 2007; Buttler et al. 2008; Auerswald et al. 2010; Pittarello et al. 2016a; Carnevalli et al. 2019; Svensk  
2915 et al. 2022). Thus, these features should be included in the management of cattle grazing to better  
2916 redistribute nutrients across the pastures. The results of this study highlight the potential of Highland  
2917 cattle grazing to become an efficient tool to effectively translocate part of the ingested N, which could  
2918 be accounted for in livestock management, and help in the restoration of former open pastures in the  
2919 long-term. However additional research is needed to assess the effect of such grazing management to  
2920 counteract N deposition in the shrublands, including long term effects on nutrients pools.

## 2921 **CHAPTER VII: General discussion**

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2922

2923 The goal of this thesis was to evaluate whether Highland cattle can become a management tool  
2924 to reduce *A. viridis* encroachment in subalpine pastures. This was achieved through an experiment  
2925 conducted for four years in the Swiss and Italian Alps, during which Highland cattle herds grazed in  
2926 highly *A. viridis*-encroached pastures. During this experiment, we assessed the forage potential of *A.*  
2927 *viridis* leaves (Chapter II) as well as Highland cattle feeding behavior (Chapter III) in order to understand  
2928 to what extent Highland cattle could forage on *A. viridis* and actively damage it. We monitored Highland  
2929 cattle using GPS collars to understand their grazing and movement behavior in the harsh conditions of  
2930 highly encroached pastures (Chapter IV), and we tested management strategies with the implementation  
2931 of attractive points to increase their impact on *A. viridis* stands (Chapter V). Finally, we estimated the  
2932 N translocation made by Highland cattle grazing to understand the potential impacts produced by this  
2933 livestock management on the long-term (Chapter VI).

2934

2935

### 2936 **The forage potential of *A. viridis* leaves**

2937

2938 Chapter II investigated the forage nutritional value of *A. viridis* leaves, through the description of  
2939 the seasonal variation of their chemical composition. The leaves displayed high crude protein and  
2940 macro- and micro- elements concentrations that are essential for livestock health (Collins et al. 2017;  
2941 Mahieu et al. 2021), and could therefore be integrated as complementary forage resource in addition to  
2942 a grass-based diet. Thanks to measurements conducted throughout the summer season, *A. viridis* leaves  
2943 were identified to be most interesting for nutritive purposes for livestock in June and July, with  
2944 decreasing digestibility over the season. In addition, *A. viridis* leaves showed a mitigation effect on CH<sub>4</sub>  
2945 emission by cattle, with a maximized effect at the beginning of the season. Therefore, this study  
2946 emphasized the potential to integrate *A. viridis* leaves for robust livestock foraging on encroached  
2947 vegetation conditions, as it had been previously described for other woody species (Terranova et al.  
2948 2018). Indeed, these results could be applied not only to Highland cattle but also to other robust species  
2949 and breeds which were previously shown to be able to forage on *A. viridis* (Pauler et al. 2022b). Other  
2950 measurements on the chemical composition of the buds and the bark of *A. viridis* could be carried out  
2951 in order to deepen the knowledge on *A. viridis* forage potential. Indeed, Dexter cattle and Engadine  
2952 sheep have been reported to feed on the buds, with sheep actively debarking the shrub as well (Zehnder  
2953 et al. 2017). The phenolic compounds of these plant parts have already been investigated by Stević et  
2954 al. (2010), but no full description on their forage quality was made yet.

2955

2956 While this chapter allowed a comprehensive assessment of the feeding potential of *A. viridis*,  
2957 providing a direct link with livestock nutritional requirements is complex. Indeed, nutritive needs of  
2958 livestock highly depends on animal condition, i.e. its maintenance, age, growth, reproduction or  
2959 lactation. Therefore, it is crucial to include other source of feed intake with complementary forage  
2960 quality in order to prevent potential loss in weight leading to health problems and a reduction of  
2961 productivity, above all at the end of the summer grazing season, when *A. viridis* leaves were shown to  
2962 decrease their overall good nutritional quality.

2963

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2967 **Feeding behaviour of Highland cattle**

2968

2969 Chapter III disentangled the diet composition of Highland cattle through the direct observation of  
2970 their feeding behaviour under different vegetation conditions. This cattle breed selected a mix of  
2971 different plant species, with herbaceous plants of high forage quality as well as woody plants, which  
2972 composed up to 46% of their diet. The *A. viridis* accounted for 12% of Highland cattle diet on average,  
2973 and its consumption was dependent to its abundance. Therefore, in highly encroached pastures, Highland  
2974 cattle consume more of *A. viridis* leaves, depending on the availability of other forage resources. While  
2975 some species composing *A. viridis* understory vegetation were avoided by Highland cattle, such as  
2976 *Adenostyles alliariae* (Gouan), some ferns such as *Dryopteris dilatata* (Hoffm.) were recorded to be  
2977 positively selected despite being known to be potentially toxic (Iussig et al. 2015a, **Figure 31**). This  
2978 behaviour is particularly interesting, as Highland cattle were able to intensively defoliate plants growing  
2979 under *A. viridis* stands in addition to directly grazing the shrub, which could facilitate the process of  
2980 subalpine grassland restoration on the long term.



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

3006 Because Highland cattle diet is composed by a mix of various species, providing a mosaic of high  
3007 quality vegetation and woody plants is essential to preserve their health and avoid reducing meat  
3008 production, as mentioned in the previous chapter. In 2022, livestock weight measurements were  
3009 performed on all Highland cows, heifers, and calves before and after the grazing season (72 days) in the  
3010 site of Bovonne, Switzerland (VD). The results (unpublished) showed an average weight gain of  
3011 21.75 kg for adult cows ( $\geq 2.5$  years) and 44.7 kg (i.e.  $0.62 \text{ kg day}^{-1}$ ) for heifers and calves ( $< 2.5$  year)  
3012 during the grazing season. Interestingly, these results are very much in line with those by Berry et al.  
3013 (2002), who measured an average weigh gain of  $0.69 \text{ kg day}^{-1}$  for Highland calves after 77 grazing days

3014 but in un-encroached subalpine pastures. In the same study, the comparison with dairy cattle highlighted  
3015 the ability of Highland cattle to utilize poor nutritive quality pastures without losing productivity, while  
3016 more productive breeds tend to lose weight under those difficult conditions. Therefore, *A. viridis*  
3017 encroached pastures provided sufficient forage resources for Highland cattle growth, without negatively  
3018 affecting their productivity.

3019

3020

### 3021 **Highland cattle grazing patterns and their effect on *A. viridis* encroached pastures**

3022

3023 Chapter IV described the distribution patterns of Highland cattle in *A. viridis* encroached pastures,  
3024 underlying their ability to exploit every areas of the paddocks. Indeed, while Highland cattle preferred  
3025 to graze in open areas with higher pastoral value vegetation, they also grazed in areas with high *A. viridis*  
3026 cover composed by poorer forage quality vegetation. In accordance with the findings of Pauler et al.  
3027 (2020a), they were able to travel and graze in the steepest slopes and in areas far from any water source,  
3028 which is of high interest as *A. viridis* shrubs mainly thrive in steep slopes and remote areas. Therefore,  
3029 Highland cattle were able to effectively penetrate the denser *A. viridis* stands, as it has been observed  
3030 for smaller species such as goats or sheep (Pauler et al. 2022b).

3031

3032 While these findings described the influence of topographical, vegetation and management factors  
3033 determining grazing behaviour, they did not include the possible effects related to weather conditions  
3034 during the summer season. In dry and warm conditions, cattle may be subjected to heat stress and will  
3035 tend to look for shaded locations that could increase the time they spent in highly encroached areas  
3036 (Dash et al. 2016). Therefore, weather could impact their spatial distribution during the day as well as  
3037 their activity (grazing or resting). In addition, heavy rainfalls might highly impact the use of steeper  
3038 slopes, where it might be more difficult to move. While the following years did not depicted any drastic  
3039 change in Highland cattle spatial distribution patterns, the limitation of this study to the summer season  
3040 of 2019 should also be considered.

3041

3042 Highland cattle showed a high potential to impact *A. viridis* stands as they grazed in highly-  
3043 encroached areas. Their impact on the shrubs and the understory vegetation was further investigated in  
3044 Chapter V, where Highland cattle were lured into the denser parts of the paddocks. In this chapter, we  
3045 demonstrated the attractiveness of molasses-based blocks as they increased the time cattle spent in  
3046 *A. viridis* stands. While previous studies have shown that molasses-based blocks could attract cattle in  
3047 underused areas (Bailey and Welling 1999), this study demonstrated that they could attract Highland  
3048 cattle despite being placed in areas with steep slopes and high shrub density. Their increased grazing  
3049 and trampling around attractive points generated considerable impacts on both *A. viridis* shrubs and the  
3050 surrounding vegetation (57.3% decrease), increasing the amount of bare soil (59.1% increase). Indeed,  
3051 the understory vegetation was mainly grazed or trampled and several branches of *A. viridis* shrubs were  
3052 found broken after grazing. By placing molasses-based blocks at the base of the shrubs, the mechanical  
3053 effects produced by livestock were thus enhanced. In addition, because the attraction had an effect up to  
3054 50 m around the molasses-based blocks, these impacts on vegetation were carried out on a rather large  
3055 scale instead of being restricted to small areas.

3056

3057 On the long term, such management could allow effective grassland restoration effects on the  
3058 denser parts of the encroached-pastures, with a slow opening of the canopy that could allow light to  
3059 reach the herbaceous levels and new light-demanding plants to settle in the gaps created by livestock  
3060 grazing and trampling. However, implementing attractive points over several years requires adapted  
3061 strategies, and rotational management, as well as a regular relocation of attractive points is needed to

3062 avoid the over-utilization of targeted areas. Indeed, an excessive exploitation of certain areas could lead  
3063 to excessive trampling with possible localized erosion on steep slopes. Pietola et al. (2005) demonstrated  
3064 that highly visited sites (such as water sources) with high trampling led to a reduced porosity in the soil  
3065 and consequently higher risks of erosion, even at low grazing intensity. This risk could also be increased  
3066 during particularly rainy years, and special care should be given during those events. Moreover, while  
3067 livestock supplements are widely used by farmers, they are usually placed in accessible zones in order  
3068 to improve animal diet or to facilitate regular inventories or health care (Pittarello et al. 2016a). With  
3069 the additionnal aim to reduce *A. viridis* cover, the set up of molasses-based blocks is more complicated.  
3070 Indeed, it requires the transportation of heavy blocks in steep and dense areas that are hardly accessible,  
3071 and needs a sustainable installation that can last the whole grazing period without being moved by the  
3072 herd. It is therefore important to take into account these practical challenges when discussing possible  
3073 management strategies, which need to be adapted to site-specific conditions.

3074  
3075

## 3076 **Nitrogen translocation**

3077

3078 Chapter VI described the N content of herbaceous vegetation, *A. viridis* leaves and Highland cattle  
3079 dung pats, and used these data to make an estimation of the N translocation fluxes in the studied areas.  
3080 In this chapter, we confirmed that the vegetation under *A. viridis* stands displayed higher N content in  
3081 comparison to adjacent open areas. Moreover, the N content of Highland cattle dung pats was higher in  
3082 comparison to the content measured for other full-grazing beef cattle breeds in previous studies,  
3083 probably due to the high N and tannin content of *A. viridis* leaves they were ingesting. Indeed, Angus  
3084 cattle have shown lower N content in their dung despite having similar crude protein content in their  
3085 diet (Koenig and Beauchemin 2013a). With increasing grazing time spent in highly encroached areas,  
3086 where the N content in the vegetation is high, Highland cattle excreted higher N concentrations. Finally,  
3087 the estimations on the N fluxes confirmed that Highland cattle actively transported N from steeper and  
3088 denser areas to open and flat adjacent pastures. Indeed, as previously assessed by other studies with  
3089 different breeds, dung pats (and therefore N) tend to accumulate in flattest areas that are mainly used as  
3090 resting spots for cattle (Jewell et al. 2007; Schnyder et al. 2010). Moreover, while Highland cattle grazed  
3091 primarily high-quality forage vegetation found in open and flat areas, which is in accordance with the  
3092 findings of Chapter IV, the N ingestion in these areas was lower than the N deposition. In contrast, *A.*  
3093 *viridis* encroached and steep areas displayed the lowest N ingestion, yet it exceeded N deposition,  
3094 leading to an overall N depletion. Nutrient availability is particularly important for the restoration of  
3095 former open pastures, and Highland cattle grazing could thus help fertilizing nutrient-poor areas, while  
3096 contributing to the reduction of N accumulation under *A. viridis* stands. However, while this study  
3097 focused on N translocation due to the N accumulation generated by *A. viridis* shrubs, similar estimations  
3098 could be done for other nutrients to provide detailed nutrient traslocation related to Highland cattle  
3099 grazing. For example, Koch et al. (2018) focused on the translocation of P, as this nutrient has great  
3100 effect on plant species composition, and is a good indicator of other nutrient levels. In addition,  
3101 particular care should be given to Highland cattle ability to modify plant available nutrient, as an over-  
3102 utilization of some areas could lead to excessive nutrient accumulation on the long term. In that regard,  
3103 the effectiveness of molasses-based blocks in attracting cows farther from resting areas could be used  
3104 to avoid this possible negative impact. In addition, management strategies such as rotational grazing, or  
3105 fencing and water trough placement could help preventing the overuse of specific resting areas (Jewell  
3106 et al. 2007).

3107

3108 While the calculations used in Chapter VI are a common method for nutrient estimations and are  
3109 increasingly precise thanks to the use of GPS-tracking (Koch et al. 2018), they are limited to the data

3110 gathered from literature when direct field measurements were not possible. For example, cattle urine  
3111 collection is particularly challenging with grazing animals, and could not be carried out in remote  
3112 mountain areas. In this study, a maximum of comparable studies with similar grazing conditions were  
3113 gathered to provide the most precise missing information for the calculation of N fluxes. However, the  
3114 possibility of a bias remains, either linked to specific field conditions or breed-dependant traits. In  
3115 addition, while grazing and resting activity periods were discriminated through the use of two different  
3116 variables (i.e., activity calculated by activity sensors and distance travelled by the cow), these  
3117 estimations could be lightly biased as well. Indeed, periods mainly attributed to grazing periods may see  
3118 a few animals resting, and inversely, as not all cows were equipped with GPS collars. Finally, there is  
3119 room for improvement as well in the estimation scales, as vegetation intake and excretions were  
3120 determined at the vegetation patch level. While vegetation is relatively homogeneous in these patches,  
3121 variations in livestock distribution patterns may occur at a finer scale due to the local presence of water  
3122 troughs, or due to localized slope shifts.

3123

3124

### 3125 **Long-term restoration objective**

3126

3127 This project was carried out for four years, and allowed us to make a comprehensive analysis of  
3128 the potential for Highland cattle to graze in *A. viridis* encroached pastures. However, the restoration of  
3129 plant and animal biodiversity is a long-term process, and several years may be needed to assess the effect  
3130 of management practices on vegetation composition changes. In high elevations specifically, ecological  
3131 responses of plant species are slower due to the short growing season (Körner 2003). The restoration of  
3132 herbaceous grassland species is also highly dependent on the presence of seed sources, as the range for  
3133 seed dispersal is spatially limited (Barbaro et al. 2001). Therefore, Highland cattle grazing in subalpine  
3134 pastures may take several years to efficiently restore former open grasslands with light-demanding, slow  
3135 growing and less competitive species. Elias et al. (2018) have demonstrated the recovery of formerly  
3136 shrub-encroached areas in Germany after they were grazed by goats over seven years, with improved  
3137 light conditions that allowed light-demanding species to settle, thanks to the reduced shrub cover.  
3138 Similar results were found by Silva et al. (2019), who measured an increased species richness after  
3139 several years of goat grazing in shrub-grassland habitats. Consequently, Highland cattle grazing could  
3140 also show similar positive effects on plant composition over the next years, through the slow opening  
3141 of the canopy of *A. viridis* stands, the seed dispersal through dungs and fur transportation, and the  
3142 effective redistribution of nutrients within grazed paddocks. This medium to long-term process may be  
3143 particularly true for the most encroached areas, where a strong grazing impact is needed to allow the  
3144 opening of the canopy and the installation of new grassland species. If repeated over the long term, such  
3145 management could thus lead to a more heterogeneous vegetation composition, with pastures  
3146 progressively composed by a mosaic of open pastures with higher forage quality and smaller stands of  
3147 *A. viridis*. In one of our experiment study (Val Vogna, Italy), typical species of nutrient-rich pastures  
3148 (e.g. *Phleum rhaeticum*) have already been observed to grow in the newly opened areas, where attractive  
3149 points were placed, only two years after their implementation (**Figure 32**). In addition, an un-published  
3150 work was carried out in 2019 to evaluate the seed germination in Highland cattle dung pats. This was  
3151 done by sampling several Highland cattle dung pats and placing them in a greenhouse after vernalization.  
3152 While very few seeds had grown, probably due to vernalization protocol issues, a majority of them were  
3153 graminoid plants (77%) such as *Poa chaixi*, *Carex sp.*, *Avenella flexuosa*, and the rest were legumes  
3154 (23%, mainly *Lotus alpinus* and *Trifolium pratense*). Therefore, there is a potential for Highland cattle  
3155 to transport seeds of valuable grass and legume species through their dung excretions. An in-depth study  
3156 on the seeds carried by Highland cattle, both through the dungs and through their furs, could help  
3157 emphasize their ability to bring them in newly opened areas. In the future, the monitoring of the plant

3158 communities of the paddocks used in this project could be done using the permanent linear transects  
3159 placed in the vegetation patches in 2019. This prospect has already been planned in the near future  
3160 (2024, 5 years after the first grazing season), and could be continued over the following years.  
3161



3162  
3163 **Figure 32** Picture of the vegetation of newly open *A. viridis* stands, two years after the placement of  
3164 molasses-based blocks. Val Vogna, Italy, 2022. Several individuals of *Phleum rhaeticum* are present,  
3165 where very few were recorded in the botanical surveys of 2020. In the picture it is also possible to see  
3166 several broken branches of *A. viridis* on the ground, thanks to Highland cattle grazing impacts.  
3167

3168

### 3169 **Result communication**

3170

3171 To successfully restore *A. viridis*-encroached pastures, it is however essential for Highland cattle  
3172 herds to continue grazing in the pastures on the long term, in order to keep constraining *A. viridis*  
3173 expansion and apply sufficient damages to the shrubs. With the end of the scientific project, this is  
3174 achieved through the persuasion of farmers that such management is worth maintaining on the long-term.  
3175 Indeed, the implementation of paddocks in remote areas is difficult and time-consuming. It involves the  
3176 set up of fences and water troughs in steep slopes, the monitoring of the herd during the summer season,  
3177 in addition to potential unforeseen events that could request immediate care (e.g., animal health issues,  
3178 water shortage, etc.). Therefore, farmers with pre-existing activities, such as dairy farms, may not have  
3179 the resources to maintain this type of management without the help of a funded project. Personal goals  
3180 and perspectives of the farmers, as well as a change in personnel on farm may also intervene in the  
3181 management decisions. In the paddock of Champlong, Highland cattle grazing stopped after the first  
3182 year due to the farmers' will to reduce the herd size, which was not compatible with the project  
3183 objectives. Yet, farmers are aware of forage losses due to shrub-encroachment, and management  
3184 attempts such as mechanical cuts are regularly set up to try reducing the shrub cover. Moreover, the gain  
3185 weight witnessed in one of the study area (Bovonne, VD) is an important argument to convince Highland  
3186 cattle owners that cows can graze in highly encroached areas without losing productivity. Therefore,  
3187 communicating the potential of Highland cattle management to farmers is essential to ensure the  
3188 continuity and spread of these restoration projects. Regular presentations of the results during the



3189 experiment to key stakeholders is needed as they do not take part to the scientific assessments of the  
3190 management practices, and long-term prospects may be difficult to grasp. During our project, this was  
3191 achieved through public agricultural events (Salon des alpages 2019 and 2022), during more targeted  
3192 events with professional interventions to field days (Journée herbagère AGRIDEA-ADCF, cours  
3193 montagne AGRIDEA), or through the publication of the results in local agricultural journals (AgriHebdo  
3194 and Gest'Alpe). By reaching a wide range of stakeholders (farmers, agricultural advisors, agricultural  
3195 schools, park and forest managers, local associations, etc.), the transmission of information goes beyond  
3196 the boundaries of the scientific community, and is delivered to actors that play a fundamental role in  
3197 pasture management. This could also help unlocking the implementation of political measures to finance  
3198 service herds and compensate the costs linked to such management systems. Direct payments for  
3199 particular agricultural practices allow farmers to be compensated for their contribution to the provision  
3200 of ecosystem services to society (El Benni et al. 2012). Future policies supporting service herds could  
3201 thus be set up to help reducing *A. viridis* encroachment in alpine and subalpine pastures through diverse  
3202 management techniques, including robust livestock cattle grazing.

