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# Taxonomic and functional approaches of trophic interactions between large herbivores and plant communities in a mountain ecosystem

Marjorie Bison

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# THÈSE

Pour obtenir le grade de

## DOCTEUR DE L'UNIVERSITÉ GRENOBLE ALPES

**préparée dans le cadre d'une cotutelle entre l'*Université Grenoble Alpes* et l'*Université de Tromsø (UiT)***

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Présentée par


## Marjorie BISON


Thèse dirigée par **Anne LOISON**
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préparée au sein du **Laboratoire d'Ecologie Alpine – UMR CNRS 5553** et du **Laboratoire de Biologie de l'UiT**

dans **l'Ecole Doctorale Chimie et Sciences du Vivant**

# Approches taxonomique et fonctionnelle des interactions trophiques entre grands herbivores et communautés végétales dans un écosystème de montagne

Thèse soutenue publiquement le **8 décembre 2015**,
devant le jury composé de :

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*"The more I learn, the more I realize how much I don't know" - Albert Einstein*

Here we are. The writing of this part announces the approaching end of the compilation of the thesis manuscript.
This three-year work would not have been realized without the help of many people, and highlights once again the collective nature of research.

My first special thanks are directed towards my two supervisors, Anne Loison and Gilles Yoccoz, for taking me in and guiding me with patience through the ambushes of a research work. As every PhD student that I met, my motivation has not always been constantly at its highest point, but the different projects, ideas of exploration and explorations of ideas were always here to emerge from my torpor. It has been a great privilege to work with you. Thank you very much for allowing me to put my hand into the huge available databases from the Bauges Massif, for directing me towards certain directions while letting me freedom of action, for all the statistical helps, for all the ideas, for all the proof readings, and sorry for my insistence sometimes. Your advices and knowledge have always been more than helpful and welcome. I greatly appreciated your complementarity, you form a good supervisor duo!
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that, at the end of the field campaigns, some of us would have been able to reach the Armene pasture blindfolded. Antoine, Jean-No, Nathalie, Annie, Luc, Seb, Isabelle, Pascal, Josefa, Jean-François, Anne, François, David, Bruno, Etienne, Marie, Rémy, Christiane, Mathilde, M-P and Tiph, I spent good times with you. Thanks a lot for your help and the envy of a well-done work. Antoine, Luc and M-P, you have been more than precious for the plant determination and the knowledge of the Armene pasture. M-P, you have been a perfect teacher for my learning of plant functional trait measurements, and plant storage in the field.
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Special thanks to Mathieu for not having been afraid by the end of a PhD.

I am sorry for the people I forgot. It was not on purpose. Thank you as well.

**“If we didn’t have the failure, what fun would climbing be” - Daniel Woods, professional climber.**

Doing a PhD is pretty much like climbing. The belayer is you supervisor, and you need to be sure that he/she could carry you if you fall, but also that he/she could guide and advise you during the ascent. Trust needs to be established between both of you. You have to think about the position of your hands and feet. If, additionally, you need to oversee you belayer, just don’t go.
Quickdraws are your background, and you should know how and when to use it at the right time. Sometimes during the ascent, you will meet other climbers, arriving on routes next to you. These people are highly important, as they are able to completely change your point of view and give you cues to overcome a difficult move, or a small slippery hold that you don’t know how to use. This is exactly the same in research, you need to be open to new ideas and be able to change your way of thinking.
Climbing and PhD are both a matter of balance: while the first one needs well-positioned hands and feet, the second one needs a well-shared time between your personal life and your work. Climbing and PhD are also both a matter of determination and temerity. For each, barriers and failures need to be kept in perspective to be able to go on or to discover your own limits. Easier said than done.
At the end of the route, you reach the belay stance, but sometimes not. In both cases, you should enjoy the view. It is now time to move on to another piece of the puzzle.

# ABSTRACT

Given the key role of large herbivores on species and functional plant diversity, we aimed at better understanding the relationship between herbivory and plant communities mainly at a fine-scale, in order to reconcile objectives of population management and plant conservation. For this purpose, we used both taxonomic and functional approaches, and studied interactions at the inter- and intra-specific levels. We combined information coming from three databases: (1) diet data from DNA-metabarcoding applied on chamois (*Rupicapra rupicapra*), roe deer (*Capreolus capreolus*) and mouflon (*Ovis gmelini musimon*) faeces from the Bauges Massif, (2) characteristics of plant communities (plant composition, biomass, phenology), (3) plant functional traits. Analyses of intra-specific variability of the three large herbivores allowed us to upscale the niche variation hypothesis (NVH) of Van Valen from the intra- to the inter-specific level, i.e. we observed a positive relationship between the species niche breadth and among-individual variation. Then, based on two chamois subpopulations living in pastures, one living in sympatry with the mouflon and the other living in allopatry, we revealed the absence of negative effects of the introduced mouflon population on native chamois population diet, both for the taxonomic and functional dietary niche. Analyses of diet selection criteria allowed us to highlight differences in choice criteria between chamois and mouflon in some seasons, which helped to partially explain the partial taxonomic and functional niche partitioning of the two species. Furthermore, the proposed scenario of the evolution of diet selection over the year for both species were consistent with ungulate-specific morpho-physiological features. Finally, contrary to the literature where no studies could discriminate the direct and indirect effects of functional traits on diet selection because of correlations, we used path analyses, which allowed us to show that in most cases, biomechanical traits had a direct effect on diet choices, whereas chemical traits had indirect effects. Furthermore, from a methodological point of view, we advised to use nitrogen fecal indices only to study the evolution of species-specific and location-specific population long-term diet quality, but not to compare diet quality between species, nor to study slight fluctuations at the intra-seasonal level. The complementarity of the approaches allowed us to better account for the structuration of herbivore communities, which should help to better assess the actual state and the evolution of relationships among individuals, species and their environment.

**Key-words:** ungulates, intra- and inter-specific interactions, taxonomic and functional approaches, DNA metabarcoding, NIRS, Bauges Massif, diet selection

# RÉSUMÉ

Etant donné le rôle clé des grands herbivores sur la diversité végétale spécifique et fonctionnelle, notre objectif était de mieux comprendre la relation entre l'herbivorie et les communautés végétales principalement à une échelle spatiale fine, afin de concilier des objectifs de gestions des populations et de conservation de la flore. Pour cela, nous avons abordé cette problématique en intégrant des approches taxonomique et fonctionnelle, et en étudiant les interactions aux niveaux inter- et intra-spécifiques. Nous avons combiné les informations de trois bases de données : (1) des données de régime alimentaire issus d'analyses d'ADN metabarcoding réalisées sur des faeces de chamois (*Rupicapra rupicapra*), chevreuil (*Capreolus capreolus*) et mouflon (*Ovis gmelini musimon*) du Massif des Bauges, (2) des données sur les caractéristiques des communautés végétales (composition floristique, biomasse, phénologie), (3) des données de traits fonctionnels des plantes. L'analyse de variabilité intra-spécifique des trois grands herbivores nous a permis de valider l'hypothèse de variation de niche (NVH) de Van Valen au niveau intra-spécifique mais aussi au niveau inter-spécifique, soit une relation positive entre la largeur de niche de l'espèce et sa variabilité intra-spécifique. Ensuite, grâce à deux populations de chamois d'alpage, l'une vivant en sympatrie avec le mouflon et l'autre vivant en allopatrie, nous avons pu mettre en évidence l'absence d'effets négatifs de la population introduite de mouflon sur le régime alimentaire de la population native de chamois, autant au niveau de la niche alimentaire taxonomique que fonctionnelle. L'analyse des critères de sélection alimentaire nous a finalement permis de mettre en avant des différences de critères de choix entre les deux espèces à certaines saisons, expliquant ainsi partiellement le partitionnement partiel des niches taxonomique et fonctionnelle des deux espèces. Par ailleurs, les scénarios proposés d'évolution de la sélection alimentaire au cours de l'année pour les deux espèces concordaient avec les caractéristiques morphologiques spécifiques à l'espèce d'ongulé. Finalement, contrairement à la littérature où aucune étude n'était capable de discriminer les effets directs et indirects des traits fonctionnels à cause de corrélations, nous avons pu, grâce à des analyses de pistes, démontrer que, dans la plupart des cas, les traits biomécaniques avaient un effet direct sur le choix alimentaire alors que les traits chimiques n'avaient qu'un effet indirect. Enfin, d'un point de vue méthodologique, nous conseillons l'utilisation d'indices d'azote fécaux uniquement dans l'étude de la variation à long terme de la qualité des régimes de manière spécifique à chaque espèce et chaque site, et non pas pour comparer des qualités de régime entre espèces ni pour étudier les variations fines à l'échelle intra-saisonnière. La complémentarité des approches nous a permis de mieux appréhender la structuration des communautés d'herbivores, et devrait nous aider à mieux évaluer l'état actuel et l'évolution des relations entre individus, entre espèces et avec leur environnement.

**Mots-clés :** ongulés, interactions intra- et inter-spécifiques, approche taxonomique et fonctionnelle, ADN metabarcoding, NIRS, massif des Bauges, sélection alimentaire

# LIST OF PAPERS

**Paper I.** Bison M., Ibanez S., Redjadj C., Boyer F., Coissac E., Miquel C., Rioux D., Saïd S., Maillard D., Taberlet P., Yoccoz N.G., Loison A. *Upscaling the niche variation hypothesis from the intra- to the inter-specific level*. Oecologia, 2015.

**Paper II.** Bison M., Dupeyras P.A., Redjadj C., Miquel C., Rioux D., Taberlet P., Maillard D., Yoccoz N.G., Loison A. *Taxonomic and functional niches differentiation between two large herbivore species.* In prep. for Ecology or Journal of Ecology.

**Paper III.** Bison M., Ibanez S., Puijalon S., Dehédin A., Langrand E., Redjadj C., Miquel C., Rioux D., Taberlet P., Maillard D., Loison A., Yoccoz N.G. *The relative contribution of biomechanical and biochemical traits in plant selection by ungulates over the year.* In prep. for Functional Ecology.

**Paper IV.** Ancin Murguzur F.J., Smis A., Bison M., Struyf E., Bråthen K.A. *Over the frontiers: a single NIRS calibration for sub-arctic and temperate ecosystems.* In prep. for PeerJ.

**Note.** Bison M., Redjadj C., Miquel C., Rioux D., Taberlet P., Maillard D., Yoccoz N.G., Loison A. *Comparison between two methods for estimating diet quality in large herbivores: NIRS-derived fecal nitrogen vs dietary nitrogen from ingested plants.*

# OTHER COMMUNICATIONS

**Oral:**

**ECOVEG.** 25-27 March 2015. *Quels traits fonctionnels comme critères de sélection alimentaire chez le chamois ?*

**Ecology and Behavior.** 18-21 May 2015. *Taxonomic and functional niche differentiation between two ungulate species*

**Poster:**

**Doctoral School EDCSV, Grenoble.** *Upscaling the Niche Variation Hypothesis from the intra- to the inter-specific level.* Bison M., Boyer F., Coissac E., Ibanez S., Maillard D, Miquel C., Rioux D., Saïd S., Taberlet P., Yoccoz N.G., Loison A.

**Popularizing "our" science:**

**Meeting with hunters.** *Apport de l'ADN des faeces sur la connaissance d'espèces d'ongulés.*

**Research in the Bauges Massif.** *Interactions fonctionnelles entre grands herbivores et communautés végétales : présentation des résultats.*

**Herbivorie Info n°18.** *Le choix des plantes par les ongulés.*

**Popularizing the science of others researchers (internship in the Industrial, Technical and Scientific Culture Center of Grenoble):**

- Des champignons sur la falaise
  http://www.echosciences-grenoble.fr/actualites/des-champignons-sur-la-falaise
- Francis Hallé, botaniste et militant
  http://www.echosciences-grenoble.fr/actualites/francis-halle-botaniste-et-militant
- Faire travailler son esprit critique avec le CorteX
  http://www.echosciences-grenoble.fr/actualites/faire-travailler-son-esprit-critique-avec-le-cortex
- A la recherche des ondes gravitationnelles avec Frédérique Marion
  http://www.echosciences-grenoble.fr/actualites/la-recherche-des-ondes-gravitationnelles-avec-frederique-marion
- Au détour d'une comète : la mission Rosetta avec Wlodek Kofman
  http://www.echosciences-grenoble.fr/actualites/au-detour-dune-comete-la-mission-rosetta-avec-wlodek-kofman
- Les détectives du changement climatique
  http://www.echosciences-grenoble.fr/actualites/les-detectives-du-changement-climatique
- Du fossile aux géoparcs
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# INTRODUCTION

1

*« Qui cherche à façonner le monde,*
*Je vois, n'y réussira pas.*
*Le monde, vase spirituel, ne peut être façonné.*
*Qui le façonne le détruira.*
*Qui le tient le perdra.*
*[…] »*

*Lao-tseu*
*Tao-tö king*

## Chapter I: How to get excited about ecology

### 1.1 Picture of the actual world

According to the Nobel price Paul Crutzen, we are currently at the heart of the Anthropocene era, which began at the dawn of the industrial revolution (end of 18th century) and left behind the Holocene era. Even if the concept is still debated by the scientific community (Lewis and Maslin 2015), the origin of the idea is clear: human activity is global and has great implications on environmental changes, from biogeochemical cycles to the evolution of life (Lewis and Maslin 2015).

Given that the "natural" world cannot be viewed without incorporating the action of humans, the relationship and the question of how to interact with Nature is essential, but varies according to culture and society. By the beginning of the previous sentence, my thoughts are already constrained by the idea of a difference of essence between Humanity and Nature. This vision of our modern science, initiated during the 17th century by Galileo, lead to the idea of independency of Nature against humans (Larrère & Larrère 1997). Even if humans are part of the Nature, we – people having grown up within the "modern western" society – still feel outside of it. We tend to think that we are able to describe it with mathematical laws and regard it as a tool for which we can define a value. Diversity in nature is therefore considered in terms of benefits brought for human populations (ecosystem services, Maris 2011). This point of view is rooted in our Western society, but does not exist everywhere. For example, for Amazonian people (Kayapos indians), humanity is not restricted to humans, but includes the air they breathe, the plants they feed on, the animals they hunt, etc (Klein 2010).

Given that action of humans causes global changes leading to biodiversity loss that might affect the dynamic and functioning of ecosystems (Halpern et al. 2008, Cardinale et al. 2012, Newbold et al. 2015, McGill 2015), the "modern society" tries to maintain an environment as stable as possible by reaching a compromise with the economic and industrial development. Hence, we realize that the "protection/management of Nature" only exists with the emergence of industrial

societies (Larrère 2013). Philosophers come to the conclusion that it is not the Nature that dominates humans, neither humans that dominate the Nature, but that a new interaction arises that we do not know how to define (Larrère & Larrère 1997, Maris 2007). Hence, it appears necessary to integrate a new vision of our interaction with the environment, in order to propose solutions to ongoing ecosystem changes.

The first step is to clearly identify the causes of ecosystem changes to better understand ecosystem functioning and their response to environmental fluctuations. The term "ecosystem functioning" includes ecosystem properties, ecosystems goods and ecosystem services (Christensen et al. 1996). In the following, we will refer to ecosystem properties when referring to "ecosystem functioning". It includes the size of compartments (e.g. pools of carbon or organic matter) and the processes involving fluxes of biomass and energy between trophic levels and the environment (Hooper et al. 2005). As stated by Naeem et al. (1999), "functioning" means "showing activity". When an ecosystem is altered, for example in terms of species identities, community composition or diversity, but also in terms of abiotic conditions, its rate of plant production, rate of decomposition, carbon storage or nutrient cycling are some processes that can be deteriorated (Naeem et al. 1999). The stability of the ecosystem can consequently be modified.

Climate and land use are the most influential drivers of changes in biodiversity in temperate regions (Boulangeat et al. 2014). Species distribution and diversity are affected by climate change (Parmesan 2006, Lenoir et al. 2010, Gottfried et al. 2012). Land use change has also been shown to have a strong influence on vegetation structure and diversity (Foley et al. 2005) through two scenarios: the land abandonment leading to the closure of open areas (Gehrig-Fasel et al. 2007); and the intensification of agriculture leading to species extinctions (Hodgson et al. 2005). Land use is also associated with habitat fragmentation (Wallis De Vries et al. 1998) and destruction (Wright & Muller-Landau 2006), such as forest conversion to agriculture, river channelization or road settlements. Four other factors can affect the biodiversity, and they act at a shorter time-scale than climate change: pollution such as nitrogen deposition (Thuiller 2007, Zvereva et al. 2008) or chemical contaminants (Bickham et al. 2000, Ricciardi et al. 2009); biological invasions, species introductions and reintroductions (Wilcove et al. 1998, Thuiller 2007, Hahn & Orrock 2014); the increase of carbon dioxide levels in the atmosphere (Thuiller 2007); and the overexploitation leading to resource depletion (Lotze et al. 2006). All those impacts and their interactions contribute to affect the biotic and abiotic environment of a lot of species (Wilcove et al. 1998, Hahn 2014). While the major consequences of climate and land use changes on biodiversity are generally associated with a loss of species, the increase of some population census size is conversely important (Buntgen et al. 2014). Indeed, some species take full advantage of the new environmental conditions associated with the increase of temperature, land use changes, decrease of predators, limitations of hunting quotas, or introductions in environments where they are released from predators ("enemy release hypothesis", Maron & Vilà 2001). Therefore, the increase of population density is also a major threat to biodiversity, which is usually overlooked.

Many authors agree to say that we are on the way of an environmental crisis (Ehrlich & Mooney 1983, Ceballos et al. 2002, Pereira et al. 2010), confirmed by a recent article of Ceballos et al. (2015) stating that current extinction rates are far higher than the natural average background rates in Earth's history. These erosions are not limited to a sharp decrease and to a

homogenization (Clavel et al. 2010) of the species distribution (endemic species extinction and exotic species introduction, Mack et al. 2000, Mooney & Hobbs 2000) but also lead to the degradation of ecosystem functioning in their wholeness (such as dynamic of fire, carbon sequestration, biogeochemical cycles, Estes et al. 2011). This can, in turn, affect the services provided by the Nature to Humanity, such as the use of biodiversity for medicinal purposes, feeding and non-feeding resource supply or energy production; the esthetic, ethical or spiritual value of Nature which are an important aspect to motivate people to protect Nature; the ecological value of biodiversity. Ecosystems can become unbalanced and functionally altered (such as poor nutrient recycling, poor resilience) (Schröter et al. 2005), at different degrees depending on the ecosystem type (Thuiller 2007) and sensitivity. In this context, scientists try to understand at which scales the changes happen, its origins, its maintenance and try to predict the evolution of systems taking into account the past and actual states, notably to bring facts, ideas and elements of decisions for policy-making processes (Thuiller 2007).

## 1.2 From community ecology to functional ecology

### a) Describing the communities

The first steps of ecology have been characterized by descriptive and classification tasks. What is a species? How can we classify them? How many species are living in a given place?
The word “species” has received considerable attention in biology for hundreds of years. It all began with the idea of classifying and giving names to plants, animals, etc in order to make oneself understood and to communicate, but also to get a more comprehensible pattern of the diversity observed in Nature and to order it. At the beginning, the species concept did not require any precise definition and all the animals that looked like a sheep were called a sheep. Then, with the discovery of new unidentified organisms, things started to get more complicated. It called for precisely define what a species was. But since the word “species” appeared in the scientific literature, its meaning has been evolving into a multitude of definitions (24 species concepts, Mayden 1997). The biological species concept (BSC) has been introduced by Mayr in 1957. It is still the most used and popular concept: a species is defined as a “group of interbreeding natural populations that are reproductively isolated from other such groups”. Moreover, offspring have to be fertile. The main problem with BSC is that most of the life on the planet does not reproduce sexually but asexually (cloning, vegetative reproduction) (Ereshefsky 2010) and inter-specific hybridizations are common, e.g. within plants. Following the BSC concept of species, asexual organisms would actually not form species. The phylogenetic species concept (PSC) is complementary as it is not only used to sort organisms but also consider their evolution. Many other species concepts are found in the literature. We could see this pluralist approach as sterile discussion among scientists, but it actually illustrates the multitude of research approaches and the points of view used to approach the organization of living organisms. Each concept has its limits and weaknesses, but the main question is: “What does best fit the biological question?” Those old concerns are still questioned and some researchers keep working on the way of sorting living organisms (de Queiroz 2007). As stated above, one of the old questions in ecology also deals with the evaluation of biodiversity. Species are considered as one of the fundamental units in ecology. With the definition of the

“biodiversity”, we can easily conceive why. The term “biodiversity” has been defined as “the variability among living organisms from all sources including, inter-alia, terrestrial, marine and other aquatic ecosystems, and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems” (definition from the Convention of Biological Diversity, Rio de Janeiro, 1992). Given the central place given to the species-level, the multitude of species concepts remains problematic in the case of biodiversity assessment: depending on the species definition and the classification we choose, it will give different estimates of biodiversity. Recently, Cadotte et al. (2010) used the two concepts stated above (BSC and PSC) to propose a new assessment method of biodiversity index based on phylogeny. They integrated the species richness (number of species in a community), their abundance and their evolutionary ecology. Consequently, they assumed that a community composed of three closely related species would be less diverse than a community composed of three phylogenetically distant species. In addition, different indices can be used to estimate the diversity: the species richness (number of species in a community), the Gini-Simpson index and the Shannon index (both take into account the richness and the evenness), all grouped into the Hill numbers considered as a general approach to measure diversity (Hill 1973, Chao et al. 2014). Finally, the scale of the taxonomic diversity measurement has been discussed by Whittaker in 1960, who proposed different measurements, such as the local diversity (α-diversity), regional diversity (γ-diversity) and the between site diversity index (β-diversity). The choice of the classification, the index and the scale of diversity measurements can influence the estimation of diversity, and these questionings are still at the heart of community ecology studies.

b) Characterizing the assemblage of species

The following questions concern the assemblages of species within communities. Why are there more species in some places than in others? How do species interact and coexist within a community? Does one facilitate the development of another? Is there competition? What are the relative influences of temperature and soil characteristics in the complexity of plant communities? How does composition evolve temporally?

**Box 1** – Ecological niche

Ecological niche is defined as a hypervolume with n-dimensions (Hutchinson 1957) which holds all the environmental conditions that allow a species to have a positive growth rate (Grinnell 1917). This complete hypervolume is called the “fundamental niche”, however it cannot be estimated as it is not possible to test all the conditions where the species could persist (Panzacchi et al. 2014). The niche that we observe is the “realized niche” and is shaped by the biotic interactions and the limitations of dispersion. Therefore, the realized niche can fluctuates with changes in the environment. Other concepts are linked to the ecological niche. The “Grinnellian niche” (Grinnell 1917) refers to the species requirements essential for its survival, while the “Eltonian niche” (Elton 1927) refers to how a species impacts its local environment (the “function”). In both cases, the concept of resources is central, whatever the niche definition.

The aims of community ecology as a scientific discipline are to answer those questions and to elucidate the processes underlying the composition pattern of communities. In this context,

approaches mainly focus on the ecological interactions among organisms and with their abiotic environment. The interactions among organisms (competition, facilitation, predation, parasitism, symbiosis, mutualism, neutralism, commensalism) happen on a single or several axis of the species ecological niche (box 1) such as food, time or space.

Species can compete on the spatial axis, but not on the food axis if food resources used by the species are not limiting. Facing the global environmental changes discussed above, species need to adapt their habitat, diet selection or their activity rhythm; or to move or to die. Similarly to the ecological niche, interactions among species can be summarized as a hypervolume with n-dimensions, where *n* represents the number of interactions a species maintained with the *n* other species. During this thesis, I mainly focused on the dietary (or feeding) niche of species. Without additional information, the word "niche" should be interpreted as "dietary (or feeding) niche".

### c) Finding general rules in community structuration

More recent questions have emerged to understand how the assemblage of species works. What are the functions of the species in the community? How does a certain assemblage of species influence the ecosystem processes? How can we generalize our local conclusions to more general pattern at a wider scale?

The taxonomic vision does not reflect the function of organisms and does not allow to give general principles about community assembly (Cornwell & Ackerly 2009, Spasojevic and Suding 2012, Mason et al 2012), neither to predict the abundance of species (Shipley et al. 2006, Laughlin et al 2012), nor to understand the influence of organisms on ecosystem functioning (Diaz & Cabido 2001, Lavorel & Garnier 2002). About 25 years ago, the functional approach of communities has been introduced (Lavorel & Garnier 2002, Cornelissen et al. 2003, Violle et al. 2007) in a context where ecologists were wondering if universal laws could also govern ecology (Lawton 1999, McGill et al. 2006).

Based on individuals, the functional ecology describes the organisms with their biological characteristics (e.g. vegetative height, specific leaf area, root density) and their functions (e.g. light interception, resource intake, nutrient and water absorption) within their environment instead of describing them with their taxonomic identity (Calow 1987). Following the review of "traits" definitions given by Violle et al. (2007), that details how the meaning of the term "trait" varies among studies, we used the definition of traits at the individual-level following Garnier & Navas (2013), i.e. "a trait is any morphological, physiological or phenological feature measurable at the level of individual only, from the cell to whole-organism level, without reference to the environment or any other level of organization". According to this definition, a trait is not influenced by environmental factors or other level of organization (Violle et al. 2007). Species that are taxonomically different can actually be similar in terms of functions and biological characteristics (morphological, chemical, phenological, biomechanical measurements). These features that have a direct impact on the fitness (survival, growth or reproduction) are called functional traits (Lavorel et al. 1997, Violle et al. 2007). The value of a functional trait is the result of compromises among the different functions of the plants (Diaz & Cabido 1997). The use of independent functional traits allows describing general plant functional strategies useful for the understanding of ecosystem functioning (Lavorel et al.

1997). For example, leaf dry matter content (LDMC) is negatively correlated with specific leaf area (SLA): species with strong LDMC (weak SLA) are composed of a low density of foliar tissue, a low photosynthetic rate not allowing a high resource intake but a high conservation ability, and in turn a slow growth rate. These species are called “conservative” species. The opposite are the “exploitative” species and are dominant in fertile environment (Grime et al. 1997, Reich et al. 1999).

In absolute terms, taking into account the individual variability would theoretically allow completely overcoming the species concept (Albert et al. 2010, Albert et al. 2012). However, this requires a huge amount of work and because a species trait is usually the mean trait value measured from some individuals, it does not overcome the species concept. The use of well-chosen functional traits can however reveal general functions and strategies not determined with the single taxonomic approach. However, nowadays, the importance of intra-specific variability is highly studied (Albert et al. 2012, Violle et al. 2012, Albert et al. 2015) and its omission could lead to misinterpretations of ecosystem functioning. This is discussed hereafter in *1.3*.

The functional approach is useful as indicator of population/community structure, dynamics and assembly at local (Kraft et al. 2008, Angert 2009) and biogeographic scales (Swenson 2010, Siefert 2013), to quantify functional diversity of communities (de Bello et al. 2009), to describe the relationships between traits (Reich et al. 1997, Wright et al. 2004, Onoda et al. 2011), to describe the distribution of traits according to environmental gradients (Thuiller et al. 2004, Albert et al. 2010), to relate functioning of ecosystems and services associated (Diaz et al. 2007a), to explain the relationship between traits and fundamental/realized niche (McGill et al. 2006), to mechanistically understand trophic network (Ibanez et al. 2013a) or to predict community response to disturbance (Deraison et al. 2015). For example, plant functional traits such as plant height, or leaf mass, are well correlated to herbivory pressure (Diaz et al. 2001). Although the relationship between traits and herbivory pressure is usually non-linear, it is possible to predict the response of easily measurable plant traits to grazing, even in communities that are taxonomically diverse. Indeed, cattle grazing tends to favor annual over perennial plants, short plants over tall plants, rosette and stoloniferous rather than tussock architecture, prostate rather than erect forms (Diaz et al. 2007b). Consequently, the functional approach allows a more mechanistic understanding of the forces shaping the communities and their dynamics, and to generalize results across organisms and ecosystems (McGill et al. 2006).

Nowadays, researchers are trying to reveal general ecological theories of community assembly (Pavoine & Bonsall 2011) through the combination of indices of diversity based on traits (functional richness, functional evenness, functional divergence, functional dispersion, Mason et al. 2005, Villéger et al. 2008, Laliberté & Legendre 2010), taxonomy and phylogeny (Pavoine & Bonsall 2011 and references therein). Especially, several studies tried to relate the diversity of traits with species diversity (Mayfield et al. 2005, Holdaway & Sparrow 2006, Grime 2006, Villéger et al. 2010). Some of these studies demonstrate the absence of relationship between trait and species diversity (Mason et al. 2008), which emphasizes their complementary use. In this thesis, we particularly used the twofold taxonomic and functional approach in the study of

the coexistence between chamois (*Rupicapra rupicapra*) and mouflon (*Ovis gmelini musimon*) on the feeding axis of the ecological niche (paper II). Indeed, knowing the plant species eaten by two species is helpful to study the inter-specific competition, as the food limitation acts at the plant species level. If two primary consumers compete for the same plant species, according to the competitive exclusion concept, they can be forced to feed on different plant species (taxonomic niche), but reach the same energy requirements (functional niche), which would not impact their dynamic. The complementary use of these two approaches at different scales could sharply change our vision of community structure.

## 1.3 Downscaling ecological studies at the intra-specific level in order to better understand the dynamic of communities and ecosystem functioning

Most of the studies on niche in community ecology focus on the mean trait of species, suggesting that individuals behave in the same way (Layman et al. 2015). For example, when investigating the inter-specific interactions between chamois and sheep, La Morgia & Bassano (2009) limited their overlap measurement to the mean diets of species. However, as already stated by Darwin (1859), individuals can differ because of genetic diversity or phenotypic plasticity (Byars et al. 2007), or because biotic interactions alter their trait value (e.g. competition, Gross et al. 2009). For example, in the case of plant species, the inclusion of intra-specific variability can help to distinguish populations that differ in their trait values along environmental gradients (Albert et al. 2010). Summarizing data with species-mean values overestimates the contribution of rare species (Paine et al. 2011). Therefore, the omission of intra-specific variability can lead to misinterpretation of community functioning (Jung et al. 2010). For instance, aboveground net primary productivity (ANPP) increased with plant genotypic diversity because of higher niche complementarity, in an experimentally low diverse community (Crutsinger et al. 2006). If the objective of a study is to determine the factors influencing the ANPP, then the omission of intra-specific variability could prevent a correct interpretation of results. In the study of La Morgia & Bassano (2009), if the overlap measurement had been measured between all pairs of individuals instead of between the mean diets of chamois and sheep, they could have been able to determine whether only a subset of chamois individuals were affected by sheep. This information can be particularly important to solve management problems and change our vision of species conservation (Bolnick et al. 2003). Indeed, protecting the habitat of a species based on the mean habitat preferences whereas the species displays a strong among-individual variation is not adapted.

Because natural selection, and then adaptation, acts at the individual level, studying the intra-specific variability is essential to understand how populations adapt to their environment and figure out the evolution of their realized niche (Tinker et al. 2008, Pires et al. 2013, Salvidio et al. 2014). When food resource decreased in autumn, salamander populations increased their population niche breadth thanks to a strong diet specialization of individuals on alternate food items (Salvidio et al. 2014). Without this multi-level approach, authors would have not been able to explain the mechanism responsible of the population niche breadth increase. These improvements could also help to better predict how a population or a species would numerically, spatially and behaviorally respond to environmental changes (Bolnick et al. 2011). For example, if one of the resources used by an herbivore population with a high among-

individual variability decreases, only a subset of individuals would be impacted. Without among-individual variation measurement, predictions about the evolution of the population would have led to suggest that all the individuals suffered from the decrease of the resource, and we would have predicted a decrease of the whole individual's fitness instead of only some individuals. Hence, including the intra-specific variability in models describing the population dynamic could help to improve the predictive power of the study (Bolnick et al. 2003).

Intra-specific variation can also affect ecological interactions and in turn community assembly (Hughes et al. 2008, Bolnick et al. 2011), both in low diversity communities (Crutsinger et al. 2006, Hughes et al. 2008) and in more diverse systems (Cornwell & Ackerly 2009, Jung et al. 2010, Paine et al. 2011). For instance, the increase in the resource diversity available to herbivores increased the arthropod richness thanks to associations between particular herbivores and particular host-plant genotype, hence favoring the number of ecological interactions between plants and herbivores (Crutsinger et al. 2006). In a context of increasing spatial overlap among species due to an increase of population size, a stronger inter-specific competition could differentially impact the individuals of a targeted species according to its degree of among-individual variation. Indeed, it is only a subset of individuals (fig.1a) or all the individuals (fig.1b) that could be affected by an overlapping species (in red in fig.1). Hence, intra-specific variation promotes species coexistence by limiting similarity with competing species and by adjusting the trait values of individuals and species to their abiotic requirements (environmental filtering, Jung et al. 2010). Individual-level data appear as a more sensitive indicator of niche differentiation and environmental filtering than species-mean data (Jung et al. 2010).

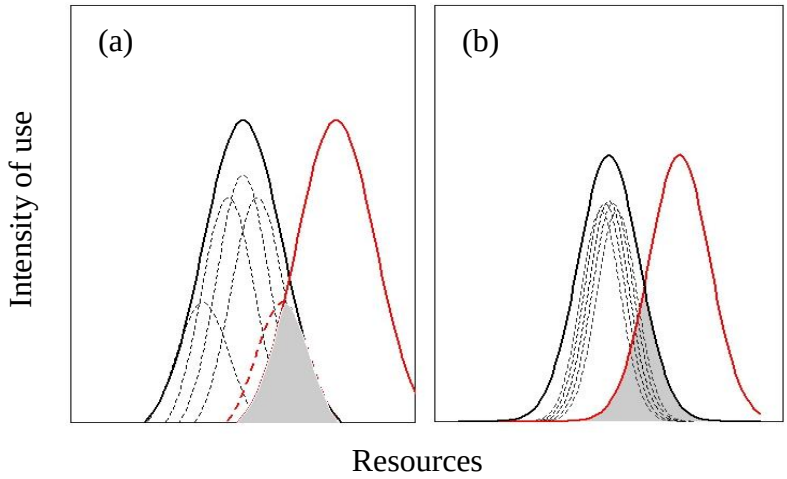



**Figure 1.** Schematic representation of the impact of a potential competitor (in red) on two populations (in grey, a and b) contrasted in their degree of inter-individual variation. Dotted lines represent the individuals within each species. Inter-individual variation is higher in the left case (a).

Given the importance of intra-specific variability in communities response to environmental changes, in biodiversity assemblage or in network functioning (Dupont et al. 2014, Willmer & Finlayson 2014, Tur et al. 2015), an increasing number of researchers try to take it into account.

For example, Tur et al. (2014) downscaled the understanding of plant-pollinator network at the intra-specific level and showed that the different parameters (linkage density, connectance, nestedness, interaction diversity) describing the structure of networks significantly changed in response of a high degree of individual specialization.

Including the intra-specific variability within ecological studies is not only useful to explain spatial or fine-temporal community composition variation, but also to explore evolutionary processes at a larger temporal scale. Indeed, in an evolutionary perspective, because environmental filters act at the individual-level, different survival, growth and reproduction can be observed among individuals leading to changes in allele frequencies, which in turn may modify the evolution of the population through natural selection (Bolnick et al. 2003).

# Chapter II: Theoretical context

## 2.1 Large herbivores as a good study model

### a) Their central place in ecosystems

Large herbivores are of economic, social, cultural and ecological importance (Gordon et al. 2004) and their study and management is therefore of special interest. In an economical perspective, herbivores are also of hunting interest for sport and trophy hunters and generate annually large economic benefits (Palazy et al. 2012). Diversity and emblematic aspect of some herbivores also favor tourism (Cederna & Lovari 1985). Also, large herbivores bring a cultural component to ecosystem services by the maintenance of several cultures (for example, Sami people from Scandinavia still rely on reindeer domestication).

Given their intermediate position, densities of large herbivores are controlled by the resource availability ("bottom up" effect), predators (natural and human predation) ("top-down" effect), illness ("top-down" effect) and competitors ("transversal" effects). Larger herbivore species which require greater plant abundance are more prone to be limited by food availability, whereas smaller herbivore species which require higher plant nutrient content would be more sensitive to predation (Olff et al. 2002, Hopcraft 2010). In predator-free environments, environmental stochasticity (Saether 1997) and density-dependence (Skogland 1985, Coulson et al. 2000) acting partly through food limitation, but also maternal care (Loison et al. 2004) and cohort effect (Gaillard et al. 2003), play an important role in the complex dynamic of herbivore populations.

In addition to be regulated by these factors, herbivores can strongly impact them (resource availability, predators and competitors) through feedback effects.
As herbivores as a whole consume over 20% of the annual net primary productivity (Agrawal 2011 and references therein, different rates of damage by large herbivores in forest for specific trees in Danell et al. 2006) and have a strong impact on the biomass of primary producers in terrestrial and marine ecosystems (Poore et al. 2012), they play a major role in the regulation of plant diversity and ecosystem functioning (Olff & Ritchie 1998, Belovsky & Slade 2000). However, predicting the consequences of herbivores on plant diversity is difficult as studies lead to contrasting results, ranging from positive (Belsky 1992, Collins et al. 1998) to negative (Milchunas et al. 1998, Wardle et al. 2001) or neutral effects (Stohlgren et al. 1999, Adler et al. 2005). Indeed, different parameters are involved in the impact of large herbivores: feeding ecology (grazer/browser gradient), intensity of herbivory pressure and type of physical disturbance (e.g. trampling, rooting behavior, fraying with antler, Latham 1999). Besides, site characteristics such as differences in productivity among locations (Bakker et al. 2006) and the evolutionary history of grazing (Milchunas et al. 1988) also modulate the response. For example, grazer herbivores, supposed to be less selective, should impact dominant species (Milchunas & Lauenroth 1993) and increase plant diversity at higher productivity but decrease diversity at low productivity (Bakker et al. 2006). This is the case of the bison (*Bos bison*), which maintains plant diversity in productive systems in meadows of North-America (Collins et al. 1998). Moreover, the predictions are not straightforward as the relationship between

herbivory pressure and plant diversity is non-linear. A moderate grazing pressure has been shown to enhance a higher plant diversity than a high and low grazing (Hobbs & Huenneke 1996, Rooney & Waller 2003, Boulangeat et al. 2014, Ganjurjav et al. 2015), which corresponds to the intermediate disturbance hypothesis explaining plant diversity (Connell 1978) even if the theoretical base of this hypothesis has been questioned recently (Violle et al. 2010).

By their grazing and browsing action, herbivores are a useful tool for landscape management. They can speed up or slow down successional processes, for example by maintaining open habitats and preventing forest establishment (Menoni et al. 2008), or enhancing the forest settlement by promoting the selective grazing on particular plant species (Prins 1998). Hence, domestic herds can be used to restore brushwoods grasslands (Garnier & Navas 2013) and avoid the closure of open areas. For example, in the Bauges Massif (our study site), cows are taken up to the Armene pasture in summer to prevent the spread of the green alder. A good adequacy between the knowledge of the functional structure of the vegetation and the grazing diet, is important for the success of operations of herbivore introduction (Dulphy 1995, Garnier & Navas 2013). Indeed, the introduction of a specific herbivore is not possible everywhere, especially because they can have negative effects on plant diversity in unproductive area (Bakker et al. 2006) or in area that are not used to recent strong grazing pressure (Milchunas et al. 1988).

Aside from the direct energy and material flows between plants and herbivores or predators and herbivores, herbivores also generate trophic cascade through vegetation changes on invertebrates (Martin et al. 2010), small mammals (Smit et al. 2001) or birds (Cardinal et al. 2012). For example, changes in density of forest cover through intensive browsing by white-tailed deer (*Odocoileus virginianus*) lead to the homogenization of songbird community (Cardinal et al. 2012). Herbivores also influence indirectly ecosystem processes such as alteration of nutrient cycles (Wardle et al. 2004, Garibaldi et al. 2007), influences on net primary production (Wardle et al. 2004), modification of abiotic disturbance such as fire (Hobbs 1996), promotion of ground-level light availability in grassland ecosystems through the removal of plant biomass of superior competitors (highest plant species) (Borer et al. 2014).

These effects can act at different spatial and temporal scales (Hobbs 1996) and have in turn different consequences. For example, herbivores can favor the development of grazing-resistant or avoided plant species which in turn reduces the diversity of the plant communities at the landscape-scale, whereas the nutrient release by faeces should improve nutrient cycling and increase the diversity at local-scales (Crawley 1997).

### b) Large herbivores in Europe

Rapid increases in large herbivore densities (Putman et al. 1996, Loison et al. 2003, Milner et al. 2006, Maillard et al. 2010, IUCN red list) observed during the last thirty years in Europe led to a large increase of studies on plant-ungulate relationships (Coulson 1999, Côté et al. 2004, Ward 2005, Shelton et al. 2014). In order to draw a parallel with the beginning of the introduction, we are going to specifically address the main factors involved in these variations of population densities. First, alpine and Nordic environments are specifically affected by lower

snowfall, early melting and decrease of vegetal productivity (Hamel et al. 2009). These warmer winters lead to the decrease of animal winter mortality while early springs reduce the critical period of search for food (Mysterud et al. 2007, Pettorelli et al. 2007). However, these consequences should be viewed cautiously, given that a same cause can have multiple, and sometimes opposite, consequences. Early springs are not necessarily beneficial for animals because of the asynchrony between the peak of vegetation biomass and the time to build up fat reserves (Pettorelli et al. 2007, Post et al. 2008). This mismatch in timing has also been demonstrated for herbivorous mammals and food plants (Inouye et al. 2000), parasitoids and their host insects (Van Nouhuys & Lei 2004) or insect pollinators with flowering plants (Visser et al. 2005, Harrington et al. 1999). Altitudinal displacement of tree line at the highest elevations, caused by climate warming and by changes in land use during the 20$^{th}$ century (Gehrig-Fasel et al. 2007), increases the availability of high quality vegetal biomass, which can be beneficial to sustain energetic requirements and improve fitness. In addition, the decrease of predator densities and the changes in the way population are managed (Apollonio et al. 2010), through hunting plans or species introduction, largely contribute to the increase of ungulate populations in France and Europe. This contrasts with the overall decline of large herbivores at the global scale (Hopcraft et al. 2010, Ripple et al. 2015). Luo et al. (2014) demonstrated that the modifications of environmental components used by ungulates, such as plant availability, resource quality or other habitat parameters, dramatically altered the spatial distribution of species on the Tibetan Plateau. Modelling results show that species could lose more than fifty percent of their habitat range but the percentage varies with species and location (18% of range contraction in African mammals, Thuiller et al. 2006), whereas some of them might occupy new distribution area through dispersal (Luo et al. 2014).

### c) The motives that lead us to initiate this work

Following the increase of large herbivore distribution area in Europe (Loison et al. 2003, Milner et al. 2006, Maillard et al. 2010, IUCN red list), spatial overlap among species – including domestic herds – increases and can lead to changes in intra- and inter-specific interactions. If competition appears or strengthens among individuals within a species and among species, individuals could suffer a decrease of fitness that could lead to changes in population dynamics. For example, this could be problematic for native species suffering the consequences of increasing densities of introduced species (Forsyth & Hickling 1998, Lovari et al. 2014, Ferretti et al. 2015). In parallel, their increase can also become an issue because of a stronger impact on composition, structure and dynamic of vegetal communities (Hobbs & Huenneke 1996), and can also lead to agricultural and forest damages. In order to better understand the structuration of herbivore community, the impact of large herbivores species on ecosystems and give cues to population and habitat management, it appeared necessary to determine how spatially co-occurring herbivores select and partition food at the intra- and inter-specific level. To achieve these goals, we focused our work on the large herbivore community of the Bauges Massif where four ungulate species can be found (chamois, roe deer, deer, mouflon) including one introduced species (mouflon in 1950s, Darmon et al. 2007).

## 2.2 Diet as a linkage between two trophic levels

### a) A matter of spatio-temporal scale

Diet of animals is the last step of a multiple spatio-temporal scale selection process (Johnson 1980, Senft et al. 1987). The selection by an animal requires that a resource be over or under-consumed relatively to its availability in the environment.
Species or populations occur within a distribution area (1st-order selection), where organisms define their home range (2nd-order selection) and select feeding station (vegetal communities) (3rd-order selection) in which they select item (4th-order selection) (fig.2). The selection at each scale impacts the selection at the lower-scale.

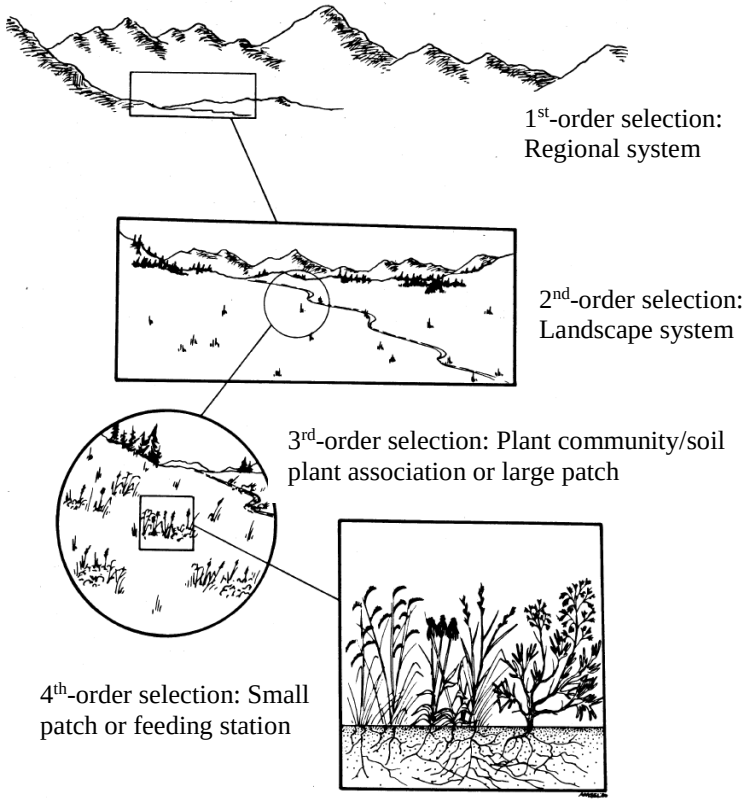



**Figure 2.** From Senft et al. (1987) and Johnson (1980), Ecological hierarchy of foraging by large herbivores.

Predicting the feeding behavior of animals have been and is still a major goal in ecology (Pyke et al. 1977). One of the most famous theories is the "optimal foraging theory" (OFT), firstly developed by McArthur & Pianka (1966) and then approached by several other authors (Pyke et al. 1977, Stephens & Krebs 1986). Originally stated for carnivores, it predicts that individuals should maximize their energy intake per time unit while minimizing the costs related to searching, handling and digestion processes. Ruminants have to make decisions on a gradient opposing two behavioral options: maximize the time of searching behavior to maximize energy gained ("energy maximizers") or minimize the time of searching and be less selective for forage in order to save time for other tasks ("time minimizers", Schoener 1971, Bergman et al. 2001,

Fortin et al. 2003). With this vision, trade-offs are consequently required between choosing an optimal diet (in terms of quantity and quality), and lose less energy as possible with displacements and forage handling, and allocate the right time to patches (Searle et al. 2005). Hence, this theory includes different scales of selection and different constraints related to the animal features (temporal, energetic and cognitive). This theory of optimal foraging has been described in an environment where the only constraint was the heterogeneous distribution of resources. However, abiotic and biotic constraints such as inter- and intra-specific interactions, predation (Grignolio et al. 2007, McAthur et al. 2014) and disturbance are known to play a major in the animal decisions. Moreover, this principle may not be specific to energy but also to other plant biomechanico-physico-chemical characteristics (Chapin et al. 1980, Massey et al. 2009, Dostaler et al. 2011, Zweifel-Schielly et al. 2012). Hence, these additional factors should be included in the analyses to better fit to the "real" environment encountered by species.

The temporal variability in the diet selection is also an important aspect of ecological interactions (Wam et al. 2010), as the quality and quantity of forage fluctuates over the year (Duncan 2005). In addition to habitat changes, herbivores are also constrained by their annual cycle and their concurrent requirements (Kaske & Growth 1997) during gestation, lactation, growth of young or fat storage before winter. For example, some studies suggest that herbivores should select proteins in summer for body growth and digestible carbohydrates in winter when the thermoregulation is high (Berteaux et al. 1998, Dostaler et al. 2011).

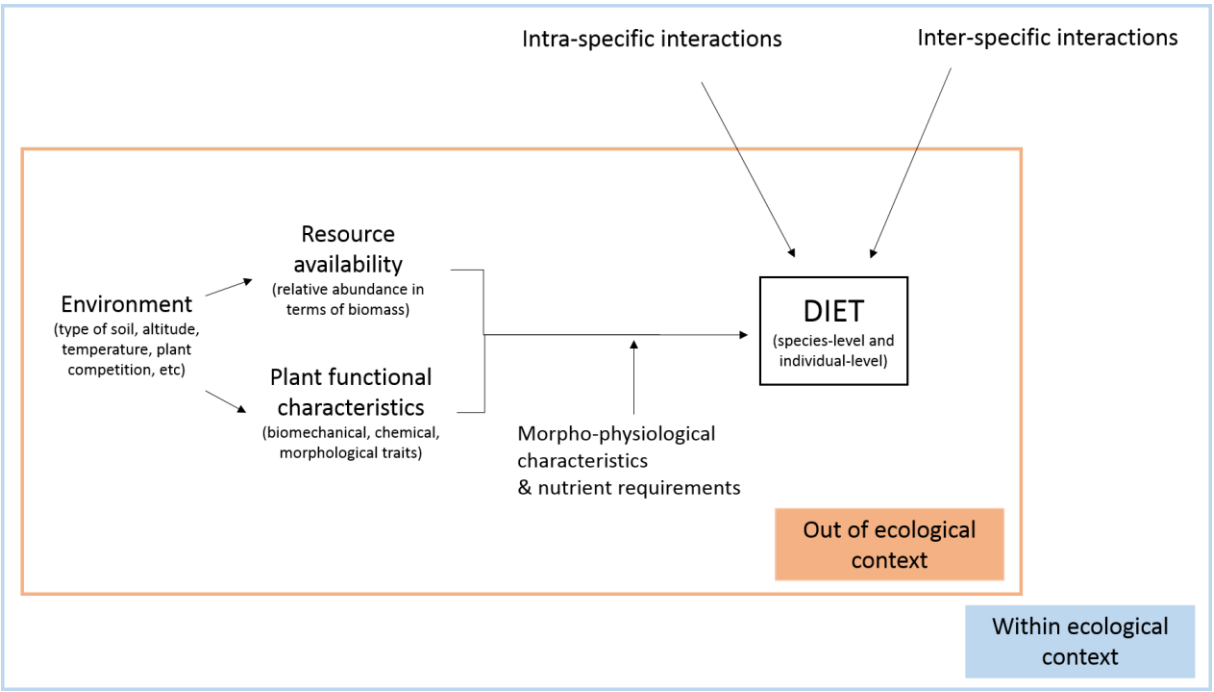



**Figure 3.** Illustration of the factors influencing the diet of animals.

In this work, we focused mainly on the item scale, and we also used information of available resources at the scale of vegetal communities when we analyzed the scale of diet selection. Habitat selection at broader scale was the topic of Gaëlle Darmon's PhD (Darmon 2007) on the same study site, and is currently pursued by Antoine Duparc for his PhD. While other factors

such as animal characteristics (morphological, physiological, cognitive and social traits), the permanent presence of predators, the knowledge animals have gained about their previous diet (Provenza 1995) and the precise spatial distribution, organization and accessibility (Dumont & Petit 1998) of plants are important in the decisions of individuals to choose where to forage, we focused on local foraging decisions and local characteristics of plant abundance and traits that influence those decisions (fig.3). We also incorporated the temporal dynamic in our analyses.

In the following paragraphs, I will specifically describe the factors involved in plant assemblages in individual diet (fig.3).

### b) Animal morpho-physiological characteristics and nutrient requirements

Herbivores have to face the paradox of living in a green world (box 3) without having the morpho-physiological characteristics enabling them to feed on all available plant species (Hofmann 1989, Polis 1999, Clauss et al. 2003b). In general, plants are low in nutritional value and hard to digest, because of the tough fibrous thick cell wall (lignin, cellulose, hemicellulose) and/or toxicity due to secondary compounds. Herbivores differ in their ability to cope with these constraints. They are therefore exposed to exert a trade-off between nutrient requirements and digestive constraints, in order for them to meet the nutritional demands required for growth, survival and reproduction (Hanley 1997).

In this work, we will focus on the group of the ungulates belonging to the large herbivores (box 2) (Asher & Helgen 2010). Ungulates represent a highly diversified guild. The highest diversity is found in Africa (Fritz 1994, Fritz et al. 2002) and has been widely studied in community ecology (duToit & Cumming 1999, Fritz & Loison 2006). Especially, we will work on the foregut fermenters, more commonly referred to ruminants (Artiodactyles). Contrary to hindgut fermenters (e.g. horses, rhinoceros, micro-mammals, rabbits) for which the digestive process is monogastric (one stomach chamber) and where the microbial fermentation happens in the large intestine and caecum, cellulose digestion of ruminant diet (foregut fermenters) through microbial fermentation occurs in the reticulo-rumen composed of four stomach chambers. This physiology gives them digestive and nutrient absorption advantages as micro-organisms in the rumen of foregut fermenters can break down the plant material before it arrives in the small intestine where nutrients are absorbed (Feldhamer 2007). On the contrary, food goes directly to small intestine in hindgut fermenters, without being well degraded, and these animals consequently have to forage in large quantities. In environments where the quantity of food is limited, ruminants are more adapted as they have a more efficient digestive system (Feldhamer 2007).

**Box 2 –** What is an herbivore?

An herbivore is an animal that gets its energy from eating plants. Herbivores can be found in different classes such as mollusks, insects, birds, reptiles, fish, marsupials and mammals (Hopcraft et al. 2010). Herbivores can be divided (artificially) into two groups relatively to their size. Large herbivores are defined as having a body weight > 2kg (Fritz & Loison 2006), which includes mostly ungulates and marsupials, but also some large rodents or birds.

Ruminant species differ on many aspects: the size, musculature and composition of their rumen, the size of the salivary glands, the type of salivary binding proteins, the size and strength of their jaw, their body size (Hofmann 1989, Clauss et al. 2008a). All these morpho-physiological characteristics play a role in the demarcation of their feeding niche, which explains how they coexist on the dietary axis and share resources. For example, the two most employed ruminant classification ranks them on two gradients: the first one describes the resource they use (grazer/browser, Hofmann 1989, fig.4), the second one describes the morpho-physiology of their rumen (moose-type/cattle-type, Clauss et al. 2003b). Browsers, such as roe deer, are selective animals feeding mainly on lignified plant organs (fruits, seeds, buds) and forbs and are able to deal with toxic component and anti-nutrients. Their rumen ("moose-type") is less adapted than the "cattle-type" (see hereafter) to digest fiber (Clauss et al. 2010b). Their salivary glands contain certain proteins able to bind with tannins. It allows the animals that feed on highly nutrient rich plants having high anti-herbivore deterrents (high polyphenol content as tannins) to digest the plant crude protein (measured as nitrogen content, CP=6.25*nitrogen content), without they form indigestible complexes with tannins released from vacuole during chewing (Verheyden et al. 2011). Grazers, such as mouflon, have a diet rich in grasses (Hofmann 1989), and are supposed to favor quantity rather than quality. The high capacity and strong musculature of their rumen ("cattle-type") allow them to overcome the physically resistant diet by being efficient to digest fiber and cell wall (Clauss et al. 2008a, 2010). The size of their salivary gland is smaller than the ones of browsers suggesting a smaller need for the synthesis of salivary tannin-binding protein and a lower ability to digest lignified plant rich in tannins. This is in agreement with the low content of tannins in grass species (Shipley 1999). (This is discussed in the Note). Finally the intermediate feeders, such as chamois, are able to adapt their strategy between the two extremes of the gradient. This diversity in diet categories and diet morpho-physiologies may contribute to the diversity and coexistence of large herbivores.

This simplified vision of ruminant classification could lead us to think that the cattle-type has to adopt a grazer behavior. However, this is not always the case, as it has been demonstrated for the mouflon (Marchand et al. 2013).

The evolution of ruminants can be related to the type of habitat in which they occured. Paleontological studies showed that the main driver of the speciation of new feeding type would have been the increase of the availability of open habitats (Perèz-Barberia et al. 2001). The oldest common ancestor of large herbivore reported so far is a browser inhabiting the forests of the middle of Oligocene era (-34 to -23 millions of years) (Perèz-Barberia et al. 2001). Emergence of meadows inside forests after climate warming events would have opened the way to the evolution of intermediate feeders, then grazers (Perèz-Barberia et al. 2001).

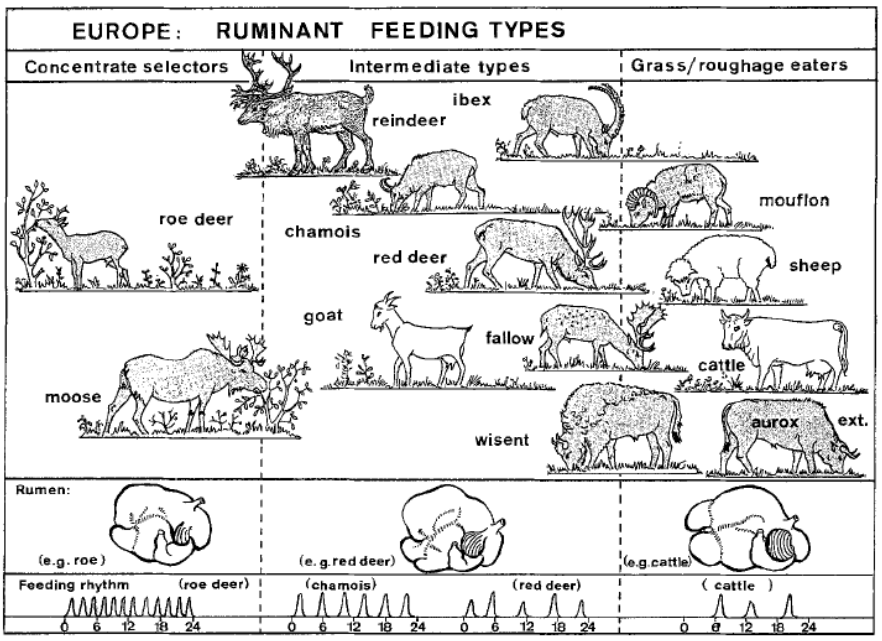



**Figure 4.** From Hofmann (1989), classification of large herbivores according to their feeding type and rumen morpho-physiology, and their consequences on activity rhythm.

As evoked hereinabove, herbivores also differ in the number of plant species they are able to ingest. They are ranked on a gradient going from generalists, which include many plant species in their diet, to specialists, which only feed on a few plant species (Freeland 1991, Shipley et al. 2009). The low number of specialists species among mammals has been suggested by two hypotheses: one plant cannot provide all the required nutrients ("nutrient constraint hypothesis") and the detoxification system of mammalian herbivores cannot detoxify a high concentration of secondary compounds present in a single plant species ("detoxification limitation hypothesis") (Dearing et al. 2000). A distinction between "obligate" and "facultative" specialists or generalists allows to differentiate the cases where specialization is due to an evolutionary process and is the result of behavioral and/or morphological adaptation ("obligate"), or if specialization is the result of local availability of resources, and the time and spatial temporal scale used ("facultative") (Shipley et al. 2009). Those variations in the dietary specialization play a role in the explanation of the dietary diversity in herbivores and hence their ability to coexist.

In addition to be determined by the morpho-physiological characteristics, we stated that diets of animals were also constrained by their nutrient requirements. The nutrient requirement depends mainly on the body size of animals. Indeed, the metabolic rates are higher for a small herbivore than for a bigger one (Demment & Van Soest 1985, White & Seymour 2003). Consequently, small herbivores have to focus on diets of high quality in order to quickly reach their needs. On the contrary, larger herbivores can use forage of lower quality (Demment and Van Soest 1985) due to their reduced relative energy requirements and their longer passage rates and therefore thorough digestion (Clauss et al. 2003a). To compensate the low quality forage that is low in nutritional value, they ingest larger quantity of food thanks to their larger gut size. Many authors tried to relate the body size directly with diet. However, conclusions are

diverse and still debated (McArthur 2014). Whereas some studies find a relationship between body size and percentage of grass intake (Jarman 1974, Demment & Van Soest 1985, Gagnon & Chew 2000), others contradict these predictions (Hofmann 1989, Mysterud et al. 2001, Codron et al. 2007d). Conclusions of several studies stress the need to realize further tests on relationships between plant physical properties and functional morphology (Codron et al. 2007d) or include other animals factors such as the intake and pre-digestion food processing (Steuer et al. 2014).

The trade-off between morpho-physiological constraints and nutrient requirements is consequently reflected by a trade-off between resource quantity and quality. Although spatially co-occuring herbivores rely on the same food resources, they can face different digestive and metabolic constraints that lead them to differentially use the resources, and allow the coexistence. We describe hereafter the importance of resource availability, plant functional characteristics and ecological factors (intra- and inter-specific interactions) in the diet selection by herbivores.

c) Resource availability (relative abundance in terms of biomass)

According to the optimal foraging theory (OFT) described hereinabove, the morpho-physiological constraints and the nutrient requirements, animals have to exert a trade-off between forage quantity and quality. Given that the most nutritious plants tend to be the least available (Hansen et al. 2009), preferences for plants of high quality requires more searching time. In turn, lower energy is needed for the digestive process. On the contrary, a focus on quantity does not require to spend energy for searching plant but requires energy to digest high biomass plants of low quality plant.

In order to determine how large herbivores compose their diet, we need to define to which extent food availability influences the choice. Here, we define plant availability as the relative abundance of the plant in terms of biomass. If herbivores favor quantity rather than quality, we should observe a linear relationship between the relative abundance of a plant in the diet and the relative abundance of that plant in terms of biomass in the environment.

Few studies examine the response of free-ranging large herbivores to available relative abundance of plants in terms of biomass (Alm et al. 2002, Forsyth et al. 2005). Forsyth et al. (2005) showed that deer avoided the most abundant species, and preferred the low abundances in terms of relative biomass. Other exploration has been done with experimental trials on sheep (Parsons 1994) but conclusions were not straightforward as many other factors other than resource availability (in terms of percentage of covered area) influenced the selection (e.g. plant composition, previous diet, preferences).

To our knowledge, most of the studies that reported resource availability to be important in the field focused on the patch-scale or higher scale to explain the spatial distribution of animals (Bergman et al. 2001, Fortin et al. 2003, Fryxell et al. 2004, Zweifel-Schielly et al. 2009, van Beest et al. 2010). A multi-scale approach has been used in Bee et al. (2008) where they showed that red deer selected vegetation patches according to the patch quality, and then selected plant species within this patch. However, no studies clearly reported the multi-scale approach of diet selection in the field by taking into account the real relative abundance of plants in terms of biomass and not the general quality of the patches. Consequently, we are not aware of the

importance of the relative abundance of a plant in the field on the diet selection process at a fine-scale (i.e. selection of an item within the feeding patch), and if the strength of the selection is the same as at a coarse-scale (i.e. selection of a patch within seasonal home range). We could assume different alternative hypotheses: (1) animals exert a strong selection for a patch where the preferred resources are highly abundant and then feed on these plants according to their relative abundance within the patch (no selection within the patch), (2) animals select a patch according to other characteristics (e.g. safety, temperature) and are highly selective for the preferred resources within the patch. This requires that the food availability is heterogeneously distributed. In the case of a homogeneous distribution of the resource, (3) no selection would be observed for a patch, but animals could be selective within the patch for preferred plant species. This question has been approached by Dupeyras (2014) and is detailed in the *Summary of results*. Additional information about the selectivity of chamois and mouflon are given in paper II and III.

d) Plant functional characteristics

If herbivore do not forage according to resource availability, then a selection could be observed for certain plant species. For there to be a selection, it requires that plant species differ in their physical, chemical and nutritional characteristics, thereafter affecting their handling and processing rate by herbivores (Pyke et al. 1977). Therefore, the functional approach is the appropriated way to define the diet selection criteria through chemical, biomechanical or physical functional traits (the usefulness of the functional approach has been described in the chapter I). Nowadays, standardized protocols are available to allow robust comparisons among studies (Cornelissen et al. 2003, Perez-Harguindeguy et al. 2013) and we used them for our measurements.

Animals must acquire five major nutritional categories from their environment in order for them to grow, survive and reproduce (Robbins 1983). These are energy, protein, water, minerals and vitamins (Robbins 1983). Proteins are important constituents of the animal body (e.g. cell wall components, enzymes, hormones, lipoproteins in fat transport, antibodies) and are involved in the growth and reproduction of individuals (Robbins 1983). Their content in plants is measured through their nitrogen content. When multiplied by 6.25, the nitrogen content is termed the crude protein content (Robbins 1983). The other most limiting nutrients for herbivores are digestible energy which plays role in metabolism and thermoregulation (Robbins 1983).
As animals need to reach a certain threshold of energetic requirements for growth and reproduction (Robbins 1983), it is easily conceivable that the selection should go toward plant traits that satisfy their metabolic requirements. For example, 14-18% of crude protein has been estimated as the optimum percentage for maximum body growth of deer (French et al. 1956, Magruder et al. 1957). For the female elk, 8% of crude protein were required (Van Soest 1994, Cook 2002).
However, plants constrain the use of all these optimal resources by defensive traits usually deterrent for herbivores (box 3), such as lignin which reduces cell wall digestibility or secondary plant compounds that can be toxic (terpenoids, alkaloids, phenolics compounds, Robbins 1983, Tixier et al. 1997, Sauvé & Côté 2007). Hence, large herbivores have to cope

with different plant defense strategies and make a trade-off between nutrient intake and plant defenses to get a balanced diet that satisfy their nutritional needs.
Other chemical traits, such as the alkaloid content in plants synthetized by endophytic fungi can also strongly impact the large herbivore growth and survival through intoxication (Vicari & Bazely 1993, Miles et al. 1998). To our knowledge, those traits have never been tested in dietary choices experiments, but only studied because of their impact on animals (Miles et al. 1998).

**Box 3** – Green World Hypothesis (Hairston et al. 1960) and "Plant Self-Defense Hypothesis" (Terborgh 2005)
Herbivores encounter a large plant diversity but are not able to feed on all the green biomass available. The "Green world hypothesis" (Hairston et al. 1960) states that herbivores are mainly kept in check by predators, parasites or pathogens ("top-down" control), preventing herbivores to strongly damage the vegetation. At the same time, the discovery of plant secondary compounds (such as phenolics, alkaloids, terpenoids, glycosides) led to the statement that such costly investments were involved in anti-herbivore defenses. The "Plant Self-Defense Hypothesis" (Terborgh 2005) consequently argues that the world is green also because plant can defend themselves against herbivores through chemical or physical (spines, thorns) properties ("bottom-up" control).