3203

3204

## 3205 **Perspectives**

3206

3207 While this project included GPS technology, which allowed a better monitoring of Highland  
3208 cattle behaviour in such environments, additional technological devices could help the real-time  
3209 monitoring of the herds and reduce the workload needed for livestock management. For example, the  
3210 implementation of virtual fencing technologies could relieve farmers from fencing installation in remote  
3211 areas. Indeed, virtual fencing is of increasing interest in agriculture, as it has the potential to improve  
3212 farmers work by increasing flexibility and reducing the labour and costs of livestock management  
3213 (Umstatter 2011). This method is particularly interesting for an adapted management along the grazing  
3214 season and to provide faster reaction to changing circumstances, in comparison to physical fences.  
3215 Several trials have been conducted on cattle herds, and positive results show their ability to rapidly  
3216 respond to virtual fencing (Campbell et al. 2020). However, this technology is not widely spread among  
3217 farmers and a few products are available on the market, mostly due to regulations in many European  
3218 countries, where these devices are not yet authorized, such as in Switzerland (Aaser et al. 2022).  
3219 Additional trials are necessary to test virtual fencing in mountain areas and potentially help farmers for  
3220 livestock control in remote areas to preserve these open habitats of great nature conservation value.

3221

3222 In addition, the utilisation of satellite and drone images could help monitor *A. viridis* cover and  
3223 keep track of Highland cattle effect on the canopy. Indeed, satellite and drone technology is increasingly  
3224 used for vegetation mapping and monitoring as it provides aerial images that can be analyzed to detect  
3225 different plant communities (James and Bradshaw 2020). It has been used in several contexts, such as  
3226 the detection of invasive plants, or in agriculture for the estimation of crop coverage and health (Pajares  
3227 2015). For example, Olariu et al. (2022) have used very high resolution drone imagery to map woody  
3228 plant encroachment in grasslands in Texas (USA). They were able to accurately classify different woody  
3229 plant species and provide precise vegetation mapping using machine learning algorithms. Drone  
3230 technology has also been used to monitor leaf phenology and ontogeny at the tree-crown level (Wu et  
3231 al. 2021). Therefore, this technology could be applied in *A. viridis*-encroached pastures to follow leaf  
3232 seasonal changes and also potentially track Highland cattle grazing impacts that can generate the drying  
3233 out of impacted branches.

3234

3235 Robust breed grazing in *A. viridis*-encroached areas in subalpine pastures could also be  
3236 complemented by regular manual/mechanical cutting of the woody shrubs. Mechanical cuts alone may

3237 not be efficient to effectively stop the invasion of shrubs, because of the difficulty to access the areas  
3238 and because it may not always help restore grass resources (Camacho et al. 2008). However, a  
3239 combination of cutting and grazing could be efficient on the long term. Indeed, in a six year study  
3240 conducted in the French Pre-Alps by Barbaro et al. (2001), the combination of manual cuttings and  
3241 livestock grazing was efficient in reducing shrub invasion and restoring grasslands, whether it was grazed  
3242 by goats, sheep, or cattle. In their study, the number of species was doubled in four years after shrub  
3243 cleaning by cuts and grazing, which led to a restoration of species-rich grasslands. While it is an  
3244 additional labour for farmers to perform these cuttings at a regular basis, associations that aim to help  
3245 protecting the environment could help by providing workforce. In the canton of Vaud in Switzerland,  
3246 the association “Alpes Vivantes” has previously organized cuttings of *A. viridis* in subalpine pastures,  
3247 and discussions have been initiated with them to perform cutting interventions in the encroached areas  
3248 in Bovonne as a follow up of the current project.

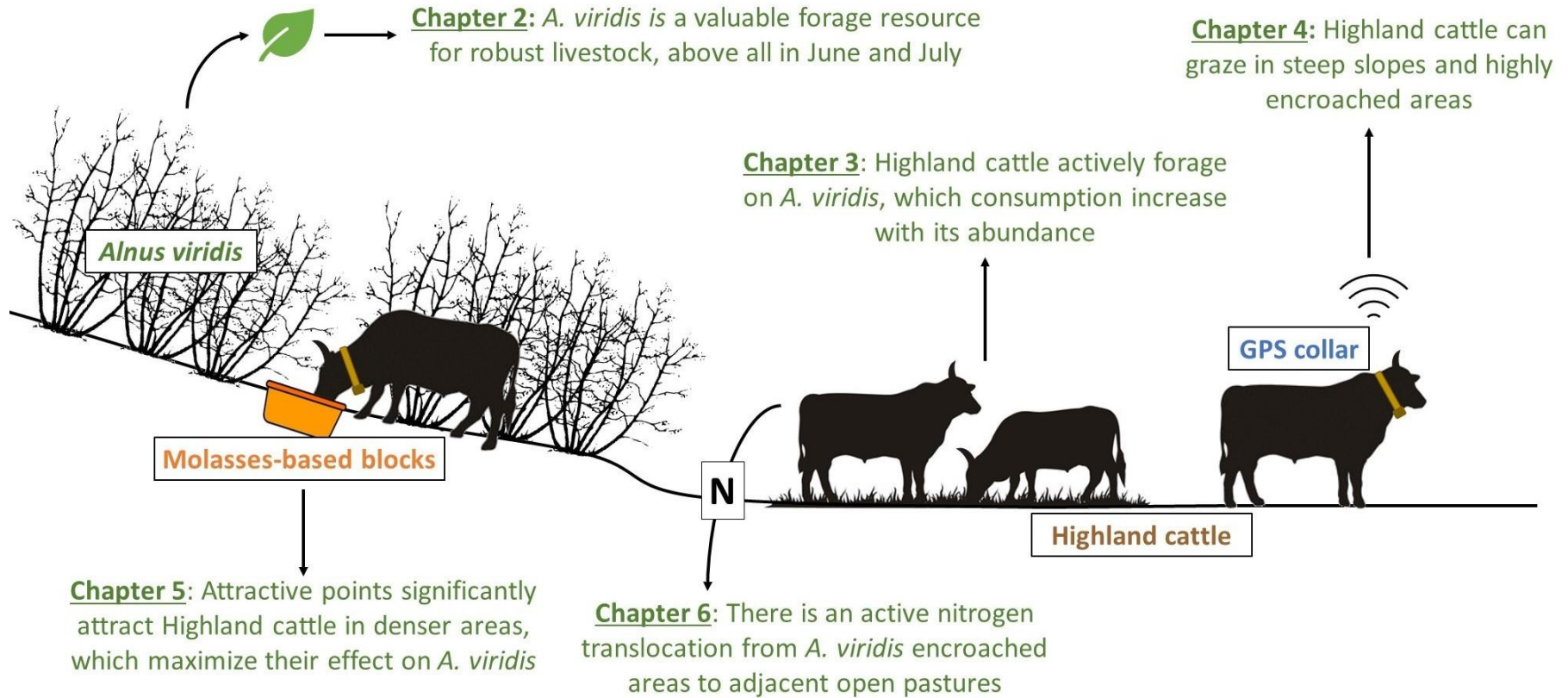
3249  
3250 Finally, the results of this project could be useful for other grazing breeds and species. Indeed,  
3251 several studies have highlighted the efficiency of goats and sheep to apply damages on *A. viridis* shrubs  
3252 and other invasive woody plants (Elias and Tischew 2016; Elias et al. 2018; Pauler et al. 2022b). They  
3253 are used by farmers as service herds to counteract the propagation of undesirable species. The knowledge  
3254 gained on the forage potential of *A. viridis* leaves, the effect of molasses-based blocks, as well as the N  
3255 translocation evaluated in this project, could be used to implement other management strategies with  
3256 different livestock categories, and provide future alternatives for farmers.

3257  
3258

## 3259 **Conclusion**

3260  
3261 The present thesis showed how Highland cattle can become an efficient management tool to  
3262 counteract *A. viridis* encroachment (**Figure 33**). Indeed, they could effectively damage the shrubs  
3263 through grazing, in addition to applying severe mechanical damages through trampling and the breaking  
3264 of branches. Thanks to their low weight and their adaptation to low forage quality vegetation, Highland  
3265 cattle were able to graze in highly encroached and steep areas. These effects can be enhanced by the  
3266 placement of attractive points in the form of molasses-based blocks. These impacts, combined with their  
3267 effective rebalance of N between encroached areas and open pastures could lead to a progressive  
3268 restoration of former grassland vegetation in the long-term. Nowadays, Highland cattle are being  
3269 increasingly reared in many alpine countries, and could therefore become a trending management tool to  
3270 protect grasslands against *A. viridis* encroachment.

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



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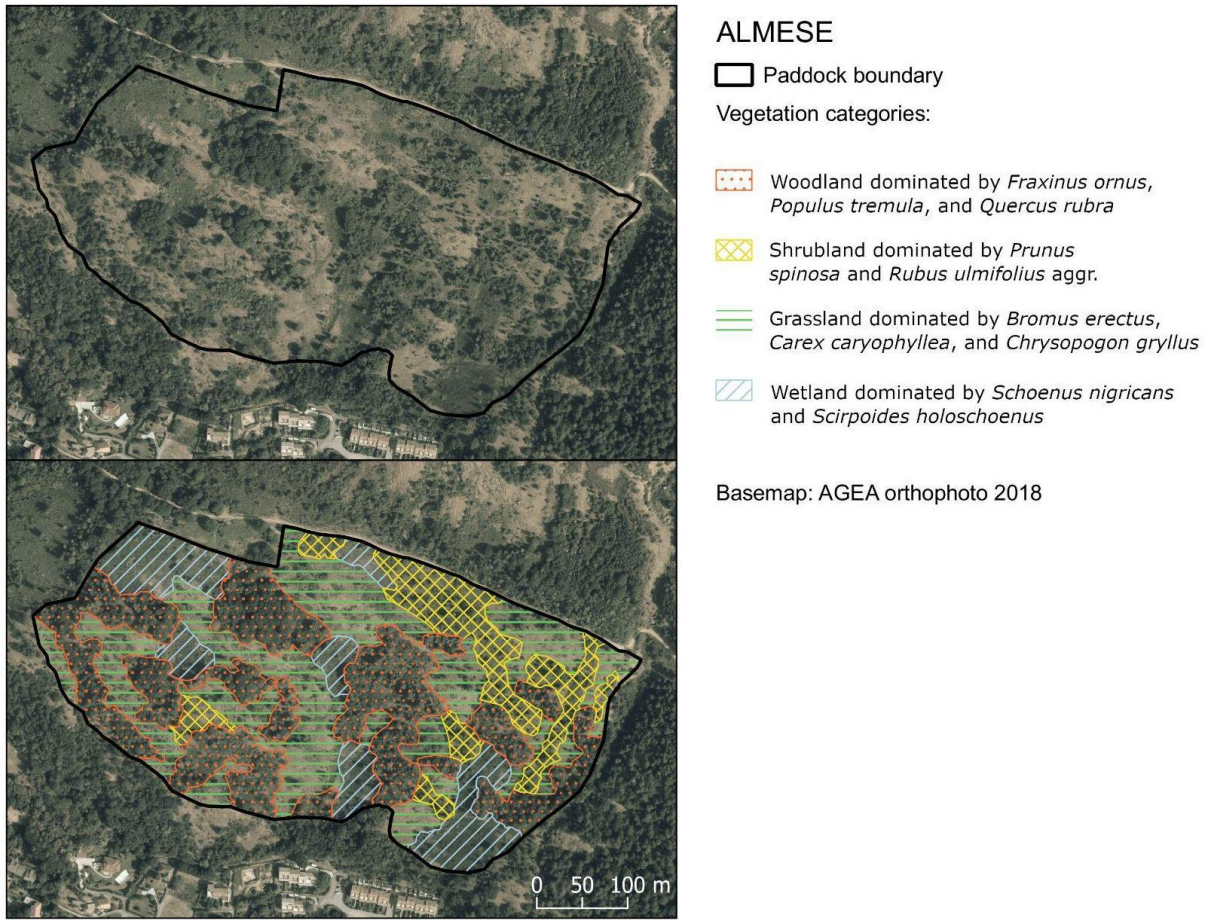
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Figure 33 Summary of the main findings of the RobustAlps project

3273 **SUPPLEMENTARY MATERIAL**

3274 **Supplementary materials of CHAPTER III**

3275



3276

3277 **Figure S1** Aerial photograph and vegetation map of Almese study area



**TORRETTE**

□ Paddock boundary

Vegetation categories:

▤ Woodland dominated by *Acer pseudoplatanus*,  
*Fraxinus excelsior*, and *Larix decidua*

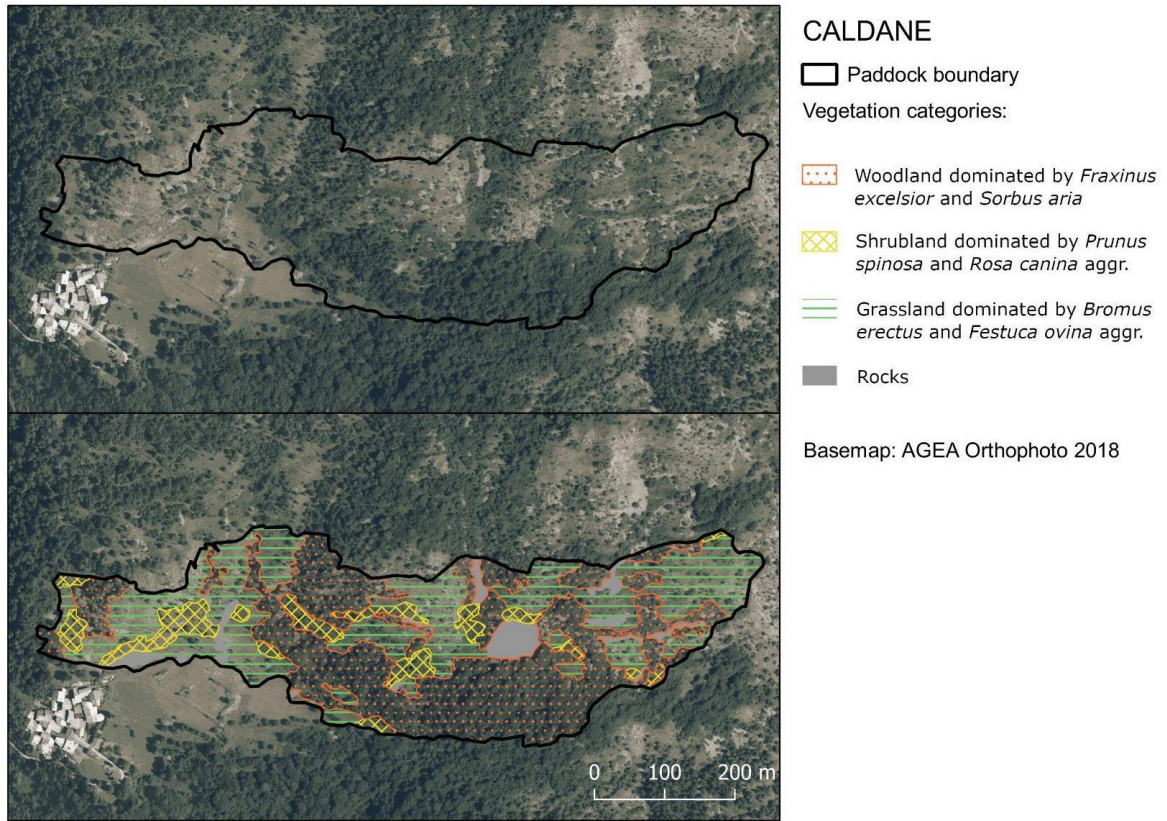
▨ Shrubland dominated by *Rubus idaeus*

Basemap: AGEA Orthophoto 2018

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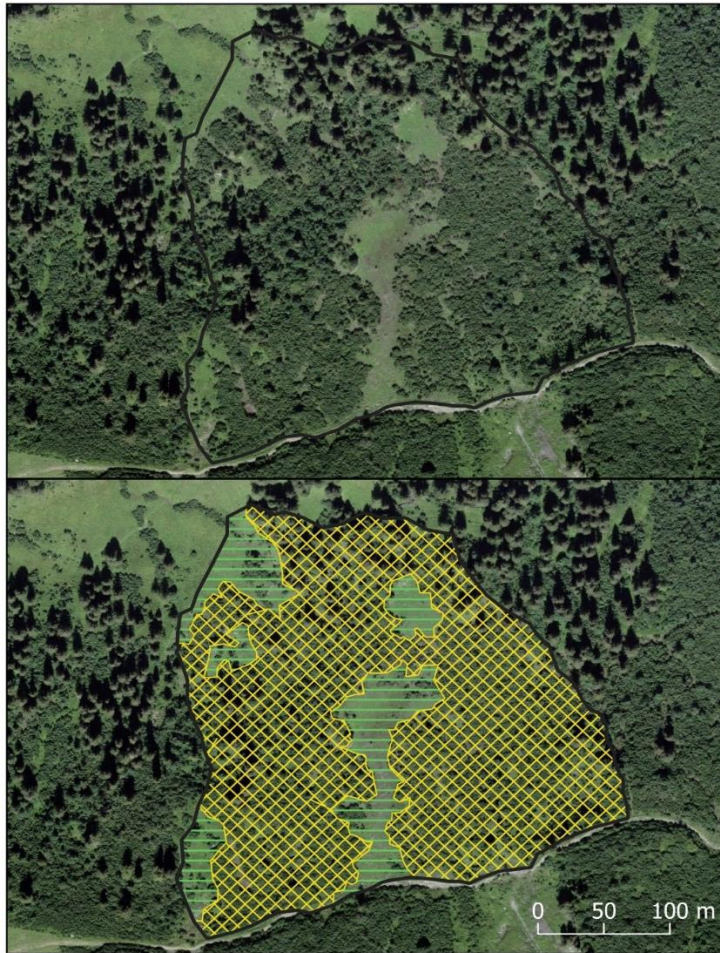
**Figure S2** Aerial photograph and vegetation map of Torrette study area



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**Figure S3** Aerial photograph and vegetation map of Caldane study area



## BOVONNE

□ Paddock boundary

Vegetation categories:

▨ Shrubland dominated by *Alnus viridis*

▨ Grassland dominated by *Alchemilla xanthochlora* and *Calamagrostis villosa*

Basemap: SWISSIMAGE 2020  
©swisstopo

3282

3283

**Figure S4** Aerial photograph and vegetation map of Bovonne study area

3284 **Table S1** List of the woody (W) and herbaceous (H) plant species recorded in the paddocks of the four  
3285 study areas (i.e., Almese, Torrette, Caldane, and Bovonne) during the direct observations, with the  
3286 corresponding number of observation sessions, number of cows that met the species, and the proportion  
3287 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	<i>Ailanthus altissima</i>	W	42	5	0.19	0.00
Almese	<i>Alnus glutinosa</i>	W	3	1	0.02	0.02
Almese	<i>Betula pendula</i>	W	24	7	0.24	0.33
Almese	<i>Calluna vulgaris</i>	W	342	10	1.47	0.16
Almese	<i>Cedrus atlantica</i>	W	15	3	0.09	0.00
Almese	<i>Celtis australis</i>	W	85	6	0.88	1.39
Almese	<i>Clematis recta</i>	W	19	3	0.16	0.38
Almese	<i>Crataegus monogyna</i>	W	151	11	1.16	0.58
Almese	<i>Euonymus europaeus</i>	W	15	3	0.07	0.23
Almese	<i>Ficus carica</i>	W	6	1	0.02	0.00
Almese	<i>Frangula Alnus</i>	W	212	9	1.61	3.35
Almese	<i>Fraxinus excelsior</i>	W	27	5	0.15	0.27
Almese	<i>Fraxinus ornus</i>	W	211	10	1.76	2.87
Almese	<i>Hedera helix</i>	W	7	2	0.02	0.00
Almese	<i>Juniperus communis</i>	W	18	4	0.14	0.00
Almese	<i>Ligustrum vulgare</i>	W	121	8	1.07	0.78
Almese	<i>Malus</i> sp.	W	12	2	0.10	0.19
Almese	<i>Populus tremula</i>	W	211	9	1.06	1.79
Almese	<i>Prunus avium</i>	W	6	4	0.03	0.10
Almese	<i>Prunus mahaleb</i>	W	25	3	0.34	0.42
Almese	<i>Prunus serotina</i>	W	39	7	0.29	0.35
Almese	<i>Prunus spinosa</i>	W	764	11	5.38	2.87
Almese	<i>Pteridium aquilinum</i>	H	161	8	1.07	0.00
Almese	<i>Pyrus pyraeaster</i>	W	11	2	0.08	0.18
Almese	<i>Quercus pubescens/petraea</i>	W	30	8	0.18	0.31
Almese	<i>Quercus rubra</i>	W	49	7	0.65	1.12
Almese	<i>Robinia pseudoacacia</i>	W	36	4	0.20	0.17
Almese	<i>Rosa canina</i> aggr.	W	63	9	0.45	0.35
Almese	<i>Rubus ulmifolius</i> aggr.	W	1415	12	9.22	2.59
Almese	<i>Sorbus aria</i>	W	3	1	0.04	0.05
Almese	<i>Ulmus minor</i>	W	138	10	1.39	1.86
Torrette	<i>Acer pseudoplatanus</i>	W	244	3	2.05	1.83
Torrette	<i>Alnus incana</i>	W	4	2	0.06	0.00
Torrette	<i>Athyrium filix-femina</i>	H	27	2	0.26	0.00
Torrette	<i>Betula pendula</i>	W	3	1	0.02	0.00
Torrette	<i>Corylus avellana</i>	W	489	3	4.37	0.40
Torrette	<i>Dryopteris affinis</i>	H	6	1	0.03	0.00
Torrette	<i>Dryopteris dilatata</i>	H	3	1	0.01	0.00
Torrette	<i>Dryopteris filix-mas</i>	H	131	3	1.09	0.46
Torrette	<i>Fagus sylvatica</i>	W	26	2	0.24	0.00
Torrette	<i>Fraxinus excelsior</i>	W	466	3	3.49	2.05
Torrette	<i>Gymnocarpium dryopteris</i>	H	2	1	0.02	0.00
Torrette	<i>Laburnum alpinum</i>	W	55	3	0.35	0.00
Torrette	<i>Larix decidua</i>	W	22	2	0.15	0.00
Torrette	<i>Lonicera alpigena</i>	W	1	1	0.04	0.04
Torrette	<i>Lonicera xylosteum</i>	W	95	3	1.64	1.46
Torrette	<i>Polystichum aculeatum</i>	H	2	2	0.01	0.00
Torrette	<i>Populus alba</i>	W	12	2	0.08	0.06
Torrette	<i>Prunus avium</i>	W	21	2	0.10	0.03
Torrette	<i>Quercus petraea</i>	W	12	1	0.08	0.00
Torrette	<i>Ribes uva-crispa</i>	W	26	3	0.15	0.01
Torrette	<i>Rosa canina</i> aggr.	W	63	3	0.41	0.05
Torrette	<i>Rubus idaeus</i>	W	1354	3	30.70	39.54
Torrette	<i>Rubus ulmifolius</i> aggr.	W	1	1	0.01	0.01
Torrette	<i>Salix alba</i>	W	4	2	0.04	0.04

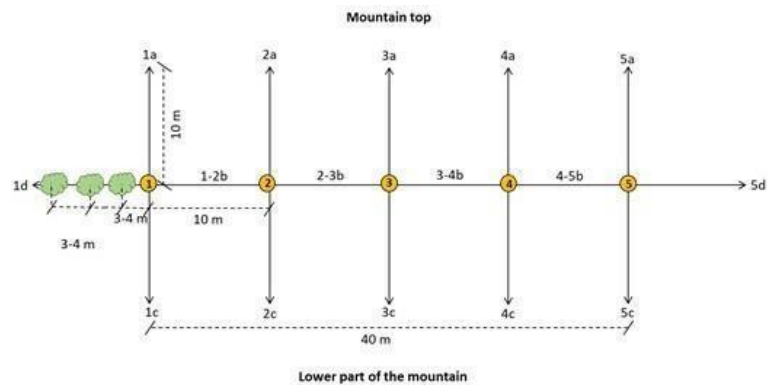


Torrette	<i>Salix caprea</i>	W	8	2	0.05	0.00
Torrette	<i>Salix purpurea</i>	W	1	1	0.00	0.00
Torrette	<i>Sorbus aria</i>	W	59	2	0.40	0.07
Torrette	<i>Sorbus aucuparia</i>	W	15	2	0.08	0.02
Torrette	<i>Tilia platyphyllos</i>	W	5	2	0.06	0.15
Torrette	<i>Ulmus minor</i>	W	2	2	0.01	0.00
Caldane	<i>Acer pseudoplatanus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

3289 **Supplementary materials of CHAPTER V**

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3293 **Figure S5** Scheme of the 16 cross-shaped vegetation transects performed in attractive points and control  
3294 areas. Yellow numbers represent the attractive points (molasses-based blocks) or the control points,  
3295 depending on the area. The green figures represent the *Alnus viridis* shrubs selected in the external  
3296 transects which are only linked to one molasses-based block (i.e. not the central transects labeled as 1-  
3297 2b, 2-3b, 3-4b, and 4-5b).

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3301 **Figure S6:** Intense defoliation on small *Alnus viridis* shrubs having 1-2 meters of height.

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**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.



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**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.



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



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**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.

3322 **Table S2** Composition of molasses-based blocks. The blocks contained 2-3% of residual moisture.

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<b>Components (%)</b>		<b>Additives per kg (mg)</b>	
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%		

3335 **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  
 3336 groups. Plant species nomenclature follows Aeschimann et al. (2004).

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

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3342 **Table S4** Statistical results of the analyses made on spatial distribution (generalized linear model), soil cover (herbaceous cover and fraction of bare soil), *Alnus*  
 3343 *viridis* leaves and understory plant functional groups (generalized least square model).  
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Spatial distribution				Vegetation cover			<i>A. viridis</i> leaves			Functional groups					
Variables	10 m	50m		Variables	Herbaceous cover	Bare soil	Variables			Variables	Tall herbs	Forbs	Woody plants		
	<i>Df</i>	<i>p</i>	<i>p</i>		<i>Df</i>	<i>p</i>	<i>p</i>		<i>Df</i>	<i>p</i>		<i>Df</i>	<i>p</i>	<i>p</i>	
<b>Molasses blocks (MB)</b>	1	0.8589	0.2513	<b>Molasses blocks (MB)</b>	1	3.65E-05	2.41E-03	<b>Molasses blocks (MB)</b>	1	2.718E-10	<b>Molasses blocks (MB)</b>	1	0.01051	0.007926	2.88E-07
<b>Year (Y)</b>	1	0.4713	0.0058					<b>Distance (D)</b>	1	0.4238					
<b>MB x Y</b>	1	2.26E-05	0.0237					<b>MB x D</b>	1	0.01019					

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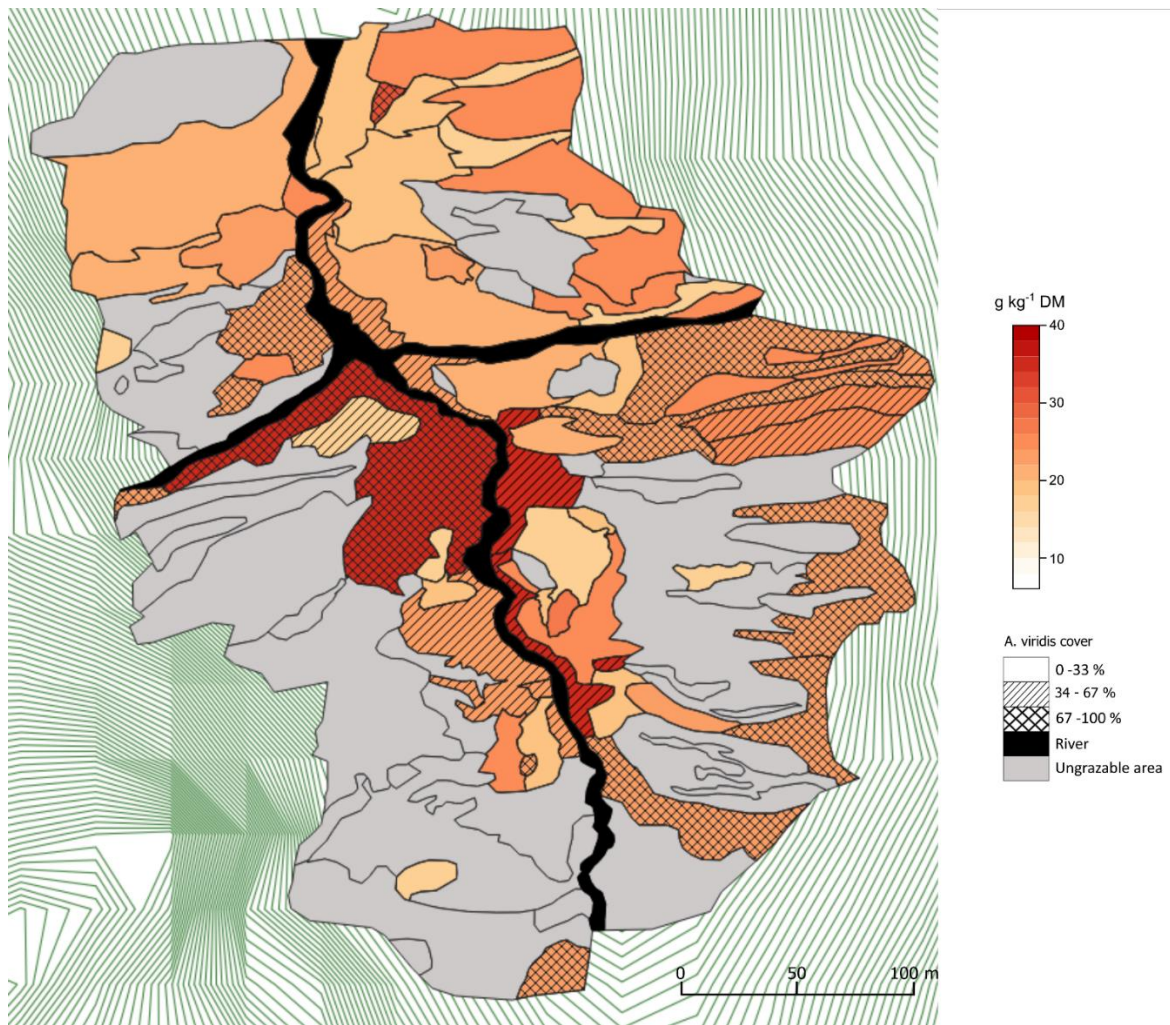
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3347 **Table S5** Statistical results of the additional analyses (Anovas) made on the average RR in each area of the paddocks (i.e. 3 control vs. 3 molasses-based blocks  
 3348 areas, one control and one MB area from each paddock: 6 data points in total).  
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Vegetation cover				<i>A. viridis</i> leaves			Functional groups				
Variables	Herbaceous cover	Bare soil		Variables			Variables	Ferns and tall herbs	Medium and small herbs	Woody plants	
	<i>Df</i>	<i>p</i>	<i>p</i>		<i>Df</i>	<i>p</i>		<i>Df</i>	<i>p</i>	<i>p</i>	
<b>Molasses blocks (MB)</b>	1	0.0313	0.05649	<b>Molasses blocks (MB)</b>	1	0.0479	<b>Molasses blocks (MB)</b>	1	0.126	0.024	0.006349

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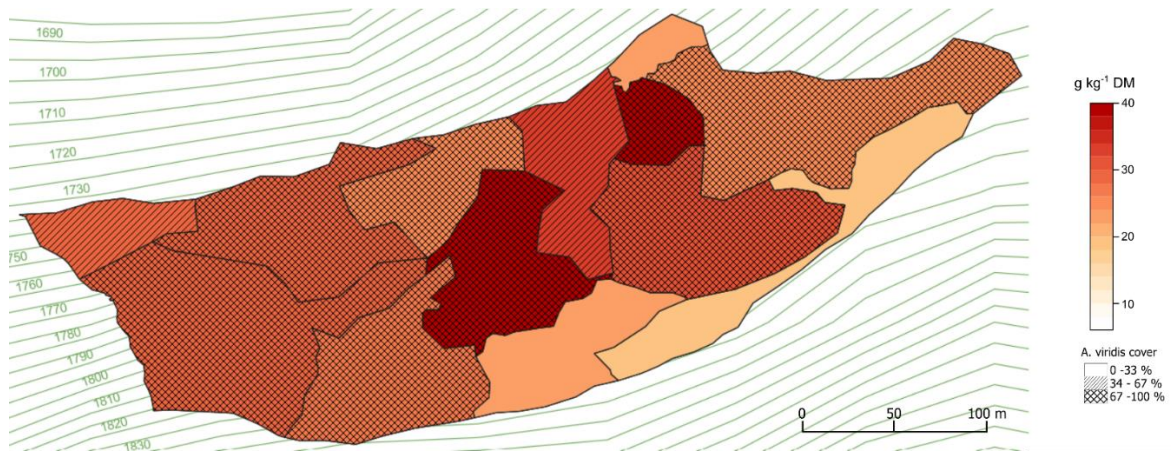


3353 **Figure S11** Nitrogen content (g kg<sup>-1</sup> DM) of the herbaceous vegetation and *Alnus viridis* cover (%) of  
3354 all vegetation patches of paddock 1.



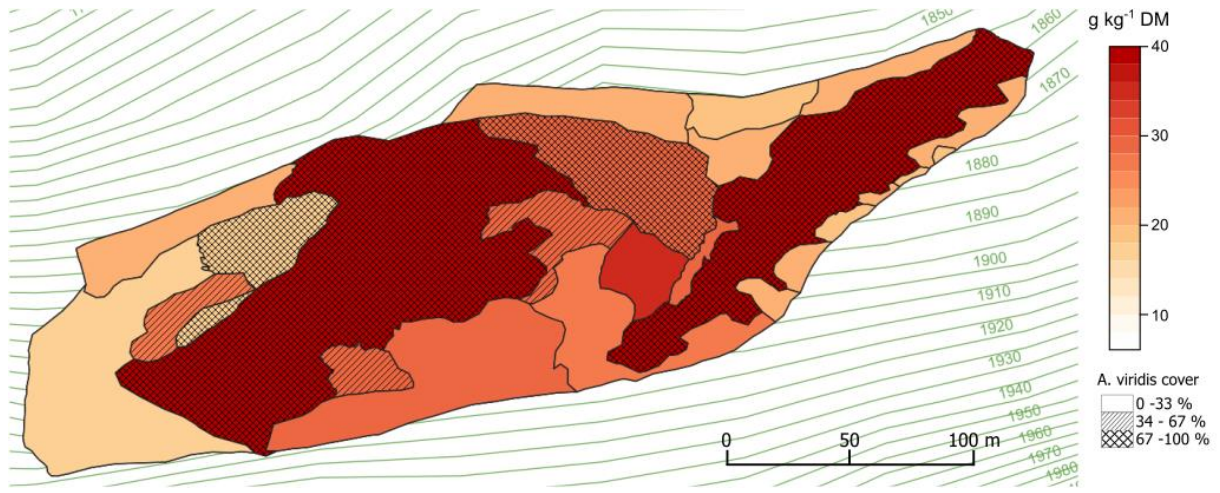
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**Figure S12** Nitrogen content ( $\text{g kg}^{-1}$ ) of the herbaceous vegetation and *Alnus viridis* cover (%) of all vegetation patches of paddock 2