While we explained that herbivores should achieve a trade-off between plant quantity and quality, the diet selection could also be influenced by other factors such as the plant visibility, the mechanical ease of plant harvesting and chewing, and the plant digestibility.
First, visibility can be measured through plant height; size, number and color of inflorescence; and plant spatial distribution. The hypothesis that visibility could play a role in diet selection led us to include these plant functional trait measurements in our field work (see *Material & Methods*). Few studies have been realized on the importance of inflorescence in diet selection criteria by large herbivores. The ones that we found focused on specific plant species (Gomez et al. 2000, Gomez et al. 2003): ibex (*Capra pyreneica*) tend to prefer *E. mediohispanicum* with a higher number of flowers (Gomez et al. 2003). However, there seems to be a lack of work on larger plant communities. In addition, we hypothesized that herbivores could be more attracted by plants with large colorful flowers as they could be easier to spot. Finally, we suggested that the distribution of plants measured by their sociality could favor the forage intake of some plant species: as individuals of social plants are grouped, they could be more easily visible and attract animals.
Secondly, we hypothesized that the mechanical ease of plant harvesting and chewing, and the plant digestibility could influence the diet selection. These three mechanisms are mainly influenced by plant fiber composition (Searle & Shipley 2008). Because grasses contain higher fiber content than non-woody browses, herbivores should spend longer time chewing and digesting a unit mass of grass than non-woody browse (Choong et al. 1992). Similarly, even though ruminants are able to extract energy from plant cell wall composed of cellulose and hemicellulose, thanks to the microbial fermentation in the rumen (Hofmann 1989), highly fibrous plant reduce digestibility. In turn, this can influence the food retention time in the gastro-intestinal tract and the plant intake rate (Hanley 1982). Species could have evolved to maximize energy intake in order to allow a higher level of metabolism which gives competitive

advantages (McNab 2006). As energy maximization is permitted by a high food intake or a high digestive efficiency (Clauss et al. 2010b), the estimation of digestibility of eaten plants could give information about the animal digestive strategy. During this work we used biomechanical traits (tensile strength and leaf punch toughness) as proxy of leaf harvesting and chewing. These biomechanical traits could actually also be involved in the assessment of plant quality as a plant easy to harvest, chew and digest would allow the herbivore saving energy and would allow an easier nutrient release from vacuole valuable for animals.
Finally, the question of digestibility could be explored at the plant-level and not only at the leaf-level, as previously described. The percentage of dry weight twig/leave on total dry weight could also play a role in diet selection if the animal is not able to feed on the most nutritious and digestible part of a plant, i.e. the leaves. For instance, the proportion of green leaf in the sward can modify the intake of green leaf by wildebeest and topi in Africa: the higher the proportion of green leaves in sward, the higher the proportion of green leaves in diet and the higher the intake rate (Murray & Illius 2000). Consequently, we suggested that a plant allocating more energy to the leaves instead of twigs could be preferred as digestibility of the whole plant would be increased.

The questions of diet selection have been experimentally approached with insects (Coley 1983, Choong 1996, Perez-Harguindeguy et al. 2003, Ibanez et al. 2013a) and some large herbivores (white-tailed deer *Odocoileus virginianus*: Berteaux et al. 1998, Sauvé & Côté 2005, Dostaler et al. 2011; roe deer *Capreolus capreolus*: Verheyden-Tixier & Duncan 2000, Pollock et al. 2007; fallow deer *Dama dama*: Bergvall & Leimar 2005; domestic sheep: Cingolani et al. 2004, Massey et al. 2009, table 1) for which experimental studies allow a controlled design of traits. However the mechanisms of diet selection are poorly understood for wild animals as it requires precise information on diet, on resource availability and a large plant traits database. The studies that tried to demonstrate the factors influencing the herbivore choices in the field dealt with red deer (Forsyth et al. 2005, Verheyden-Tixier et al. 2008, Lloyd et al. 2010, Zweifel-Schielly et al. 2012) or eland (Watson & Owen-Smith 2002). Often, previous works focused on either chemical or physical plant functional traits such as protein and carbohydrates (Chapin 1980, Berteaux et al. 1998), silica (McNaughton et al. 1985, Massey 2009), spines and thorns (Cooper & Owen-Smith 1986) or plant secondary compounds (Bergvall & Laimar 2005). In other cases, a higher number traits were combined, but they were analyzed separately in order to determine which ones were positively or negatively correlated the plant eaten (Tixier et al. 1997, Forsyth et al. 2005, Zweifel-Schielly 2012). However, plant functional traits measured are generally correlated, which might confound the relationship of an independent variable on the response variable (Smith et al. 2009, Ray-Mukherjee et al. 2014, Ruffell et al. 2015). In order to identify the direct and indirect effects of plant functional traits in the process of diet selection, we tested the validity of causal models relating plant functional traits and diet using path analysis (Wright 1921). Especially, we tested whether biomechanical (plant toughness) and chemical (LDMC, nitrogen content) had direct or indirect effects on diet selection, and whether the selection varied between season and species (chamois and mouflon in spring, summer and autumn). Hypothesis, results and discussion are described in the paper III, and in the *Summary of Results*.

Today, the understanding of regional-scale ecosystem functioning is becoming an important topic in ecology (Sutherland et al. 2013). However, large scale studies lack extensive and intensive data that would allow to unify the different parts of ecosystems at different temporal and spatial scales. Hence, it appears necessary to find cost-effective, rapid and accurate methods to deal with plant functional trait measurements with large sample size. For the study of plant nutrient stoichiometry, the NIRS method appears as one of the promising methodology to overcome the problems stated above: (1) time and cost efficient method which overcomes the wet chemistry methodological limitations, (2) the ability to cope with large sample size that would allow the increase of the sampling scale of studies. However, it remains to test the transferability of the method in different geographical regions and for different plant nutrients. This question is studied in the *Summary of Results* and in the *Synthesis, perspectives and directions*.

**Table 1.** Review of selected traits by wild and domestic large herbivores (SLA: Specific Leaf Area). Asterisks "*" refer to the traits measured in the thesis. Their measurement method is described in chapter IV, section *4.2*.

| | References | Herbivore | Selection toward… | Others traits tested |
|---|---|---|---|---|
| Plant structure * | Holt et al. 1994 | Not specified | The superior resource competitor for height | |
| | Cingolani et al. 2004 | Domestic sheep | Short plants | Height, SLA, leaf toughness |
| | Pollock et al. 2007 | Red deer and goats (experimental trial) | Large leaf area for both species, leaves with low stem strength and low divaricating index | Divarication index, leaf area, stem strength, stem phenolics |
| Nutrient*, sugar and silica content | Tixier et al. 1997 | Roe deer | Plants rich in soluble sugars | Digestibility, nitrogen, fiber, soluble sugars and silica content, phenolic and terpenes compounds |
| | Berteaux et al. 1998 | White-tailed deer (experimental trial) | Diets high in energy and low in protein in winter | |
| | Massey et al. 2009 | Domestic sheep (experimental trial) | Plants with low silica content | |
| | Verheyden-Tixier et al. 2008 | Red deer | Diet rich in soluble sugars | Nitrogen, fiber and tannins content |
| | Dostaler et al. 2011 | White-tailed deer (experimental trial) | Plant rich in nitrogen in summer and autumn | Fiber content, nitrogen content, dry matter digestibility (DMD) |
| | Zweifel-Schielly et al. 2012 | Red deer | Plants rich in protein and with a high protein:fiber ratio in summer | Water, protein, tannins, fiber, content |

| | | | | |
|---|---|---|---|---|
| Fiber content | Tixier et al. 1997 | Roe deer | Plant low in fiber content | Digestibility, nitrogen, fiber, soluble sugars and silica content, phenolic and terpenes compounds |
| | Watson & Owen-Smith 2002 | Eland | Diet low in fiber content | Nitrogen, phosphorus, calcium, magnesium, potassium, fiber, total phenols, condensed tannins content |
| | Forsyth et al. 2005 | Red deer | Plants with low fiber content in winter (hemicellulosis, cellulosis, lignin) | Nitrogen, phosphorus, fiber, condensed tannins content, SLA |
| | Sauvé & Côté 2005 | White-tailed deer (experimental trial) | Plants with low fiber content in winter | Crude proteins, total phenols, fiber, condensed tannins content |
| | Zweifel-Schielly et al. 2012 | Red deer | Plants with low fiber content in spring and summer | Water, protein, tannins, fiber, content |
| Specific leaf area | Lloyd et al. 2010 | Red deer and domestic sheep (experimental trial) | Deer: Plants with high SLA<br>Sheep: Leaf tensile strength, SLA, leaf nitrogen content | SLA, tensile strength, nitrogen and phosphorus content, mass of phytoliths (as a proportion of leaf dry weight) |
| | Mkhize et al. 2014 | Goat (experimental trial) | Plants with broad-leaves with long shoot and no spines | Broad/fine leaves, long/short shoot, nitrogen, condensed tannins, fiber content |
| Leaf toughness* | Cingolani et al. 2004 | Domestic sheep | Plants with high toughness until 5 N $mm^{-1}$ and then selectivity decreases with low toughness | Height, SLA, leaf toughness |
| Secondary compounds | Bergvall & Leimar 2005 | Fallow deer (experimental trial) | Food of low tannins concentrations | |
| | Sauvé & Côté 2005 | White-tailed deer (experimental trial) | Plants with low condensed tannins content in winter | Crude proteins, fiber, total phenols |

e) Inter-specific interactions

Positive, negative or neutral interactions for a resource can occur between large herbivores. These ecological factors can modify the niches of animals and in turn structure the community. We describe them hereafter. As we work on the feeding axis of the niche, we will limit our examples and explanations to the food resources. However, resources can also be spatial, temporal or climatic for example.

Positive interactions describe the positive effect of a species on another. For example, grazing facilitation has been observed in different cases: among wild large herbivores (Wegge et al. 2006, Waldram et al. 2008), and between wild and domestic ungulates (Gordon 1988, Hobbs et al. 1996, Odadi et al. 2011). Facilitation can occur through increased resource access or quality of resources (Arsenault & Owen-Smith 2002). For example, the grazing by cattle during winter on the Isle of Rum enhances the proportion of green biomass, and these areas are preferred by the red deer in the following spring (Gordon 1988). Also, the presence of white rhinoceros (*Ceratotherium simum*), by its grazing activity on tall grass swards, facilitated the short grass grazers (impala, wildebeest, zebra) use (Waldram et al. 2008).

Negative interactions describe the adverse effect of a species on another. The competition is considered as the major driver of herbivore community organization (Schoener 1983, Gordon & Illius 1989, Arsenault & Owen-Smith 2002). If two species spatially co-occurring are competing for a limiting resource, the subordinate species would be excluded, as the niches of two species cannot completely overlap (except if resource is abundant enough). This has been called the "principle of competitive exclusion" (Gause 1934, Hardin 1960). Competition can be direct, called "interference", when the presence of one species directly impacts the behavior of another species through visual or acoustic disturbance. For example, a species (chamois) can shift its feeding station if a superior competitor (mouflon) is present (Chirichella et al. 2013). Herbivores can also compete at the patch scale indirectly through changes in plant composition and diversity ("exploitation") (Huntly 1991, Murray 2000). As we are interested in the item scale, competition can occur at the plant species or plant part level. By pre-empting the resources of another species, the superior competitor can lead native animals to feed on lower quality resources (Jenkins and Wright 1987), to spend more time searching for food (Kie et al. 1991), or to decrease the quantity of resources ingested (lower food intake rate, Lovari et al. 2014). This could result in changes in individual growth, survival and reproduction (Lindström et al. 1999, Lummaa et al. 2002, Pettorelli et al. 2007, Richard et al. 2010, Ferretti et al. 2015) which in turn could influence the population dynamics of the inferior competitor (Sinclair & Norton-Griffiths 1982, Forsyth & Hickling 1998, Forsyth 2000, Mishra 2004).

Positive and negative interactions can therefore modify the dietary niche of a species through niche expansion/shrinkage or niche displacement (fig.5). Even if competition occur on the dietary axis, the shift can also be done on others axis of the ecological niche (temporal or spatial) and enhance coexistence. On the opposite, competition can also make disappear the inferior competitor through a degradation of fitness.

Neutral interactions can also occur when the resource is not limiting and/or because species niche are partitioned on other unmeasured variable. For example, two species can feed on the same plant species but at different period of the day, and consequently prevent interference competition. Darmon et al. (2012, 2014) demonstrated that mouflon had no effects on the spatial

and temporal (activity rhythm) niche axis of the chamois, in the Bauges Massif. Indeed, as daily activity rhythm were species-specific, mouflon had no behavioral interference on chamois. Besides, even if chamois and mouflon were occupying the same pasture in spring, they choose different plant communities (preference for the meadows dominated by *Carex furruginea* for mouflon, and fallen rocks and meadows dominated by *Sesleria* and *Carex sempervirens* for chamois). However, even though mouflon did not have negative impact on the spatial and temporal niche of the chamois, we could hypothesize an exploitative competition for forage between both ungulates, as they both track high quality food. The coexistence on the dietary niche axis is explored in the paper II.

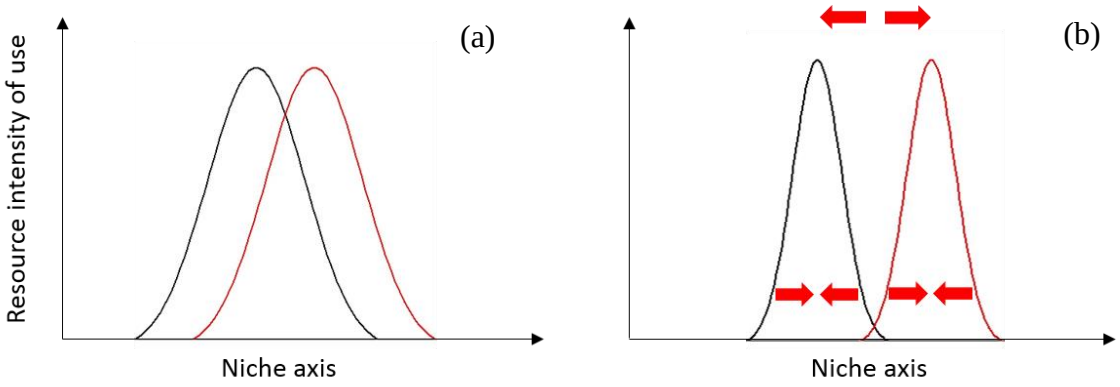



**Figure 5.** Illustration of the niche dynamics. (a) Overlap of the niches. If resource is not limiting, species can coexist. If resource is limiting, inter-specific competition can be enhanced. (b) To avoid competition, species niche of one or both species can narrow and/or shift.

The overlap measurement has been widely used to measure the strength of inter-specific interactions (Jenkins & Wright 1987, Homolka 1993, Singer & Norland 1994, Bertolino et al. 2009). However, a high overlap of resources can indicate competition if resource is limiting, or an absence of competition if resource is abundant enough. The best option to quantify the strength of interactions is to compare the studied population in two conditions between two similar environments: a site where it lives in allopatry or in sympatry with another species. This setting has been used in the paper II for the study of the impact of mouflon on chamois.

f) Downscaling the study of diet at the individual-level

All the previous description has been done at the species-level to explain how different factors can influence the diets of species, as the inter-specific differences on the dietary axis of the ecological niche play a major role in the species assemblage in a community. The same factors can impact the diets of individuals within a species. This statement implies that individuals can potentially differ one from another. Indeed, Charles Darwin, in its publication of *On the Origin of Species* (Darwin 1859), already stated that a high variability could be observed among the individuals of a species and it was its one requirement for the natural selection to occur. The study of the diet of each individual in itself is not particularly relevant. However, studying the degree of among-individual variability and how it varies with intrinsic species characteristics (e.g. body mass, sex, experience) or ecological factors (such as intra- and inter-specific

interactions, environmental heterogeneity) can help to improve our understanding of population dynamic and community structure (Hughes et al. 2008). The interest of including individual specialization in community ecology studies is explained in the chapter I. In this section, I describe how the factors of fig.3 influence among-individual variation (see Araujo et al. 2011 for a complete review on the ecological causes of individual specialization).

Bolnick et al. (2003) have brought up to date the inter-individual differences by defining the "individual specialization" (mainly called among-individual variability or among-individual variation in this work). It can be measured with different indices (Bolnick et al. 2002) and define an individual as a specialist if its niche is narrower than the population niche for reasons not attributable to the sex, age, or discrete morpho-physiological characteristics (fig.6, Bolnick et al. 2003). This definition was used to exclude obvious classifications such as in case of sexual dimorphism (Layman et al. 2015). However recent studies (Smith et al. 2015, Snowberg et al. 2015) showed that subtle differences between sexes or morphological variants can be important determinants of individual specialization (Layman et al. 2015). Hence, different hypotheses arose in the literature to explain the drivers of diet variability and feeding strategy among individuals.

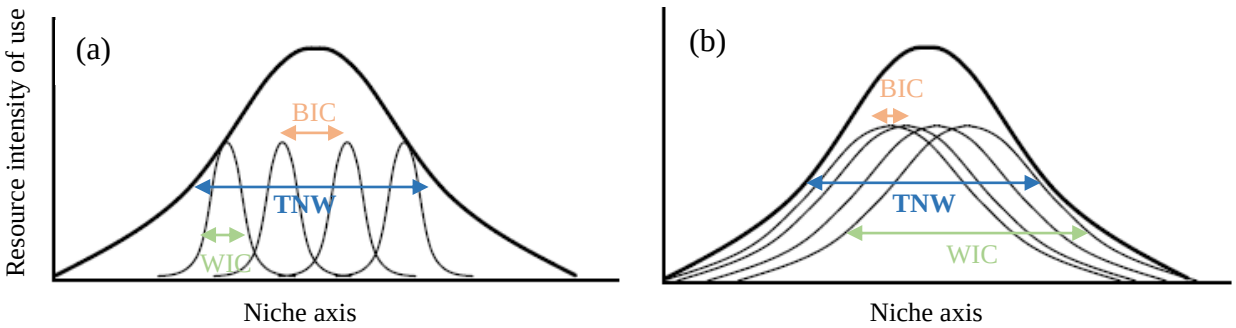



**Figure 6.** Illustration of two possible degree of among-individual variation with (a) strong among-individual variation, (b) low among-individual variability (inspired from Bolnick et al. 2003). TNW, WIC and BIC are described in the box 4 hereafter.

**Box 4 –** Parallel with α, β and γ-diversity

The total niche width (TNW) of a population or a species refers to the γ-diversity as it measures the diversity of the diet of the mean population. The within individual component (WIC) refers to the α-diversity as it measures the average of individual niche widths, calculated as the diversity of the diet of each individual. The between individual component (BIC) refers to the β-diversity as it measures the variance in mean resource use among individuals (Bolnick et al. 2003). (TNW=WIC+BIC, fig.6)

Intrinsic factors such as body mass (Luna et al. 2013), sex (Smith et al. 2015), reproductive status (Belovsky & Jordan 1978), age (Dostaler et al. 2011), physio-morphology (Snowberg et al. 2015), experience (Provenza et al. 2003) or dominance (Goss-Custard & Durell 1988) can influence the nutrient requirements of each individual and its ability to detect/handle/digest optimal resources. As described above at the species-level, these characteristics will lead

individuals to make a trade-off between resource availability (relative abundance in terms of biomass), plant quality, plant digestibility or plant visibility.

Ecological factors such as intra- and inter-specific interactions also influence among-individual variation (fig.7).

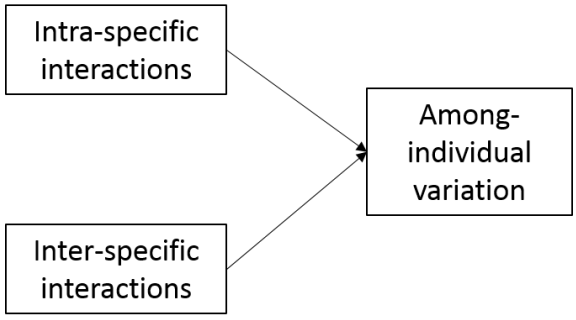



**Figure 7.** Ecological factors influencing among-individual variation.

The signs of the arrows are not straightforward to predict. In order to facilitate the reasoning, we will take the example of competition as the interaction. We will also limit the explanation to the case of exploitative competition. Indeed, other kind of competition such as interference competition can differently influence among-individual variation (Svanbäck et al. 2011).
The decrease of preferred resources caused by environmental changes, increase of population density or inter-specific competition can lead to different evolution of the degree of among-individual variation according to:

(1) The degree of among-individual variability at time $t_0$ (or pattern of rank-preference variation, see Araùjo et al. 2011): for instance, if the inter-individual variability is low and the few preferred resources become limiting, some individuals should shift on less-preferred resources which would increase among-individual variability (fig.3.a in Araùjo et al. 2011). On the contrary, if the among-individual variation is high, we can suggest that the decrease of preferred foods would lead the animals to converge on the same alternative available resources, and reduce among-individual variation (fig.3.b in Araùjo et al. 2011).
(2) The plasticity of feeding behavior of animals: individual niche width can be limited by an upper bound if functional trade-offs (cognitive or physical constraints) prevent the evolution of a generalist strategy (Taper & Case 1985, Wilson & Turelli 1986). In that case, if the preferred resources decrease, individuals cannot expand their niche width on sub-optimal resources but can only change their niche position which leads to the increase of among-individual variation (Araùjo et al. 2011). Hence, according to the ability of the animals to ingest a certain number of plant species and to its behavioral and morpho-physiological skills to shift on other resources, the among-individual variability will vary.
(3) The diversity and abundance of available resources: ecological opportunities (diversity and abundance of available resources) are supposed to influence among-individual variation, i.e. higher levels of ecological opportunity favor individual specialization (Roughdarden 1974, Araùjo & Gonzaga 2007, Gerardo Herrera et al. 2008).

Although the evolution of among-individual variation depends on all these factors, conclusions of several studies lead to two general patterns. The increase of (1) intra-specific competition induces among-individual variation through individuals using different subset of resources (Araùjo et al. 2008, Svanbäck et al. 2004, 2007), (2) inter-specific competition reduces niche width though the decrease of variation in resource use among individuals (inverse of ecological release, box 5) (Van Valen 1965, Knudsen et al. 2007, Costa et al. 2008, Bolnick et al. 2010). However, as said before, this need to be taken carefully as inter-specific competition can lead to niche displacement of the focal species and have no impact on among-individual variability for example, or the ecological release can also lead to a decrease of individual specialization because of the increase of individual niche widths for example in Bolnick et al. (2010, box 5).

Out of their ecological context, few studies tried to explain the differences in among-individual variability among different species. Svanbäck et al. (2015) demonstrated with different size classes of Eurasian perch that the trophic position of a population could impact the among-individual variability. However, we found no studies comparing species of the same trophic level differing in their population niche width. In paper I (Bison et al. 2015), we tested two hypotheses: the niche variation hypothesis (NVH, Van Valen 1965) and the sociality hypothesis, to explain the differential diet breadths observed among ungulate species. We extended the NVH to the inter-specific level (because it is originally stated at the intra-specific level) and we tested whether generalist species with wider niche display higher among-individual variability (box 5). The sociality hypothesis posits that other mechanisms could influence the level of among-individual variation: more social species could indeed display lower among-individual variability if all individuals belonging to given social groups share similar diets. The tests of these hypotheses were possible because in our study, the generalist species was the most social, and the specialist species the least social (see fig.1 from paper I for hypotheses).

Having regard to the two last sections (*2.2.c* and *2.2.d*), we highlight that intra- and inter-specific interactions are constantly evolving and interacting before species reach coexistence and stability within a community. In case of competition, as it is considered as a major force structuring community assemblage even if highly debated (Murray & Illius 2000), the niche expansion of a superior competitor can (1) displace the niche of the inferior competitor and/or (2) make decrease the niche breadth of the inferior competitor. In the first case, species can in turn compete with another species. In the second case, it can enhance intra-specific competition, which can lead to increase species niche breadth through individual specialization, or through increase of all individual niche widths (box 5). This niche breadth expansion may in turn cause overlap with another species, and so forth. This dynamic underlines the need to consider the temporal variability, and hence study population niches at different time scales and at the intra- and inter-specific level.

**Box 5 –** Decoupling of individual and population niche widths

When a population/species is released from a competitor, the total niche width of the population/species can increase through two different paths: all individuals increase their niche width (WIC) by adding new high-value preys to their diet (fig.8 upper path), or between-individual variation (BIC) increases while niche width remain constant (Niche Variation Hypothesis, fig.8 lower path) (Bolnick et al. 2010).

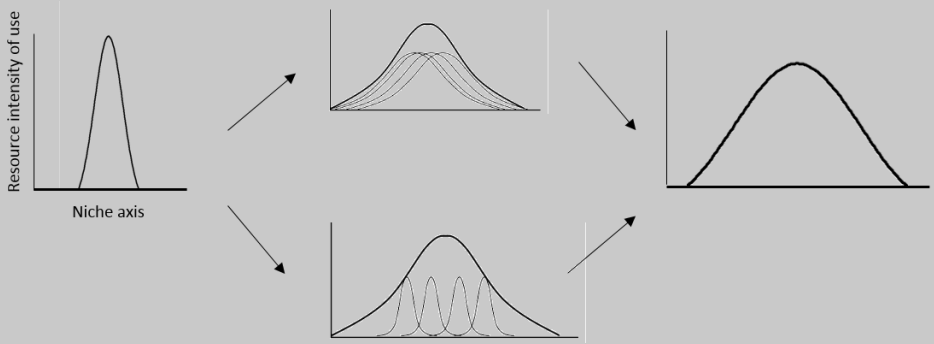



**Figure 8.** Illustration of the increase of population niche width through two different paths.

Specialization can take a wide range of formats: individuals can be more or less specialized within a population (fig.9a), individuals can be clustered (fig.9b) or nested (fig.9c). In this work, we only explored the clustering of individuals (see Summary of Results *6.1.a*). However, the type of specialization can influence the interactions among individuals and in turn have consequences on population dynamic that remain to be determined (Araùjo et al. 2009). For example, in the case (b), if individuals, e.g. young individuals, form a cluster because of similar requirements, then the decrease of their preferred resource could strongly affect the population dynamic as all the young individuals would be impacted. In the case (c), interactions among individuals would be asymmetric if the resources are limiting.

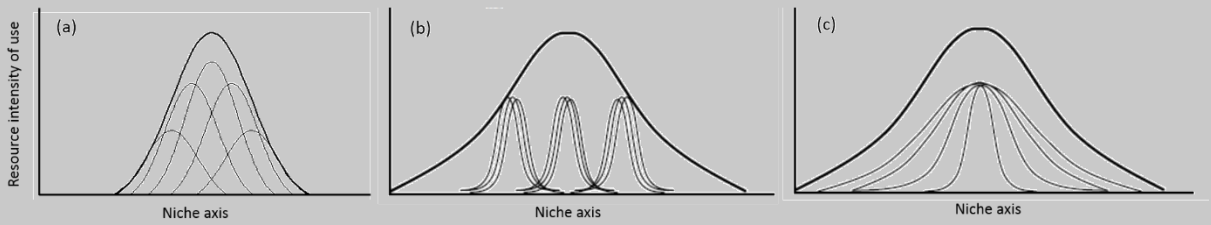



**Figure 9.** Alternative ways in which individuals can specialize (inspired from Araùjo et al. 2009).

## 2.3 Objectives

The aims of this three-year work was to improve our understanding on ecosystem structure and functioning through the study of interactions between large herbivores and vegetal communities at the intra- and inter-specific levels (fig.10). At the intra-specific level, we were interested in how sociality affect the feeding behavior of individuals (paper I). At the inter-specific level, we focused on whether introduced species can modify the native community of herbivores by trophic interactions (paper II) and we tried to identify diet selection criteria of large herbivore at relatively fine scale (paper III). Finally, we explored the usefulness of NIRS methodology in ecological studies (paper IV and note).

The results from papers and additional analyses are organized as below:

1) From community to individuals (paper I)
    - Does sociality drive the inter-specific variability of populations?
    - How does intra-specific variability in diet vary with resource availability?

2) The relative importance of food quantity and quality in the large herbivore diet selection (papers II-III)
    - How do large herbivores with high metabolic rate survive in poor environment such as pastures?
    - Methodological considerations
    - At which scale do herbivores make their diet selection?

3) How do introduced species influence plant-herbivore interactions? Are introduced species compulsory harmful for ecosystems? (papers II-III)
    - What is the impact of introduced mouflon on the native chamois trophic niche?
    - How do species coexist?

4) The unexpected importance of biomechanical traits in large herbivore diet selection criteria (paper III)
    - Does plant chemical content better explain diet selection criteria than other traits?
    - Is the relationship between biomechanical traits and chemical traits (C:N ratio, LDMC) consistent across growth forms?
    - What are the traits correlated to leaf punch toughness?
    - What about the relationship between diet and other traits?

5) A bit of methodology: is the use of NIRS relevant and useful for estimating diet and plant quality? (paper V and VI)
    - Are diet quality estimations with NIRS consistent with measurements on plant functional traits ingested?
    - Is the estimation of N, C and P with NIRS as robust as classical methods? Can we create three general calibration models (one for each chemical component) usable for different ecosystems? (paper in prep. not included in the thesis, Ancin Murguzur F.J, Smis A., Bison M., Struyf E., Bråthen K.A.)

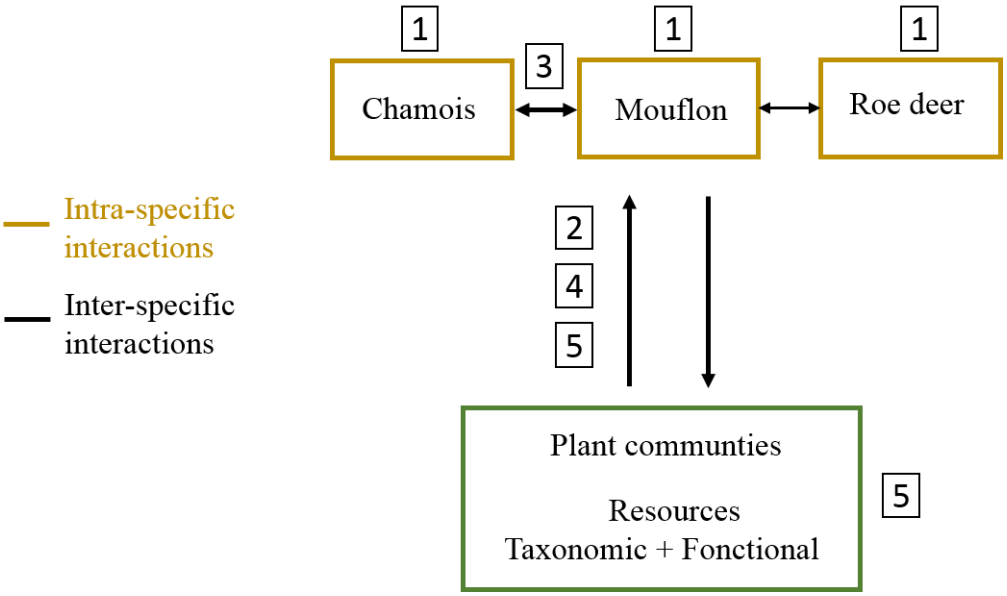



**Figure 10.** Connections between the topics approached in the thesis. In yellow are the intra-specific interactions within large herbivores. In black are the inter-specific interactions among large herbivores (arrows between boxes of ungulates) and between large herbivores and plant communities. The number corresponds to the questions asked hereabove.

For hypotheses and predictions, see the corresponding papers.

# Chapter III: Roe deer, chamois and mouflon in the Bauges Massif

## 3.1 Bauges Massif

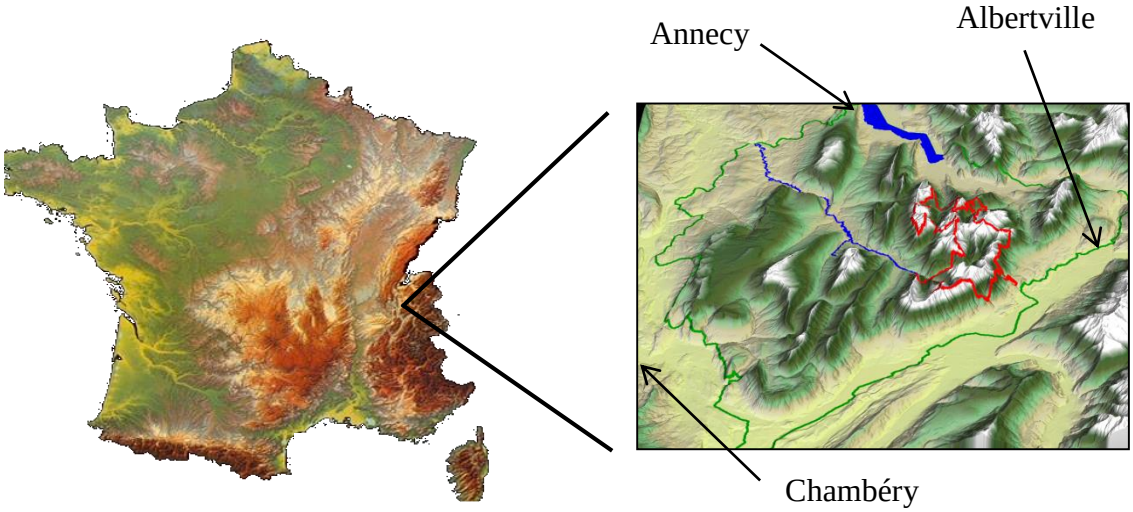



**Figure 11.** Localization of the Bauges Massif in France. In red is the Bauges Game and Wildlife Reserve.

"Physically, without being as wild and magnificent as the neighbouring alpine Maurienne, Tarentaise and Haut-Faucigny Massifs, the Bauges Massif displays a certain originality in its aspect, its soil composition, its plants, its animals, its climate, its watercourses, its stratigraphic curiosities. It is a world rich in natural gifts and full of appealing beauty". Translated from Louis Morand (Les Bauges. Histoire et Documents. 1889)

The Bauges Massif (156 km²) is situated between the departments of Savoie and Haute-Savoie. It has a roughly triangular shape, with the towns of Chambéry, Albertville and Annecy as corners (fig.11).

The morphology of the Massif is remarkable with steep rocky cliffs (mostly urgonian limestones) alternating with grassy smooth slopes. Besides being a Natural regional park, it also received the "geopark" label acknowledging his geological features. The altitude goes from 250 to 2217m (sub-montane, montane and subalpine levels), dominated by the Arcalod summit. The mixture of calcareous cracks and old glacial basin that can be found on the Margeriaz slopes, where the wind blows vertically to climb cliffs and falls forward in short vegetation, evoke a fantastic world. During foggy days, the fog fills the empty space delimited by the cliffs of the Armene pasture, and let the rocky knolls appear, as independent towers carrying some vegetation.

**Figure 12.** Arces summit from the ascent of Armenaz summit. We can see the Belledonne Massif behind the sea of clouds.

There is no gold in the Bauges Massif, or at least, not the gold that we first think about. It's the green gold that we find. Since the last period of glaciation, the massif has been covered by extensive forests where we find the main woody species are fir, spruce, pine, yew, some of them being of economic forestry interest. Deciduous species such as beech, oak, maple, ash, elm, alder, rowan and others grow in the Bauges forests. Pastures harbor specific species from the alpine environment. The Bauges Massif is a wonderful place for botanists with more than 1500 plant species. Regarding animals, there are passing wolves (no pack established so far), and large populations of ungulates such as chamois, roe deer, mouflon, red deer and wild boar. Mouflon has been introduced in 1950s (Darmon et al. 2007) and co-occur in pastures with the chamois, and in forest with the other ungulates. In the Bauges Game and Wildlife Reserve (5,170 ha), chamois and mouflon are the two most abundant species (Darmon et al. 2012) and have no natural predators, except for golden eagles or wolves that may anecdotally depredate new borns and sick animals. Hunting occurs from September to January.
The Bauges mountains constitute a transitional area between the internal Alps and the lowland areas in the west and is subjected to a continental climate with an oceanic influence (Loison et al. 1999) causing large precipitations (> 1850 mm/year) favorable to high plant diversity. Temperature goes from 0°C in winter to 17°C in summer, on average. The green meadows are replaced by the plant yellowing in autumn (September-November) and a thick layer of snow covers the ground in winter (November-April), mostly permanently above 1000m.

**Figure 13.** Two different views of the Armenaz pasture in summer.

## 3.2 This work takes place in an already well-studied ecosystem

The Bauges Game and Wildlife Reserve (~5200ha) is included in the Bauges Massif and is co-managed by three public institutions: National Forest Office (ONF), National Agency for Hunting and Wildlife (ONCFS) and the Bauges National Park (PNR Bauges). The Reserve was first created in 1913 to stop the sharp decrease of chamois populations. Today, one of the main objectives of the Reserve is to improve knowledge on mountain wildlife through applied researches. Especially, four sites (Armenaz and Charbonnet in pastures, Coutarse and Bellevaux in forest) have been targeted to more fully study chamois populations and other ungulates (mouflon and roe deer). They are long-term study sites for chamois population demography studies, as animals have been equipped with collars since 1985, and with GPS since 2003.

Different projects have been hold on ungulates from the Bauges Massif, for example about genetic of populations (Cassar 2007, Loison et al. paper in prep), epidemiology (Loison et al. 1996, Pioz 2006, Pioz et al. 2008), demography (Loison et al. 1999, Garel et al. 2009, 2011, Bleu et al. 2014, 2015), movement (Loison et al. 2008, Gaudry et al. 2015, Tablado et al. in prep.), colonization process of the introduced species (Darmon et al. 2007), habitat and diet selection (Darmon et al. 2012, Redjadj et al. 2014), species niche partitioning (Darmon et al. 2012, 2014), or human disturbance effects (Tablado et al. in prep).

Studies focusing on the spatial axis of the ecological niche demonstrated that chamois and mouflon living in the same pasture could coexist by choosing different plant communities, at least in spring (Darmon et al. 2012). As for spatial studies, mouflon did not behaviorally interfere with chamois when the activity rhythm was studied, thanks to GPS equipped with activity sensor recording motion data (i.e. animal's head movements) (Darmon et al. 2014). These studies suggest the absence of competition between chamois and mouflon, partly allowed by species-specific requirements and behavior. It is in this context that we were consequently interested in testing whether and how ungulates could coexist on the dietary axis of the ecological niche, at the inter- and intra-specific levels. Indeed, spatial distribution and habitat selection of ungulates can fluctuate over the year, and chamois and mouflon could be more prone to be spatially aggregated in some seasons (strong spatial overlap in July contrary to august for example, Darmon 2007). This could lead to exploitative competition for food resources, as both species track high-quality plants. Approaching those questions was possible owing to existing databases on ungulates diet. During her PhD, Claire Redjadj (2010, Redjadj

et al. 2014) determined the evolution of the diet composition and quality of chamois, roe deer, mouflon and red deer over the year, thanks to DNA-metabarcoding and NIRS on faeces samples (Rayé et al. 2011), and micro-histology and NIRS on rumen samples. C. Redjadj and A. Duparc (then in Master 2) also estimated the biomass, phenology and composition of plant communities on two pastures of the Bauges Massif, in order to include resource availability in the studies (Duparc et al. 2012, Redjadj et al. 2012). Hence, these huge databases have been the starting point of my PhD.
All these results and databases provided a framework particularly useful for my thesis to continue the exploration of trophic interactions between large herbivores and plant communities at the intra- and inter-specific levels, and are also relevant for the other ongoing PhDs.
Indeed, the current PhD of Antoine Duparc aims at determining the impact of human activities and degree of inter-specific coexistence on the spatial occupancy of ungulates in mountain. These questions about human disturbance take part in a bigger project which led to an additional partnership with the laboratory of environment, dynamics and mountain lands (EDYTEM) in order to understand the sociological and ecological aspects of human-wildlife interactions, e.g. during the practice of winter sports, but also during hunting or other recreational activities in the Bauges Massif. Finally, the PhD of Tiphaine Lefebvre aims to explore the impacts of herbivory (chamois and cows) on the biomass and quality (nitrogen, carbon, phenols, lignin) of alpine plant communities, and on the soil through the decomposition rate of litter.

Besides, the Reserve takes part in the “Zone Atelier Alpes” (ZAA) which aims at understanding the climate-human-environment dynamics, through long-term studies on several topics such as the dynamic of biological diversities in the Alps, the ecosystem functioning and the provided services, the inter-relationships between ecological systems and human activity system.

## 3.3 Studied species

The studied species are of high interest as they are range on the browser-grazer, specialist-generalist, and solitary-gregarious gradients (table 2).

Because population sizes of ungulates in open and forest areas are difficult to estimate with precisions, we prefer to not give estimates of population size that could be wrong, but we can say that there are more chamois (>2500) than mouflon, red deer and roe deer. Monitoring methods, based on repeated censuses (Loison et al 2006) are aimed at identifying populations trends, and indicate that populations of chamois may have reach a plateau since 1990 (based on abundance index), mouflon are increasing and roe deer are stable (pers. comm.).

Mouflon have been introduced in 1950’s from the zoological park of Chambord (see Darmon et al. 2007 for the mouflon colonization, and Marchand 2013 for details on mouflon phylogeny).

Chamois and mouflon are physiologically adapted to steep slopes of open areas. However, mouflon is not well-adapted to snow cover because of the absence of a membrane between the two fingers of the foot, unlike chamois which hoofs act as a snowshoe. While mouflon migrate in the valley during winter, chamois can stay all year round in pastures. Yet, some chamois also

occupies the forest (see the thesis of Claire Redjadj 2010), and for most part, seem to stay in the forest all year round (ongoing analyses, Duparc PhD). Roe deer mainly inhabits forest (see fig.14 for the localization of studied sites and faeces sampled).

Sexual dimorphism differ between ungulate species. Sexual dimorphism can be assessed by differences in body mass or physical attributes sex-specific.
It is not easy task for inexperienced observers to visually differentiate male from female chamois. Indeed, male and female have horns and the difference between sexes is based on the angle of the horn's hook. In addition, even if the body mass is heavier for males than females (Garel et al. 2009), the difference is not high enough to easily differentiate them from a considerable distance. Additional cues such as the general stature of the animal (such as neck and head size) and its behavior (such as calves following their mother, lactation, urination) can help the identification.
Visually, it is easy to differentiate female and male roe deer during the period when males have antlers. When they lose them in autumn, it becomes more difficult as the body mass ratio between male and female is also low (1.10 for Trois Fontaines population, Gaillard et al. 1993).
Finally, the sexual dimorphism is more pronounced for the mouflon. Females do not have horns (or little ones), contrary to males. The sexual dimorphism in terms of body mass is important as females weigh between 25 and 35 kg whereas males weigh between 35 and 50 kg (Vallance 2007).

For most sexual dimorphic ungulates, sexual segregation occurs outside the mating season (Cransac et al. 1998, Bonenfant et al. 2004 and references therein). This spatial segregation could be explained by different responses to the predation risk ("predation risk hypothesis"), different energetic requirements ("forage selection hypothesis") or different activity rhythm ("activity budget hypothesis") (Main 1996, Ruckstuhl & Neuhaus 2002).

The "predation risk hypothesis" predicts that females with calves should make a different trade-off between patch quality and security than males. Females with calves are more vulnerable to predation. Hence, they should choose secure sites (for example steep areas) of lower quality to ensure the calves survival (breeding success, Main 1996), which would in turn decrease their diet quality. On the contrary, because the breeding success of males is related to their body mass, they would be more prone to choose sites of higher resource quantity and quality to increase their body growth and secondary sex characteristics development (Main 1996). In addition, habitat segregation should peak during calving, when calves are more subject to predation (Bonenfant et al. 2004).
Males and females, even subtly (see hereabove), differ in their body mass. The "forage selection hypothesis" describes the consequences of sex body mass differences, and in turn physiological requirements, on habitat segregation and hence on diet quality (Main 1996, Ruckstuhl & Neuhaus 2002). Indeed, as females have a smaller body size and body mass than males, they have higher energetic requirements and they should be more selective in terms of resource quality than quantity, and choose high quality food habitats (Main 1996) (see section *2.2.b* for allometric relationships between energetic requirements and gut size). However, this "forage

selection hypothesis" has been questioned as several studies show no differences in habitat quality used by both sexes (Ruckstuhl & Neuhaus 2002 and references therein).
The "activity budget hypothesis" do not predict habitat segregation but social segregation. Because of body size dimorphism, males and females could differ in their activity budgets. For example, as males are bigger than females and supposed to feed on lower quality plants, the time dedicated to rumination should be higher than for females. A mixed group would be less synchronized because of differences in activity budgets between sexes. This would led to less stable groups because neighbors would compromise the optimal activity of each individual (Conradt 1998). Hence, the best strategy would be that animals with similar activity budgets form groups, which would also be efficient to decrease predation risk in open areas (Ruckstuhl & Neuhaus 2002).
Consequently, by impacting the habitat selection, sex can influence the diet selection and hence diet quality. In this thesis, we did not take into account any sex effect. However, further analyses should explore the differences in diet quality between males and females at different periods of the year and try to determine which hypotheses could explain these differences.

Domestic species (cows) occupy the studied pastures of Armenaz and Charbonnet in summer and beginning of autumn (june to September) since 2004, partly to prevent the spread of the green alder in the pasture. They can occasionally physically interact with chamois when they come through enclosures.

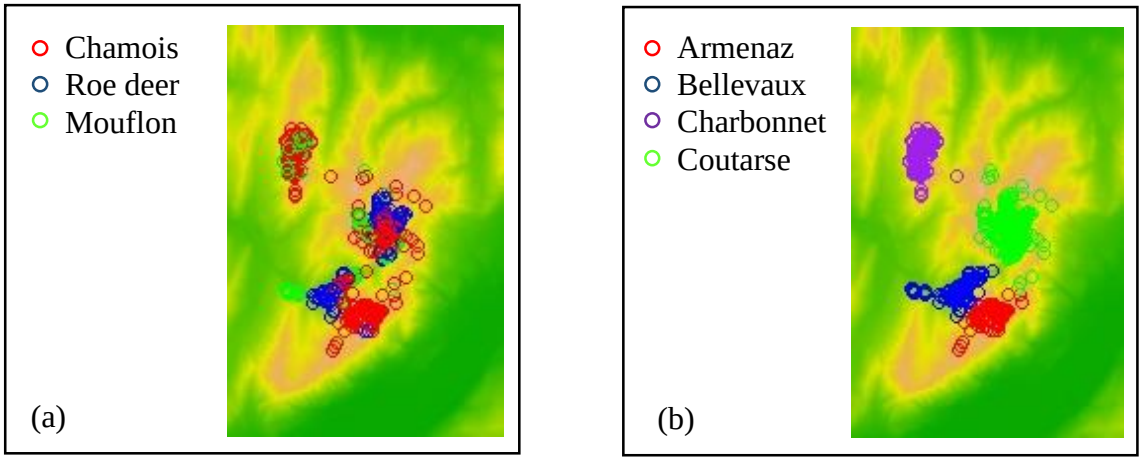



**Figure 14.** (a) Localization of chamois, roe deer and mouflon faeces sampled in the Bauges Game and Wildlife Reserve in 2007 and 2008, (b) Localization of the sites where the faeces of the three ungulates have been sampled in 2007 and 2008.

**Table 2.** Ecological and biological characteristics of studied animals.

| | Roe deer<br>*Capreolus capreolus* | Chamois<br>*Rupicapra rupicapra* | Mouflon<br>*Ovis gmelini musimon* |
|---|---|---|---|
| Family | Cervidae | Bovidae | Bovidae |
| Sub-family | Capreolinae | Caprinae | Caprinae |
| Artiodactyle/ Perissodactyle ? | Artiodactyle | Artiodactyle | Artiodactyle |
| Number of youngs | 1-3 | 1 | 1-2 |
| Habitat | Forest | Pasture | Forest and pasture |
| Sociality | Solitary | Social (low stability) | Social (high stability) |
| Body mass | 15 to 35 kg [a] | 35 to 60 kg [a] | 35 to 60 kg (according to the sex) [a] |
| Masseter muscle | 44.2 ± 11.6 g [b] | 58.9 ± 4.0 g [b] | 143.2 g [b] |
| Diet category | Browser [c] | Intermediate [d] | Grazer/ Intermediate [e] |
| Digestive morpho-physiology | Moose-type [f] | Intermediate [g] | Cattle-type/ Intermediate [h] |
| Niche breadth | Specialist [i] | Intermediate [i] | Generalist [i] |

(a) ONCFS, (b) Clauss et al. 2008a, (c) Hofmann 1989, Tixier et al. 1996, Redjadj et al. 2014, (d) Hofmann 1989, Garcia-Gonzalez et al. 1996, Bertolino et al. 2009, La Morgia et al. 2009, Redjajd et al. 2014, (e) Hofmann 1989, Cransac et al. 1997, Marchand et al. 2013, Redjadj et al. 2014, (f) Kamler 2001, Clauss et al. 2009, Clauss et al. 2010b, (g) Clauss et al. 2010a, (h) Kamler 2001, Clauss et al. 2009, Clauss et al. 2010a, (i) Hofmann 1989, Clauss et al. 2003b, Bison et al. 2015

# Chapter IV: Overview of databases

## 4.1 DNA-metabarcoding as the method to identify the diets (papers I-II-III-Note)

The composition of diet in wildlife can be determined in several ways:

- Through direct field or controlled experiment observation (Tixier et al. 1997, Massey 2009)
- Through identification of herbivory impacts on plant species (browse surveys) (Lembke 2005)
- Through rumen analyses (Staines 1976, Jackson 1980, Forsyth 2005, Redjadj 2014)
- Through faeces analyses (Garcia-Gonzalez & Cuartas 1996, Bertolino et al. 2009)

Two methods can be used to determine the composition of rumen and faeces: microhistology (Jenkins 1987) or DNA-metabarcoding (Taberlet et al. 2007, Rayé et al. 2011, Pompanon et al. 2012).

The morphological diagnosis used for microhistology is tedious. First, it requires a high-level of technical expertise. Second, some plant morphological keys are only usable for specific development stage or specific genous, and the resolution can be difficult below family or genous. There are also some cryptic species that are morphologically indistinguishable. Species identification can be wrong because of the phenotypic plasticity of the used feature. Finally, many fragmented cuticles are so small that they cannot be visually recognizable, which in turn under-estimate the diet species richness (Pärtel 2014).

DNA-metabarcoding method solves a significant part of those issues. The molecular barcoding is a technique which allows, with a small non-damaged DNA sequence, to determine the species in question, crossing the determined sequence with a reference database. This technique has been implement by Hebert in 2003 and was developed to differentiate animal species. Instead of sequencing the whole genome, Hebert and its team discovered that the mitochondrial gene of the cytochrome c oxidase had a strong species discriminative power. Indeed, mitochondria accumulate enough mutations to differentiate species, but do not allow the differentiation of two conspecific individuals. This technique offers an access to the « hidden » biodiversity, i.e. not visible through direct or miscroscope observation. In the case of large herbivores rumen and faeces analyses, DNA-metabarcoding needs to be adapted to plant species differentiation. Taberlet and his team (Taberlet et al. 2007) have used a short chloroplast DNA fragment, the P6 loop of the *trn*L (UAA) intron, as minimalist barcode (Rayé et al. 2011) for plant identification. Thanks to universal primers being capable of binding to the chloroplast intron, it is possible to amplify the DNA signal through PCR (Polymerase Chain Reaction). Then the amplicons are sequenced and compared to a reference database for identification. However, other issues are encountered with DNA-metabarcoding. This is a method which requires time to select the right gene to sequence in order to differentiate the species of interest. Besides, sequenced plants are not always identified or indexed in the database, which makes sequence assignment not always feasible. Finally, sequencing errors can lead to wrong species identification. Still, from a general point of view, it appears to be a powerful tool, that has now been used in many studies (e.g. Soininen et al. 2009, Valentini et al. 2009a, b, Rayé et al. 2011,

Ibanez et al. 2013b, Bison et al. 2015, to name only some where LECA is involved as examples) as this is a relatively low-cost method which gives a high taxonomic resolution (Rayé et al. 2011) not necessarily achieved with microhistology. This is particularly useful for the study of trophic networks as it can provide reliable results of diet composition (Rayé et al. 2011). However, biases can be found in the frequency of sequences detected in faeces because of the differential digestibility of plants in the digestive tract, which itself varies with species. Indeed, DNA fragments of some eaten plants cannot be recovered in faeces due to a high degradation (Rayé et al. 2011). To calibrate the results on faeces obtained with DNA-metabarcoding, controlled experiment should be conducted (Rayé et al. 2011). Finally, as dozens of samples with several thousand sequences per PCR product can be directly characterized (Pompanon et al. 2012), it a useful approach for broad temporal and scale studies.
In this work, DNA-metabarcoding method has been applied on faeces samples. DNA extraction and taxonomic identification of plants found in faeces have been realized by Claire Redjadj between 2007 and 2011, during her PhD – see the thesis Redajdj 2010 for a detailed description of faeces sampling and Taberlet et al. 2007 for detailed DNA-barcoding method.

## 4.2 Plant functional traits measurements (papers II-III-Note)

In order to determine the diet selection criteria of large herbivores, we measured different plant functional traits that we classified in different categories (table 3): ecological traits influencing the visibility of a plant and the ease for an herbivore to find it (e.g. reproductive and vegetative height, number, size and color of inflorescence, distribution through sociality), quality traits related to animal nutrient requirements and the ease of plant harvesting, chewing and digestion (chemical traits: leaf nitrogen content, leaf carbon content, leaf phosphorus content, leaf dry matter content, biomechanical traits: punch toughness, tensile strength).

### a) Sampling design

Plants have been sampled in the Bauges Massif on the Armene pasture at altitudes ranging from 1700 to 2000 m and in the Bellevaux forest at altitudes ranging from 700 to 1000 m.
The pasture consisted of eight vegetal communities (Duparc et al. 2012, Appendix 4). A total of 87 plant species have been sampled in spring after snow melt (beginning of June), in summer during the vegetation peak (mid-july) and in autumn during senescence (mid-September) (fig.15).
Plant biomass and specific composition of the plant communities of the Armene pasture have been estimated between 2007 and 2010 with the BOTANAL method (Lavorel et al. 2008, Redjadj et al. 2012).
Plant species have been sampled according to their abundance in the different vegetal communities: (1) when the abundance of a species in each community was less than 10%, we sampled the plant species in the community where it was the most abundant; (2) when the abundance of a species in each community was more than 10%, we sampled the plant species in each of those communities. We assumed that the abundance of a plant in a community is the result of environmental pressures. Hence, we considered that the more a plant is abundant in a community the better it is suited to the local environmental biotic and abiotic conditions

optimally expressing its traits (Vile et al. 2006, Cingolani et al. 2007). Hence, each plant species has been sampled in one or two communities and each plant community has been visited.
For each community, one GPS point has been firstly drawn randomly at the average altitude of the community, if possible outside the cow pens. Then, individuals were sampled in a 90 m² quadrat around the GPS point. When possible, the distance of 5 meters was respected to avoid clones or genetically close individuals. Two communities were situated inside the cow pens. Sampling therefore occurred in this area. The same locations have been used for each sampling season. Because of the difficulty to find targeted plant species in forest, sampling locations have been based on a Bauges Forest Inventory and not on random GPS points.

**Figure 15.** Pastures in (a) spring (June 2013), (b) summer (July 2013), (c) autumn (September 2013).

b) Traits measurements

- Visibility traits

Vegetative height

The vegetative height was defined as the distance between the ground and the highest point of the highest leave, without taking into account an exceptional branch (fig.16). It has been measured on 15 individuals using a tape measure to the nearest 1cm.
Special case for basal plant rosette: for plants whom most of the leaves were basal rosette, with proportionally few photosynthetical area on the twig, we measured the vegetative height as the distance between the ground and the top of the rosette. For plants with one third of rosette leaves and two third of twig leaves, we measured the vegetative height as the distance between the ground and the highest leave of the main twig.

Reproductive height

The reproductive height, or flower height, was defined as the distance between the ground and the highest point of the flower (fig.16). It has been measured on the same 15 individuals as for the vegetative height, using a tape measure to the nearest 1cm.

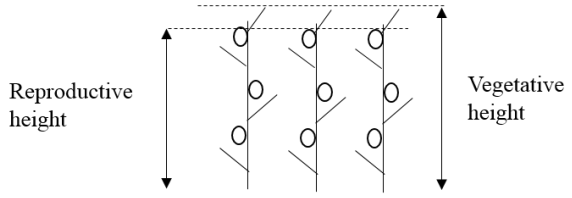



**Figure 16.** Schematic view of the reproductive and vegetative height measurements.

The following term « inflorescence » meant bud, flower or fruit, depending on the phenology.

Inflorescence size

The length, width and height of 10 inflorescences per species have been measured to the nearest 1mm using a caliper (fig.17). During flowering period, only open flower were taken into account. During fruiting period, only fruits were measured. If the inflorescences were individualized or not closely clustered, the length, width and height of a medium inflorescence was quantified, and the volume was multiplied by the number of inflorescences of an individual. If they were clumped, the whole inflorescence was measured.

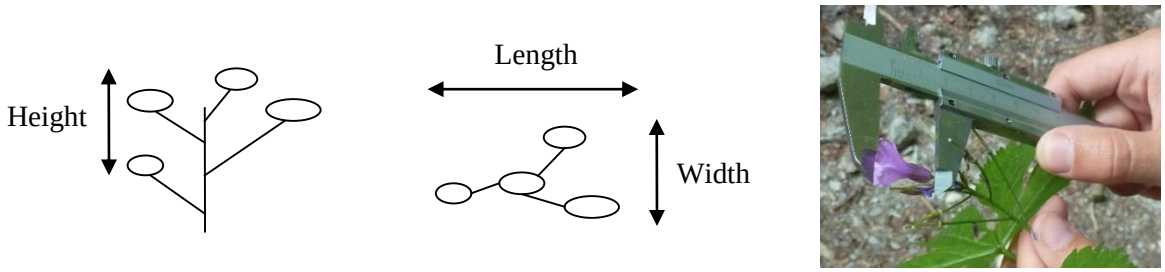



**Figure 17.** Schematic view of the inflorescence length, width and height measurements, and a picture of height measurement.

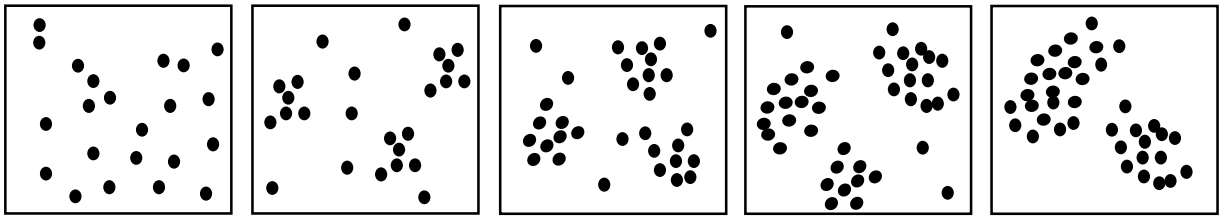
Color of inflorescence

The colors of 10 inflorescence per species have been recorded from a human visual perception, in a crude-way (yellow, red, green, brown, purple, white).

Sociality

The sociality has been defined at the population-level, contrary to the others traits that were measured at the individual-level. This measure did not fit the definition of traits given above, but for the sake of simplicity, we kept this information here.

Sociality was defined as the spatial distribution pattern of individuals on the studied area (Braun-Blanquet et al. 1952): 1-isolated individuals, 2-individuals spread in small isolated groups, 3-individuals in tighter groups, 4-individuals in more or less dense colonies, 5-individuals in compact settlements (fig.18).



1-isolated individuals | 2-individuals spread in small isolated groups | 3-individuals in tighter groups | 4-individuals in more or less dense colonies | 5-individuals in compact settlements

**Figure 18.** Illustration of the different levels of sociality.

- Quality traits: Chemical traits

For each plant species, we sampled 10 young leaves (i.e. the most relatively new and developed leaves) on 10 robust and well-developed individuals. Damaged plants by herbivores or pathogens were excluded.

Leaf Dry Matter Content (LDMC)

Limbs were kept in wet tissue from the field study site to the lab, in order to keep them hydrated. They were weighted to obtain the fresh mass. Then, they were stored in a 60°C oven for 48h, and weighted again to obtain the dry mass. The ratio between dry and fresh mass gave the leaf dry matter content (inverse of leaf water content).

This operation has also been done on the twigs and petiole to determine the twig dry matter content (TDMC) and petiole dry matter content (PDMC).

The 10 limbs were distributed in 3 eppendorfs (3 individuals in the first, 3 in the second and 4 in the third) and grinded with tungsten balls. Leaf veins were not taken off except for the species which main rachis was well-apparent (*Heracleum sphondyllium*, *Rumex arifolius*, *Rumex alpinus*). The choice of keeping veins was motivated by our wish to mimic as closely as possible the leave uptake from a large herbivore that cannot avoid veins.

Leaf Nitrogen Content (LNC) and Leaf Carbon Content (LCC) (estimated with classical method)

Between 3 and 5 mg of grinded dried plants were put in aluminium cups. The cups were placed in the elementary scanner (Elementary sensor, Flash EA 1112, Thermo Electron Corporation in the LECA Grenoble) and underwent a "Flash" dynamic burning (combustion). The gaz produced went through a catalytic oxidization and then, the NOx was reduced in $N_2$ through a reduction column. The oxygen and $SO_2$ in excess were trapped. Then, the elements were separated in a chromatography column. $H_20$ was trapped. Gaz detection was performed with a catharometer (universal sensor). The output values were obtained in % of N and C and were then weighted by the dry mass in order to obtain the C and N absolute values in the measured organ.

Leaf Phosphorus Content (LPC) (estimated with classical method)

Quantitative analysis of phosphorus content in leaves with molybdate blue has been done in the Center of functional and evolutive ecology (CEFE) in Montpellier. First, the dried plant sample was weighted and then mineralized. Then, the phosphorus in the sample associated with the molybdate to create a phosphomolybdic complex in a highly acidic environment. This complex was reduced by ascorbic acid giving the blue coloration. 880 nm was the appropriate wavelength for the optic density measurement. The relationship between absorbance and phosphorus concentration followed the Beer-Lambert law. The optic densities obtained were proportional to phosphorus content. Phosphorus concentrations were expressed in µg/ml.

NIRS estimations

Leaf nitrogen, carbon, phosphorus and silica content were also estimated with Near-InfraRed Spectroscopy (NIRS) to study the NIRS applicability (See *Material and Methods: NIRS measurements* for details). Calibration has been done through the Bauges plants for nitrogen, carbon and phosphorus. Silica measurements had to be cautiously interpreted as calibration has been done with Norwegian plants. Moreover, forbs had usually low amounts of Si, and the predictions were not highly reliable. Except for Alchemilla alpina, which could have reasonably high values, the rest of the forbs had Si concentrations below 0.5 (as a general rule). See *Material and Methods: NIRS measurements* for the calibration and validation models, and the transferability of calibration models from sub-arctic to temperate ecosystems.

Percentage of inflorescences/leaves/twigs dry weight on total dry weigth

Three entire individuals per species have been sampled and directly dried. Inflorescences, leaves and twigs have been separately weighted to obtain the percentage of inflorescences/leaves/twigs dry weight on total dry weigth. This measurement was done to evaluate the energy allocation in plant.

- Quality traits: Biomechanical traits

Among the techniques commonly used in ecological studies that include biomechanics (Sanson et al. 2001), we used the punching and tearing tests, which are respectively considered as tests of compression and tension.

We used a sophisticated machine (Instron 5942, Canton, MA, USA) from the LEHNA (Laboratoire d'Ecologie des Hydrosystèmes Naturels et Anthropisés, Puijalon S.) in Lyon. For the punch trial (fig.20, fig.21), a flat-ended cylindrical steel rod (2 mm diameter) was mounted onto the moving head of the machine, and went through a stationary base with a hole setting the leaf. For the tearing trial (fig.20, fig.21), two pliers were mounted both on the moving head and at the base of the device to maintain the leaf. For both trials, the force was applied at a constant speed (10 mm $s^{-1}$), irrespective of the resistance.

Punch strength

It corresponds to the leaf hardness: maximum force per unit punch area required to punch a hole through the leaf lamina (in N $m^{-2}$, fig.19, fig.20, fig.21). The specific punch strength is the ratio of punch strength to leaf thickness (Read & Sanson 2003, in N $m^{-2}$ $m^{-1}$). This measure has only be done on the lamina part of the leaves.

Punch toughness (Work to punch)

It corresponds to the energy required to punch a hole through the leaf lamina or, the total work required to fracture a leaf per unit punch area (in J $m^{-2}$, fig.19, fig.20, fig.21). The specific punch toughness is the ratio of punch toughness to leaf thickness (Read & Sanson 2003, in J $m^{-2}$ $m^{-1}$). This measure has only be done on the lamina part of the leaves.

The punch toughness is preferred to punch strength as it takes into account how the material deforms and breaks (Sanson et al. 2001).

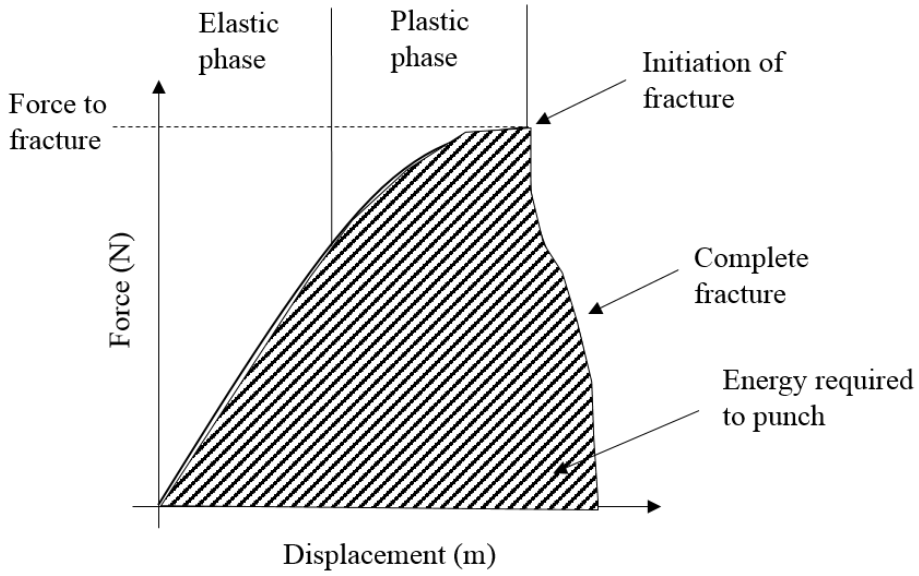



**Figure 19.** Evolution of the force in function of the displacement. During the elastic phase, the material return to its original dimensions if the force is removed. During the plastic phase, the material will deform permanently. The figure and the legend are inspired from Sanson et al. 2001.