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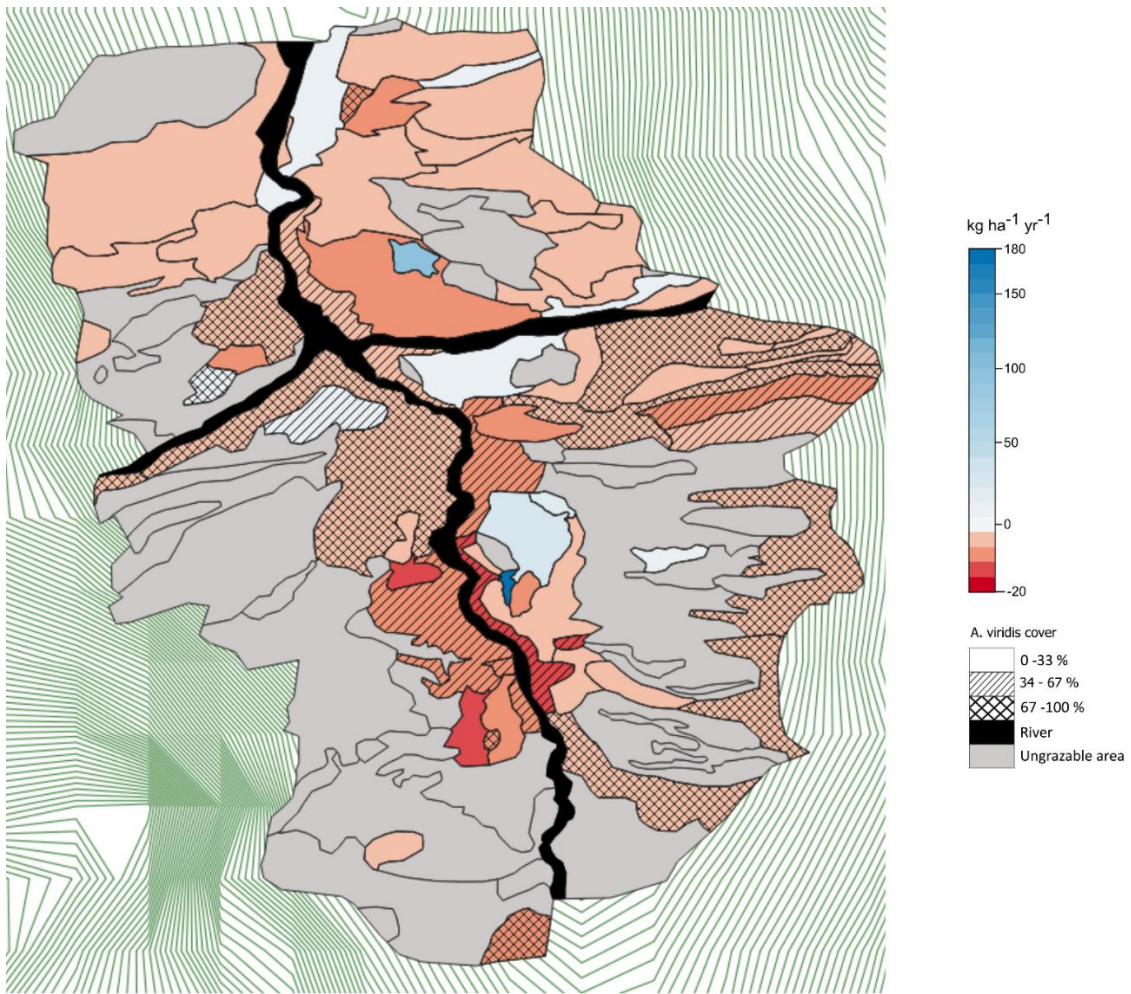
**Figure S13** Nitrogen content ( $\text{g kg}^{-1}$ ) of the herbaceous vegetation and *Alnus viridis* cover (%) of all vegetation patches of paddock 3.



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

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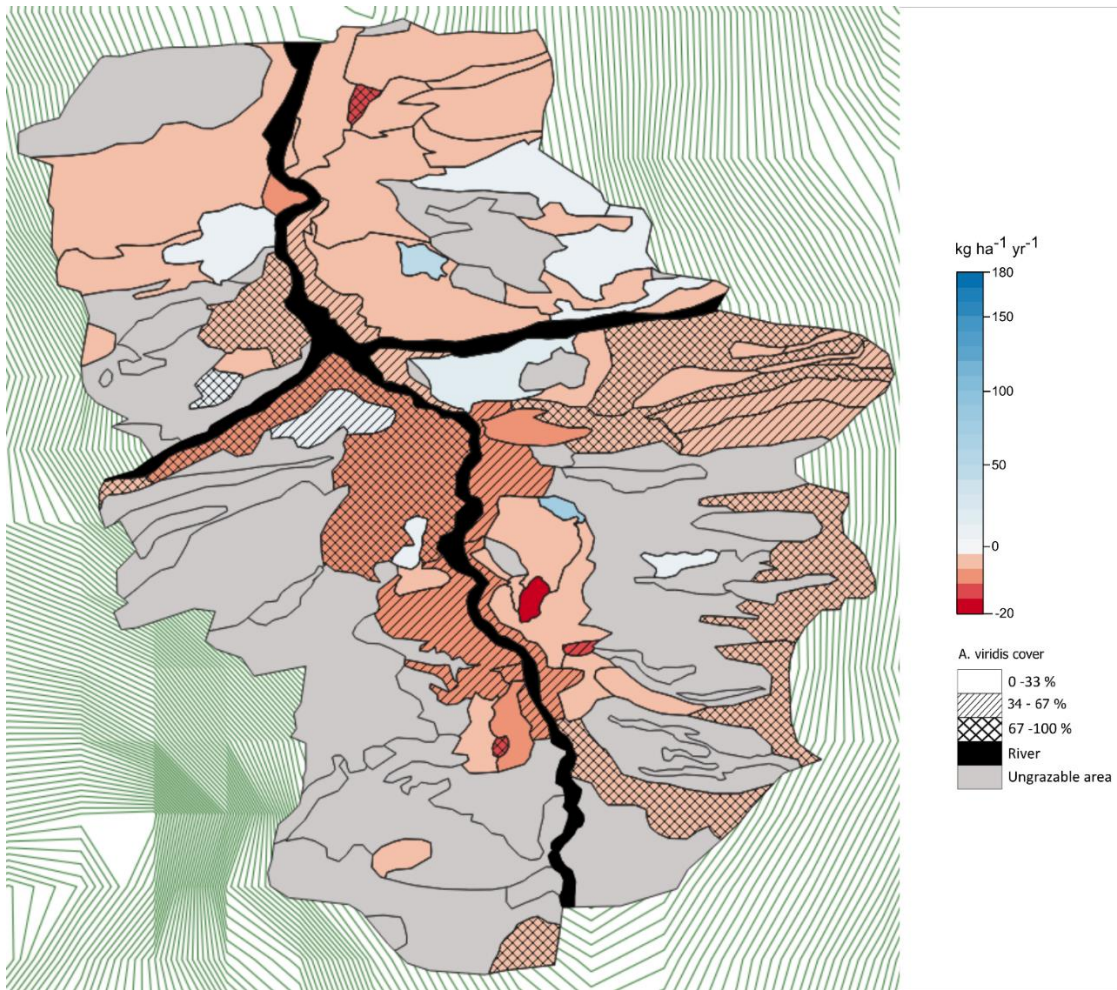


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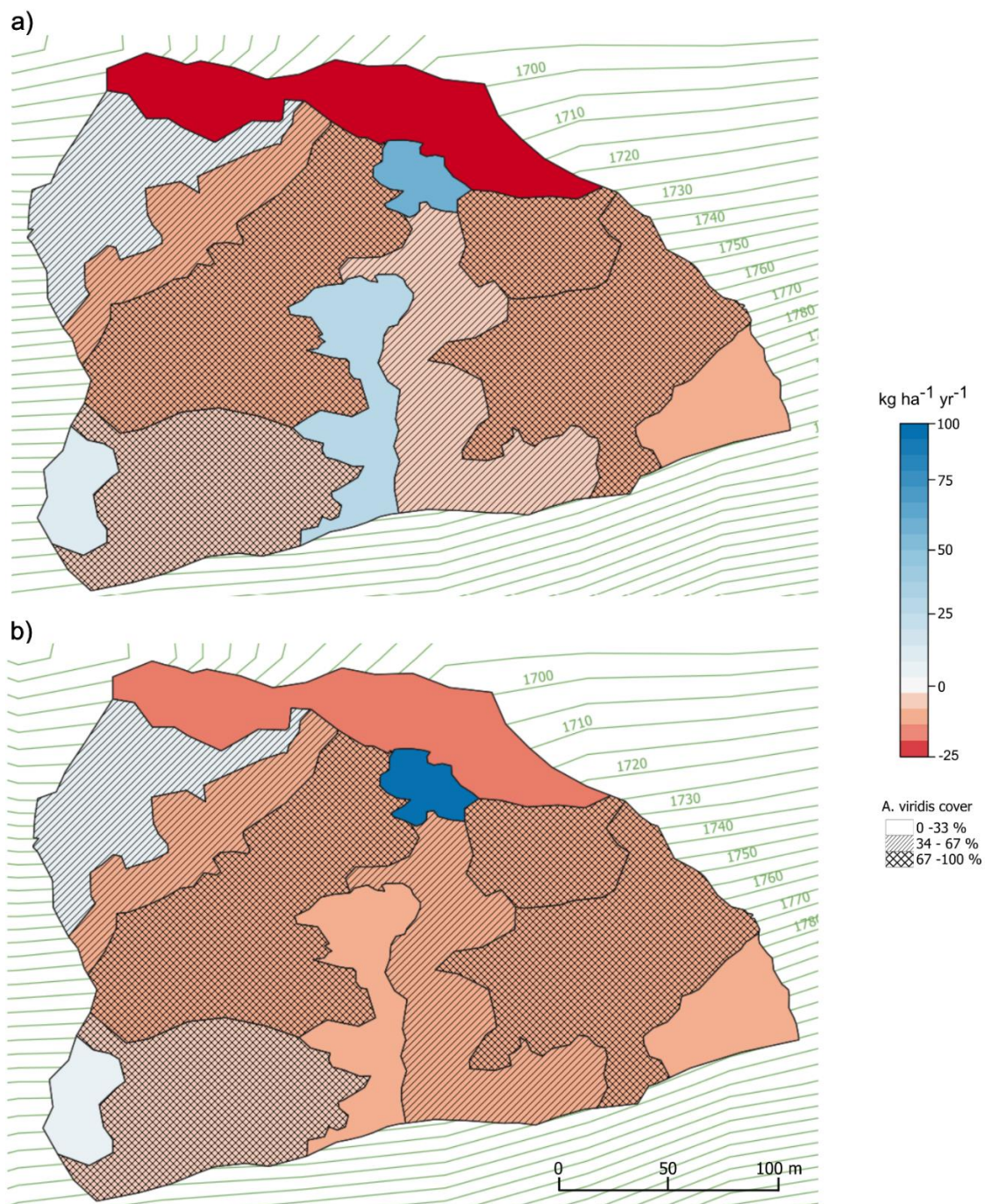
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**Figure S15** Nitrogen fluxes (50% quantile, kg ha<sup>-1</sup> yr<sup>-1</sup>) of the vegetation patches of paddock 1 in 2019.



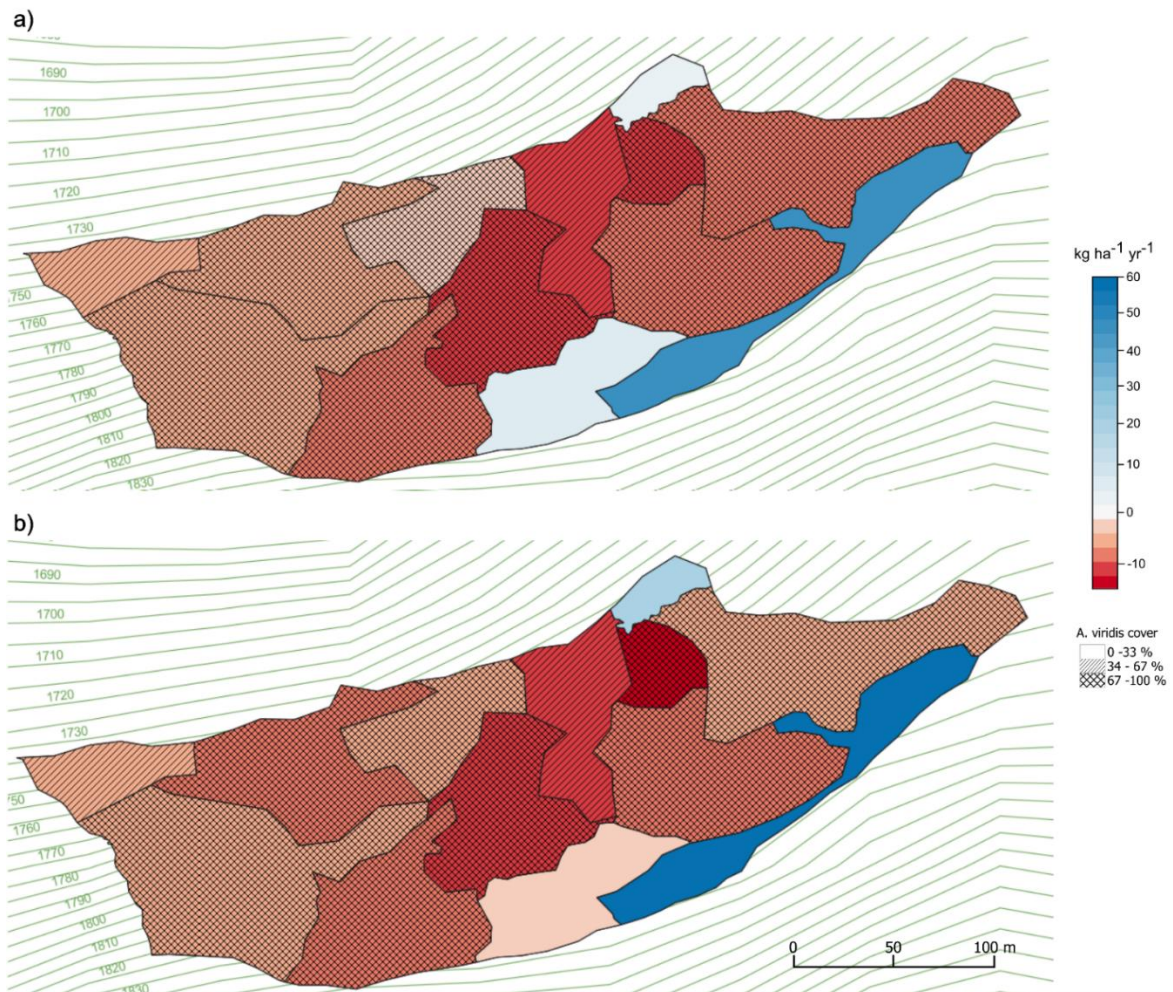
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**Figure S16** Nitrogen fluxes (50% quantile, kg ha<sup>-1</sup> yr<sup>-1</sup>) of the vegetation patches of paddock 1 in 2020.



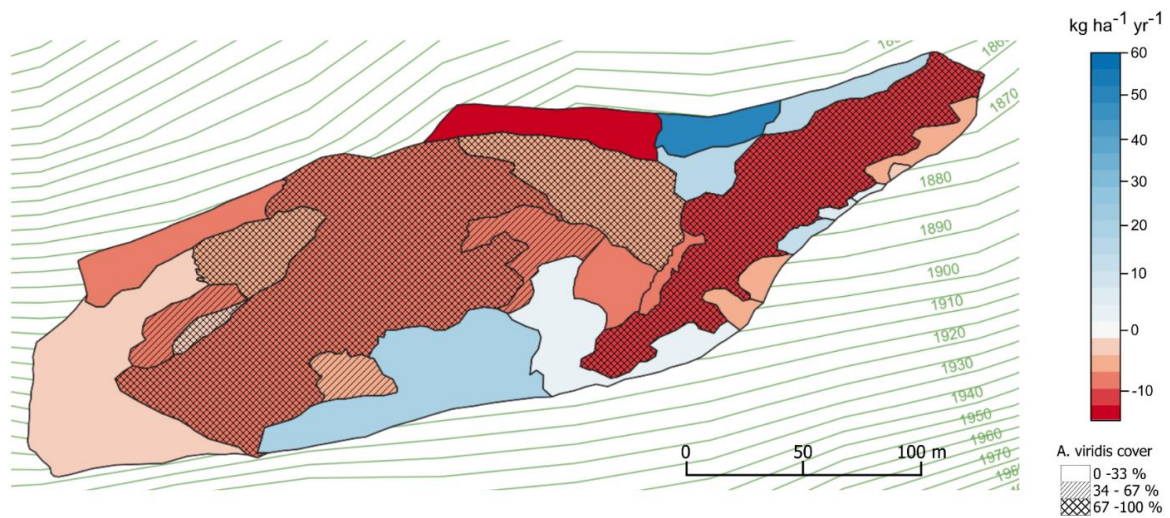
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**Figure S17** Nitrogen fluxes (50% quantile,  $\text{kg ha}^{-1} \text{ yr}^{-1}$ ) of the vegetation patches of paddock 2 in: a) 2019, and b) 2020.



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 3378 **Figure S18** Nitrogen fluxes (50% quantile,  $\text{kg ha}^{-1} \text{yr}^{-1}$ ) of the vegetation patches of paddock 3in: a)  
 3379 2019, and b) 2020.

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 3383 **Figure S19** Nitrogen fluxes (50% quantile,  $\text{kg ha}^{-1} \text{yr}^{-1}$ ) of the vegetation patches of paddock 4 in  
 3384 2020.

3385 **REFERENCES**

3386

3387 Aaser MF, Staahltoft SK, Korsgaard AH, et al (2022) Is Virtual Fencing an Effective Way of  
3388 Enclosing Cattle? Personality, Herd Behaviour and Welfare. *Animals* 12:842.  
3389 <https://doi.org/10.3390/ani12070842>

3390 Abegg M, Brändli U, Cioldi F, et al (2020) Swiss national forest inventory NFI. Result tables and  
3391 maps of the NFI surveys 1983-2017 (NFI1, NFI2, NFI3, NFI4) on the internet. In: *Swiss Natl.*  
3392 *For. Invent.* Birmensdorf, Swiss Fed. Res. Inst. WSL. [https://www.lfi.ch/resultate/resultate-](https://www.lfi.ch/resultate/resultate-en.php?prodNr=32&prodItNr=201803&lang=en)  
3393 [en.php?prodNr=32&prodItNr=201803&lang=en](https://www.lfi.ch/resultate/resultate-en.php?prodNr=32&prodItNr=201803&lang=en). Accessed 16 May 2020

3394 Aber JD, Nadelhoffer KJ, Steudler P, Melillo JM (1989) Nitrogen Saturation in Northern Forest  
3395 Ecosystems. *Bioscience* 39:378–386. <https://doi.org/10.2307/1311067>

3396 Aeschmann D, Lauber K, Moser D-M, Theurillat J-P (2004) *Flora alpina*

3397 Agabriel J (2010) *Alimentation des bovins, ovins et caprins: besoins des animaux, valeurs des*  
3398 *aliments: Tables Inra, Editions Q. Versailles, France*

3399 Agnusdei MG, Mazzanti A (2001) Frequency of defoliation of native and naturalized species of the  
3400 Flooding Pampas (Argentina). *Grass Forage Sci* 56:344–351. [https://doi.org/10.1046/j.1365-](https://doi.org/10.1046/j.1365-2494.2001.00283.x)  
3401 [2494.2001.00283.x](https://doi.org/10.1046/j.1365-2494.2001.00283.x)

3402 Alados CL, Saiz H, Nuche P, et al (2019) Clearing vs. Burning for restoring Pyrenean grasslands after  
3403 shrub encroachment. *Geogr Res Lett* 45:441–468. <https://doi.org/10.18172/cig.3589>

3404 Alaoui A, Willmann E, Jasper K, et al (2014) Modelling the effects of land use and climate changes  
3405 on hydrology in the Ursern Valley, Switzerland. *Hydrol Process* 28:3602–3614.  
3406 <https://doi.org/10.1002/hyp.9895>

3407 Allison CD (1985) Factors Affecting Forage Intake by Range Ruminants: A Review. *J Range Manag*  
3408 38:305. <https://doi.org/10.2307/3899409>

3409 Alonso-Amelot ME, Oliveros-Bastidas A, Calcagno-Pisarelli MP (2007) Phenolics and condensed  
3410 tannins of high altitude *Pteridium arachnoideum* in relation to sunlight exposure, elevation, and  
3411 rain regime. *Biochem Syst Ecol* 35:1–10. <https://doi.org/10.1016/j.bse.2006.04.013>

3412 Álvarez-Martínez J, Gómez-Villar A, Lasanta T (2016) The Use of Goats Grazing to Restore Pastures  
3413 Invaded by Shrubs and Avoid Desertification: A Preliminary Case Study in the Spanish  
3414 Cantabrian Mountains. *L Degrad Dev* 27:3–13. <https://doi.org/10.1002/ldr.2230>

3415 Ammar H, López S, González JS, Ranilla MJ (2004) Chemical composition and in vitro digestibility  
3416 of some Spanish browse plant species. *J Sci Food Agric* 84:197–204.  
3417 <https://doi.org/10.1002/jsfa.1635>

3418 Angelidis A, Crompton L, Misselbrook T, et al (2019) Evaluation and prediction of nitrogen use  
3419 efficiency and outputs in faeces and urine in beef cattle. *Agric Ecosyst Environ* 280:1–15.  
3420 <https://doi.org/10.1016/j.agee.2019.04.013>

3421 Anthelme F, Cornillon L, Brun J-J (2002) Secondary succession of *Alnus viridis* (Chaix) DC. in  
3422 Vanoise National Park, France: coexistence of sexual and vegetative strategies. *Ann For Sci*  
3423 59:419–428. <https://doi.org/10.1051/forest:2002016>

3424 Anthelme F, Grossi J-L, Brun J-J, Didier L (2001) Consequences of green alder expansion on  
3425 vegetation changes and arthropod communities removal in the northern French Alps. *For Ecol*  
3426 *Manage* 145:57–65. [https://doi.org/10.1016/S0378-1127\(00\)00574-0](https://doi.org/10.1016/S0378-1127(00)00574-0)

3427 Anthelme F, Michalet R, Barbaro L, Brun J (2003) Environmental and Spatial Influences of Shrub  
3428 Cover (*Alnus viridis* DC.) on Vegetation Diversity at the Upper Treeline in the Inner Western  
3429 Alps. *Arctic, Antarct Alp Res* 35:48–55. <https://doi.org/10.1657/1523->



- 3430 0430(2003)035[0048:EASIOS]2.0.CO;2
- 3431 Anthelme F, Villaret J, Brun J (2007) Shrub encroachment in the Alps gives rise to the convergence of  
3432 sub-alpine communities on a regional scale. *J Veg Sci* 18:355–362.  
3433 <https://doi.org/10.1111/j.1654-1103.2007.tb02547.x>
- 3434 Auerswald K, Mayer F, Schnyder H (2010) Coupling of spatial and temporal pattern of cattle excreta  
3435 patches on a low intensity pasture. *Nutr Cycl Agroecosystems* 88:275–288.  
3436 <https://doi.org/10.1007/s10705-009-9321-4>
- 3437 Bailey DW, Brown JR (2011) Rotational grazing systems and livestock grazing behavior in shrub-  
3438 dominated semi-arid and arid rangelands. *Rangel Ecol Manag* 64:1–9.  
3439 <https://doi.org/10.2111/REM-D-09-00184.1>
- 3440 Bailey DW, Gross JE, Laca EA, et al (1996) Mechanisms That Result in Large Herbivore Grazing  
3441 Distribution Patterns. *J Range Manag* 49:386. <https://doi.org/10.2307/4002919>
- 3442 Bailey DW, Jensen D (2008) Method of Supplementation May Affect Cattle Grazing Patterns. *Rangel  
3443 Ecol Manag* 61:131–135. <https://doi.org/10.2111/06-167.1>
- 3444 Bailey DW, Mosley JC, Estell RE, et al (2019) Synthesis Paper: Targeted Livestock Grazing:  
3445 Prescription for Healthy Rangelands. *Rangel Ecol Manag* 72:865–877.  
3446 <https://doi.org/10.1016/j.rama.2019.06.003>
- 3447 Bailey DW, Welling GR (2007) Evaluation of low-moisture blocks and conventional dry mixes for  
3448 supplementing minerals and modifying cattle grazing patterns. *Rangel Ecol Manag* 60:54–64.  
3449 <https://doi.org/10.2111/05-138R1.1>
- 3450 Bailey DW, Welling GR (1999) Modification of Cattle Grazing Distribution with Dehydrated  
3451 Molasses Supplement. *J Range Manag* 52:575. <https://doi.org/10.2307/4003626>
- 3452 Bailey DW, Welling GR, Miller ET (2001) Cattle use of foothills rangeland near dehydrated molasses  
3453 supplement. *J Range Manag* 54:338–347. <https://doi.org/10.2307/4003101>
- 3454 Bakker ES, Olf H, Boekhoff M, et al (2004) Impact of herbivores on nitrogen cycling: Contrasting  
3455 effects of small and large species. *Oecologia* 138:91–101. <https://doi.org/10.1007/s00442-003-1402-5>  
3456
- 3457 Barbaro L, Dutoit T, Cozic P (2001) A six-year experimental restoration of biodiversity by shrub-  
3458 clearing and grazing in calcareous grasslands of the French Prealps. *Biodiveristy Conserv*  
3459 10:119–135
- 3460 Bartolomé J, Plaixats J, Piedrafita J, et al (2011) Foraging Behavior of Alberes Cattle in a  
3461 Mediterranean Forest Ecosystem. *Rangel Ecol Manag* 64:319–324.  
3462 <https://doi.org/10.2111/REM-D-09-00160.1>
- 3463 Battaglini L, Bovolenta S, Gusmeroli F, et al (2014) Environmental sustainability of Alpine livestock  
3464 farms. *Ital J Anim Sci* 13:431–443. <https://doi.org/10.4081/ijas.2014.3155>
- 3465 Bernal M, Llorens L, Julkunen-Tiitto R, et al (2013) Altitudinal and seasonal changes of phenolic  
3466 compounds in *Buxus sempervirens* leaves and cuticles. *Plant Physiol Biochem* 70:471–482.  
3467 <https://doi.org/10.1016/j.plaphy.2013.06.012>
- 3468 Berry NR, Jewell PL, Sutter F, et al (2002) Selection, intake and excretion of nutrients by Scottish  
3469 Highland suckler beef cows and calves, and Brown Swiss dairy cows in contrasting Alpine  
3470 grazing systems. *J Agric Sci* 139:437–453. <https://doi.org/10.1017/S002185960200271X>
- 3471 Berry NR, Jewell PL, Sutter F, et al (2001) Effect of concentrate on nitrogen turnover and excretion of  
3472 P, K, Na, Ca and Mg in lactating cows rotationally grazed at high altitude. *Livest Prod Sci*  
3473 71:261–275. [https://doi.org/10.1016/S0301-6226\(01\)00183-X](https://doi.org/10.1016/S0301-6226(01)00183-X)

- 3474 Besharati M, Taghizadeh A (2011) Effect of Tannin-Binding Agents (Polyethylene Glycol and  
3475 Polyvinylpyrrolidone) Supplementation on In Vitro Gas Production Kinetics of Some Grape  
3476 Yield Byproducts. *ISRN Vet Sci* 2011:1–8. <https://doi.org/10.5402/2011/780540>
- 3477 Binkley D, Cromack jr. K, Baker DD (1994) Nitrogen Fixation by Red Alder: Biology, Rates, and  
3478 Controls. *Biol Manag Red Alder* 57–72
- 3479 Bjelke U, Boberg J, Oliva J, et al (2016) Dieback of riparian alder caused by the *Phytophthora alni*  
3480 complex: Projected consequences for stream ecosystems. *Freshw Biol* 61:565–579.  
3481 <https://doi.org/10.1111/fwb.12729>
- 3482 Boege K, Marquis RJ (2005) Facing herbivory as you grow up: the ontogeny of resistance in plants.  
3483 *Trends Ecol Evol* 20:441–448. <https://doi.org/10.1016/j.tree.2005.05.001>
- 3484 Boscutti F, Poldini L, Buccheri M (2014) Green alder communities in the Alps: Phytosociological  
3485 variability and ecological features. *Plant Biosyst* 148:917–934.  
3486 <https://doi.org/10.1080/11263504.2013.809813>
- 3487 Brasier CM, Kirk SA, Delcan J, et al (2004) *Phytophthora alni* sp. nov. and its variants: Designation of  
3488 emerging heteroploid hybrid pathogens spreading on *Alnus* trees. *Mycol Res* 108:1172–1184.  
3489 <https://doi.org/10.1017/S0953756204001005>
- 3490 Bregant C, Sanna GP, Bottos A, et al (2020) Diversity and pathogenicity of *Phytophthora* species  
3491 associated with declining alder trees in Italy and description of *Phytophthora alpina* sp. nov.  
3492 *Forests* 11:1–16. <https://doi.org/10.3390/F11080848>
- 3493 Browne EM, Juniper DT, Bryant MJ, Beever DE (2005) Apparent digestibility and nitrogen utilisation  
3494 of diets based on maize and grass silage fed to beef steers. *Anim Feed Sci Technol* 119:55–68.  
3495 <https://doi.org/10.1016/j.anifeedsci.2004.12.001>
- 3496 Brüchert F, Gallenmüller F, Bogenrieder A, Speck T (2003) Stem mechanics, functional anatomy and  
3497 ecology of *Alnus viridis* and *Alnus glutinosa*. *Feddes Repert* 114:181–197.  
3498 <https://doi.org/10.1002/fedr.200390021>
- 3499 Bühlmann T, Caprez R, Hiltbrunner E, et al (2017) Nitrogen fixation by *Alnus* species boosts soil  
3500 nitrous oxide emissions. *Eur J Soil Sci* 68:740–748. <https://doi.org/10.1111/ejss.12457>
- 3501 Bühlmann T, Hiltbrunner E, Körner C (2014) *Alnus viridis* expansion contributes to excess reactive  
3502 nitrogen release, reduces biodiversity and constrains forest succession in the Alps. *Alp Bot*  
3503 124:187–191. <https://doi.org/10.1007/s00035-014-0134-y>
- 3504 Bühlmann T, Körner C, Hiltbrunner E (2016) Shrub Expansion of *Alnus viridis* Drives Former  
3505 Montane Grassland into Nitrogen Saturation. *Ecosystems* 19:968–985.  
3506 <https://doi.org/10.1007/s10021-016-9979-9>
- 3507 Buijs AE, Pedroli B, Luginbühl Y (2006) From Hiking Through Farmland to Farming in a Leisure  
3508 Landscape: Changing Social Perceptions of the European Landscape. *Landsc Ecol* 21:375–389.  
3509 <https://doi.org/10.1007/s10980-005-5223-2>
- 3510 Bumb I, Garnier E, Coq S, et al (2018) Traits determining the digestibility- decomposability  
3511 relationships in species from Mediterranean rangelands. *Ann Bot* 121:459–469.  
3512 <https://doi.org/10.1093/aob/mcx175>
- 3513 Burggraaf VT, Snow VO (2010) Effects of condensed tannins on nitrogen dynamics in grazed  
3514 temperate agricultural systems. In: Petridis GK (ed) *Tannins: Types, Foods Containing and*  
3515 *Nutrition*. Nova Science Publishers, Inc: New York, pp 317–326
- 3516 Buttler A, Kohler F, Gillet F (2008) The Swiss Mountain Wooded Pastures: Patterns and Processes.  
3517 *Agrofor Eur* 377–396. [https://doi.org/10.1007/978-1-4020-8272-6\\_19](https://doi.org/10.1007/978-1-4020-8272-6_19)
- 3518 Camacho O, Dobremez L, Capillon A (2008) Shrub encroachment in pastures in the Alps. *Rev*

- 3519 géographie Alp 89–100. <https://doi.org/10.4000/rga.575>
- 3520 Camerano P, Gottero F, Terzuolo PG, Varese P (2004) Tipi forestali del Piemonte. Metodologia e  
3521 guida per l'identificazione. 8:204
- 3522 Campbell DLM, Ouzman J, Mowat D, et al (2020) Virtual Fencing Technology Excludes Beef Cattle  
3523 from an Environmentally Sensitive Area. *Animals* 10:1069. <https://doi.org/10.3390/ani10061069>
- 3524 Carnevalli RA, De Mello ACT, Shozo L, et al (2019) Spatial distribution of dairy heifers' dung in  
3525 silvopastoral systems. *Cienc Rural* 49:. <https://doi.org/10.1590/0103-8478cr20180796>
- 3526 Casasús I, Bernués A, Sanz A, et al (2007) Vegetation dynamics in Mediterranean forest pastures as  
3527 affected by beef cattle grazing. *Agric Ecosyst Environ* 121:365–370.  
3528 <https://doi.org/10.1016/j.agee.2006.11.012>
- 3529 Cavallero A, Aceto P, Gorlier A, et al (2007) I tipi pastorali delle Alpi piemontesi [Pasture types of the  
3530 Piedmont Alps]. Bologna, IT
- 3531 Caviezel C, Hunziker M, Schaffner M, Kuhn NJ (2014) Soil-vegetation interaction on slopes with  
3532 bush encroachment in the central Alps - adapting slope stability measurements to shifting process  
3533 domains. *Earth Surf Process Landforms* 39:509–521. <https://doi.org/10.1002/esp.3513>
- 3534 Caviezel, Hunziker M, Kuhn NJ (2017) Green alder encroachment in the European Alps: The need for  
3535 analyzing the spread of a native-invasive species across spatial data. *CATENA* 159:149–158.  
3536 <https://doi.org/10.1016/j.catena.2017.08.006>
- 3537 Chang C-R, Lee P-F, Bai M-L, Lin T-T (2004) Predicting the geographical distribution of plant  
3538 communities in complex terrain - a case study in Fushian Experimental Forest, northeastern  
3539 Taiwan. *Ecography (Cop)* 27:577–588. <https://doi.org/10.1111/j.0906-7590.2004.03852.x>
- 3540 Chapin FS, Kedrowski RA (1983) Seasonal Changes in Nitrogen and Phosphorus Fractions and  
3541 Autumn Retranslocation in Evergreen and Deciduous Taiga Trees. *Ecology* 64:376–391
- 3542 Chapin FS, Sturm M, Serreze MC, et al (2005) Role of land-surface changes in arctic summer  
3543 warming. *Science (80- )* 310:657–660. <https://doi.org/10.1126/science.1117368>
- 3544 Chen WQ, Wang XY, Zhang YJ, Huang D (2015) Effects of the vertical and horizontal availability of  
3545 food resources: the diet selection of sheep grazing on natural grassland. *J Agric Sci* 153:322–334.  
3546 <https://doi.org/10.1017/S0021859614000355>
- 3547 Cherney DJR, Cherney JH, Lucey RF (1993) In Vitro Digestion Kinetics and Quality of Perennial  
3548 Grasses as Influenced by Forage Maturity. *J Dairy Sci* 76:790–797.  
3549 [https://doi.org/10.3168/jds.S0022-0302\(93\)77402-0](https://doi.org/10.3168/jds.S0022-0302(93)77402-0)
- 3550 Cislighi A, Giupponi L, Tamburini A, et al (2019) The effects of mountain grazing abandonment on  
3551 plant community, forage value and soil properties: observations and field measurements in an  
3552 alpine area. *Catena* 181:. <https://doi.org/10.1016/j.catena.2019.104086>
- 3553 Collins M, Nelson CJ, Moore KJ, Barnes RF (2017) Forages, Volume 1: An Introduction to Grassland  
3554 Agriculture. Wiley
- 3555 Cornelissen JHC, Lavorel S, Garnier E, et al (2003) A handbook of protocols for standardised and  
3556 easy measurement of plant functional traits worldwide. *Aust J Bot* 51:335.  
3557 <https://doi.org/10.1071/BT02124>
- 3558 Costa G, Mosimann E, Zagni C (1990) Effet de la présence nocturne au pâturage sur les performances  
3559 d'un troupeau laitier et sur ses restitutions à l'alpage. *Fourrages* 123:305–311
- 3560 Cosyns E, Claerbout S, Lamoot I, Hoffmann M (2005) Endozoochorous seed dispersal by cattle and  
3561 horse in a spatially heterogeneous landscape. *Plant Ecol* 178:149–162.  
3562 <https://doi.org/10.1007/s11258-004-2846-3>