Tear strength

It is the strength at which the sample breaks corrected by the cross-sectional area (in N $m^{-2}$, fig.20, fig.21). It describes the robustness of the material. This measure has been done on the leaves (without the veins on big leaves) or on the stems, depending on the stature and height of the plant. In order to mimic the plant intake by herbivores, we considered that an animal should not be able to feed on small leaves of short plants (e.g. *Helianthemum nummularium*) and we therefore did the tearing test on the twig. On the contrary, we supposed that on tall plants with big leaves, animals were able to physically discriminate the leaves from the twig and we only did the tearing test on leaves (e.g. *Veratrum album*).

Young modulus

It describes the stiffness of the material (i.e. resistance to distortion).

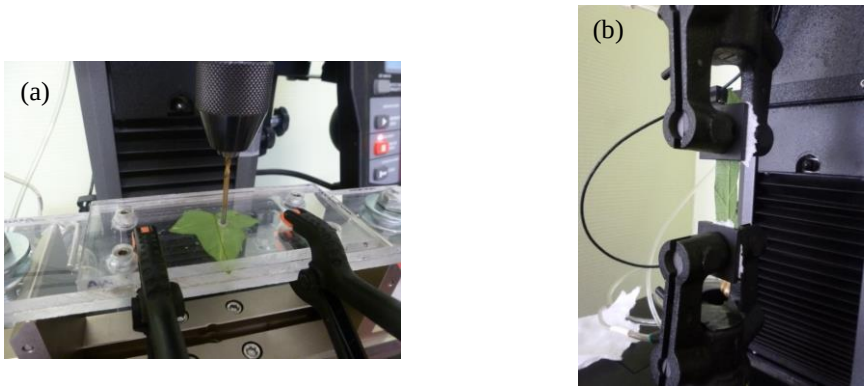



**Figure 20.** Pictures of the "torturometer" device, (a) punching test, (b) stretching trial.

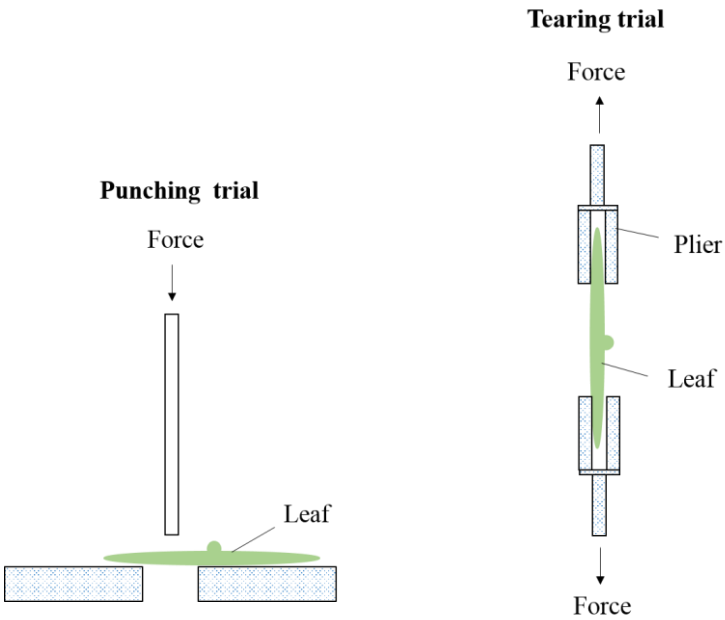



**Figure 21.** Schematic view of the punch and tear trials realized on a leaf.

- Others

<u>Phenology</u>

We identified the phenology on 10 individuals: 1-vegetative form, 2-flowering (defined as soon as one flower is opened), 3-mature (fruit and autumnal yellowing).

<u>Spinescence/pubescence</u>

The presence of spines or hairs on the leaves, twigs or inflorescences has been specified.

**Table 3.** Summary of measured traits on the field or in the laboratory.

| Category | Traits | Type of data | Unity/level |
|---|---|---|---|
| Visibility | Vegetative height<br>Reproductive height<br>Inflorescence* size<br>Number of inflorescences<br>Color of inflorescence<br>Sociality (population-level) | Continuous<br>Continuous<br>Continuous<br>Continuous<br>Categorical<br>Categorical | cm<br>cm<br>cm (height, length, width)<br><br>Yellow, White, Brown, Red<br>1, 2, 3, 4, 5 (low to highly social) |
| Quality (chemical traits) | LDMC<br><br>PDMC<br>TDMC<br>C<br>N<br>P<br>% of flower/leaf/twig dry weight on total dry weigth | Continuous<br><br>Continuous<br>Continuous<br>Continuous<br>Continuous<br>Categorical<br>Continuous | Ratio leaf fresh matter (FM) content/dry matter (DM) content<br>Ratio petiole FM/DM<br>Ratio twig FM/DM<br>%<br>%<br>% weak, medium, strong<br>% (weight of the dry flow/weight of the whole individual) |
| Quality (biomechanical traits) | Punch strength<br>Punch Toughness<br>Break strength<br>Tensile strength<br>Young modulus | Continuous<br>Continuous<br>Continuous<br>Continuous<br>Continuous | N<br>J $m^{-2}$<br>N<br>N<br>N |
| Others | Phenology<br><br>Spinescence<br>Pubescence | Continuous<br><br>Binary<br>Binary | Between 1 et 3 (vegetative, flower, fruit)<br>yes/no = 1/0<br>yes/no = 1/0 |

* “inflorescence” means bud, flower or fruit, according to the phenology

## 4.3 NIRS measurements (papers IV-Note)

NIRS measurements have been done on faeces from the ungulates of the Bauges Massif after calibration with a reference laboratory analysis (Redjajd 2010) to measure the total nitrogen, and fiber content (ADF, NDF, ADL) (Redjadj 2010).

NIRS has also been applied on 84 plant species of the Bauges Massif and on 23 plant species from Finmark to evaluate the ability of NIRS to correctly estimate nitrogen, carbon and phosphorus content, but also to evaluate the transferability of calibration models between two different geographic areas. This study was conducted by Francisco J. Ancin Murguzur and Kari-Anne Bråthen from the department of biology from the University of Tromsø, and I took part of it. As I did not include the paper in preparation (Ancin Murguzur F.J, Smis A., Bison M, Struyf E., Bråthen KA) in the thesis, I describe hereafter the main points of material and methods explained by Ancin Murguzur F.J. in the paper in prep.
All plant species were sampled at different seasons and samples measured with NIRS consisted of green non-woody parts of the leaves, in order to avoid light interference with big pieces of veins that could not be perfectly ground. Plant samples were dried at 60°C for 48h and grinded into powder using a ball mill. The powder was pressed into tablets (Ø 16mm, 1mm thick) using a hydraulic press. Tablets were dried again. A portable NIRS spectrometer has been used to scan the samples (range of 350-2500 nm wavelengths). Leaf carbon, nitrogen and phosphorus content were previously analyzed with classical methods (described in *4.2* for France analyses).
The first aim was to estimate the applicability of the NIRS method. To answer this question, calibration and validation models testing the relationship between NIRS measured and classical chemistry values have been developed for each plant constituent and each region. The most parsimonious calibration models were determined based on a high correlation coefficient ($R^2$), given a number of wavelength (k) and low root mean square of the error of the calibration (RMSEC, error between predicted and measured constituents). Each calibration model was tested against its respective validation set (25% of the total samples). The same parameters as for calibration models have been calculated to evaluate the robustness of the validation.
The second objective was to assess the transferability of each regional model to the other region. This has been achieved by predicting the nutrient contents of one region with the model of the other region. $R^2$, RMSEP and bias of the linear relationship between NIRS measured and classical chemistry values were also calculated.
The third objective was to test whether a single global model including the data from both regions could be enough robust to correctly estimate C, N, P content of both regions. $R^2$, RMSEP and bias were also used to estimate the robustness of the calibration and validation models of each constituent.

## 4.4 Resource availability (papers II-III)

Available plant diversity and biomass has been described at the scale of plant communities. Given the GPS position of faeces, available resource at the scale of 48h and seasonal home range has also been calculated.

a) Within plant communities

Thirtheen plant associations (homogeneous plant unities) have been mapped by photo-interpretation across the Bauges Game and Wildlife Reserve, and photosociological relevés allowed to determine them by using the CORINE biotope database as typology reference (Boissier 2005, CBNA 2005, European community commission 1991) in 2001. We assume that changes in relative area of plant communities were marginal between 2001 and 2007 (first faeces sample date). Previously, we wrote that the pastures consisted of eight vegetal communities. Here, thirtheen vegetal communities are described as forest areas are taken into account (mixed forest, coniferous, riparian forest, pre-forest semi-lignified, meadow).

**Table 4.** Relative abundance (in %) of each plant association within the Bauges Game and Wildlife Reserve of the Bauges Massif (data from the report of P.A Dupeyras 2014).

| Plant association | Relative abundance (%) |
|---|---|
| Mixed forest | 49.3 |
| Lawn of *Sesleria* | 19.3 |
| Scree | 8.6 |
| Mountain forage | 6.0 |
| Coniferous | 3.3 |
| Meadow | 2.8 |
| Megaphorbiaie | 2.8 |
| Lawn of *Carex ferruginea* | 2.7 |
| *Alnus* | 2.6 |
| Lawn of *Nardus stricta* | 1.0 |
| Heathland of *Rhododendron* | 1.0 |
| Riparian forest | 0.7 |
| Pre-forest semi-lignified | 0.2 |

Maps of plant communities in the pastures have been validated during Claire Redjadj's PhD. Qualitative and quantitative estimation of available resources in pasture has been done on the Armenaz and Charbonnet sites in 2007, 2008, 2009 and 2010, by Antoine Duparc and Claire Redjadj (Redjajd et al. 2012) by using the BOTANAL method (Tothill et al. 1992). The "quadrat 3D" method (or "Botacube") (Saïd et al. 2005) has been used in forest and shrubby areas (Coutarse and Charbonnet sites) to estimate biomass per strata and per plant species. These two protocols allowed to obtain data on plant composition, phenology and biomass per plant community.

300 plots have been randomly distributed within these four sites (Armenaz, Charbonnet, Coutarse and Bellevaux), which sampling resulted in the identification of 296 plant taxa. On the pastures, plant composition and biomass have been estimated during two period: between

mid-May and mid-June, and between end of June and end of July (Durparc et al. 2012). Relative abundances of plant species were not significantly different between the two periods and we always used the summer period data (end of June to end of July 2007) in our analyses, in order to fit with the hypothesis of Manly et al (2002) assumption that plant species proportion availability should not vary across the studied period.

b) Within the 48h home range

Since our goal was to identify selection criteria at a fine scale, we determined the plant characteristics in the close vicinity of each faeces. Plant found in the faeces are likely summing up several meals depending on the digestibility of plants. We considered (see Rayé et al. 2011, Steuer et al. 2011) that plants eaten during the last 48 hours were most likely to be found in the faeces. Therefore, we estimated plant availability in an area around the faeces which size amounted the average 48 hours home range of chamois and mouflon. The latter was estimated based on GPS-fixed from marked chamois and mouflon. This was the topic of P.A. Dupeyras master in 2014 (appendix 1 of paper II).

Given that faeces are not expected to be at the center of the last 48 hours home range, the relative proportion of vegetal community around faeces has been calculated as the mean relative proportion through the bootstrap method with 1000 samplings from 100 buffers randomly located around the faeces GPS location (fig.22). Consequently, each buffer has a centre more or less shifted relatively to the corresponding faeces location. Radiuses of 48h-home range buffers (r) were randomly sampled from the radius value distribution of 48h-home ranges of the summer season (fig.22).
Then, we estimated the relative proportion of plant species around faeces given the relative proportion of vegetal communities around faeces and the plant proportion within each vegetal community.

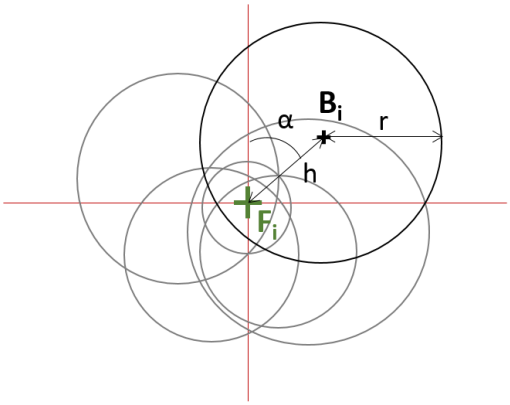



**Figure 22.** Visual representation of the method to estimate resource availability at the individual scale. $F_i$ is the localisation of the faeces, $B_i$ is the center of a buffer, with a radius $r$, staggered from a distance $h$ and an angle $\alpha$ (from Dupeyras 2014).

c) Within seasonal home range

A minimum convex polygon (MCP) of faeces on both pastures was used to determine a theoretical zone corresponding to the seasonal areas occupied by the animal shaving produced the sampled faeces. This MCP has been enlarged by a buffer whose diameter was equal to two size the maximum radius calculated from areas values of 48h home range of each species (rmax=645m for chamois and 890m for mouflons). A visual check from GPS data of individuals equipped with GPS in the same sites allowed to validate a potential zone used by the animals (Dupeyras 2014).

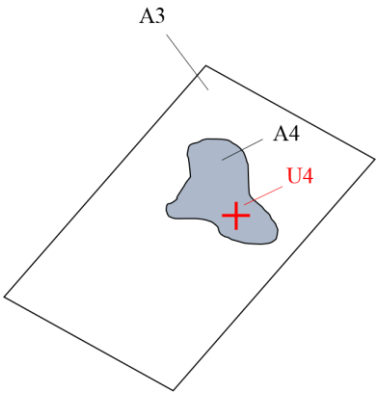



**Figure 23.** Schematic view of the different spatial scales surrounding an individual. A3: Relative abundance of available resources within the seasonal home range (*4.4.c*). A4: Relative abundance of available resources within the 48h home range (*4.4.b*). U4: Relative abundance of used resources measured in the faeces (*4.1*). Red cross corresponds to the GPS localization of the faeces. Selection at the 4$^{th}$-order corresponds to the ratio of U4 to A4. Selection at the 3$^{rd}$-order corresponds to the ratio of A4 to A3 (from Dupeyras 2014).

## 4.5 Synthetic view of the databases used for each analysis

In the first paper, we only used the information of taxonomic diets of the three ungulates (chamois, roe deer, mouflon, fig.24) to study the among-individual variation.

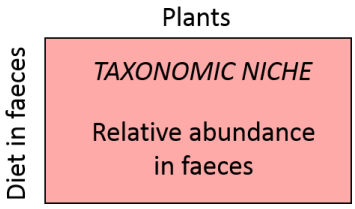



**Figure 24.** Database used in the article I (Upscaling the Niche Variation Hypothesis from the intra- to the inter-specific level).

In the second paper, we used the taxonomic and functional information of diet and habitat to study the coexistence between chamois and mouflon (fig.25).

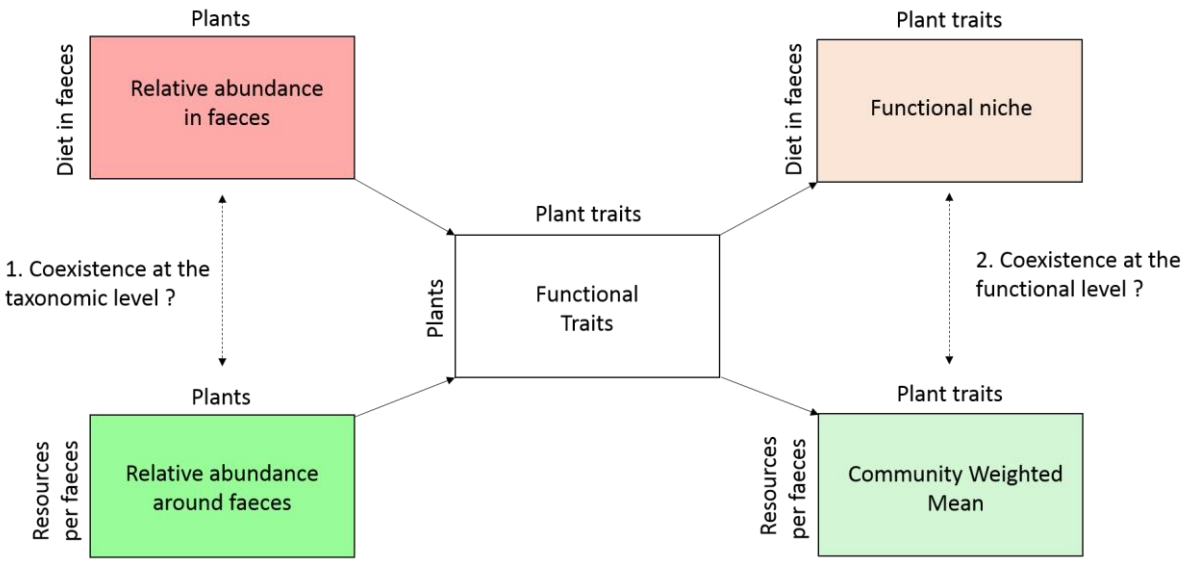



**Figure 25.** Overview of the databases and methods used. The Functional Niche (FN) and Community Weighted Mean (CWM) are calculated from the equation: $\boldsymbol{CWM} = \sum_{i=1}^{n} \boldsymbol{p_i} \times \boldsymbol{trait_i}$, where $n$ is the total number of plant species in the diet or habitat, $p_i$ is the relative abundance of species $i$ in the diet or habitat and $trait_i$ the trait value of species $i$. Questions 1 and 2 are solved with the coupled analysis of diet and habitat data, respectively at the taxonomic and functional level.

In the third paper, we used the functional niche of diet and habitat community weighted mean to determine the degree of selectivity; and the taxonomic diet of ungulates and the database of plant functional traits to determine the diet selection criteria over the year (fig.26).

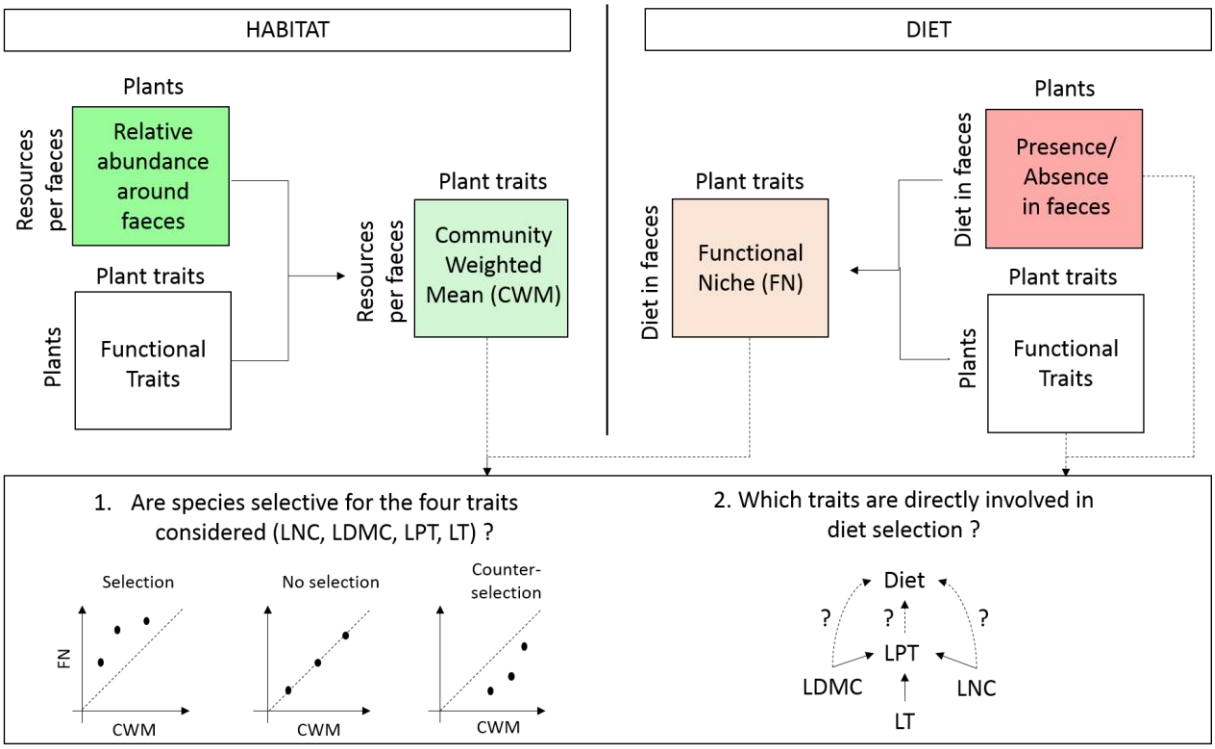



**Figure 26.** Overview of the databases used and questions asked. The Functional Niche (FN) and Community Weighted Mean (CWM) are calculated from the equation: $\boldsymbol{CWM} = \sum_{i=1}^{n} \boldsymbol{p_i} \times \boldsymbol{trait_i}$, where $n$ is the total number of plant species in the diet or habitat, $p_i$ is a proportion and is the same for each plant species $i$ in the faeces (if a faeces is composed on 10 plants, then each plant will have a proportion of 1/10),or $p_i$ is the relative abundance of plant species $i$ in the habitat and $trait_i$ the trait value of plant species $i$.

In the analysis of <u>resource selection at multiple scale</u> (Dupeyras 2014), we used the taxonomic diet of chamois and mouflon, the relative abundance of plants around faeces and at the pasture-scale (fig.27).

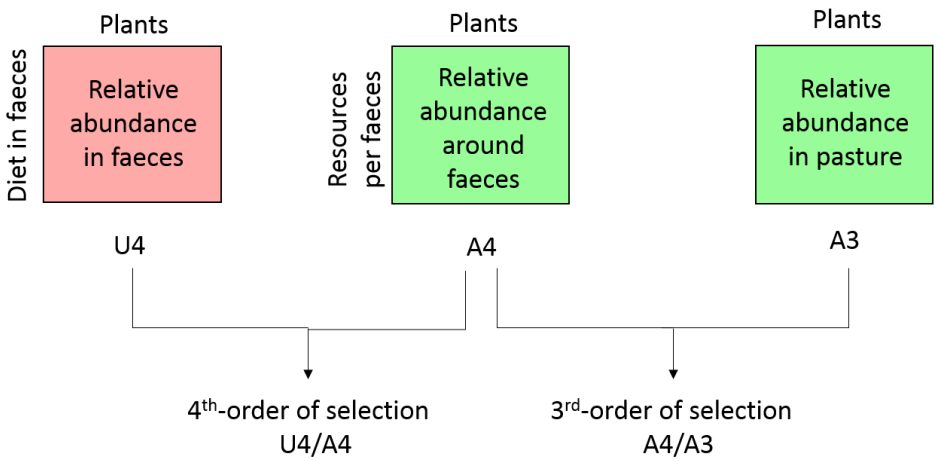



**Figure 27.** Databases used to determine the scale of resource selection.

In the fourth paper, we used the results from NIRS measurements and classical method measurements of French and Norwegian plants to determine the transferability of calibrations from a sub-arctic to temperate ecosystem (fig.28).

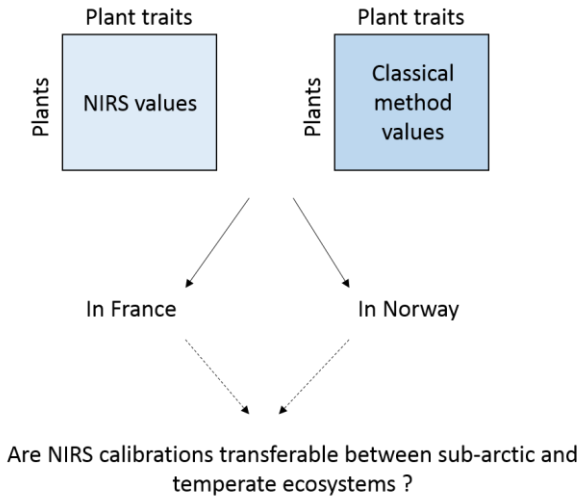



**Figure 28.** Databases used for NIRS calibrations in France and Norway.

In the note, we used the NIRS measurements on faeces and the functional niche of diets estimated from the plant functional trait database and the taxonomic diet database (fig.29).

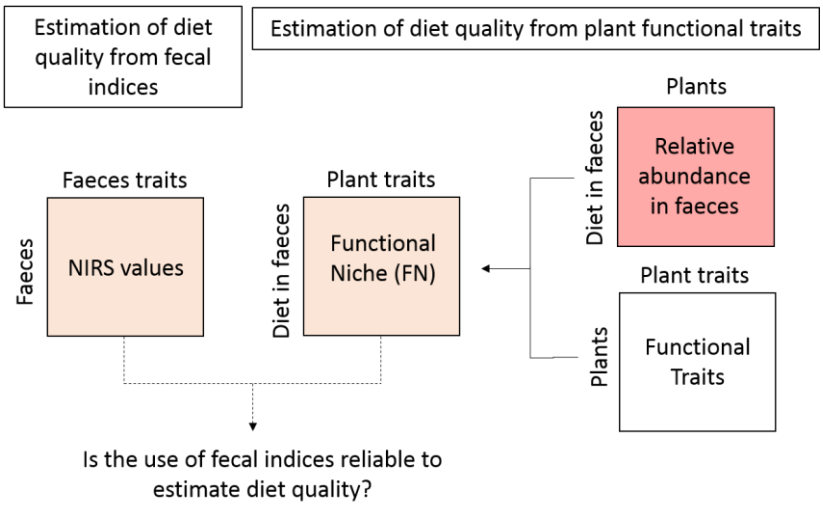



**Figure 29.** Databases used for different diet quality estimations comparison.

# Chapter V: General information about the diets

## 5.1 Database information

1076 faeces have been sampled in the Bauges Game and Wildlife Reserve, including 659, 205 and 212 faeces of chamois, mouflon and roe deer respectively. The sexes of all species, but roe deer, was identified. Faeces of chamois come from 339 females and 284 males, and faeces of mouflon from 115 females and 66 males. We did not use this information in the work presented here, but it could be used for further analyses as males and females can differ in their nutrient requirements (difference in body mass, antler and horn growth, gestation and lactation, see *3.3*).

## 5.2 Plant species identified in faeces

Respectively 326, 281 and 250 plant taxa were respectively identified in chamois, mouflon and roe deer faeces in the raw database.
After having removed plant species whose proportion was under 2.5% of DNA sequences in a faeces; 96, 79 and 70 plant taxa were respectively left in the faeces of chamois, mouflon and roe deer (see appendix 1 for the plant present in the faeces and their associated mean frequency of sequence and frequency of occurrence over the year; table 5 and table 6, and appendix 3 for rarefaction curves). Under this threshold, the sequence was considered as a barcoding mistake or as an occasional resource (Bison et al. 2015). All the analyses have been performed based on this cleaned-up database.

**Table 5.** Mean frequency of sequence (FS, %) and frequency of occurrence (FO, %) over the year of growth forms present in the diets of chamois, mouflon and roe deer (as rosodae are important in the diet, we let it in the table even if it is not a growth form denomination).

| | Chamois (FS, %) | Chamois (FO, %) | Mouflon (FS, %) | Mouflon (FO, %) | Roe deer (FS, %) | Roe deer (FO, %) |
|---|---|---|---|---|---|---|
| Deciduous shrub | 4,81 | 21,85 | 2,04 | 20,00 | 2,96 | 17,45 |
| Deciduous tree | 1,58 | 12,59 | 4,69 | 28,29 | 3,66 | 28,77 |
| Evergreen shrub | 29,49 | 65,10 | 6,04 | 32,68 | 5,96 | 11,32 |
| Evergreen tree | 2,08 | 16,84 | 8,75 | 27,80 | 4,04 | 24,06 |
| Fern | 0,14 | 0,30 | 0,00 | 0,00 | 0,00 | 0,00 |
| Forb | 21,02 | 82,25 | 12,81 | 59,02 | 19,23 | 64,15 |
| Grass | 0,43 | 4,70 | 1,33 | 16,10 | 0,08 | 0,47 |
| Leguminous | 18,82 | 67,37 | 29,35 | 62,93 | 5,56 | 20,28 |
| Rosodae | 16,97 | 47,80 | 24,18 | 63,41 | 53,46 | 92,45 |
| Sedge | 0,04 | 0,46 | 0,00 | 0,00 | 0,01 | 0,47 |
| Others | 4,61 | 4,60 | 10,81 | 5,92 | 5,03 | 4,72 |

**Table 6.** Overview of the sample size, mean number of eaten plant species per individual, and number of plants in the mean diet for the three ungulate species during each month (the other months were not included because of a too low sample size).

| | | **April** | **May** | **June** | **July** | **Aug.** | **Sept.** | **Oct.** | **Nov.** |
|---|---|---|---|---|---|---|---|---|---|
| **Chamois** | Sample size | 11 | 71 | 116 | 120 | 118 | 74 | 133 | 16 |
| | Mean number of eaten plant species/indiv. | 3.2<br>Min: 1<br>Max: 5 | 5.2<br>Min: 1<br>Max: 11 | 6.5<br>Min: 2<br>Max: 11 | 6.4<br>Min: 2<br>Max: 12 | 6.3<br>Min: 2<br>Max: 11 | 5.2<br>Min: 1<br>Max: 13 | 3.6<br>Min: 1<br>Max: 10 | 2.2<br>Min: 1<br>Max: 4 |
| | Number of plants in the mean diet | 12 | 47 | 53 | 64 | 53 | 46 | 48 | 10 |
| **Mouflon** | Sample size | 16 | 20 | 19 | 61 | 16 | 8 | 34 | 31 |
| | Mean number of eaten plant species/indiv. | 4<br>Min: 1<br>Max: 7 | 4.35<br>Min: 1<br>Max: 9 | 6.7<br>Min: 3<br>Max: 10 | 4.6<br>Min: 1<br>Max: 11 | 5.1<br>Min: 1<br>Max: 8 | 7.6<br>Min: 6<br>Max: 10 | 6.4<br>Min: 1<br>Max: 9 | 3.4<br>Min: 1<br>Max: 8 |
| | Number of plants in the mean diet | 20 | 23 | 26 | 27 | 17 | 17 | 53 | 30 |
| **Roe deer** | Sample size | 13 | 63 | 6 | 22 | 17 | 27 | 46 | 18 |
| | Mean number of eaten plant species/indiv. | 1.7<br>Min: 1<br>Max: 4 | 5<br>Min: 1<br>Max: 11 | 4.7<br>Min: 3<br>Max: 7 | 4.5<br>Min: 2<br>Max: 8 | 5.3<br>Min: 1<br>Max: 9 | 4<br>Min: 1<br>Max: 10 | 3.1<br>Min: 1<br>Max: 10 | 3<br>Min: 1<br>Max: 7 |
| | Number of plants in the mean diet | 5 | 48 | 11 | 17 | 30 | 28 | 41 | 24 |

# Chapter VI: Results from papers and additional analyses

## 6.1 From community to individuals (paper I)

### a) Does sociality drive the inter-specific variability of populations?

The sociality hypothesis (SH) positing that in social species, individuals may have a more similar diet than individuals of solitary species as all individuals feed close to each other with the same resource availability, was not supported with our data. On the contrary, the niche variation hypothesis (NVH), predicting that populations with wider niche should display higher among-individual variability, was supported at the inter-specific level (fig.2 in paper I).

We conducted additional analyses at the within species-level to determine if groups of individuals having similar foraging behavior could be identified, assuming that we were likely to find clusters of individuals with similar diets in social species (chamois and mouflon). We measured the degree of clustering with the clustering coefficient proposed by Araùjo et al. (2008). However, no clusters were found in the populations of chamois, roe deer and mouflon. The idea that we should find clusters of individuals feeding on the same plant species within the mouflon population as it is a social species where individuals spatially aggregate, and to a lesser extent the chamois population, was therefore not supported.

### b) How does intra-specific variability in diet vary with resource availability?

Our results showed that the total niche width (TNW) of all populations declined with resource availability. TNW was the lowest in winter, and the highest in summer for chamois and roe deer as the highest plant diversity occurred during this period. Mouflon had the largest niche width in autumn when it shifted from pastures to forests, resulting in a diet composed of various plants from these two environments (fig.2 in paper I).
In addition, the response of inter-individual variation to seasonal variation in TNW supported the niche variation hypothesis (NVH) at the intra-specific level, which meant that the more diverse the available resource, the higher the TNW and the higher the inter-individual variation (fig.2 in paper I).

## 6.2 The relative importance of food quantity and quality in large herbivore diet selection (papers II-III)

### a) How do large herbivores with high metabolic rate survive in poor environment such as pastures?

The question of the use of food resources by large herbivores in relation to their availability is a recurrent question in ecology (Wam et al. 2010). It opposes two hypotheses: the forage-abundance hypothesis where ungulates feed in response to the abundance of forages and the selective-quality hypothesis where the selectivity of feeding depends on the nutrient quality of forages (Weckerly 1994).