- 3563 Cromsigt JPGM, Kemp YJM, Rodriguez E, Kivit H (2018) Rewilding Europe's large grazer  
3564 community: how functionally diverse are the diets of European bison, cattle, and horses? *Restor*  
3565 *Ecol* 26:891–899. <https://doi.org/10.1111/rec.12661>
- 3566 Daccord R, Arrigo Y (2001) Teneurs en constituants pariétaux. In: Agroscope (ed) *Revue suisse*  
3567 *d'agriculture* vol.33 N°2. pp 73–80
- 3568 Daget P, Poissonet J (1971) Une méthode d'analyse phytologique des prairies. *Ann Agron* 22:5–41
- 3569 Dakskobler I, Rozman A, Seliškar A (2013) Forest and scrub communities with green alder (*Alnus*  
3570 *viridis*) in Slovenia. *Hacquetia* 12:95–185. <https://doi.org/10.2478/HACQ-2013-0012>
- 3571 Dash S, Chakravarty AK, Singh A, et al (2016) Effect of heat stress on reproductive performances of  
3572 dairy cattle and buffaloes: A review. *Vet World* 9:235–244.  
3573 <https://doi.org/10.14202/vetworld.2016.235-244>
- 3574 Dawson JD, Funk DT (1981) Seasonal change in foliar nitrogen concentration of *Alnus glutinosa*. *For*  
3575 *Sci* 27:239–243. <https://doi.org/https://doi.org/10.1093/forestscience/27.2.239>
- 3576 Dawson JO (2008) Ecology Of Actinorhizal Plants. In: Pawlowski K, Newton WE (eds) *Nitrogen-*  
3577 *fixing Actinorhizal Symbioses*. Springer Netherlands, Dordrecht, pp 199–234
- 3578 De Kort H, Vandepitte K, Bruun HH, et al (2014) Landscape genomics and a common garden trial  
3579 reveal adaptive differentiation to temperature across Europe in the tree species *Alnus glutinosa*.  
3580 *Mol Ecol* 23:4709–4721. <https://doi.org/10.1111/mec.12813>
- 3581 Della Marianna G, Gusmeroli F, Pierik ME, et al (2012) Esperienze Di Pascolo Con Bovini Di Razza  
3582 Highland Scozzese Nella Montagna Alpina: Etologia E Prelievi Alimentari. In: *Quaderno*  
3583 *SOZOOALP: Sistemi Agro-Zootecnici Biologici Ed Eco-Compatibili In Ambiente Montano*. pp  
3584 93–106
- 3585 Díaz S, Lavorel S, McIntyre S, et al (2007) Plant trait responses to grazing - A global synthesis. *Glob*  
3586 *Chang Biol* 13:313–341. <https://doi.org/10.1111/j.1365-2486.2006.01288.x>
- 3587 El Benni N, Finger R, Mann S, Lehmann B (2012) The distributional effects of agricultural policy  
3588 reforms in Switzerland. *Agric Econ (Czech Republic)* 58:497–509.  
3589 <https://doi.org/10.17221/215/2011-agricecon>
- 3590 Eldridge DJ, Bowker MA, Maestre FT, et al (2011) Impacts of shrub encroachment on ecosystem  
3591 structure and functioning: Towards a global synthesis. *Ecol Lett* 14:709–722.  
3592 <https://doi.org/10.1111/j.1461-0248.2011.01630.x>
- 3593 Elias D, Hölzel N, Tischew S (2018) Goat paddock grazing improves the conservation status of shrub-  
3594 encroached dry grasslands. *Tuexenia* 38:1–19. <https://doi.org/10.14471/2018.38.017>
- 3595 Elias D, Tischew S (2016) Goat pasturing—A biological solution to counteract shrub encroachment on  
3596 abandoned dry grasslands in Central Europe? *Agric Ecosyst Environ* 234:98–106.  
3597 <https://doi.org/10.1016/j.agee.2016.02.023>
- 3598 Espunyes J, Lurgi M, Büntgen U, et al (2019) Different effects of alpine woody plant expansion on  
3599 domestic and wild ungulates. *Glob Chang Biol* 25:1808–1819. <https://doi.org/10.1111/gcb.14587>
- 3600 Estel S, Kuemmerle T, Alcántara C, et al (2015) Mapping farmland abandonment and recultivation  
3601 across Europe using MODIS NDVI time series. *Remote Sens Environ* 163:312–325.  
3602 <https://doi.org/10.1016/j.rse.2015.03.028>
- 3603 Estermann BL, Wettstein HR, Sutter F, et al (2003) Effect of calving period on herbage intake and  
3604 nutrient turnover of Simmental and Angus suckler cows with Angus sired calves grazing  
3605 subalpine and alpine pastures. *Livest Prod Sci* 79:169–182. [https://doi.org/10.1016/S0301-](https://doi.org/10.1016/S0301-6226(02)00152-5)  
3606 [6226\(02\)00152-5](https://doi.org/10.1016/S0301-6226(02)00152-5)

- 3607 Estermann BL, Wettstein HR, Sutter F, Kreuzer M (2001) Nutrient and energy conversion of grass-fed  
 3608 dairy and suckler beef cattle kept indoors and on high altitude pasture. *Anim Res* 50:477–493.  
 3609 <https://doi.org/10.1051/animres:2001109>
- 3610 Ewald KC (2001) The neglect of aesthetics in landscape planning in Switzerland. 54:.  
 3611 [https://doi.org/https://doi.org/10.1016/S0169-2046\(01\)00140-2](https://doi.org/https://doi.org/10.1016/S0169-2046(01)00140-2)
- 3612 Faccioni G, Sturaro E, Ramanzin M, Bernués A (2019) Socio-economic valuation of abandonment and  
 3613 intensification of Alpine agroecosystems and associated ecosystem services. *Land use policy*  
 3614 81:453–462. <https://doi.org/10.1016/j.landusepol.2018.10.044>
- 3615 Farmer RE, Maley ML, Stoehr MU, Schnekenburger F (1985) Reproductive characteristics of green  
 3616 alder in northwestern Ontario. *Can J Bot* 63:2243–2247. <https://doi.org/10.1139/b85-318>
- 3617 Fraser MD, Theobald VJ, Griffiths JB, et al (2009) Comparative diet selection by cattle and sheep  
 3618 grazing two contrasting heathland communities. *Agric Ecosyst Environ* 129:182–192.  
 3619 <https://doi.org/10.1016/j.agee.2008.08.013>
- 3620 Galloway JN, Aber JD, Erisman JW, et al (2003) The nitrogen cascade. *Bioscience* 53:341–356.  
 3621 [https://doi.org/10.1641/0006-3568\(2003\)053\[0341:TNC\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2003)053[0341:TNC]2.0.CO;2)
- 3622 Giger-Reverdin S, Domange C, Broudiscou LP, et al (2020) Rumen function in goats, an example of  
 3623 adaptive capacity. *J Dairy Res* 87:45–51. <https://doi.org/10.1017/S0022029920000060>
- 3624 González-Hernández MP, Starkey EE, Karchesy J (2000) Seasonal variation in concentrations of fiber,  
 3625 crude protein, and phenolic compounds in leaves of red alder (*Alnus rubra*): Nutritional  
 3626 implications for cervids. *J Chem Ecol* 26:293–301. <https://doi.org/10.1023/A:1005462100010>
- 3627 Gowda JH, Palo RT, Udén P (2019) Seasonal variation in the nutritional value of woody plants along  
 3628 a natural gradient in Eastern Africa. *Afr J Ecol* 57:226–237. <https://doi.org/10.1111/aje.12583>
- 3629 Grigorieva EA, Matzarakis A, de Freitas CR (2010) Analysis of growing degree-days as a climate  
 3630 impact indicator in a region with extreme annual air temperature amplitude. *Clim Res* 42:143–  
 3631 154. <https://doi.org/10.3354/cr00888>
- 3632 Gusmeroli F, Della Marianna G, Puccio C, et al (2007) Indici foraggeri di specie legnose ed erbacee  
 3633 alpine per il bestiame caprino. *SOZOOALP* 4:73–82
- 3634 Han W, Tang L, Chen Y, Fang J (2013) Relationship between the relative limitation and resorption  
 3635 efficiency of nitrogen vs phosphorus in woody plants. *PLoS One* 8:1–11.  
 3636 <https://doi.org/10.1371/journal.pone.0083366>
- 3637 Happe PJ, Jenkins KJ, Starkey EE, Sharrow SH (1990) Nutritional Quality and Tannin Astringency of  
 3638 Browse in Clear-Cuts and Old-Growth Forests. *J Wildl Manage* 54:557.  
 3639 <https://doi.org/10.2307/3809349>
- 3640 Harrington JA, Kathol E (2009) Responses of shrub midstory and herbaceous layers to managed  
 3641 grazing and fire in a North American Savanna (Oak Woodland) and prairie landscape. *Restor*  
 3642 *Ecol* 17:234–244. <https://doi.org/10.1111/j.1526-100X.2008.00369.x>
- 3643 Harris SL, Clark DA, Laboyrie PJ (1998) Birdsfoot trefoil - an alternative legume for New Zealand  
 3644 dairy pastures. *Proc New Zeal Grassl Assoc* 99–103. <https://doi.org/10.33584/jnzg.1998.60.2314>
- 3645 Hautier Y, Niklaus PA, Hector A (2009) Competition for Light Causes Plant Biodiversity Loss After  
 3646 Eutrophication. *Science* (80- ) 324:636–638. <https://doi.org/10.1126/science.1169640>
- 3647 Haynes RJ, Williams PH (1993) Nutrient Cycling and Soil Fertility in the Grazed Pasture Ecosystem.  
 3648 *Adv Agron* 49:119–199. [https://doi.org/10.1016/S0065-2113\(08\)60794-4](https://doi.org/10.1016/S0065-2113(08)60794-4)
- 3649 Hedtcke J, Posner J, Rosemeyer M, Albrecht K (2009) Browsing for conservation: Springtime forage  
 3650 value of midstory shrubs of degraded oak savannas in southern Wisconsin. *Renew Agric Food*

- 3651 Syst 24:293–299. <https://doi.org/10.1017/S1742170509990160>
- 3652 Hejman M, Hejmanová P, Pavlů V, Thorhallsdottir AG (2016) Forage quality of leaf fodder from  
3653 the main woody species in Iceland and its potential use for livestock in the past and present.  
3654 Grass Forage Sci 71:649–658. <https://doi.org/10.1111/gfs.12224>
- 3655 Hejmanová P, Stejskalová M, Hejman M (2014) Forage quality of leaf-fodder from the main broad-  
3656 leaved woody species and its possible consequences for the Holocene development of forest  
3657 vegetation in Central Europe. Veg Hist Archaeobot 23:607–613. <https://doi.org/10.1007/s00334-013-0414-2>
- 3659 Hiltbrunner E, Aerts R, Bühlmann T, et al (2014) Ecological consequences of the expansion of N<sub>2</sub>-  
3660 fixing plants in cold biomes. Oecologia 176:11–24. <https://doi.org/10.1007/s00442-014-2991-x>
- 3661 Hofmann RR (1989) Evolutionary steps of ecophysiological adaptation and diversification of  
3662 ruminants: a comparative view of their digestive system. Oecologia 78:443–457.  
3663 <https://doi.org/10.1007/BF00378733>
- 3664 Homburger H, Lüscher A, Scherer-Lorenzen M, Schneider MK (2015) Patterns of livestock activity  
3665 on heterogeneous subalpine pastures reveal distinct responses to spatial autocorrelation,  
3666 environment and management. Mov Ecol 3:. <https://doi.org/10.1186/s40462-015-0053-6>
- 3667 Homburger H, Schneider MK, Hilfiker S, Lüscher A (2014) Inferring Behavioral States of Grazing  
3668 Livestock from High-Frequency Position Data Alone. PLoS One 9:e114522.  
3669 <https://doi.org/10.1371/journal.pone.0114522>
- 3670 Houle G (2001) Reproductive costs are associated with both the male and female functions in *Alnus*  
3671 *viridis* ssp. *crispa*. Ecoscience 8:220–229. <https://doi.org/10.1080/11956860.2001.11682648>
- 3672 Howell C (2008) Consolidated list of environmental weeds in New Zealand. DOC Res Dev Ser 292:42
- 3673 Hunziker M, Caviezel C, Kuhn NJ (2017) Shrub encroachment by green alder on subalpine pastures:  
3674 Changes in mineral soil organic carbon characteristics. CATENA 157:35–46.  
3675 <https://doi.org/10.1016/j.catena.2017.05.005>
- 3676 Huss-Daniel K (1997) Tansley Review No. 93.. Actinorrhizal symbioses and their N<sub>2</sub> fixation. New  
3677 Phytol 136:375–405. <https://doi.org/10.1046/j.1469-8137.1997.00755.x>
- 3678 Iussig G, Lonati M, Probo M, et al (2015a) Plant species selection by goats foraging on montane semi-  
3679 natural grasslands and grazable forestlands in the Italian Alps. Ital J Anim Sci 14:484–494.  
3680 <https://doi.org/10.4081/ijas.2015.3907>
- 3681 Iussig G, Renna M, Gorlier A, et al (2015b) Browsing ratio, species intake, and milk fatty acid  
3682 composition of goats foraging on alpine open grassland and grazable forestland. Small Rumin  
3683 Res 132:12–24. <https://doi.org/10.1016/j.smallrumres.2015.09.013>
- 3684 James K, Bradshaw K (2020) Detecting plant species in the field with deep learning and drone  
3685 technology. Methods Ecol Evol 11:1509–1519. <https://doi.org/10.1111/2041-210X.13473>
- 3686 James TDW, Smith DW (1978) Seasonal changes in the major ash constituents of leaves and some  
3687 woody components of trembling aspen and red osier dogwood. Can J Bot 56:1798–1803.  
3688 <https://doi.org/10.1139/b78-215>
- 3689 Jarvis SC (1994) The pollution potential and flows of nitrogen to waters and the atmosphere from  
3690 grassland under grazing. In: Ap Dewi, I., Axford, R. F. E., Marai, I. F. M. and Omed H (ed)  
3691 Pollution in Livestock Production Systems. CABI International, Wallingford, Oxon (CABI), pp  
3692 227–239
- 3693 Jayanegara A, Wina E, Soliva CR, et al (2011) Dependence of forage quality and methanogenic  
3694 potential of tropical plants on their phenolic fractions as determined by principal component  
3695 analysis. Anim Feed Sci Technol 163:231–243. <https://doi.org/10.1016/j.anifeedsci.2010.11.009>

- 3696 Jewell PL, Käuferle D, Güsewell S, et al (2007) Redistribution of phosphorus by cattle on a traditional  
 3697 mountain pasture in the Alps. *Agric Ecosyst Environ* 122:377–386.  
 3698 <https://doi.org/10.1016/j.agee.2007.02.012>
- 3699 Kebreab E, France J, Beever DE, Castillo AR (2001) Nitrogen pollution by dairy cows and its  
 3700 mitigation by dietary manipulation. *Nutr Cycl Agroecosystems* 60:275–285.  
 3701 <https://doi.org/10.1023/A:1012668109662>
- 3702 Koch B, Edwards PJ, Blanckenhorn WU, et al (2015) Shrub encroachment affects the diversity of  
 3703 plants, butterflies, and grasshoppers on two Swiss subalpine pastures. *Arctic, Antarct Alp Res*  
 3704 47:345–357. <https://doi.org/10.1657/AAAR0013-093>
- 3705 Koch B, Homburger H, Edwards PJ, Schneider MK (2018) Phosphorus redistribution by dairy cattle  
 3706 on a heterogeneous subalpine pasture, quantified using GPS tracking. *Agric Ecosyst Environ*  
 3707 257:183–192. <https://doi.org/10.1016/j.agee.2017.10.002>
- 3708 Koenig KM, Beauchemin KA (2013a) Nitrogen metabolism and route of excretion in beef feedlot  
 3709 cattle fed barley-based backgrounding diets varying in protein concentration and rumen  
 3710 degradability<sup>1,2</sup>. *J Anim Sci* 91:2295–2309. <https://doi.org/10.2527/jas.2012-5652>
- 3711 Koenig KM, Beauchemin KA (2013b) Nitrogen metabolism and route of excretion in beef feedlot  
 3712 cattle fed barley-based finishing diets varying in protein concentration and rumen degradability. *J*  
 3713 *Anim Sci* 91:2310–2320. <https://doi.org/10.2527/jas.2012-5653>
- 3714 Kohler F, Gillet F, Gobat J -M., Buttler A (2004) Seasonal vegetation changes in mountain pastures  
 3715 due to simulated effects of cattle grazing. *J Veg Sci* 15:143–150. <https://doi.org/10.1111/j.1654-1103.2004.tb02249.x>
- 3717 Kohler F, Gillet F, Reust S, et al (2006) Spatial and seasonal patterns of cattle habitat use in a  
 3718 mountain wooded pasture. *Landsc Ecol* 21:281–295. <https://doi.org/10.1007/s10980-005-0144-7>
- 3719 Köhler M, Elias D, Hiller G, et al (2020) Restoration of orchid-rich dry calcareous grasslands by  
 3720 rotational goat pasturing. *Tuexenia* 40:201–223. <https://doi.org/10.14471/2020.40.009>
- 3721 Körner C (2012) *Alpine Treelines. Functional Ecology of the Global High Elevation Tree Limits*,  
 3722 Springer B
- 3723 Körner C (2003) *Alpine Plant Life: functional plant ecology of high mountain ecosystems*. Springer  
 3724 Berlin Heidelberg, Berlin, Heidelberg
- 3725 Kowarik I, Säumel I (2007) Biological flora of Central Europe: *Ailanthus altissima* (Mill.) Swingle.  
 3726 *Perspect Plant Ecol Evol Syst* 8:207–237. <https://doi.org/10.1016/j.ppees.2007.03.002>
- 3727 Kumar R, Vaithyanathan S (1990) Occurrence, nutritional significance and effect on animal  
 3728 productivity of tannins in tree leaves. *Anim Feed Sci Technol* 30:21–38.  
 3729 [https://doi.org/10.1016/0377-8401\(90\)90049-E](https://doi.org/10.1016/0377-8401(90)90049-E)
- 3730 Laiolo P, Dondero F, Ciliento E, Rolando A (2004) Consequences of pastoral abandonment for the  
 3731 structure and diversity of the alpine avifauna. *J Appl Ecol* 41:294–304.  
 3732 <https://doi.org/10.1111/j.0021-8901.2004.00893.x>
- 3733 Lamoot I, Meert C, Hoffmann M (2005) Habitat use of ponies and cattle foraging together in a coastal  
 3734 dune area. *Biol Conserv* 122:523–536. <https://doi.org/10.1016/j.biocon.2004.09.009>
- 3735 Lançon J (1978) Les restitutions du bétail au pâturage et leurs effets (1ère partie). *Fourrages* 75:55–88
- 3736 Landolt E, Bäumler B, Ehrhardt A, et al (2010) *Flora Indicativa – Ökologische Zeigerwerte und*  
 3737 *biologische Kennzeichen zur Flora der Schweiz und der Alpen*
- 3738 Lantinga EA, Keuning JA, Groenwold J, Deenen PJAG (1987) Distribution of excreted nitrogen by  
 3739 grazing cattle and its effects on sward quality, herbage production and utilization. In: Van Der