➔ Taxonomic niche

According to our results from paper II, diets of chamois and mouflon appeared to be conservative, at least for the main plant species making up the diet in spring and summer, within the daily home range. By the word "conservative", I mean that whatever the availability (except 0) the proportion of plant intake (but not the selection!) was constant (fig.30). Moreover, no significant relationship were found between the proportion of plants in the diet and the local plant availability. Our results also underlined the strong avoidance of chamois and mouflon for the most abundant plant species occurring in the pastures, i.e. *Carex sempervirens* and *Sesleria caerulea*. On the contrary, they focused on rare evergreen plant species, such as the evergreen shrub *Helianthemum nummularium* or on leguminous rich in nitrogen and easily digestible such as *Onobrychis montana* or *Lotus corniculatus*.

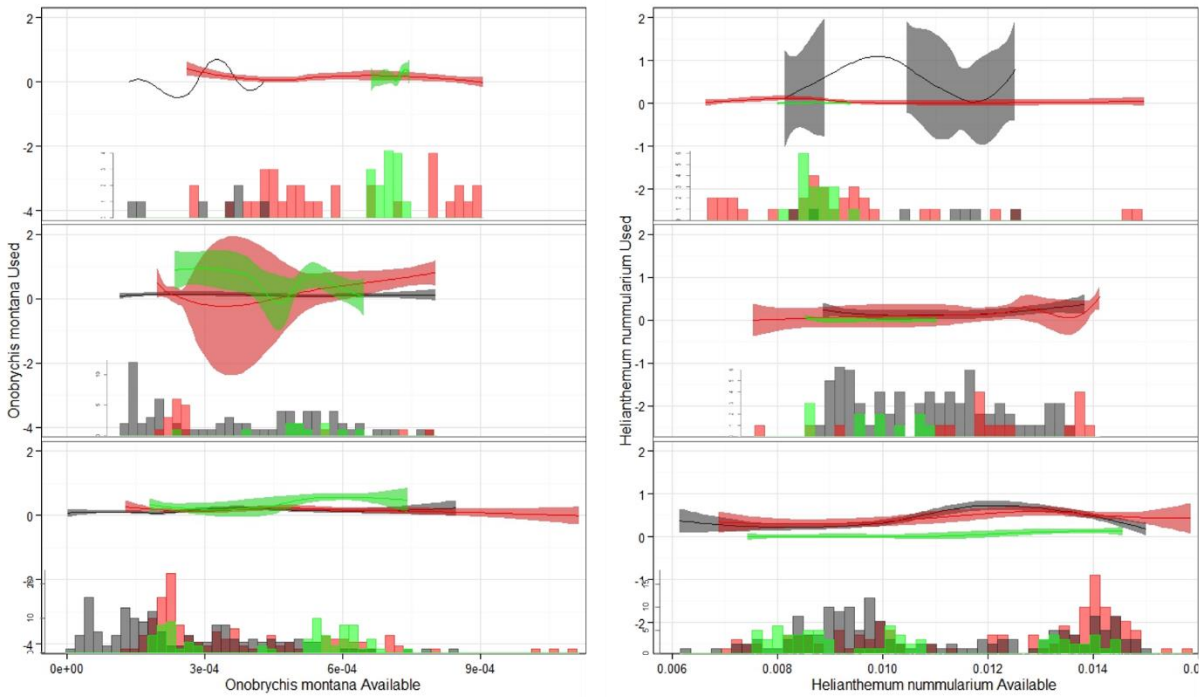



**Figure 30.** Effect of plant availability (relative abundance in terms of biomass in the field) on proportion in the diet for two of the most abundant plant species in the diets of chamois and mouflon (*Onobrychis montana* on the left and *Helianthemum nummularium* on the right). In green: mouflon population, in red: chamois in sympatry, in black: chamois in allopatry.

This selectivity observed both for chamois and mouflon was not completely in agreement with previous studies. Indeed, La Morgia & Bassano (2009), Bertolino et al. (2009), Garcia-Gonzalez et al. (1996) recorded that diets of chamois (*Rupicapra rupicapra* for the two first studies, *Rupicapra pyreneica* for Garcia-Gonzalez et al.) were composed of a large proportion of grasses (respectively about 30.2% in August to 46.9% in September, 48% in autumn to 67% in spring, 13.7% in autumn) in addition to forbs, in line with the "intermediate feeder" chamois diet type. Like in our study, both La Morgia & Bassano (2009) and Bertolino et al. (2009)

underlined the presence of Cistaceae (the family of *Helianthemum nummularium*) in the diets, but in lower proportions.

Even though mouflon have been classified as grazers in many studies (see references in Marchand et al. 2013), our study confirmed the non-compulsory grassy diet of mouflon as demonstrated in Marchand et al. (2013) (fig.31). In the Bauges Massif, their diet in pasture was mainly composed of forbs, with a low proportion of grasses (*Dactylis glomerata* especially). Hypotheses about the unusual diet compositions of chamois and mouflons are given in the *Synthesis, perspectives and directions*.

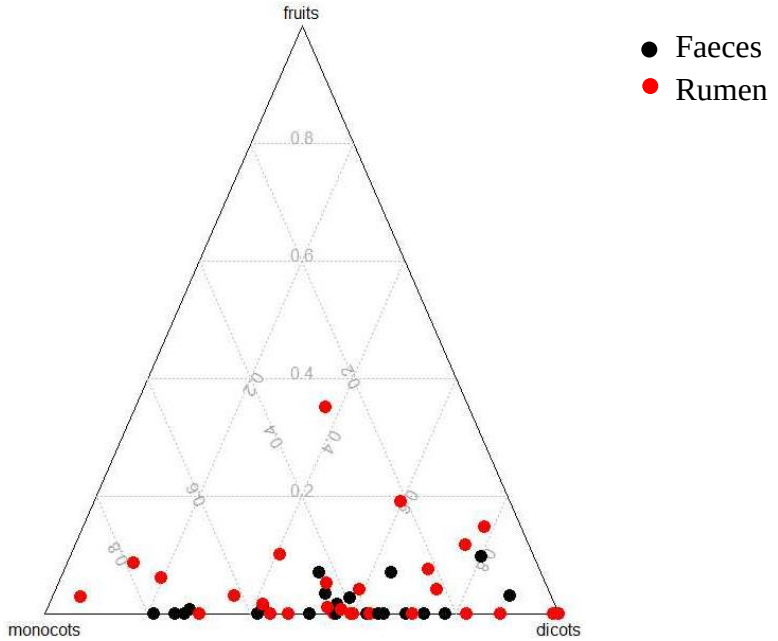



**Figure 31.** Ternary plot of the diet compositions of mouflon populations reviewed in Marchand et al. 2013.

➔ Functional niche

Functional niche of diets was described with the analogous of the CWM (Community Weighted Mean) metric, using three plant functional traits in relation with diet quality (nitrogen, phosphorus and leaf dry matter content). It also reflected the weak importance of functional availability (CWM available). Indeed, whatever the available nitrogen, phosphorus or water, animals were able to build their functional niche in a constant way, in spring and summer (fig.3 in paper II). In autumn, availability influenced the functional niche, but we hypothesized that during this period, selection could not compensate for the general decrease in the quality of plants available (fig.3 in paper II).

As stated in the introduction, 14-18% of crude protein has been estimated as the optimum percentage for maximum body growth of deer (French et al. 1956, Magruder et al. 1957). In our study, the diets of chamois and mouflon contained from 14% in autumn to 24% in spring of crude protein, which was greatly above the threshold of the deer. It suggested that chamois and mouflon, with their high metabolic rate, due to their low body mass (Demment & Van Soest 1985, White & Seymour 2003) selected for and found enough of a high quality diet. Hence,

chamois and mouflon were able to reach their energy requirements whatever the availability. Moreover, we showed that they were able to survive in poor environments such as pastures mainly composed of grass species by selecting rare plants of higher quality than the average available vegetation (diet rich in nitrogen, rich in water, easily digestible; fig.2 in paper III).

➔ Functional diversity

Although the preferences did not depend on the environment (CWM of the diet independent of the available CWM), we wondered whether the functional diversity (variation of functional trait value) of the diet depended on the functional diversity of the environment (48h home range around faeces). For that, we calculated the functional dispersion index (FDis, Laliberté & Legendre 2010), which estimates the mean distance between species trait and the CWM. For each season, we tested the relationship between the FD of the diet and the available FD, as in paper II for the CWM.

Except for LDMC in autumn where there was a positive relationship (slope=0.91, $p<0.05$), the functional diversity of the diet estimated with functional dispersion did not longer depend on the functional diversity of the available plant in the environment (fig.32, $p>0.05$).

***Main result:***

Chamois and mouflon fed on plants of high quality rather than on plants of high biomass and of low-quality. We assumed that it allowed them to reach their energetic requirements for growth and reproduction.

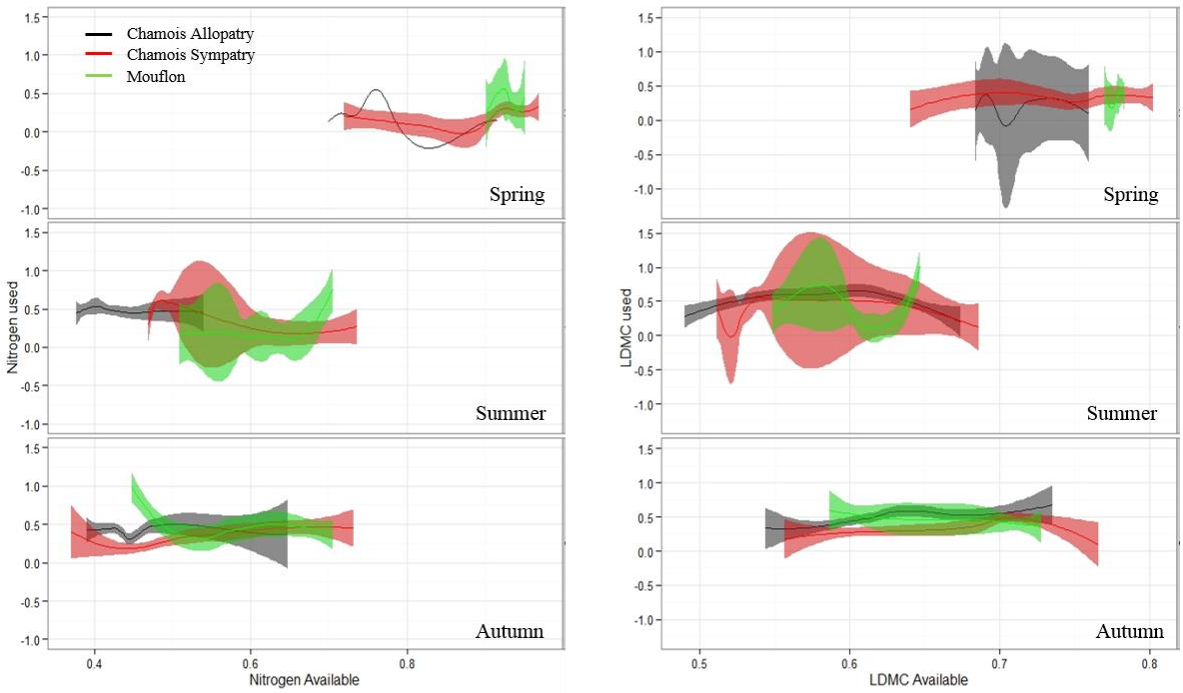



**Figure 32.** Effect of habitat FD (calculated as functional dispersion) on diet FD for nitrogen content (on the left) and LDMC (on the right).

b) Methodological considerations

In this work, the carbon content in plants had a relatively low coefficient of variation compared to nitrogen, phosphorus and water content. Consequently, even if Elser et al. (2000) advise the use of nutrient ratios, we decided to not include the C:N and C:P ratio in our analyses as their variations tracked the N and P variability. Moreover, we thought that the interpretation of single components was easier, as we are not aware of optimum ratio values required for chamois, mouflon and roe deer growth and reproduction.

In addition, the carbon content measured with the CHN analyzer included the indigestible and digestible carbohydrates. As the carbohydrates on which herbivores rely on are the digestible ones, which constitutes the proxy of energy, the total carbon content value does not give precise information on what is really used by the animal. Indeed, the lignin, considered as deterrent for herbivores as it is indigestible (Robbins 1983), is highly composed of carbon and could explain a large proportion of the total carbon content. The carbon content measured was not adapted to test the hypothesis that in winter, ungulates (white-tailed deer) would focus on plant rich in energy (Berteaux et al. 1998). For their part, they estimated the energy through the percentage of dry matter digestibility in food.

Finally, Elser et al. (2000) advised to use N:P ratio for animal studies. For the same reason as before, we preferred to focus on the two components independently, as we did not know the optimum values that large herbivores need to reach for their development.

c) At which scale do herbivores make their diet selection?

Additional analyses on the feeding selection scale by chamois and mouflon have been realized by P.A. Dupeyras (2014, M2 internship). The hypotheses are detailed in *2.2.c*.

The results showed that a large part of the key-resources (resources on the right of the red axis x = 1 on fig.33) of both chamois and mouflon had a selection pattern similar as “SP1” (selection pattern 1 on the fig.34). Key-resources were selected at the 48h home range ($4^{th}$-order selection) and not at the seasonal home range ($3^{rd}$-order selection) (fig.34). Therefore, animals did not choose sites where the preferred resources were abundant ($1^{st}$ hypothesis rejected). The most abundant plant species (*Sesleria caerulea*, *Carex sp.*) were eaten in low proportion or not eaten and led to pattern “SP2” or “SP3”. Hence, those patterns suggested that the home range selection did not depend on forage resources but on other factors such as sociality, fear or body physiology.

In addition, “SP1” highlighted a strong inter-individual variability in the proportion of key-resources eaten, which was in line with the significant among-individual variation observed in paper I.

***<u>Main result:</u>***

Herbivores selected their diet at the fine-scale ($4^{th}$ order of selection), i.e. within the 48h home range.

While availability did not seem to be the strongest determinant shaping the diet, the selection of plants should, at least partly, depends on functional traits. We used this approach to determine the plant characteristics involved in the diet selection hereafter (*6.4*).

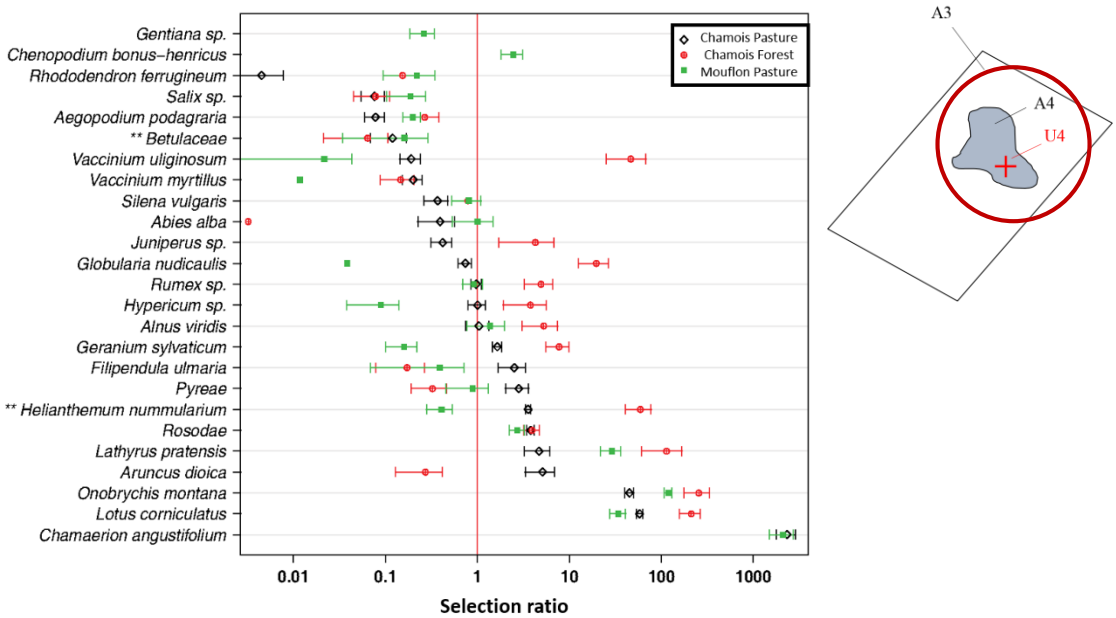



**Figure 33.** Mean selection ratio for the 25 key-resources in the diets of chamois and mouflons. Vertical red line (x = 1) corresponds to the axis where selection is neutral (from Dupeyras 2014). Selection ratios need to be interpreted carefully as they can be high in two cases: when the resource is rare and highly used, or when the resource is abundant and overused.

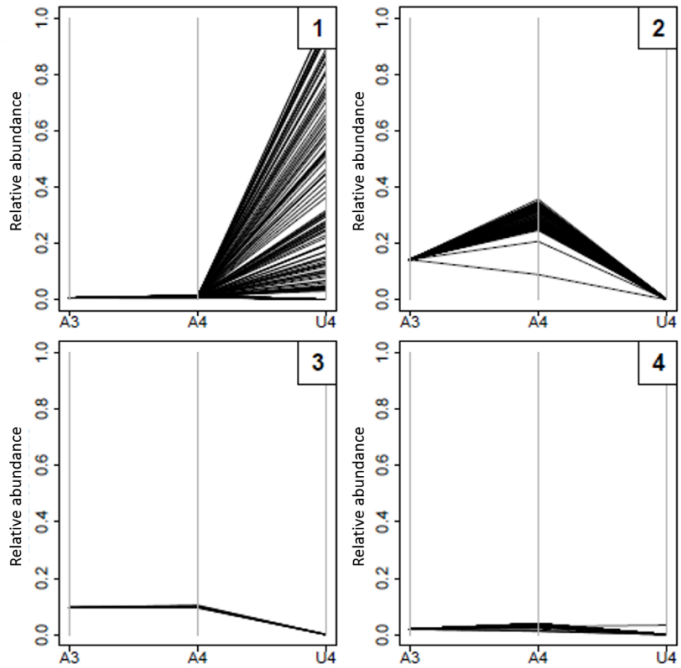



**Figure 34.** Selection pattern (SP) at the different orders of selection. 1: *Helianthemum nummularium* from chamois in pasture, 2: *Sesleria caerulea* from mouflon in pastures, 3: *Carex sp*. From chamois in pasture, 4: Betulaceae from chamois in forest. A3: Relative abundance of available resources within the seasonal home range (4.3.c). A4: Relative abundance of available resources within the 48h home range (4.3.b). U4: Relative abundance of used resources measured in the faeces (4.1). Each individual is represented by two lines linking the relative abundance of the used resource at two spatial scale (A3 and A4) (from Dupeyras 2014).

## 6.3 How do introduced species influence plant-herbivore interactions? Are introduced species always harmful for ecosystems? (papers II-III)

### a) What is the impact of introduced mouflon on the native chamois trophic niche?

Our results demonstrated a neutral interaction between chamois and mouflon on the trophic axis of the ecological niche. Chamois in sympatry did not remarkably change its taxonomic niche in the presence of mouflon.
The additional analyses on functional diversity (functional dispersion, fig.32) revealed some differences between the two chamois subpopulations. Depending on the season and the traits, functional diversity of the diet of chamois in sympatry was always equal or lower than for the chamois in allopatry. As these differences were not explained by availability, we could hypothesize that the presence of mouflon influenced the diet mixing of chamois.
However, the mean diet quality (calculated with CWM) of chamois in sympatry was always equal or higher than for the chamois in allopatry.
These information were in agreement with previous findings on activity rhythm and habitat selection (Darmon 2012, 2014) for which interactions between chamois and mouflon were marginal. These results confirmed the idea that introduced species are not necessarily harmful for co-occurring species of the same trophic level, even when both species are at relatively high densities.

***Main result:***
Introduced mouflon does not have negative impact on native chamois, neither on the taxonomic (ingested plant species) nor on the functional (diet quality) niches.

### b) How do species coexist?

The absence of negative effect of mouflon on chamois dietary niche may be due to (1) species-specific taxonomic and functional dietary niches, (2) non-limiting resource availability, (3) relatively low population density of introduced mouflon compared to the chamois preventing forced competition (Forsyth & Hickling 1998) for food (even though we are not aware of the absolute densities of chamois and mouflon in the pastures, we nevertheless know that chamois densities are similar between the sympatric and the allopatric sites, and the density of mouflon are lower than the density of chamois in the sympatric site), or (4) a spatial partitioning at a fine-scale during the three seasons preventing competition for shared resources. The population density estimates have to be interpreted carefully as population densities are difficult to assess.

#### (1) Species-specific taxonomic and functional dietary niches?

Our results (paper II) showed that, depending on the season, chamois and mouflon differed more or less in their diets in terms of taxonomic and functional niche position (paper II). Comparisons of the mean scores of chamois and mouflon populations on the first axis of the NSCA (fig.2 in paper II) revealed no differences in spring, but significant differences in summer and autumn, suggesting a partial taxonomic niche partitioning between both ungulates. The differences in functional niches between both species were less visible, but mouflon had a higher diet quality than chamois in autumn.

In spring, the absence of taxonomic and functional niche partitioning could be explained by a lower richness of developed plant species available, leading the animals to feed on the same first plantlets. In addition, as chamois and mouflon were partially spatially segregated during this period (Darmon et al. 2012), exploitative competition would have a weaker effect on populations.
In summer and autumn, the partial taxonomic and functional niche partitioning could be explained by differences in diet selection criteria, but not necessarily. Indeed, ungulates can have the same diet selection criteria (for example leaf nitrogen content) but have different taxonomic niche if they forage on different plant species to avoid competition, and different functional niche if ungulates exploit different range of values depending on their needs (Behmer & Joern 2008). Besides, both species can have different functional niches based on nitrogen, phosphorus and leaf dry matter content (as in paper II), but other plant functional trait could actually act as the main diet selection criteria.
Results from paper III showed that this partial niche partitioning can be partly related to different diet selection criteria between ungulates (see *6.4.a*).

(2, 3, 4) What about the others suggested factors?
Even though a partial niche partitioning was observed between chamois and mouflon, it does not mean that non-limiting resource availability, herbivore density or spatial partitioning (see *6.3.a*) are not acting in the coexistence.
Indeed, as we still observed a taxonomic overlap between both species in autumn and summer, we hypothesize that (1) the shared resources are not limiting and prevent competition, or (2) resources are limiting and there is exploitative competition, but, as they are suboptimal resources, it does not impact chamois diet quality (fig.2 in paper II).
To test the effect of mouflon population density on the strength of the niche partitioning between chamois and mouflon, we should investigate different sites varying in their mouflon population density.
Finally, a partial spatial partitioning of ungulates was observed in spring in Darmon et al. (2012), and it could also be active in summer and autumn and prevent competition on the shared food resources.

## 6.4 The unexpected importance of biomechanical traits in the large herbivore diet selection criteria (paper III)

“All models are wrong but some are useful” claimed Georges Box 30 years ago. We tried to emphasize some useful models to determine the plant functional traits involved in the food selection by ungulates. As correlation does not imply causation, as multicollinearity can be found between independent variables and might confound the relationship of an independent variable and the response variable (Smith et al. 2009, Ray-Mukherjee et al. 2014, Ruffell et al. 2015), and as relations between variables can be direct or indirect, we tested the validity of causal models relating plant functional traits and diet using path analysis (Wright 1921).

a) Do plant chemical content better explain diet selection criteria than other traits?

The results from paper III highlighted the major role of biomechanical traits in the food selection of chamois over the year (see fig.4 in the paper III). The pattern was slightly different for the mouflon. Indeed, in spring, none of the measured traits were responsible of diet composition, while LNC was preferred in summer and leaf toughness was avoided in autumn (see fig.4 in the paper III). In spring, when the mean leaf nitrogen content of plants is high in average and variability is low (Robbins 1983), selectiveness is likely to be relatively less important than in other seasons (Weckerly & Kennedy 1992). Still, the most abundant plant species did not dominate the diet, and ungulates selected plants on the basis of the four traits considered (Figure 2). Unmeasured traits might drive the selection criteria, or the too low sample size might prevent any significant pattern for the path Leaf Punch Toughness → Diet in spring. In summer, mouflon would select the plants with high nitrogen content in order to support nutritional demands, particularly for yearling growth (Crête & Huot 1993, Parker et al. 2009, Dostaler et al. 2011), horn development and body mass maintenance (Asleson et al. 1997), but also for lactating females that have to meet the protein demand for milk production (Reese & Robbins 1994) and to replenish their body condition before a new breeding cycle and winter (Crete & Huot 1993, Gerhart et al. 1997). Finally, in autumn, as plant quality decreases, mouflon focused on easily chewed plants.

Then comes the question: "Why do we not observe the same pattern of selection for chamois over the year?" Morphological traits of ungulate might be involved in the differences observed in diet selection criteria. Mouflon have more muscular rumen and stronger masseter muscles than chamois (Clauss et al. 2008a). Given these features, mouflon would have a higher ability to overcome the forage resistance both orally (mouth) and internally (rumen). Hence, in summer, when plants are still easy to chew or digest for the mouflon, they prefer to focus on the nitrogen content than on the leaf toughness. On the contrary, as masseter muscle mass of chamois are twice lower than mouflon (Clauss et al. 2008a), we hypothesized that the work required to chew or digest is the most important criteria for the choice over the year. For example, leaf punch toughness and nitrogen content are lower for *Helianthemum nummularium* (preferentially selected by chamois) than *Onobrychis montana* (preferentially selected by mouflon). Each of these plants builds up a large proportion of chamois and mouflon diets respectively and play a role in taxonomic diet differentiation, especially in summer and autumn (Bison et al. in prep).

Our results disclosed the importance of mechanical resistance, a criteria herbivore strongly select against, and therefore could have evolved in plants to protect leaves from herbivory (e.g. Coley 1983, Choong 1996, Wright & Vincent 1996, Pérez-Harguindeguy et al. 2003, Clissold 2007, Ibanez et al. 2013a).

***Main result:***

In the three seasons for chamois and in autumn for mouflon, biomechanical traits were more directly involved in diet selection than biochemical traits. Such patterns were not able to be detected in other studies because of correlations among traits.

b) Is the relationship between biomechanical traits and chemical traits (C:N ratio, LDMC) consistent across growth forms?

Testing the correlations between traits, we observed that strategies of plants are not as perfectly defined as theory predicts for the leaf economic spectrum (LES). For example, we found a positive correlation between punch toughness and leaf dry matter content during the three seasons, when all growth forms were included in the model (such as found in Ibanez et al. 2013a). However, when focusing on forbs, punch toughness was not related to leaf dry matter content anymore (fig.35) in spring (p-value = 0.18), summer (p-value = 0.08) and autumn (p-value = 0.91). The absence of correlation was already observed in some other studies (Perez-Harguindeguy et al. 2003, Deraison et al. 2015, unpublished data Ibanez S.). When feeding on forbs, this allows the animals making opposite selection toward two traits usually correlated.

The same observation was highlighted for the relationship between LDMC and C:N. While they were generally correlated at large scale ($p<0.05$ when all the growth forms were included in the analysis) (Wright et al. 2004), they were independent when linear relations were tested for each growth form separately. This resulted in the idea that correlations depend on the scale (Gross et al. 2007) and vary with the pool of species. For instance, Deraison et al. (2015) found no correlation between LDMC and C:N because of a high proportion of forbs in their samples. In such cases, it becomes possible to discriminate the effects of LDMC and C:N on the diet selection process.

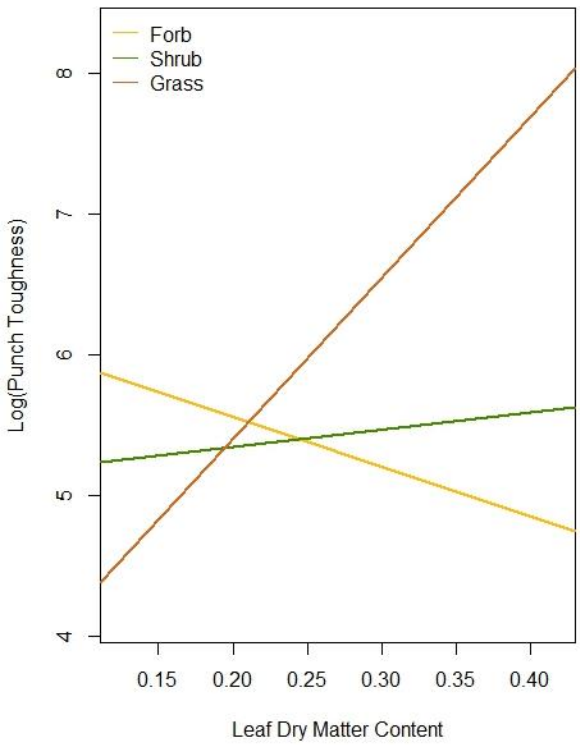



**Figure 35.** Relationship between punch toughness and leaf dry matter content for three growth forms in July.

c) What are the traits correlated to leaf punch toughness?

About 60% of the leaf punch toughness variability was explained by leaf dry matter content (LDMC), leaf nitrogen content (LNC) and leaf thickness (LT). The remaining 40% could be related to the organization, type and density of fiber content (Choong 1992, Onoda et al. 2011). Indeed, Choong et al. (1992) showed for dicotyledonous trees that the sclerophylly index – proxy of digestibility –, defined by Loveless (1961) as the ratio of crude fiber to crude protein, was correlated with the leaf toughness, particularly for the former.
In addition, fiber content was supported to be a strong diet selection criteria for red deer (Forsyth 2005, Zweifel-Schielly et al. 2012), white-tailed deer (Sauvé & Côté 2005) and eland (Watson & Owen-Smith 2002) as fibers reduce the digestibility of food to ruminants (Van Soest 1994) and makes nutrient access more difficult (Clissold 2007).

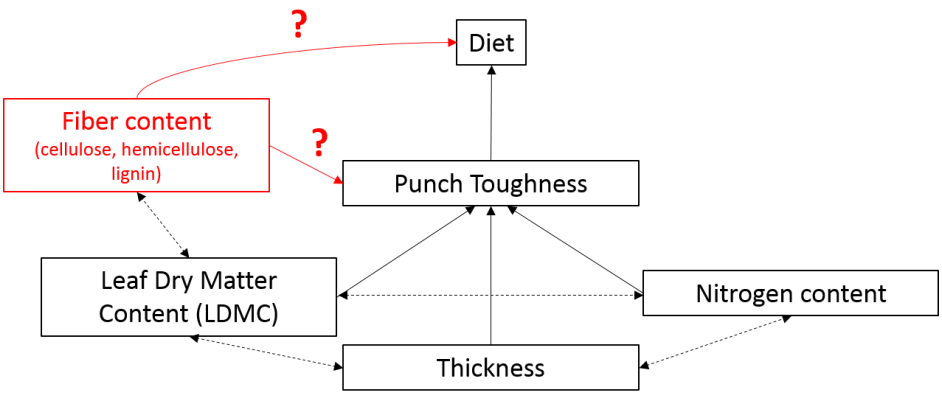



**Figure 36.** Hypothetical path analysis including plant fiber content as a trait influencing punch toughness.

Even though fiber content is correlated with LDMC (Al Haj Khaled et al. 2006, $r = 0.63$, $p<0.05$ for grass species), we hypothesize that fiber content could have a direct effect on diet selection, in addition to an indirect effect through LDMC. Further estimations of fiber content should be done to assess their influence on biomechanical traits measurements and how they impact diet selection.

Other anatomical properties of cell walls, veins organization, and cuticule/epidermis features could also be involved in the toughness measurement (Lucas et al. 2004, Read & Stokes 2006, Sanson 2006, Peeters et al. 2007, Onoda et al. 2008).

***Main result:***

The leaf nitrogen content, leaf dry matter content and leaf thickness accounted for 60% of variation of biomechanical traits. The remaining percentage could partly be related to plant fiber content.

d) What about the relationships between diet and other traits?

We did not include all the measured plant functional traits in the path analyses for two reasons: first, it would have led to a highly complex path diagram, and second, causal relationships among these other traits and diet are difficult to predict.

In the paper III, we tested the effects of quality traits on diet (biomechanical and chemical traits). In the introduction (*2.2.d*), we proposed that plant traits related to visibility could also influence diet choice. Hereafter we present plots relating the mean frequency of occurrence of plants in the diets of chamois in function of the plant "visibility" traits (vegetative height and inflorescence volume). We did not present the boxplot showing the frequency of occurrence of plants in the diet in function of sociality or phenology, as no trends were observed.

To calculate the inflorescence volume of each individual, we first measured the volume of a medium inflorescence (width*height*length) and then we multiplied this number by the number of clusters of inflorescence on the individual. In the case of *Arnica montana* for example, there was one inflorescence and we only measured the volume of this inflorescence. For *Heracleum sphondyllium* for instance, for which flowers are clustered, we first measured the volume of a medium cluster, and we multiplied this number by the number of clusters of inflorescence. Inflorescence means bud, flower or fruit, depending on the phenology.

No strong tendencies were observed between the frequency of occurrence in the diet and either of the two visibility traits (vegetative height and inflorescence volume). We hypothesized that their effects on diet selection of chamois were not major. However, more robust statistical analyses taking into account all the individuals, and not the mean frequency of occurrence of plants in the diets as presented here, should be done. In addition, the inflorescence volume should be interpreted with the information of the percentage of individuals having an inflorescence. Indeed, the inflorescence volume has been calculated from the plants having an inflorescence. Consequently, a plant can display a high inflorescence volume in our database, but as being the only individual amongst the 10 samples to have an inflorescence. This parameter can also play a role in the visibility and should be investigated.

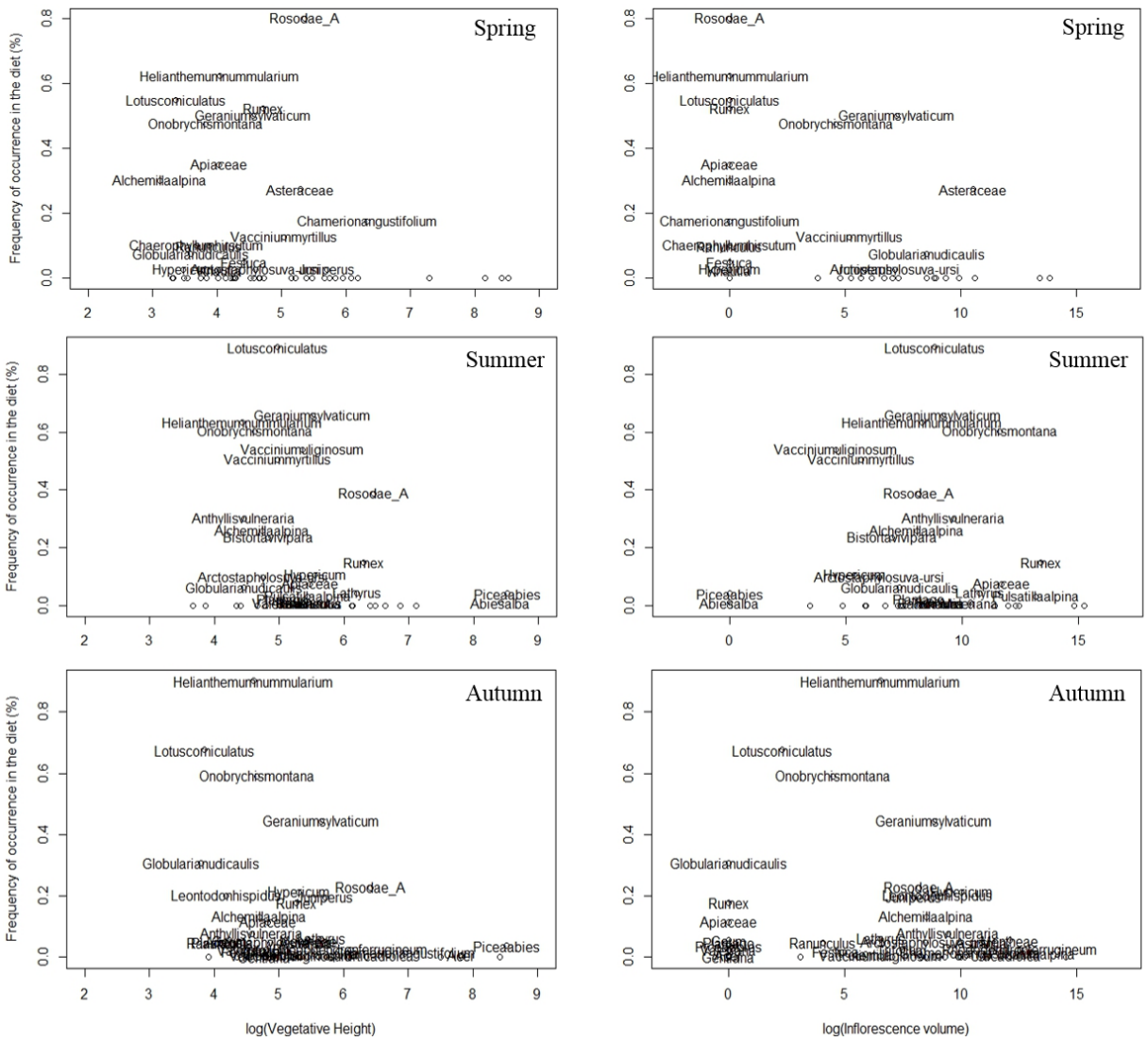



**Figure 37.** Mean frequency of occurrence of plants in the diets of chamois as a function of vegetative height and inflorescence volume in spring, summer and autumn. Dots without names are the plants present in the pasture but not eaten.

## 6.5 A bit of methodology: is the use of NIRS relevant and useful for estimating diet and plant quality? (papers IV-Note)

a) Are diet quality estimations with NIRS consistent with measurements on plant functional traits ingested?

In the previous analysis of this thesis, the quality of the diets has been estimated through the plant quality of ingested plants. This required a precise diet database and an extensive sampling and measurement of plant functional traits. In several studies, fecal indices (fecal nitrogen content for instance) appeared as a useful tool to determine the diet quality of populations and individuals. However, the relevance of fecal proxies of diet quality has been questioned by some authors (Robbins 1983, Robbins et al. 1987, Hobbs 1987, Servello et al. 2005).

At the inter-seasonal level, our results showed that FN (fecal nitrogen) cannot be used as a reliable estimator of DN (dietary nitrogen estimated with the nitrogen values of ingested plants) as only 38% and 14% of variation of DN were explained by FN for chamois and mouflon respectively. At the intra-seasonal level, no more significant relationships were observed. Different causes could be at play to interpret the differences in the slopes and intercepts of the relationships between FN and DN between both ungulates: (1) the activity level of tannin-binding salivary protein differ among species, which in turn affects the fecal nitrogen release differently between the two species (see *2.2.b* for the effect of tannin on nitrogen), (2) the diets of chamois and mouflon vary in their tannin content, which in turn, as previously, affects the fecal nitrogen release differently between the two species , (3) as a part of the fecal nitrogen come from undigested microbial nitrogen, the microbial rumen community could diverge among ungulates and differently affect the nitrogen release. Further analysis should be conducted to better detect the causes that are at the sources of the differences in relationships between DN and FN for different ungulates with different feeding types. Diets more rigorously and clearly described in terms of botanical and chemical compositions, in addition to a good knowledge about the digestive and assimilation process of nutrients by ungulates, should bring additional robust conclusions.
As a conclusion, we advised to use fecal nitrogen index for species-specific population at a large time-scale and not to detect small changes in population response to a changing environment, nor to compare the diet quality from fecal indices of different species.

***Main result:***
Fecal nitrogen estimated with NIRS account for a low proportion of dietary nitrogen variation estimated from functional traits of ingested plants.

b) Is the estimation of N, C and P with NIRS as robust as classical methods? Can we create three general calibration models (one for each chemical component) usable for different ecosystems? (paper in prep. not included in the thesis, Ancin Murguzur F.J., Smis A., Bison M., Struyf E., Bråthen K.A.)

Results demonstrated that the cross-validation and validation models varied in their goodness of fit depending on the nutrient tested (table 7). The best models were obtained for nitrogen (N, table 7, fig.38), as in Meuret et al. (1993), de Aldana et al. (1995), Moron & Cozzolino (2002), Petisco et al. (2005). The estimation of phosphorus (P) and carbon (C) were less accurate. As mineral elements as P do not directly absorb NIR radiation, they are indirectly measured by NIRS (de Aldana et al. 1995, Chodak et al. 2008). The prediction relies on association with organic or hydrated inorganic molecules (Clark et al. 1987). As the proportion of forms in which P exists in plants (phytate, phospholipids and nucleic acids) may vary among season, species and location, the reliability and consistency of calibration models are weak (Foley et al. 1998), relatively to nitrogen. In addition, the low range of P content values contained in plant could increase the error measurement and bias the models, explaining the low accuracy of P calibrations. Finally, as the coefficient of variation obtained for P with classical reference method was high, it could be additionally responsible for the unsatisfactory NIRS models. The use of NIRS data to estimate P should be suitable for studies not requiring high accuracy (Petisco et al. 2005).

The predictive ability of a region-specific model transferred toward another region was limited for all the three nutrients (C, N, P, table 7).
We suggested different hypotheses to explain this low transferability. First, same plant species exposed to different environments would have different biochemical structures which would absorb NIR radiation at different wavelengths. Hence, the wavelengths used to build specific-location models were different, leading to less reliable estimations when predicting sample values from different location. In addition, if a calibration does not include, for example freeze-resistant plant species, then a prediction of freeze-resistant plant constituents would be biased because the model "is not aware" of how much the antifreeze molecules change the spectra. As only ten species were in common between France and Norway, the difference in plant composition between the regions could participate in the low transferability of models. Finally, as the classical methods used to evaluate N, C and P content differed between France and Norway, the low transferability ability could be explained by errors in reference values (high coefficient of variation). Samples from both locations should be analyzed concurrently with both classical methods in order to remove the bias due to the methodology.

However, the global models including the data from both regions showed similar calibration coefficients as both models taken separately (table 7, fig.38). The global model helped to overcome the specificities related to the location, plant growth form, seasonality and methodology used, making more robust the estimation of chemically unknown samples from different location, species or season. Hence, our results suggested that the use of global models should be a useful and accurate predictive tool for extensive and intensive analysis at a large scale.

***Main result:***

Estimation of nitrogen content (N) with NIRS was as robust as classical methods but models and reference values of carbon (C) and phosphorus (P) need to be improved.
A single global model could be used to estimate leaf nitrogen, carbon and phosphorus content from different ecosystems.

**Table 7.** From the paper in prep. (Ancin Murguzur et al.). Parameters of cross-validation and validation models for France and Finnmark data for the three plant nutrients (C, N, P). Additional parameters of validation models done with foreign dataset. $R^2$ cal = $R^2$ for cross-validation, RMSEC = Root Mean Standard Error of Calibration, $R^2$ validation = $R^2$ of the validation set (internal validation and foreign dataset), RMSEP = Root Mean Standard Error of the Prediction, bias = mean error between estimated and measured values.

| **Carbon** | k | $R^2$ cal | RMSEC | $R^2$ validation | RMSEP | Bias | Intercept | Slope |
|---|---|---|---|---|---|---|---|---|
| Global | 26 | 0.83 | 1.1432 | 0.88 | 1.0027 | -0.03 | 2.29 | 0.95 |
| France | 21 | 0.88 | 1.0034 | 0.89 | 0.8 | -0.04 | 1.99 | 0.96 |
| *Prediction of Finnmark with France model* | | | | 0.66 | 1.19 | 2.0519 | 18.02 | 0.59 |
| Finnmark | 16 | 0.83 | 1.0792 | 0.87 | 1.1568 | -0.19 | 3.36 | 0.93 |
| *Prediction of France with Finnmark model* | | | | 0.7 | 1.36 | 2.4295 | -3.09 | 1.09 |

| **Nitrogen** | k | $R^2$ cal | RMSEC | $R^2$ validation | RMSEP | Bias | Intercept | Slope |
|---|---|---|---|---|---|---|---|---|
| Global | 17 | 0.93 | 0.2688 | 0.95 | 0.2207 | -0.07 | 0.09 | 0.99 |
| France | 20 | 0.96 | 0.2373 | 0.93 | 0.2662 | -0.03 | 0.09 | 0.97 |
| *Prediction of Finnmark with France model* | | | | 0.86 | 0.28 | 0.42 | -0.19 | 0.96 |
| Finnmark | 18 | 0.96 | 0.1583 | 0.94 | 0.172 | -0.02 | 0.26 | 0.88 |
| *Prediction of France with Finnmark model* | | | | 0.88 | 0.38 | 0.5657 | 0.54 | 0.94 |

| **Phosphorus** | k | $R^2$ cal | RMSEC | $R^2$ validation | RMSEP | Bias | Intercept | Slope |
|---|---|---|---|---|---|---|---|---|
| Global | 13 | 0.65 | 0.0747 | 0.75 | 0.0685 | <0.01 | 0.02 | 0.89 |
| France | 6 | 0.71 | 0.0675 | 0.81 | 0.0565 | <0.01 | 0.05 | 0.8 |
| *Prediction of Finnmark with France model* | | | | 0.55 | 0.09 | 0.1222 | -0.02 | 0.653 |
| Finnmark | 10 | 0.69 | 0.0672 | 0.69 | 0.0714 | <0.01 | 0.04 | 0.84 |
| *Prediction of France with Finnmark model* | | | | 0.54 | 0.08 | 0.1324 | 0.09 | 0.97 |

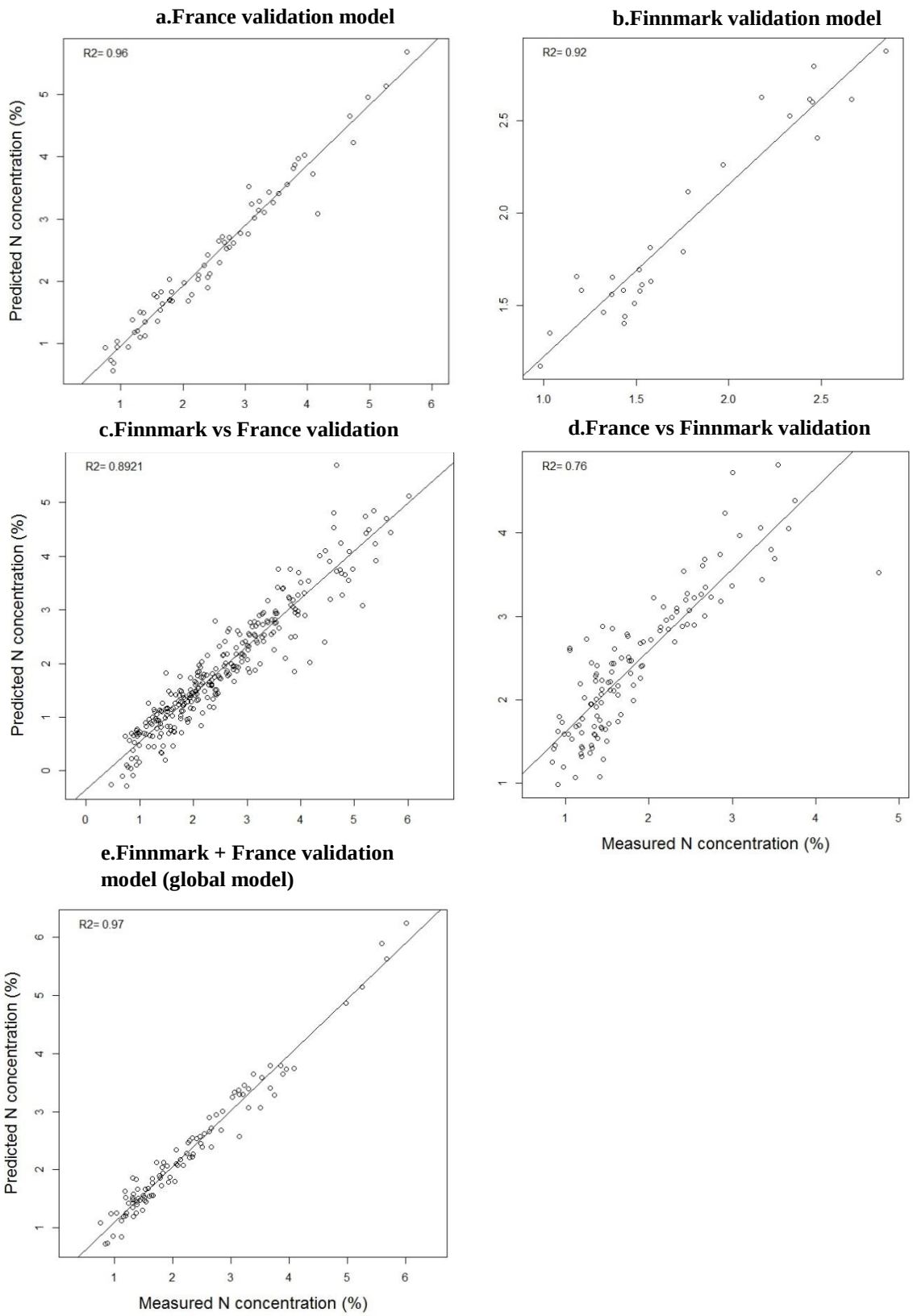



**Figure 38.** From the paper in prep. (Ancin Murguzur et al.). Validation models testing the correlation between predicted N content (%) from NIRS and measured N content (%) from classical chemistry methods, at each site (a,b), one site vs the other (c,d), for the global model (e).

# SYNTHESIS, PERSPECTIVES & DIRECTIONS

4

First, I will address some methodological considerations that should be taken into account in interpreting the results. Then, I will discuss about plant-herbivores interactions in the light of results obtained in this thesis. Finally, I would like to demonstrate that our results, in addition to provide some clarifications about trophic interactions between ungulates and plant communities, could be useful in the field of studies that attempt to predict species distribution in response to climate change.

## 7.1 Methodological considerations

### a) Questioning the reliability of diet estimation from faeces data

Usually assessed from rumen or fecal samples, browse surveys or direct observations (Pompanon et al. 2012), composition of diets can be misevaluated. In the case of fecal samples, the real diet is usually biased because of the differential digestibility among plant species (Verne & Ullrey 1984). Indeed, as plants with low fiber content are more easily digested than high fiber content plants, they are hard to identify and tend to be underestimated (Skinner & Telfer 1974). In this work, grass species that decompose slowly were poorly represented in the faeces, which reinforce our conclusions that grass species are eaten to a low extent by chamois and mouflon. However, the diets described for chamois and mouflon were not always consistent with other studies, as detailed in the summary of results (*6.2*). Different hypotheses about these differences are possible. Firstly, the grass species were under-represented in the faeces even though a large proportion (between 20 and 50% of the diet) was found in the rumen of chamois and mouflon (Redjadj et al. 2014). However, the comparison has to be done carefully as Redjadj et al. (2014) focused on the period from September to January, which has been poorly studied in our work as a very low number of faeces was available in winter. Second, diets obtained from other studies (La Morgia & Bassano (2009), Bertolino et al. (2009), Garcia-Gonzalez et al. (1996)) have been determined by microhistology, a method where the identification of highly digested small fragments is very difficult and which tends to overestimate grass species. We would need to analyze faeces both by microhistology and barcoding to properly compare the discrepancies between the two methods. Third, according to the review of Marchand (2013), diets of mouflon are not restricted to grass species, and the environment influences the diet choices. Hence, it is conceivable that mouflon from the Bauges Massif could prefer a diet rich in dicots. As chamois are classified as intermediate feeders, it was not surprising that their diet contained only few grasses.

### b) NIRS experience

One of the challenging topics in ecological studies is the identification of large-scale process driving the structure and dynamics of communities (Sutherland et al. 2013). We therefore have an urgent need for sampling and analytical methods suitable for building large databases of

plant chemical functional traits, for example. Devices such as CHN analyzer used for classical chemical analysis, e.g. to estimate plant nitrogen content, are time-consuming, costly and destructive. The use of NIRS methodology is a promising approach as it overcomes the problems of time, cost and sample destruction. Several studies, among which one presented in section *6.5.b*, have demonstrated that NIRS was a useful tool for predicting nitrogen content in plants (Petisco et al. 2005, Moron & Cozzolino 2002, de Aldana et al. 1995, Meuret et al. 1993). Additional measurements need to be done to determine why NIRS is not as accurate for estimating carbon and phosphorus content. Besides, the results showed that a single calibration for sub-arctic and temperate ecosystems gave similar results as specific models for each location for the three studied nutrients (C, N and P). This opens the way to extensive sampling whilst saving time and money: instead of having to calibrate a model for each location, a single global model could give similar results.

#### c) Fecal indices as poor estimator of diet quality

NIRS was applied on faeces sample to evaluate the diet quality of the animals that produced the faeces. Here, the reliability of NIRS was not tested by comparing it to results obtained from traditional chemical analyses, but we indirectly assessed its relevance to detect diet quality by comparing it to the average nitrogen content of ingested plants (determined by DNA-metabarcoding). The relationship between these two estimates of diet quality was poor suggesting that using NIRS nitrogen estimates was a poor proxy of diet quality at the intra- and inter-seasonal level. Fecal indices could perform poorly because of the effect of tannins on nitrogen release. However, the main shortcoming of our results is that our method to estimate ingested nitrogen from diet composition obtained from barcoding, combined with plant nitrogen, is not yet validated as a reliable method to estimate true nitrogen intake. Experimental trials controlling the initial quality or resource ingested should be used to determine the factors and the methodological bias affecting the diet quality measurements.

## 7.1 Plant-herbivores interactions

European mountain ecosystems are affected by climate warming, concomitant with the increase of large herbivore densities and changes in vegetation dynamic. In order to predict population dynamic and ungulate interactions, resource characteristics that are important for herbivores need to be determined. This involves identifying the factors explaining diet selection and coexistence among herbivores at the intra- and inter-specific level.

#### a) Thoughts about diet selection

The results of this work suggested that diets of ungulates living in alpine pasture environments (chamois and mouflon) were affected by food quality rather than quantity (paper II & III), and that the resource selection happened at the fine-scale 48h home range rather than at the broader-scale of the seasonal home range. At this coarse-scale, the selection was driven towards high biomass (and relatively low-quality) plant communities (Duparc, personal communication), and towards areas where preferred resources were locally rare. Those results contradict other studies

where ungulates selected patches of high-quality within the seasonal home-range in order to reach their energy requirements for lactation or growth (deer in winter in Zweifel-Schielly et al. 2009, moose in summer in van Beest et al. 2010). It suggested that, in our study, other factors such as sociality, fear (search for refuge sites against predators, Ruckstuhl & Neuhaus 2002) or body physiology (temperature plays a role in the habitat selection, Marchand et al. 2015) motivated the plant community choices. However, within the chosen plant communities, ungulates were highly selective and were able to compose a diet of higher quality than the average available vegetation (paper III).
While we showed that species functional niche position (calculated as CWM) was influenced by food preferences for both chamois and mouflon, we also demonstrated that the variation of functional diversity of each functional trait (estimated as functional dispersion hereabove, Laliberté & Legendre 2010) did not depend on habitat diversity. The level of diet generalization of these ungulates seemed to be species-specific, contrary to grasshoppers where the species niche widths depended on habitat functional diversity (Ibanez et al. 2013b). Even though co-occurring species differ in their niche position, such as chamois and mouflon in paper II, large and overlapping functional niche widths could limit the niche partitioning (Ibanez et al. 2013b).

Our results (paper III) pointed out that plant functional traits may be used as determinants of diet selection and that different choice criteria among ungulates can act as mechanisms at the origin of niche partitioning. Furthermore, our analysis highlighted the importance of biomechanical traits in diet selection, as already observed for insects (Gomez et al. 2008, Perez-Harguindeguy et al. 2003, Ibanez et al. 2013a). Biomechanical plant properties therefore have the potential to act as anti-herbivore defenses (Sanson et al. 2001), even against large herbivores. In addition, our results went one step further as they discriminated the direct and indirect effects of correlated functional traits of diet selection and pointed out that the usual idea that herbivores would focus their selection towards protein intake (Berteaux et al. 1998, Dostaler et al. 2011) was true but not complete: this parameter has not always a direct but an indirect effect on diet selection through biomechanical trait.

- We measured the functional traits of plants, but what about their match with herbivore functional traits?

As large herbivores are able to make a choice based on plant biomechanical features, it suggests that their morphology constrains the use of resources, and/or they are morphologically able to feed on all the resources but have preferences for soft plants. Soft plants indeed require less energy to be digested. In both cases, it involves innate or learning abilities in order for animals to make the appropriate selection (Provenza 1995, Tixier et al. 1998). The importance of morphological associations between resources and consumers has been emphasized in studies of herbivore-plant network (Ibanez et al. 2013a), prey-predator interactions (Song & Kim 2014, Spitz et al. 2014), or plant-pollinator systems (Temeles et al. 1996, Maglianesi et al. 2014). For example, Ibanez et al. (2013) showed that mandibular traits reflected the feeding preferences of grasshopper species, as grasshoppers with higher mandibular strength were able to consume tougher plants. In hummingbird-flower interactions, bird species with curved bills better reached nectar from curved flowers than straight-bill hummingbirds (Maglianesi et al. 2014). These results indicate that morphology can strongly influence resource use, which in turn allows

niche partitioning within species assemblages and reduces competition. Different studies demonstrated the link between feeding strategy of large herbivores (browser-grazer) and animal characteristics (such as type of reticulo-rumen: Clauss et al. 2003b, Clauss et al. 2009; shape of muzzle: Gordon & Illius 1988; salivary binding proteins activity: Clauss et al. 2005; teeth size and wear: Williams & Kay 2001, Mendoza et al. 2002; jaw muscle strength: Clauss et al. 2008a). For example, Clauss et al. (2003) showed that grazers are more prone to overcome a diet composed of grass than browsers, as the stronger musculature and the higher capacity of their reticulo-rumen allows digesting fibrous plants in relatively large quantity. Hence, large herbivores with strong masseter and rumen muscles should be able to better feed on tough plants than herbivores with low masseter and rumen strength for example. Those associations are suggested in our paper III. The quantitative confirmation of such associations would be consistent with the optimal foraging theory predicting that a strong match between traits of resources and consumers favors the efficiency of resource use (Pyke et al. 1977). Therefore, one of the next step in plant-large herbivore interactions would be to further explore quantitatively the relationship between the eaten plant characteristics, i.e. not only their proportion in diet, and the intrinsic animal characteristics to determine to which extent functional traits of primary producers and consumers are related and to mechanistically explain the network of trophic interactions (Clauss et al. 2008b). This kind of analysis should be done on a large ungulate community, such as found in Africa, to obtain a wide gradient of herbivore features. However, we are aware that biomechanical traits are not the only traits implied in diet selection, as diet results from a complex set of constraints. Other biochemical traits could exert additional selection pressures on diet selection (Daering et al. 2005, Iason et al. 2005) and lead some animals or species to feed on non-optimal plants in terms of biomechanical traits (Ibanez et al. 2013a). For instance, large herbivores with low jaw and rumen strength could feed on tough plants to avoid secondary compounds negative effect.

Besides, given the selected plant and the herbivore's anatomy, the cropping and chewing rate would be affected. As grasses are tougher than browses (higher fiber content, higher dry matter content, lower nitrogen content), the chewing and digestion process will take longer time (Robbins 1983, Choong et al. 1992, Wright & Illius 1995), especially if the animals has a reticulo-rumen less adapted to digest fibrous plants (Clauss et al. 2003b). However, the gain of energy animals would win on a relatively fast passage rate permitted by a higher digestibility, would be potentially offset by the loss of energy required to search for non-apparent high quality resources (Pyke 1977, "process 1" in Spalinger & Hobbs 1992).

- Including the spatial distribution of vegetation at a fine-scale in diet selection studies

Even if our results point at the importance of food quality rather than quantity in the diet selection and a frequency-independent food choice, we still need to more precisely evaluate the influence of plant availability on diet choice. A more precise spatial analysis of the available vegetation would be useful to disentangle the relationship between spatial heterogeneity of vegetation (Laca et al. 2008), resource selection and among-individual variation. For example, it has been shown that the plants neighbors can influence the food intake (Palmer et al. 2003, Bergvall et al. 2006, Bee et al. 2008). Field study showed that red deer were less likely to browse plant species when they were surrounded by less palatable plants because herbivores were avoiding low-quality patches, and more likely to browse if they were in a patch of high quality

vegetation, in agreement with the “repellent-plant hypothesis” (Bee et al. 2008). On the contrary, the “attractant-decoy hypothesis” predicts that a plant growing among plants of high quality would gain protection from herbivores, as herbivore would also feed on the other high-quality plants, but would lose protection if it is surrounded by lower quality plants (Bee et al. 2008, Bergvall et al. 2006). As, in our study, chamois and mouflon were selecting patch of high biomass where preferred resource were locally rare, we could suggest that the foraging on the preferred plant species was exacerbated by the surrounding plants of low quality, as predicted by the “attractant-decoy hypothesis”. Here, we would like to determine whether such processes influence chamois and mouflon diet choice in the wild. For that, the positions of feeding station of animals and the associated diet, in addition to precise vegetation maps describing the abundance, the spatial distribution and quality of plants at a small resolution would be required, which represents a huge amount of field work. This could help to bring robust conclusions about the relative importance of quality, quantity and spatial heterogeneity of plants in the process of individual diet selection by wild solitary large herbivores.

- Concerns

Different concerns arose from the diet selection criteria study. As we used generalized linear mixed models in the path analyses, we were not able to determine the percentage of variation of the diet explained by the variables included in the models. As the process of food selection is not driven by a single trait, but by a complex set of features, it is likely that other factors or plant functional traits not included in the model explain another large part of the variation that we did not account for. Including other variables in path analysis requires to figure out how traits co-vary. We already have a good knowledge about the relationships between some chemical and biomechanical traits (LNC, LCC, LDMC, leaf punch toughness) and plant structure (specific leaf area, height), but relatively little is known about the inflorescence traits and their link with the plant traits described previously for example. Indeed, we could easily conceive that the size or color of inflorescence would play a role in plant selection (Gomez et al. 2003) in summer as we observed a lot of headless plants on the field. As the concentration of nutrients vary among the plant organs and influence the herbivore nutrient intake (Bailey et al. 1996), information about the preferred plant parts by herbivores could also improve the investigation of resource partitioning among species, but it would require to additionally perform microhistology on faeces (Pompanon et al. 2012), direct observations or recorded movies from movie camera head or neck placed on animals.

In addition, even if the importance of biomechanical trait, relatively to the chemical defences such as secondary compounds, has been already underlined by some researchers working on herbivorous insects (Coley 1983, Lowman & Box 1983, Ohmart & Edwards 1991, Steinbauer et al. 1998) and mammals (Wright & Vincent 1996), including data on plant secondary compounds and the species-specific ability of ungulates to cope with it could help to improve our understanding of the selection of some plant species.

b) Thoughts about rules of ungulate community assembly

In our study area, competition did not drive habitat selection of chamois and mouflon at the plant community-level (Darmon et al. 2012). Even though there was no negative effect of the mouflon on the spatial axis of the ecological niche of the chamois, the large spatial overlap

observed at a broader scale led us to wonder about the competition for food resources. Our work demonstrated no negative impact of mouflon on chamois population on the taxonomic and functional feeding axis of the ecological niche (paper II). Chamois and mouflon were able to partly partition resources during the main part of the year, and even in autumn when the quality of the vegetation began to decrease. The partial taxonomic and functional niche partitioning seemed to be partly allowed by different diet selection criteria between chamois and mouflon over the year (paper III), which could be itself partly explained by morphological features of herbivores (paper III). It supports the idea that grazing ungulates with differing morphology occupy separate feeding niches (Murray & Illius 2000). Therefore, an introduced species with similar body size and similar ecological needs as native species of the same trophic level does not necessarily influence the relationships between the native species and its resources negatively, which contradicts the "usual" observed competition (Forsyth 2000, Lovari et al. 2014, Ferretti et al. 2015). Even though the coexistence has been observed on the spatial, temporal, and food axis of the ecological niche, the question of competition between both ungulates should be further considered in the context of increasing ungulate densities, of density-dependence (Garel et al. 2011), and with regard to the contrasted conclusions regarding chamois and mouflon co-occurrence (Bertolino et al. 2009, Chirichella et al. 2013).
The consistency of a coexistence pattern in chamois and mouflon interactions could be investigated through the study of other pairs of sympatric/allopatric sites for instance. Additionally, downscaling the analysis of food partitioning at the individual-level could allow determining whether competition may act a finer scale and impact only a subset of individuals.

Whereas the competition between species is usually seen as a major structuring force of communities, facilitation could also be involved in the species coexistence (Gordon 1988, Arsenault & Owen-Smith 2002, Waldram et al. 2008). In our study (paper II), we observed that chamois population in sympatry had an equal or higher diet quality than in allopatry. We suggested different hypotheses for this observation (see discussion of the paper II), and one of it was a facilitation effect of mouflon populations. Indeed, mouflon could allow a better access to suboptimal resources of high quality for chamois in sympatry, by reducing grass height or removing stems (Arsenault and Owen-Smith 2002). This effect could be predominant during the growing season (Arsenault and Owen-Smith 2002). However, competition should dominate facilitation during period of low biomass availability (Arsenault and Owen-Smith 2002). Because of a too low sample size and because mouflon shift to forest habitat in winter, we have not been able to assess the interactions between chamois and mouflon during a period of very low resource availability (in autumn, the resource availability decreases but not enough to result in competition, paper II). However, in winter, mouflon are subject to overlap with forest ungulates (roe deer and red deer, Redjadj 2010), and as individuals should be restricted to small refuge areas where vegetation is still available, the population density within food areas should increase and in turn, enhance inter- and intra-specific competition. However, this remain to be tested, as done in paper II, by comparing situations of sympatry and allopatry.

c) Why do species differ in their among-individual variability? An unsolved question

One of the questions that remained unsolved at the end of the paper I was the determination of the causes of the intra-specific variability and why the degree varied among species. Our results demonstrated that the niche variation hypothesis (NVH, Van Valen 1965) was supported at the intra- and inter-specific level: the wider the niche, the larger among-individual variation. Our hypothesis that sociality (SH) could drive inter-individual variability has been rejected. On the contrary, the most social species had the highest degree of among-individual variation and the largest population niche width. Sociality could actually be seen, not as favoring diet similarity due to the spatial overlap of individuals within a group, but as a driver of dissimilarity because of a stronger intra-specific competition within a social group (aggressiveness activity in large moose groups noticed in Molvar & Bowyer 1994) leading to stronger diet differentiation. Those results were in agreement with some experimental studies performed with fishes (Svanbäck and Bolnick 2007) for which inter-individual variation increased with population density. In contrast, solitary species such as roe deer should be less prone to intra-specific competition at the fine spatial scale, as each individuals should be able to feed on their preferred resources without pressure from congeners in their close vicinity. In addition, as roe deer are supposed to have a lower digestive plasticity than mouflon, we hypothesized that morpho-physiological characteristics could constrain the degree of among-individual variability.