- 3740 Meer HG, Unwin RJ, Van Dijk TA, Ennik GC (eds) *Animal Manure on Grassland and Fodder*  
3741 *Crops. Fertilizer or Waste?* Springer, Dordrecht
- 3742 Lantz TC, Gergel SE, Henry GHR (2010) Response of green alder (*Alnus viridis* subsp. *fruticosa*)  
3743 patch dynamics and plant community composition to fire and regional temperature in north-  
3744 western Canada. *J Biogeogr* 37:1597–1610. <https://doi.org/10.1111/j.1365-2699.2010.02317.x>
- 3745 Leng RA (1997) *Tree foliage in ruminant nutrition*. FAO Anim Prod Heal Pap
- 3746 Lindemann-Matthies P, Briegel R, Schüpbach B, Junge X (2010) Aesthetic preference for a Swiss  
3747 alpine landscape: The impact of different agricultural land-use with different biodiversity.  
3748 *Landsc Urban Plan* 98:99–109. <https://doi.org/10.1016/j.landurbplan.2010.07.015>
- 3749 Locatelli B, Lavorel S, Sloan S, et al (2017) Characteristic trajectories of ecosystem services in  
3750 mountains. *Front Ecol Environ* 15:150–159. <https://doi.org/10.1002/fee.1470>
- 3751 Lockyer DR, Whitehead DC (1990) Volatilization of ammonia from cattle urine applied to grassland.  
3752 *Soil Biol Biochem* 22:1137–1142. [https://doi.org/10.1016/0038-0717\(90\)90040-7](https://doi.org/10.1016/0038-0717(90)90040-7)
- 3753 Longhini VZ, Cardoso A da S, Berça AS, et al (2020) Nitrogen supply and rainfall affect ammonia  
3754 emissions from dairy cattle excreta and urea applied on warm-climate pastures. *J Environ Qual*  
3755 49:1453–1466. <https://doi.org/10.1002/jeq2.20167>
- 3756 Lovreglio R, Meddour-Sahar O, Leone V (2014) Goat grazing as a wildfire prevention tool: a basic  
3757 review. *iForest - Biogeosciences For* 7:260–268. <https://doi.org/10.3832/ifer1112-007>
- 3758 Luske B, van Eekeren N (2017) Nutritional potential of fodder trees on clay and sandy soils. *Agrofor*  
3759 *Syst* 92:975–986. <https://doi.org/10.1007/s10457-017-0180-8>
- 3760 MacDonald D, Crabtree JR, Wiesinger G, et al (2000) Agricultural abandonment in mountain areas of  
3761 Europe: Environmental consequences and policy response. *J Environ Manage* 59:47–69.  
3762 <https://doi.org/10.1006/jema.1999.0335>
- 3763 Mack G, Walter T, Flury C (2013) Seasonal alpine grazing trends in Switzerland: Economic  
3764 importance and impact on biotic communities. *Environ Sci Policy* 32:48–57.  
3765 <https://doi.org/10.1016/j.envsci.2013.01.019>
- 3766 MacLusky DS (1960) Some Estimates of the Areas of Pasture Fouled By the Excreta of Dairy Cows.  
3767 *Grass Forage Sci* 15:181–188. <https://doi.org/10.1111/j.1365-2494.1960.tb00176.x>
- 3768 Magurran AE (1998) *Ecological Diversity and Its Measurement*. Princeton, NJ, USA: Princeton  
3769 University Press
- 3770 Mahieu S, Novak S, Barre P, et al (2021) Diversity in the chemical composition and digestibility of  
3771 leaves from fifty woody species in temperate areas. *Agrofor Syst* 95:1295–1308.  
3772 <https://doi.org/10.1007/s10457-021-00662-2>
- 3773 Maillard A, Diquélou S, Billard V, et al (2015) Leaf mineral nutrient remobilization during leaf  
3774 senescence and modulation by nutrient deficiency. *Front Plant Sci* 6:1–15.  
3775 <https://doi.org/10.3389/fpls.2015.00317>
- 3776 Makkar HPS (2003) *Quantification of Tannins in Tree and Shrub Foliage*. Springer Netherlands,  
3777 Dordrecht
- 3778 Mallik AU, Bell FW, Gong Y (1997) Regeneration behavior of competing plants after clear cutting:  
3779 Implications for vegetation management. *For Ecol Manage* 95:1–10.  
3780 [https://doi.org/10.1016/S0378-1127\(97\)00012-1](https://doi.org/10.1016/S0378-1127(97)00012-1)
- 3781 Mandaluniz N, Aldezabal A, Oregui LM (2011) Diet selection of beef cattle on Atlantic grassland-  
3782 heathland mosaic: Are heathers more preferred than expected? *Livest Sci* 138:49–55.  
3783 <https://doi.org/10.1016/j.livsci.2010.12.002>



- 3784 Manousidis T, Kyriazopoulos AP, Parissi ZM, et al (2016) Grazing behavior, forage selection and diet  
3785 composition of goats in a Mediterranean woody rangeland. *Small Rumin Res* 145:142–153.  
3786 <https://doi.org/10.1016/j.smallrumres.2016.11.007>
- 3787 Marques D, Fachada M, Viana H (2017) Synergies Between Goat Grazing and Shrub Biomass in  
3788 Mountain Areas. In: *Sustainable Goat Production in Adverse Environments: Volume I*. Springer  
3789 International Publishing, Cham, pp 155–175
- 3790 Marrs RH, Watt AS (2006) Biological Flora of the British Isles: *Pteridium aquilinum* (L.) Kuhn. *J*  
3791 *Ecol* 94:1272–1321. <https://doi.org/10.1111/j.1365-2745.2006.01177.x>
- 3792 Maurer K, Weyand A, Fischer M, Stöcklin J (2006) Old cultural traditions, in addition to land use and  
3793 topography, are shaping plant diversity of grasslands in the Alps. *Biol Conserv* 130:438–446.  
3794 <https://doi.org/10.1016/j.biocon.2006.01.005>
- 3795 McMaster G (1997) Growing degree-days: one equation, two interpretations. *Agric For Meteorol*  
3796 87:291–300. [https://doi.org/10.1016/S0168-1923\(97\)00027-0](https://doi.org/10.1016/S0168-1923(97)00027-0)
- 3797 Meisser M, Deléglise C, Freléchoux F, et al (2014) Foraging behaviour and occupation pattern of beef  
3798 cows on a heterogeneous pasture in the swiss alps. *Czech J Anim Sci* 59:84–95.  
3799 <https://doi.org/10.17221/7232-cjas>
- 3800 Menke HH, Steingass H (1988) Estimation of the energetic feed value obtained from chemical  
3801 analysis and in vitro gas production using rumen fluid. *Anim Res Dev* 28:7–55
- 3802 Minson D, Milford R (1967) The voluntary intake and digestibility of diets containing different  
3803 proportions of legume and mature Pangola grass (*Digitaria decumbens*). *Aust J Exp Agric* 7:546.  
3804 <https://doi.org/10.1071/EA9670546>
- 3805 Mitlacher K, Poschlod P, Rosén P, Bakker JP (2002) Restoration of wooded meadows - A  
3806 comparative analysis along a chronosequence on Öland (Sweden). *Appl Veg Sci* 5:63–73.  
3807 <https://doi.org/10.1111/j.1654-109X.2002.tb00536.x>
- 3808 Moore TR, Matthews HD, Simmons C, Leduc M (2015) Quantifying Changes in Extreme Weather  
3809 Events in Response to Warmer Global Temperature. *Atmosphere-Ocean* 53:412–425.  
3810 <https://doi.org/10.1080/07055900.2015.1077099>
- 3811 Mordenti AL, Giaretta E, Campidonico L, et al (2021) A review regarding the use of molasses in  
3812 animal nutrition. *Animals* 11:1–17. <https://doi.org/10.3390/ani11010115>
- 3813 Mouissie A (2004) Seed dispersal by large herbivores: Implications for the restoration of plant  
3814 biodiversity. s.n.
- 3815 Navale MR, Bhardwaj DR, Bishist R, et al (2022) Seasonal variations in the nutritive value of fifteen  
3816 multipurpose fodder tree species: A case study of north-western Himalayan mid-hills. *PLoS One*  
3817 17:. <https://doi.org/10.1371/journal.pone.0276689>
- 3818 Niedrist G, Tasser E, Lüth C, et al (2009) Plant diversity declines with recent land use changes in  
3819 European Alps. *Plant Ecol* 202:195–210. <https://doi.org/10.1007/s11258-008-9487-x>
- 3820 Niinemets Ü, Tamm Ü (2005) Species differences in timing of leaf fall and foliage chemistry modify  
3821 nutrient resorption efficiency in deciduous temperate forest stands. *Tree Physiol* 25:1001–1014.  
3822 <https://doi.org/10.1093/treephys/25.8.1001>
- 3823 Nota G, Berretti R, Ascoli D, et al (2023) Plant species selection and impact on tree resprouts by semi-  
3824 free ranging pigs in a temperate deciduous forest. *Agrofor Syst* 97:121–132.  
3825 <https://doi.org/10.1007/s10457-022-00792-1>
- 3826 Nota G, Pittarello M, Ravetto Enri S, et al (2022) Feeding preferences of Highland cattle reveal their  
3827 attitude to exploit woody vegetation in mountain environments. In: Spano D, Trabucco A,  
3828 Camilli F, et al. (eds) EURAF2022 - Agroforestry for the Green Deal transition. Research and

- 3829 innovation towards the sustainable development of agriculture and forestry. *Nuoro*, pp 343–458
- 3830 Olariu HG, Malambo L, Popescu SC, et al (2022) Woody Plant Encroachment: Evaluating  
3831 Methodologies for Semiarid Woody Species Classification from Drone Images. *Remote Sens*  
3832 14:. <https://doi.org/10.3390/rs14071665>
- 3833 Öllerer K, Varga A, Kirby K, et al (2019) Beyond the obvious impact of domestic livestock grazing on  
3834 temperate forest vegetation – A global review. *Biol Conserv* 237:209–219.  
3835 <https://doi.org/10.1016/j.biocon.2019.07.007>
- 3836 Orlandi S, Probo M, Sitzia T, et al (2016) Environmental and land use determinants of grassland patch  
3837 diversity in the western and eastern Alps under agro-pastoral abandonment. *Biodivers Conserv*  
3838 25:275–293. <https://doi.org/10.1007/s10531-016-1046-5>
- 3839 Pajares G (2015) Overview and Current Status of Remote Sensing Applications Based on Unmanned  
3840 Aerial Vehicles (UAVs). *Photogramm Eng Remote Sens* 81:281–330.  
3841 <https://doi.org/10.14358/PERS.81.4.281>
- 3842 Papachristou T., Platis P., Papanastasis V., Tsiouvaras C. (1999) Use of deciduous woody species as a  
3843 diet supplement for goats grazing Mediterranean shrublands during the dry season. *Anim Feed*  
3844 *Sci Technol* 80:267–279. [https://doi.org/10.1016/S0377-8401\(99\)00056-5](https://doi.org/10.1016/S0377-8401(99)00056-5)
- 3845 Papachristou TG, Papanastasis VP (1994) Forage value of Mediterranean deciduous woody fodder  
3846 species and its implication to management of silvo-pastoral systems for goats. *Agrofor Syst*  
3847 27:269–282. <https://doi.org/10.1007/BF00705061>
- 3848 Papanastasis VP, Yiakoulaki MD, Decandia M, Dini-Papanastasi O (2008) Integrating woody species  
3849 into livestock feeding in the Mediterranean areas of Europe. *Anim Feed Sci Technol* 140:1–17.  
3850 <https://doi.org/10.1016/j.anifeedsci.2007.03.012>
- 3851 Pardini A, Nori M (2011) Agro-silvo-pastoral systems in Italy: integration and diversification.  
3852 *Pastoralism* 1:1–10. <https://doi.org/10.1186/2041-7136-1-26>
- 3853 Patra AK, Saxena J (2010) A new perspective on the use of plant secondary metabolites to inhibit  
3854 methanogenesis in the rumen. *Phytochemistry* 71:1198–1222.  
3855 <https://doi.org/10.1016/j.phytochem.2010.05.010>
- 3856 Pauler CM, Isselstein J, Berard J, et al (2020a) Grazing Allometry: Anatomy, Movement, and  
3857 Foraging Behavior of Three Cattle Breeds of Different Productivity. *Front Vet Sci* 7:.  
3858 <https://doi.org/10.3389/fvets.2020.00494>
- 3859 Pauler CM, Isselstein J, Braunbeck T, Schneider MK (2019) Influence of Highland and production-  
3860 oriented cattle breeds on pasture vegetation: A pairwise assessment across broad environmental  
3861 gradients. *Agric Ecosyst Environ* 284:106585. <https://doi.org/10.1016/j.agee.2019.106585>
- 3862 Pauler CM, Isselstein J, Suter M, et al (2020b) Choosy grazers: Influence of plant traits on forage  
3863 selection by three cattle breeds. *Funct Ecol* 34:980–992. <https://doi.org/10.1111/1365-2435.13542>
- 3865 Pauler CM, Lüscher A, Kreuzer M, et al (2022a) Robust cattle, sheep and goats in green alder shrubs –  
3866 or how to preserve mountain pastures. In: Delaby L, Baumont R, Brocard V, et al. (eds)  
3867 *Grassland at the heart of circular and sustainable food systems - Proceedings of the 29th General*  
3868 *Meeting of the European Grassland Federation, Grassland Science in Europe 27 (series).*  
3869 *European Grassland Federation, Caen*, pp 247–249
- 3870 Pauler CM, Zehnder T, Staudinger M, et al (2022b) Thinning the thickets: Foraging of hardy cattle,  
3871 sheep and goats in green alder shrubs. *J Appl Ecol*. <https://doi.org/10.1111/1365-2664.14156>
- 3872 Pierce LL, Running SW, Walker J (1994) Regional-Scale Relationships of Leaf Area Index to Specific  
3873 Leaf Area and Leaf Nitrogen Content. *Ecol Appl* 4:313–321. <https://doi.org/10.2307/1941936>

- 3874 Pietola L, Horn R, Yli-Halla M (2005) Effects of trampling by cattle on the hydraulic and mechanical  
3875 properties of soil. *Soil Tillage Res* 82:99–108. <https://doi.org/10.1016/j.still.2004.08.004>
- 3876 Pignatti S (1982) *Flora d'Italia*, Volumes 1-3. Edagricole, Bologna, IT
- 3877 Piñeiro-Vázquez A, Canul-Solís J, Alayón-Gamboa J, et al (2015) Potential of condensed tannins for  
3878 the reduction of emissions of enteric methane and their effect on ruminant productivity. *Arch*  
3879 *Med Vet* 47:263–272. <https://doi.org/10.4067/S0301-732X2015000300002>
- 3880 Piñeiro-Vázquez AT, Canul-Solis JR, Alayón-Gamboa JA, et al (2017) Energy utilization, nitrogen  
3881 balance and microbial protein supply in cattle fed *Pennisetum purpureum* and condensed tannins.  
3882 *J Anim Physiol Anim Nutr (Berl)* 101:159–169. <https://doi.org/10.1111/jpn.12436>
- 3883 Pisetta M, Montecchio L, Longa CMO, et al (2012) Green alder decline in the Italian Alps. *For Ecol*  
3884 *Manage* 281:75–83. <https://doi.org/10.1016/j.foreco.2012.06.024>
- 3885 Pittarello M, Lonati M, Gorlier A, et al (2018) Plant diversity and pastoral value in alpine pastures are  
3886 maximized at different nutrient indicator values. *Ecol Indic* 85:518–524.  
3887 <https://doi.org/10.1016/j.ecolind.2017.10.064>
- 3888 Pittarello M, Nota G, Probo M, et al (2021) Can research benefit of real-time GPS collars conceived  
3889 for livestock management by farmers? In: 1st Joint Meeting of EAAP Mountain Livestock  
3890 Farming & FAO-CIHEAM Mountain Pastures “Mountains are agroecosystems for people.” Bled,  
3891 Slovenia
- 3892 Pittarello M, Probo M, Lonati M, et al (2016a) Effects of traditional salt placement and strategically  
3893 placed mineral mix supplements on cattle distribution in the Western Italian Alps. *Grass Forage*  
3894 *Sci* 71:529–539. <https://doi.org/10.1111/gfs.12196>
- 3895 Pittarello M, Probo M, Lonati M, Lombardi G (2016b) Restoration of sub-alpine shrub-encroached  
3896 grasslands through pastoral practices: effects on vegetation structure and botanical composition.  
3897 *Appl Veg Sci* 19:381–390. <https://doi.org/10.1111/avsc.12222>
- 3898 Pontes LDS, Soussana JF, Louault F, et al (2007) Leaf traits affect the above-ground productivity and  
3899 quality of pasture grasses. *Funct Ecol* 21:844–853. <https://doi.org/10.1111/j.1365-2435.2007.01316.x>
- 3901 Pornaro C, Schneider MK, Macolino S (2013) Plant species loss due to forest succession in Alpine  
3902 pastures depends on site conditions and observation scale. *Biol Conserv* 161:213–222.  
3903 <https://doi.org/10.1016/j.biocon.2013.02.019>
- 3904 Pregitzer KS, Zak DR, Burton AJ, et al (2004) Chronic nitrate additions dramatically increase the  
3905 export of carbon and nitrogen from northern hardwood ecosystems. *Biogeochemistry* 68:179–  
3906 197. <https://doi.org/10.1023/B: BIOG.0000025737.29546.fd>
- 3907 Probo M, Lonati M, Pittarello M, et al (2014) Implementation of a rotational grazing system with large  
3908 paddocks changes the distribution of grazing cattle in the south-western Italian Alps. *Rangel J*  
3909 36:445–458. <https://doi.org/10.1071/RJ14043>
- 3910 Probo M, Massolo A, Lonati M, et al (2013) Use of mineral mix supplements to modify the grazing  
3911 patterns by cattle for the restoration of sub-alpine and alpine shrub-encroached grasslands.  
3912 *Rangel J* 35:85–93. <https://doi.org/10.1071/RJ12108>
- 3913 Probo M, Pittarello M, Lonati M, Lombardi G (2016) Targeted grazing for the restoration of sub-  
3914 alpine shrub-encroached grasslands. *Ital J Agron* 11:268–272.  
3915 <https://doi.org/10.4081/ija.2016.775>
- 3916 Qin J, Shangguan Z, Xi W (2018) Seasonal variations of leaf traits and drought adaptation strategies of  
3917 four common woody species in South Texas, USA. *J For Res*. <https://doi.org/10.1007/s11676-018-0742-2>
- 3918

- 3919 Radkowski A, Radkowska I, Bocianowski J, Cyplik A (2022) Weight Gain of Highland Cattle  
3920 Depending on the Share of Perennial Ryegrass (*Lolium Perenne* L.) in the Meadow Sward. *Ann*  
3921 *Anim Sci* 22:385–395. <https://doi.org/10.2478/aoas-2021-0045>
- 3922 Ravetto Enri S, Probo M, Renna M, et al (2020) Temporal variations in leaf traits, chemical  
3923 composition and in vitro true digestibility of four temperate fodder tree species. *Anim Prod Sci*  
3924 60:643. <https://doi.org/10.1071/AN18771>
- 3925 Reid W V., Mooney HA, Cropper A, et al (2005) Millennium Ecosystem Assessment, Island Pre.  
3926 Washington, DC.
- 3927 Richard L (1967) L'aire de répartition de l'aulne vert. In: Documents pour la carte de la végétation des  
3928 Alpes, volume 5, Laboratoire. Grenoble, pp 81–113
- 3929 Rodríguez-Barrueco C, Miguel C, Subramaniam P (1984) Seasonal fluctuations of the mineral  
3930 concentration of alder (*Alnus glutinosa* (L.) Gaertn.) from the field. *Plant Soil* 78:201–208.  
3931 <https://doi.org/10.1007/BF02277851>
- 3932 Roggero PP, Bagella S, Farina R (2002) Un archivio dati di Indici specifici per la valutazione integrata  
3933 del valore pastorale. *Riv di Agron* 36:149–156
- 3934 Sales-Baptista E, Ferraz-de-Oliveira MI (2021) Grazing in silvopastoral systems: multiple solutions  
3935 for diversified benefits. *Agrofor Syst* 95:1–6. <https://doi.org/10.1007/s10457-020-00581-8>
- 3936 Sampò S, Bergero R, Buffa G, Luppi-Mosca AM (1997) Soil fungal communities in a young and an  
3937 old *Alnus viridis* coenosis. *Mycologia* 89:837–845.  
3938 <https://doi.org/10.1080/00275514.1997.12026854>
- 3939 Schellberg J, Möselers BM, Kühbauch W, Rademacher IF (1999) Long-term effects of fertilizer on soil  
3940 nutrient concentration, yield, forage quality and floristic composition of a hay meadow in the  
3941 Eifel mountains, Germany. *Grass Forage Sci* 54:195–207. <https://doi.org/10.1046/j.1365-2494.1999.00166.x>
- 3943 Schirpke U, Leitinger G, Tasser E, et al (2013) Multiple ecosystem services of a changing Alpine  
3944 landscape: Past, present and future. *Int J Biodivers Sci Ecosyst Serv Manag* 9:123–135.  
3945 <https://doi.org/10.1080/21513732.2012.751936>
- 3946 Schirpke U, Timmermann F, Tappeiner U, Tasser E (2016) Cultural ecosystem services of mountain  
3947 regions: Modelling the aesthetic value. *Ecol Indic* 69:78–90.  
3948 <https://doi.org/10.1016/j.ecolind.2016.04.001>
- 3949 Schlegel P (2021) Minéraux et vitamines. In: Apports Aliment. recommandés pour les ruminants  
3950 (Livre vert). [www.agroscope.ch/livre-vert](http://www.agroscope.ch/livre-vert). Accessed 25 May 2023
- 3951 Schlegel P, Kessler J (2001) Minéraux et vitamines. 1–19
- 3952 Schlegel P, Wyss U, Arrigo Y, Hess HD (2016) Mineral concentrations of fresh herbage from mixed  
3953 grassland as influenced by botanical composition, harvest time and growth stage. *Anim Feed Sci*  
3954 *Technol* 219:226–233. <https://doi.org/10.1016/j.anifeedsci.2016.06.022>
- 3955 Schnyder H, Locher F, Auerswald K (2010) Nutrient redistribution by grazing cattle drives patterns of  
3956 topsoil N and P stocks in a low-input pasture ecosystem. *Nutr Cycl Agroecosystems* 88:183–195.  
3957 <https://doi.org/10.1007/s10705-009-9334-z>
- 3958 Schumacher S, Bugmann H (2006) The relative importance of climatic effects, wildfires and  
3959 management for future forest landscape dynamics in the Swiss Alps. *Glob Chang Biol* 12:1435–  
3960 1450. <https://doi.org/10.1111/j.1365-2486.2006.01188.x>
- 3961 Schwob G, Roy M, Manzi S, et al (2017) Green alder (*Alnus viridis*) encroachment shapes microbial  
3962 communities in subalpine soils and impacts its bacterial or fungal symbionts differently. *Environ*  
3963 *Microbiol* 19:3235–3250. <https://doi.org/10.1111/1462-2920.13818>

- 3964 Seidavi A, Tavakoli M, Rasouli B, et al (2020) Application of some trees/shrubs in ruminant feeding:  
3965 a review. *Agrofor Syst* 94:1353–1364. <https://doi.org/10.1007/s10457-018-0313-8>
- 3966 Senft RL, Coughenour MB, Bailey DW, et al (1987) Large Herbivore Foraging and Ecological  
3967 Hierarchies. *Bioscience* 37:789–799. <https://doi.org/10.2307/1310545>
- 3968 Silanikove N (2000) The physiological basis of adaptation in goats to harsh environments. *Small*  
3969 *Rumin Res* 35:181–193. [https://doi.org/10.1016/S0921-4488\(99\)00096-6](https://doi.org/10.1016/S0921-4488(99)00096-6)
- 3970 Silva V, Catry FX, Fernandes PM, et al (2019) Effects of grazing on plant composition, conservation  
3971 status and ecosystem services of Natura 2000 shrub-grassland habitat types. *Biodivers Conserv*  
3972 28:1205–1224. <https://doi.org/10.1007/s10531-019-01718-7>
- 3973 Singh B, Bhatt BP, Prasad P (2010) Altitudinal variation in nutritive value of adult-juvenile foliage of  
3974 *Celtis australis* L. *J Am Sci* 6:108–112
- 3975 Skoczowski A, Odrzywolska-Hasiec M, Oliwa J, et al (2021) Ecophysiological variability of *alnus*  
3976 *viridis* (Chaix) dc. green alder leaves in the bieszczady mountains (poland). *Plants* 10:1–17.  
3977 <https://doi.org/10.3390/plants10010096>
- 3978 Slade EM, Riutta T, Roslin T, Tuomisto HL (2016) The role of dung beetles in reducing greenhouse  
3979 gas emissions from cattle farming. *Sci Rep* 6:1–9. <https://doi.org/10.1038/srep18140>
- 3980 Soliva CR, Hess HD (2007) Measuring Methane Emission of Ruminants by In Vitro and In Vivo  
3981 Techniques. In: *Measuring Methane Production From Ruminants*. Springer Netherlands,  
3982 Dordrecht, pp 15–31
- 3983 Stephenson MB, Bailey DW, Jensen D (2016) Association patterns of visually-observed cattle on  
3984 Montana, USA foothill rangelands. *Appl Anim Behav Sci* 178:7–15.  
3985 <https://doi.org/10.1016/j.applanim.2016.02.007>
- 3986 Stević T, Šavikin K, Zdunić G, et al (2010) Antioxidant, Cytotoxic, and Antimicrobial Activity of  
3987 *Alnus incana* (L.) ssp. *incana* Moench and *A. viridis* (Chaix) DC ssp. *viridis* Extracts. *J Med*  
3988 *Food* 13:700–704. <https://doi.org/10.1089/jmf.2009.0111>
- 3989 Strebel N, Bühler C (2015) Recent shifts in plant species suggest opposing land-use changes in alpine  
3990 pastures. *Alp Bot* 125:1–9. <https://doi.org/10.1007/s00035-015-0145-3>
- 3991 Sunnerheim K, Palo RT, Theander O, Knutsson P-G (1988) Chemical defense in birch.  
3992 Platyphylloside: A phenol from *Betula pendula* inhibiting digestibility. *J Chem Ecol* 14:549–560.  
3993 <https://doi.org/10.1007/BF01013906>
- 3994 Susan F, Ziliotto U (2004) Effects of arboreal and shrubby components on some features of pastures.  
3995 In: *Land Use Systems in Grassland Dominated Regions: Proceedings of the 20th General*  
3996 *Meeting of the European Grassland Federation*. vdf Hochschulverlag AG, Luzern, Switzerland, p  
3997 314
- 3998 Svensk M, Nota G, Mariotte P, et al (2022) Use of Molasses-Based Blocks to Modify Grazing Patterns  
3999 and Increase Highland Cattle Impacts on *Alnus viridis*-Encroached Pastures. *Front Ecol Evol* 10:.  
4000 <https://doi.org/10.3389/fevo.2022.849809>
- 4001 Svensk M, Pittarello M, Mariotte P, et al (2023) Nitrogen translocation by Highland cattle grazing in  
4002 *Alnus viridis*-encroached pastures. *Nutr Cycl Agroecosystems*. [https://doi.org/10.1007/s10705-](https://doi.org/10.1007/s10705-023-10282-0)  
4003 023-10282-0
- 4004 Svensk M, Pittarello M, Nota G, et al (2021) Spatial Distribution of Highland Cattle in *Alnus viridis*  
4005 Encroached Subalpine Pastures. *Front Ecol Evol* 9:1–7.  
4006 <https://doi.org/10.3389/fevo.2021.626599>
- 4007 Tamminga S (2006) Environmental Impacts of Beef Cattle. In: *The John M. Airy Symposium: Visions*  
4008 *for Animal Agriculture and the Environment*. Kansas City, Missouri, pp 1–11

- 4009 Tasser E, Mader M, Tappeiner U (2003) Effects of land use in alpine grasslands on the probability of  
4010 landslides. *Basic Appl Ecol* 4:271–280. <https://doi.org/10.1078/1439-1791-00153>
- 4011 Tasser E, Walde J, Tappeiner U, et al (2007) Land-use changes and natural reforestation in the Eastern  
4012 Central Alps. *Agric Ecosyst Environ* 118:115–129. <https://doi.org/10.1016/j.agee.2006.05.004>
- 4013 Tavendale MH, Meagher LP, Park-Ng ZA, et al (2005) Methane production from in vitro incubation  
4014 of kikuyu grass, lucerne and forages containing condensed tannins. *Proc New Zeal Grassl Assoc*  
4015 147–153. <https://doi.org/10.33584/jnzg.2005.67.2576>
- 4016 Terranova M, Kreuzer M, Braun U, Schwarm A (2018) In vitro screening of temperate climate forages  
4017 from a variety of woody plants for their potential to mitigate ruminal methane and ammonia  
4018 formation. *J Agric Sci* 156:929–941. <https://doi.org/10.1017/S0021859618000989>
- 4019 Terranova M, Wang S, Eggerschwiler L, et al (2019) Dose-response effects of woody and herbaceous  
4020 forage plants on in vitro ruminal methane and ammonia formation, and their short-term  
4021 palatability in lactating cows. <https://doi.org/10.1017/S1751731119002076>
- 4022 Theodoridou K, Aufrère J, Andueza D, et al (2011) Effect of plant development during first and  
4023 second growth cycle on chemical composition, condensed tannins and nutritive value of three  
4024 sainfoin (*Onobrychis viciifolia*) varieties and lucerne. *Grass Forage Sci* 66:402–414.  
4025 <https://doi.org/10.1111/j.1365-2494.2011.00798.x>
- 4026 Thimonier A, Graf Pannatier E, Schmitt M, et al (2010) Does exceeding the critical loads for nitrogen  
4027 alter nitrate leaching, the nutrient status of trees and their crown condition at Swiss Long-term  
4028 Forest Ecosystem Research (LWF) sites? *Eur J For Res* 129:443–461.  
4029 <https://doi.org/10.1007/s10342-009-0328-9>
- 4030 Tian D, Kattge J, Chen Y, et al (2019) A global database of paired leaf nitrogen and phosphorus  
4031 concentrations of terrestrial plants. *Ecology* 100:.. <https://doi.org/10.1002/ecy.2812>
- 4032 Tian D, Yan Z, Niklas KJ, et al (2018) Global leaf nitrogen and phosphorus stoichiometry and their  
4033 scaling exponent. *Natl Sci Rev* 5:728–739. <https://doi.org/10.1093/nsr/nwx142>
- 4034 Tocco C, Probo M, Lonati M, et al (2013) Pastoral Practices to Reverse Shrub Encroachment of Sub-  
4035 Alpine Grasslands: Dung Beetles (Coleoptera, Scarabaeoidea) Respond More Quickly Than  
4036 Vegetation. *PLoS One* 8:e83344. <https://doi.org/10.1371/journal.pone.0083344>
- 4037 Umstatter C (2011) The evolution of virtual fences: A review. *Comput Electron Agric* 75:10–22.  
4038 <https://doi.org/10.1016/j.compag.2010.10.005>
- 4039 Ungar ED, Henkin Z, Gutman M, et al (2005) Inference of Animal Activity from GPS Collar Data on  
4040 Free-Ranging Cattle. *Rangel Ecol Manag* 58:256–266. [https://doi.org/10.2111/1551-5028\(2005\)58\[256:IOAAFJ\]2.0.CO;2](https://doi.org/10.2111/1551-5028(2005)58[256:IOAAFJ]2.0.CO;2)
- 4042 v. Bieberstein Koch-Weser MR (2005) Overcoming the Vertical Divide: Legal, Economic, and  
4043 Compensation Approaches for Sustainable Management of Mountain Watersheds. In: Huber  
4044 UM, Bugmann HKM, Reasoner MA (eds) *Global Change and Mountain Regions*. *Advances in*  
4045 *Global Change Research*, Springer, pp 627–636
- 4046 Valkó O, Venn S, Zmihorski M, et al (2018) The challenge of abandonment for the sustainable  
4047 management of Palaearctic natural and semi-natural grasslands. *Hacquetia* 17:5–16.  
4048 <https://doi.org/10.1515/hacq-2017-0018>
- 4049 van den Bergh T, Körner C, Hiltbrunner E (2018) *Alnus* shrub expansion increases evapotranspiration  
4050 in the Swiss Alps. *Reg Environ Chang* 18:1375–1385. [https://doi.org/10.1007/s10113-017-1246-](https://doi.org/10.1007/s10113-017-1246-x)  
4051 *x*
- 4052 Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for Dietary Fiber, Neutral Detergent Fiber,  
4053 and Nonstarch Polysaccharides in Relation to Animal Nutrition. *J Dairy Sci* 74:3583–3597.

- 4054 [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- 4055 Vandermeulen S, Ramírez-Restrepo CA, Beckers Y, et al (2018a) Agroforestry for ruminants: a  
4056 review of trees and shrubs as fodder in silvopastoral temperate and tropical production systems.  
4057 *Anim Prod Sci* 58:767. <https://doi.org/10.1071/AN16434>
- 4058 Vandermeulen S, Ramírez-Restrepo CA, Marche C, et al (2018b) Behaviour and browse species  
4059 selectivity of heifers grazing in a temperate silvopastoral system. *Agrofor Syst* 92:705–716.  
4060 <https://doi.org/10.1007/s10457-016-0041-x>
- 4061 Verburg PSJ, Johnson DW, Harrison R (2001) Long-term nutrient cycling patterns in Douglas-fir and  
4062 red alder stands: A simulation study. *For Ecol Manage* 145:203–217.  
4063 [https://doi.org/10.1016/S0378-1127\(00\)00418-7](https://doi.org/10.1016/S0378-1127(00)00418-7)
- 4064 Verdinelli M, Pittarello M, Caria MC, et al (2022) Congruent responses of vascular plant and ant  
4065 communities to pastoral land-use abandonment in mountain areas throughout different  
4066 biogeographic regions. *Ecol Process* 11:35. <https://doi.org/10.1186/s13717-022-00379-9>
- 4067 Vintu V, Samuil C, Sirbu C, et al (2011) Napoca sustainable management of *Nardus stricta* L.  
4068 grasslands in Romania's Carpathians. *Not Bot Horti Agrobot Cluj-Napoca* 39:142–145.  
4069 <https://doi.org/10.15835/nbha3926366>
- 4070 Waghorn G (2008) Beneficial and detrimental effects of dietary condensed tannins for sustainable  
4071 sheep and goat production—Progress and challenges. *Anim Feed Sci Technol* 147:116–139.  
4072 <https://doi.org/10.1016/j.anifeedsci.2007.09.013>
- 4073 Waghorn GC, Ulyatt MJ, John A, Fisher MT (1987) The effect of condensed tannins on the site of  
4074 digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. *Br J Nutr*  
4075 57:115–126. <https://doi.org/10.1079/bjn19870015>
- 4076 White PJ (2012) Long-distance Transport in the Xylem and Phloem. Elsevier Ltd
- 4077 White SL, Sheffield RE, Washburn SP, et al (2001) Spatial and Time Distribution of Dairy Cattle  
4078 Excreta in an Intensive Pasture System. *J Environ Qual* 30:2180–2187.  
4079 <https://doi.org/10.2134/jeq2001.2180>
- 4080 Whitehead DC (1970) The role of nitrogen in grassland productivity. A review of information from  
4081 temperate regions. In: Bulletin 48. Farnham Royal, UK: Commonwealth Agricultural Bureaux, p  
4082 202
- 4083 Whitehead DC (1995) Grassland Nitrogen. CAB International, Wallingford
- 4084 Wieczorkowski JD, Lehmann CER (2022) Encroachment diminishes herbaceous plant diversity in  
4085 grassy ecosystems worldwide. *Glob Chang Biol* 28:5532–5546.  
4086 <https://doi.org/10.1111/gcb.16300>
- 4087 Wiedmer E, Senn-Irlet B (2006) Biomass and primary productivity of an *Alnus viridis* stand - A case  
4088 study from the Schächental valley, Switzerland. *Bot Helv* 116:55–64.  
4089 <https://doi.org/10.1007/s00035-006-0758-7>
- 4090 Williams PH, Haynes RJ (1995) Effect of sheep, deer and cattle dung on herbage production and soil  
4091 nutrient content. *Grass Forage Sci* 50:263–271. <https://doi.org/10.1111/j.1365-2494.1995.tb02322.x>
- 4093 Wilson PJ, Thompson K, Hodgson JG (1999) Specific leaf area and leaf dry matter content as  
4094 alternative predictors of plant strategies. *New Phytol* 143:155–162.  
4095 <https://doi.org/10.1046/j.1469-8137.1999.00427.x>
- 4096 Wink M (2010) Mode of action and toxicology of plant toxins and poisonous plants. *Jul Kühn-Institut*  
4097 421:

- 4098 Wood SN (2011) Fast Stable Restricted Maximum Likelihood and Marginal Likelihood Estimation of  
 4099 Semiparametric Generalized Linear Models. *J R Stat Soc Ser B Stat Methodol* 73:3–36.  
 4100 <https://doi.org/10.1111/j.1467-9868.2010.00749.x>
- 4101 Woodfield DR, Roldan MB, Voisey CR, et al (2019) Improving environmental benefits of white  
 4102 clover through condensed tannin expression. *J New Zeal Grasslands* 81:195–202.  
 4103 <https://doi.org/10.33584/jnzg.2019.81.382>
- 4104 Woodmansee RG, Vallis I, Mott JJ (1981) Grassland Nitrogen. In: Clark, F.E. & Rosswall T (ed)  
 4105 *Ecological Bulletins : Terrestrial Nitrogen Cycles*. Oikos Editorial Office, pp 443–462
- 4106 Wu S, Wang J, Yan Z, et al (2021) Monitoring tree-crown scale autumn leaf phenology in a temperate  
 4107 forest with an integration of PlanetScope and drone remote sensing observations. *ISPRS J*  
 4108 *Photogramm Remote Sens* 171:36–48. <https://doi.org/10.1016/j.isprsjprs.2020.10.017>
- 4109 Yokoyama K, Kai H, Koga T, Aibe T (1991) in *Cow Dung , Dung Balls and Underlying Soil Affected*  
 4110 *By Paracoprid Dung. Changes* 649–653
- 4111 Zargoosh Z, Ghavam M, Bacchetta G, Tavili A (2019) Effects of ecological factors on the antioxidant  
 4112 potential and total phenol content of *Scrophularia striata* Boiss. *Sci Rep* 9:1–15.  
 4113 <https://doi.org/10.1038/s41598-019-52605-8>
- 4114 Zehnder T, Lüscher A, Ritzmann C, et al (2020) Dominant shrub species are a strong predictor of  
 4115 plant species diversity along subalpine pasture-shrub transects. *Alp Bot* 130:141–156.  
 4116 <https://doi.org/10.1007/s00035-020-00241-8>
- 4117 Zehnder T, Schneider MK, Berard J, et al (2016a) Reconciling meat production and biodiversity  
 4118 conservation on marginal pastures. In: Casasús I, Lombardi G (eds) *Mountain pastures and*  
 4119 *livestock farming facing uncertainty: environmental, technical and socio-economic challenges.*  
 4120 *Zaragoza, Spain*, pp 231–235
- 4121 Zehnder T, Schneider MK, Berard J, et al (2016b) Valorising forage resources and conserving  
 4122 ecosystem services in marginal pastures. In: Höglind M, Bakken AK, Hovstad KA, et al. (eds)  
 4123 *Grassland Science in Europe: The multiple roles of grassland in the European bioeconomy.*  
 4124 *Wageningen Academic Publishers, Ås, Norway*, pp 600–602
- 4125 Zehnder T, Schneider MK, Berard J, et al (2017) Forage quality of shrub-encroached marginal  
 4126 pastures does not impair quality of carcass and meat. In: Porqueddu C, Franca A, Lombardi G, et  
 4127 al. (eds) *Grassland Science in Europe: Grassland resources for extensive farming systems in*  
 4128 *marginal lands: major drivers and future scenarios.* *Wageningen Academic Publishers, Sassari,*  
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## Declaration of consent

on the basis of Article 18 of the PromR Phil.-nat. 19

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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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