Further analysis should be performed to go deeper in the understanding of the causes of individual specialization and why it varies among species. This would require a higher diversity of large herbivores, such as found in Africa, that would encounter no confounding effects between sociality, body size and feeding type (for example, different sociality levels for a single feeding type, Jarman 1974, Fritz and Loison 2006).

At the intra-specific level within a season, different factors such as sex, age, experience, social status, physiological requirements or preferences (Araùjo et al. 2011) can promote inter-individual variability. For example, status of individuals within a group could favor diet differences: subordinate individuals might be forced to feed on suboptimal resources (Araùjo et al. 2009). In addition, sex could partly explain among-individual variation, and the degree of among-individual variation could also vary between sexes (Nifong et al. 2015, Smith et al. 2015). Additional analysis with our data would be required to estimate the effect of sex on among-individual variation. Environmental factors could also be at play in the individual specialization. Indeed, fine-scale differences in the spatial distribution (patchiness) of resources (Durell 2000) could enhance among-individual variability.

Finally, in this work, a faeces was not associated to an individual and we were therefore not aware of its diet variation over several days or months. Even though a faeces is an approximation of an individual's diet over several days (Castle 1956, Rayé et al. 2011), an individual diet is likely to be more diversified than the diet assessed from one of its faeces. Therefore, the other ideal exploration would be to set longitudinal study in order to follow individuals over long period of time, through the genetic identification of faeces, and study the intra-individual diet variation.

d) Linking taxonomic and functional β-diversity

Within a species, it has been demonstrated that domestic and wild individuals (sheep, goats, fallow deer) display different preferences for protein to energy ratio to meet their needs (Atwood et al. 2001), but also that they can respond differently to plant secondary compounds (Provenza et al. 2003, Bergvall 2009). Hence, individual differences in diet selection criteria could also be observed for chamois and mouflon as we noticed a relatively high among-individual variation at the taxonomic level. Our analysis on diet selection criteria (paper III) took into account all the individuals and not the mean diet of the population. However, the among-individual variability was not quantitatively measured. Even if our results showed significant effects of some plant traits on diet selection (leaf toughness and nitrogen content, paper III), we hypothesize that the absence of significant relationships for other traits could be related to (1) the absence of effect for all the individuals, or (2) a strong inter-individual variation hiding a general pattern at the species level. Additional analysis on functional β-diversity (Swenson et al. 2010, Meynard et al. 2011, Villéger et al. 2013) could give insights about the among-individual variation in diet functional niches. This could help to determine whether some individuals benefit from a higher resource quality intake over others. For example, a population can show a strong among-individual variation at the taxonomic level but a low among-individual variability at the functional level, suggesting that individuals avoid intra-specific competition by feeding on different plant species, but also avoid a differential nutrient intake among individuals by all feeding on high quality plants. On the contrary, a higher functional beta-diversity would suggest an imbalance in the nutrient access among individuals, and so, differences in development, growth and reproduction. This taxonomic and functional approach of the inter-individual diet variability should be helpful to understand the variation in fitness among individuals, and in turn the consequences on population dynamic.

e) Thoughts about the effects of increasing populations densities on among-individual variation, and how it could affect population dynamic

In addition to the emergence of functional ecology, the integration of intraspecific variability is a promising approach to understand the dynamic of communities (see the thesis introduction). Our results on ungulates (paper I) also confirmed that among-individual variability is the rule rather than the exception and that morpho-physiological constraints may not be as strong a previously thought as the three species (and even the roe deer sometimes classified as “obligate non-grazer” and expected to rely on few key resources, Abbas et al. 2011, 2013, Redjadj et al. 2014) exhibited digestive plasticity. Indeed, among-individual variability is a ubiquitous phenomenon in both vertebrate and invertebrate populations such as gasteropods, fish, amphibians or reptiles (Bolnick et al. 2007) which occurs within the food axis of the ecological niche.

As we are in a context of increasing population sizes in Europe (Milner et al. 2006, Maillard et al. 2010, IUCN red list: increasing trend for roe deer, red deer, isard and ibex/unknown trend for chamois and wild boar), we are particularly interested in how individuals would respond to increasing conspecific and allospecific densities. If population density increases, will individuals tend to be more specialized in order to reduce competition? Or will they tend to

increase their niche breadth to include suboptimal resources as the preferred ones will decrease, and then enhance the homogenization of the population?

In the fig.39, we suggest different possible evolution of among-individual variation as a consequence of an increase in population density (due to a higher abundance of populations or aggregation of individuals in specific areas), through intra-specific competition (fig.39a,b) and inter-specific competition (fig.39c,d). These predictions are not exhaustive and we saw in the introduction that, in addition to the type of rank-preference variation in the focal species, many other factors can influence the direction of individual specialization, such as individual plasticity or resource availability (see section *2.2.d*, Araùjo et al. 2011). For these predictions (fig.39), we focused on cases where the preferred resources were decreasing. However, inter-specific competition could also decrease the abundance of suboptimal resources of the focal species. We hypothesized that it would slightly impact the position or niche width of focal individuals and species, and we did not explore it.

Because of the increase in ungulate population densities in Europe (Milner et al. 2006, Maillard et al. 2010, IUCN red list: increasing trend for roe deer, red deer, isard and ibex/unknown trend for chamois and wild boar), the availability of preferred resources may decrease. Hence intra- and inter-specific competition could be strengthened and in turn influence among-individual variation within competing species.
Even though the multiple outcomes of among-individual variation, we saw in the introduction that different studies on different organisms came to similar conclusions, i.e. the increase of (1) intra-specific competition induces among-individual variation through individuals using different subset of resources (Araùjo et al. 2008, Svanbäck et al. 2004, 2007, fig.40), (2) inter-specific competition reduces niche width though decrease variation in resource use (the inverse of ecological release) (Van Valen 1965, Knudsen et al. 2007, Costa et al. 2008, Bolnick et al. 2010, fig.40). In turn, changes in among-individual variation caused by the shift of some individuals on suboptimal resources would lead to decrease their diet quality. As the diet quality affects body condition (Prins 1996, WallisDeVries 1998, Stewart et al. 2005), it would in turn impact the survival, growth and reproduction of individuals (fitness) (Kie 2003, Cook et al. 2004, fig.40). Consequently, a feedback loop would decrease the density of populations (fig.40). This pattern would be in agreement with the phenomenon of density-dependence (Saether 1997, Coulson et al. 2000, Stewart et al. 2005, Bonenfant et al. 2009 and references therein) and inter-specific competition (Forsyth & Hickling 1998, Latham 1999, Richard et al. 2010, Lovari et al. 2014, Ferretti et al. 2015) regulating population densities in a predator-free environment.

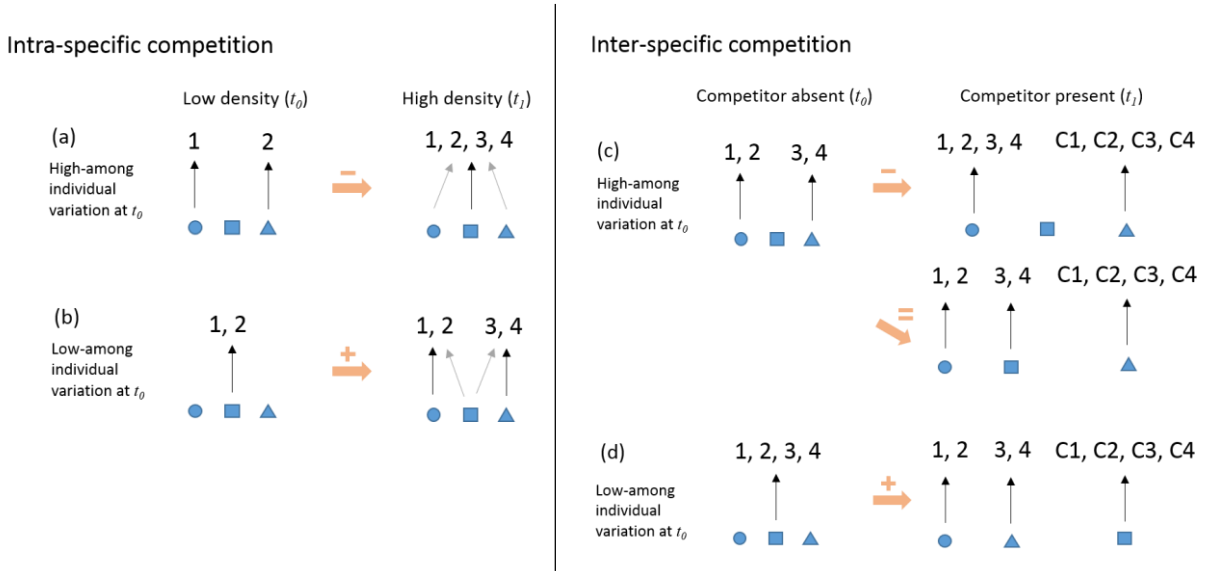



**Figure 39.** Inspired from Araùjo et al. 2011. Illustration of how intra- and inter-specific competition caused by an increase in population density can affect the among-individual variability of a focal species (represented by the individuals 1, 2, 3, 4). Arrows represent direct consumption of a resource by a consumer (arrows in grey represent consumption of suboptimal resources in low quantity). "Plus" or "Minus" indicate the sign of the effect on the degree of among-individual variation. (a) At low density, individuals differ in their rank-preference resources and therefore have different diets. At higher density reducing the preferred resources, all individuals converge to the same alternative resource and the degree of among-individual variability decreases. (b) At low density, individuals have the same preferred resources. At higher density, the preferred resource becomes scarce and individuals specialize on alternative resources, increasing the diet variation. (c) Competitor does not overlap with the focal species and individuals of the focal species have distinct preferred resources. Because of the increase in population density of the competitor, its niche width increases and overlaps with some individuals of the focal species, which enhances inter-specific competition. Individuals of the focal species are forced either (1) to converge to the same resources (circle) because the alternative resource (square) is of low quality or difficult to find, decreasing the degree of among-individual variability, or (2) individuals of the focal species impacted by competition shift on the alternative resource to avoid intra-specific competition. (d) Competitor does not overlap with the focal species and individuals of the focal species have the same preferred resources (low among-individual variation). Because of the increase in population density of the competitor, its niche width increases and overlaps with some individuals of the focal species, which enhances inter-specific competition. In order to avoid intra-specific competition, individuals of the focal species specialize on different alternative resources.

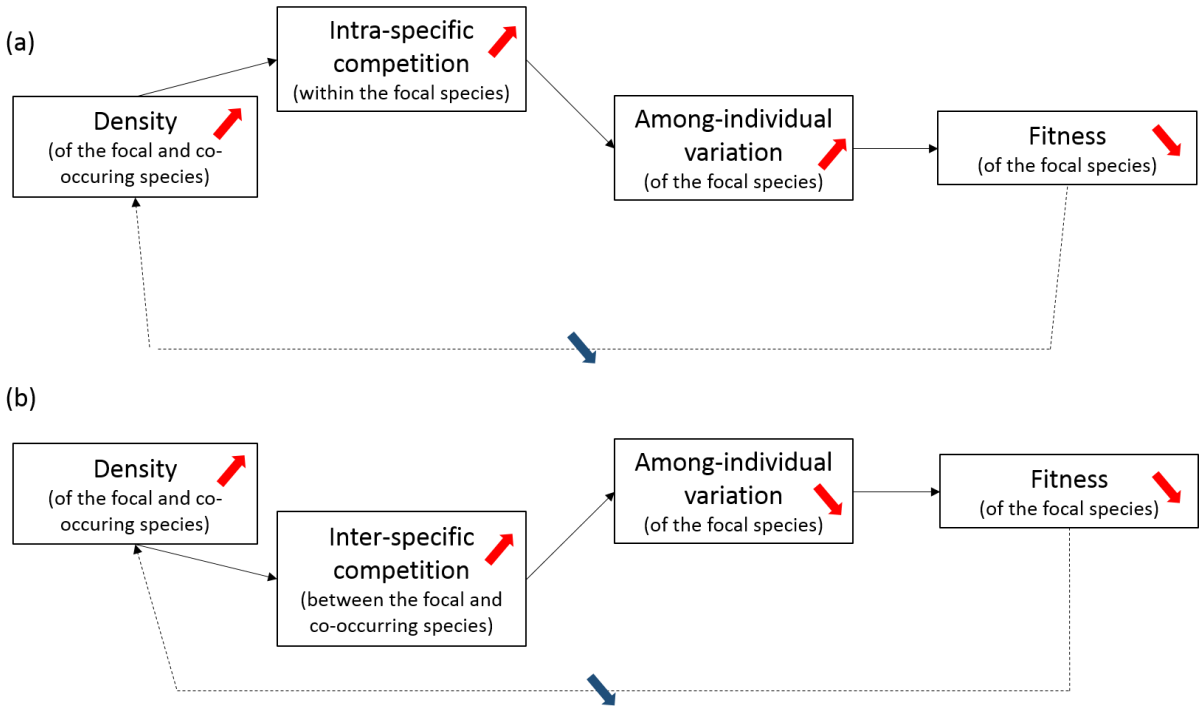



**Figure 40.** Hypothetical causal models about how density could influence among-individual variation and fitness of the focal species through intra- (a) and inter-specific competition (b).

How density affects among-individual variation is not well understood. Indeed, tracking the evolution of among-individual variation as a function of population density requires that long-term extensive and intensive faeces samplings, in addition to population censuses, be done for co-occurring species within a community (Nicholson et al. 2006). In addition, as habitat characteristics (vegetation composition and structure) can fluctuate in response to herbivory pressure or abiotic environmental changes (temperature, precipitation), it can lead to variable response of animals not only dependent on density (Kie et al. 2003).
Otherwise, we could investigate the effect of density on among-individual variation by comparing sites differing in their population densities. However, it would require that sites have the same carrying capacities in order that habitat quality would not confound the effect of density-dependence (Kie et al. 2003).

Studies trying to determine or predict how population density affects population dynamic, through density-dependence (Stewart et al. 2005, Bonenfant et al. 2009 and references therein) or inter-specific competition (Forsyth & Hickling 1998, Richard et al. 2010, Lovari et al. 2014, Ferretti et al. 2015), usually focus on a single impacted species. In order to predict how multiple populations would regulate by considering both intra- and inter-specific interactions, it would require to use a network-based approach of food webs including multiple species from a community (Montoya et al. 2006, Ings et al. 2009, Blüthgen et al. 2010). Indeed, the ecological network approach would be useful to better understand how large herbivores are structured according to food resources, but also how the diversity and density of ungulates and resources would affect the stability of the system (Thebault et al. 2005, 2010, Narwani & Mazumder

2012). We saw in this thesis that an individual-based approach could improve our understanding of community structuration. Therefore, downscaling the trophic interactions networks to the individual-level (Ings et al. 2009, Dupont et al. 2011, Tur et al. 2014, Dupont et al. 2014) would be necessary to explore the evolution of network stability in time in response to changes in ungulates population densities or in plant community composition and abundance.

## 7.3 Integrating functional interactions between large herbivores and plant communities into species distribution models

In the face of global change, species distributions models seek to develop quantitative models able to predict to which extent a species can adapt in the future (Thuiller et al. 2013, Wisz et al. 2013). However, these models lack the incorporation of biotic interactions between species (such as competition, predation or facilitation) that vary in time and space (Poisot et al. 2012) and that influence species distribution patterns (Guisan & Thuiller 2005, Van der Putten et al. 2010, Thuiller et al. 2013, Wisz et al. 2013). Hence, combining trophic network and species distribution models could be particularly relevant to predict spatial and temporal variation in community composition (Pellissier et al. 2013).

In the following paragraphs, we intend to show how our results on functional interactions between large herbivores and plant communities could be used in species distribution models.

Given our results, species distribution models could concern large herbivores or plant communities. However, as they are linked through trophic interactions, they can mutually affect themselves through feedback loops, which make models complicated. In the first case where plant species limit the herbivores distribution, we will assume that the fluctuations of the vegetation composition and relative abundance depends on other factors than herbivore selection, such as climate and land use change. In the second case where herbivores limit plant species, we will assume that the herbivory pressure is constant on the same functional traits and is not influenced by the vegetation changes.

### a) Plant species can limit the herbivores distribution

As large herbivores have to cope with changing environment where available food resources fluctuate in terms of composition and relative abundance, predicting if an ungulate population could persist in such a fluctuating environment could be useful for conservation program or population management.

In this thesis, we showed that ungulate diet selection is partly dependent on plant functional trait such as leaf toughness and leaf nitrogen content, suggesting that functional traits of food plants can determine the distribution of herbivores. Indeed, if the diet selection criteria of herbivores are known in terms of plant functional traits, it should then be possible to ignore local species composition and predict the diet of large herbivores in new environment from the knowledge of plant functional traits and herbivore preferences. Even though we are aware that ungulate population dynamics are not only driven by bottom-up controls (food resources) but also by top-down or transversal effects, the knowledge of their diet quality within a changing environment could partly help to predict their persistence.

However, this approach would require information on both the traits and the relative proportion and distribution of plant species in the studied landscape and their evolution in time, in order to determine precise functional vegetation maps. Moreover, knowledge about the physiological limits of ungulates, such as the lower boundary of energetic needs required to survive and reproduce or how they respond to temperature increase (Marchand et al. 2015), should be assessed to model their spatial distribution response to environmental changes (Thuiller et al. 2013).

Finally, in order to be confident with our results on diet selection criteria and determine if they can be generalized and used for other field of studies such as species distribution modelling, additional studies in other areas differing in their plant species composition should be first conducted.

b) Herbivores can limit plant species distribution

Climate and land use change (intensification or abandonment of domestic grazing) are two drivers expected to strongly influence the vegetation in temperate ecosystems (Boulangeat et al. 2014). Their simultaneous impacts on biodiversity at regional scales in the Ecrins National Park have been tested (Boulangeat et al. 2014). We could hypothesize that large wild herbivores could also mitigate or emphasize the previous effects. Indeed, as densities of wild large herbivores are increasing in different regions of the world over recent decades (Côté et al. 2004, Milner et al. 2006, Maillard et al. 2010, IUCN red list), they can have significant impact on vegetation and on above- and below-ground communities, leading to conservation issues (Rooney 2001, Mysterud 2006). Hence, a new approach taking into account the triple-effect of climate, land use and wild herbivores could be investigated to predict more specifically the dynamic of vegetal communities. The model could take into account the functional and taxonomic preferences of herbivores, their habitat selection and their intensity of resource use.

In 1983, in the end of the first chapter of his book “Wildlife feeding and nutrition”, Robbins said that “the application of much of the wildlife nutrition data to field management is both an art and a science because of the lack of adequate knowledge of many control mechanisms determining the outcome of any manipulation”. Finally, he concluded that many challenges need to be met, especially the questions about competition, winter feeding of wildlife, diet formulation, effects of habitat manipulation, predator-prey interactions, etc. Forty-two years later, I feel more optimistic, about the reliability of data we are now obtaining about wildlife feeding ecology that can be useful for managers. The literature cited and the work realized here show that progress has been made when it comes to understand how species and individuals interact, how animals feed during periods of low resource availability, what are the factors implied in the diet choices but also to improve the methodology and have access of more precise diet data (DNA-metabarcoding). A large part of the amazing databases used in this thesis remain to be explored (e.g. diets from forest environment, inflorescence trait measurements, food selection ratios) and should bring additional cues for the understanding of ecosystem functioning.

I finally hope that I convinced you that both taxonomic and functional approaches, and among-individual and interspecific interactions studies, can bring complementary visions highly valuable for our understanding of ecological processes, and that some of our results can be relevant for conservation and management practices.

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# ABBREVIATIONS

**ANPP.** Aboveground net primary productivity
**BIC.** Between individual component
**BSC.** Biological species concept
**C.** Carbon
**CP.** Crude protein
**CWM.** Community weighted mean
**DM.** Dry matter
**DN.** Dietary nitrogen
**DNA.** Desoxyribonucleic acid
**FD.** Functional diversity
**FM.** Fresh matter
**FN.** Fecal nitrogen
**GPS.** Global positioning system
**LDMC.** Leaf dry matter content
**LNC.** Leaf nitrogen content
**LPT.** Leaf punch toughness
**LCC.** Leaf carbon content
**LT.** Leaf thickness
**MCP.** Minimum convex polygon
**N.** Nitrogen
**NIRS.** Near-infrared spectroscopy
**NSCA.** Non-symmetric correspondence analysis
**NVH.** Niche variation hypothesis
**OFT.** Optimal foraging theory
**P.** Phosphorus
**PCA.** Principal component analysis
**PCR.** Polymerase chain reaction
**PDMC.** Petiole dry matter content
**PSC.** Phylogenetic species concept
**RMSEC.** Root mean standard error of calibration
**RMSEP.** Root mean standard error of the prediction
**SH.** Sociality hypothesis
**SLA.** Specific leaf area
**TDMC.** Twig dry matter content
**TNW.** Total niche width
**WIC.** Within individual component

# LIST OF APPENDICES

**Appendix 1 – Table A1.** Mean frequency of sequences (FS, %) and mean frequency of occurrence (FO, %) over the year of plants found in the faeces of chamois, mouflon and roe deer in the Bauges Game and Wildlife Reserve. Numbers in bold have a frequency higher than 5%.

**Appendix 2 – Table A2.** List of plant species used for functional trait measurements. The associated frequences correspond to the relative proportion of the plant in the Armenaz and Charbonnet pastures. The total line gives the number of plant species sampled per season and the sum of the frequencies for each pasture.

**Appendix 3 – Figure A1.** Rarefaction curves* for the three species (chamois, roe deer and mouflon) and the four seasons (spring, summer, autumn and winter).

**Appendix 4 – Figure A2.** Map of vegetal communities in the Armene pasture.

**Appendix 1 – Table A1.** Mean frequency of sequences (FS, %) and mean frequency of occurrence (FO, %) over the year of plants found in the faeces of chamois, mouflon and roe deer in the Bauges Game and Wildlife Reserve. Numbers in bold have a frequency higher than 5%.

| | Chamois (FS, %) | Chamois (FO, %) | Mouflon (FS, %) | Mouflon (FO, %) | Roe deer (FS, %) | Roe deer (FO, %) |
|---|---|---|---|---|---|---|
| *Abies alba* | 0,29 | 1,97 | **6,74** | **14,63** | 2,89 | **10,38** |
| *Acer* | 0,35 | 3,64 | 0,90 | **11,71** | 0,42 | 3,77 |
| *Alchemilla alpina* | 0,99 | **12,90** | 0,54 | **7,32** | 0,50 | **6,60** |
| *Alnus viridis* | 1,15 | **9,56** | 1,44 | **11,22** | 1,37 | **7,55** |
| *Alopecurinae* | 0,01 | 0,30 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Anthriscus cerefolium* | 0,01 | 0,30 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Anthyllis vulneraria* | 0,57 | **8,65** | 0,25 | 3,90 | 0,01 | 0,47 |
| *Apiaceae* | 1,30 | **12,14** | 3,78 | **24,88** | 0,58 | **6,13** |
| *Arabis alpina* | 0,00 | 0,15 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Arctostaphylos uva-ursi* | 0,28 | 3,79 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Aruncus dioicus* | 1,31 | **6,22** | 0,00 | 0,00 | 0,96 | **12,74** |
| *Asteraceae* | 2,57 | **27,77** | 0,40 | **6,83** | 1,69 | **17,45** |
| *Asterales* | 0,02 | 0,46 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Astrantia* | 0,14 | 2,58 | 0,05 | 0,98 | 0,00 | 0,00 |
| *Athyrium vidalii* | 0,14 | 0,30 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Avenella flexuosa* | 0,02 | 0,46 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Betula pendula* | 0,02 | 0,46 | 0,22 | 2,93 | 0,00 | 0,00 |
| *Betulaceae* | 0,28 | 3,03 | 4,06 | **19,02** | 1,80 | **12,26** |
| *Bistorta vivipara* | 0,53 | **7,59** | 0,06 | 1,95 | 0,00 | 0,00 |
| *Brassicaceae* | 0,00 | 0,00 | 0,04 | 0,49 | 0,00 | 0,00 |
| *Calamagrostis varia* | 0,00 | 0,00 | 0,02 | 0,49 | 0,00 | 0,00 |
| *Campanula* | 0,01 | 0,15 | 0,04 | 0,98 | 0,10 | 0,94 |
| *Campanulaceae* | 0,06 | 1,37 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Cardamine pentaphyllos* | 0,00 | 0,00 | 0,03 | 0,49 | 0,00 | 0,00 |
| *Carduinae* | 0,03 | 0,46 | 0,03 | 0,49 | 0,24 | 1,42 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Chaerophyllum hirsutum* | 0,12 | 0,91 | 0,03 | 0,98 | 0,03 | 0,47 |
| *Chamerion angustifolium* | 1,91 | **9,26** | 0,81 | **9,27** | 0,96 | **9,43** |
| *Clinopodium vulgare* | 0,01 | 0,15 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Cornus sanguinea* | 0,00 | 0,00 | 0,07 | 0,49 | 0,39 | 3,77 |
| *Coronilla vaginalis* | 0,00 | 0,00 | 0,02 | 0,49 | 0,00 | 0,00 |
| *Dactylis glomerata* | 0,05 | 0,76 | 0,90 | **13,66** | 0,00 | 0,00 |
| *Deschampsia cespitosa* | 0,00 | 0,00 | 0,01 | 0,49 | 0,00 | 0,00 |
| *Dryas octopetala* | 0,04 | 0,76 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Dryopteridaceae* | 0,00 | 0,00 | 0,40 | 4,39 | 0,00 | 0,00 |
| *Epilobium montanum* | 0,06 | 0,91 | 0,00 | 0,00 | 0,19 | 3,77 |
| *Equisetum* | 0,14 | 0,91 | 0,02 | 0,49 | 0,49 | 2,36 |
| *Euphorbia* | 0,07 | 0,91 | 0,61 | 3,90 | 0,02 | 0,47 |
| *Fagus sylvatica* | 0,04 | 0,76 | 0,09 | 0,98 | 0,03 | 0,47 |
| *Festuca* | 0,08 | 1,67 | 0,11 | 1,46 | 0,08 | 0,47 |
| *Filipendula ulmaria* | 0,87 | 3,79 | 0,26 | 1,46 | 4,04 | **14,15** |
| *Fraxinus excelsior* | 0,14 | 0,76 | 0,28 | 2,44 | 1,40 | **10,38** |
| *Galium* | 0,10 | 1,97 | 0,23 | 2,93 | 0,22 | 4,25 |
| *Gentiana* | 0,01 | 0,15 | 2,08 | **12,68** | 0,01 | 0,47 |
| *Geranium robertianum* | 0,06 | 1,06 | 0,05 | 0,98 | 0,10 | 1,89 |
| *Geranium sylvaticum* | **5,34** | **33,84** | 0,73 | **8,78** | 1,42 | **12,26** |
| *Geum* | 0,16 | 3,19 | 0,04 | 0,49 | 0,93 | 4,72 |
| *Globularia nudicaulis* | 2,45 | **21,24** | 0,44 | **5,85** | 0,20 | 1,89 |
| *Hedera helix* | 0,00 | 0,00 | 0,02 | 0,49 | 0,00 | 0,00 |
| *Hedysarum hedysaroides* | 0,93 | **5,01** | 0,00 | 0,00 | 0,00 | 0,00 |
| *Helianthemum nummularium* | **27,34** | **63,58** | 4,88 | **27,32** | 5,83 | **11,32** |
| *Hippocrepis emerus* | 0,00 | 0,00 | 0,00 | 0,00 | 0,11 | 0,47 |
| *Hordelymus europaeus* | 0,24 | 2,28 | 0,30 | 2,44 | 0,00 | 0,00 |
| *Hypericum* | 0,94 | **11,68** | 0,09 | 1,95 | 0,00 | 0,00 |
| *Juniperus* | 1,78 | **9,41** | 0,04 | 0,49 | 0,10 | 0,47 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Knautia* | 0,09 | 1,37 | 0,08 | 1,46 | 0,00 | 0,00 |
| *Larix decidua* | 0,07 | 0,91 | 0,04 | 0,98 | 0,10 | 1,89 |
| *Lathyrus* | 0,03 | 0,61 | 0,00 | 0,00 | 0,51 | 4,72 |
| *Lathyrus pratensis* | 0,80 | **7,74** | 2,57 | **17,56** | 0,08 | 2,36 |
| *Leontodon hispidus* | 0,62 | **9,71** | 0,12 | 1,95 | 0,00 | 0,00 |
| *Linaria alpina* | 0,00 | 0,00 | 0,02 | 0,49 | 0,00 | 0,00 |
| *Lonicera* | 0,01 | 0,30 | 0,12 | 1,95 | 0,15 | 1,42 |
| *Lotus corniculatus* | **7,62** | **54,02** | 2,49 | **28,29** | 1,00 | **10,38** |
| *Luzula sylvatica* | 0,04 | 0,46 | 0,00 | 0,00 | 0,01 | 0,47 |
| *Lysimachia* | 0,17 | 2,58 | 0,02 | 0,49 | 0,09 | 2,36 |
| *Melica uniflora* | 0,05 | 0,30 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Mentheae* | 0,13 | 2,73 | 0,04 | 0,98 | 0,00 | 0,00 |
| *Monolepis asiatica* | 0,00 | 0,00 | 1,95 | **12,68** | 0,00 | 0,00 |
| *Onagraceae* | 0,05 | 0,91 | 0,04 | 0,49 | 0,03 | 0,47 |
| *Onobrychis montana* | **8,78** | **36,72** | **24,02** | **53,17** | 3,71 | **8,96** |
| *Ononis natrix* | 0,01 | 0,15 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Oxalis* | 0,00 | 0,00 | 0,00 | 0,00 | 0,04 | 0,94 |
| *Phyteuma spicatum* | 0,05 | 0,91 | 0,00 | 0,00 | 0,39 | **6,60** |
| *Picea abies* | 0,44 | 3,95 | 1,25 | **10,73** | 0,00 | 0,00 |
| *Pinus sylvestris* | 0,07 | 0,46 | 0,15 | 1,95 | 0,00 | 0,00 |
| *Plantago* | 0,04 | 0,91 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Plantago alpina* | 0,10 | 1,52 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Polygonatum verticillatum* | 0,00 | 0,15 | 0,00 | 0,00 | 0,01 | 0,47 |
| *Potentilla* | 0,03 | 0,76 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Primula* | 0,00 | 0,00 | 0,05 | 1,46 | 0,00 | 0,00 |
| *Primulaceae* | 0,00 | 0,00 | 0,04 | 0,98 | 0,00 | 0,00 |
| *Prunella* | 0,01 | 0,30 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Prunus* | 0,00 | 0,00 | 0,00 | 0,00 | 0,08 | 1,42 |
| *Pulsatilla alpina* | 0,06 | 1,06 | 0,17 | 2,44 | 0,00 | 0,00 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Pyreae* | 1,29 | **12,14** | 0,61 | **7,80** | 1,15 | **14,62** |
| *Ranunculus* | 0,04 | 0,76 | 0,00 | 0,00 | 0,40 | 4,72 |
| *Ranunculus tuberosus* | 0,21 | 3,64 | 0,14 | 2,93 | 0,23 | 2,83 |
| *Rhamnus alaternus* | 0,00 | 0,00 | 0,02 | 0,49 | 0,00 | 0,00 |
| *Rhinanthus alectorolophus* | 0,04 | 0,76 | 0,00 | 0,00 | 0,53 | 3,77 |
| *Rhododendron ferrugineum* | 0,06 | 1,37 | 1,08 | **8,29** | 0,03 | 0,47 |
| *Rosodae* | **16,97** | **47,80** | **24,18** | **63,41** | **53,46** | **92,45** |
| *Rosularia alpestris* | 0,04 | 0,61 | 0,03 | 0,49 | 0,00 | 0,00 |
| *Rubiaceae* | 0,01 | 0,15 | 0,00 | 0,00 | 0,01 | 0,47 |
| *Rumex* | 3,40 | **23,37** | 3,64 | **20,49** | 5,78 | **27,36** |
| *Salix* | 0,77 | **7,13** | 0,84 | **8,29** | 0,46 | **6,60** |
| *Sambucus* | 0,19 | 0,46 | 0,16 | 2,44 | 0,82 | **10,85** |
| *Sanguisorba minor* | 0,13 | 2,12 | 0,43 | 4,88 | 0,73 | **6,60** |
| *Saxifraga aizoides* | 0,00 | 0,00 | 0,16 | 0,49 | 0,00 | 0,00 |
| *Saxifraga oppositifolia* | 0,06 | 1,21 | 0,14 | 0,49 | 0,00 | 0,00 |
| *Saxifraga paniculata* | 0,00 | 0,15 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Saxifraga rotundifolia* | 0,20 | 2,43 | 0,03 | 0,49 | 0,43 | 4,25 |
| *Sedum* | 0,02 | 0,30 | 0,09 | 0,98 | 0,00 | 0,00 |
| *Sempervivum tectorum* | 0,01 | 0,30 | 0,12 | 1,95 | 0,00 | 0,00 |
| *Silene latifolia* | 0,03 | 0,46 | 0,00 | 0,00 | 0,09 | 0,47 |
| *Silene vulgaris* | 0,39 | 3,79 | 0,67 | 4,39 | 0,49 | 2,36 |
| *Solanoideae* | 0,00 | 0,00 | 0,00 | 0,00 | 0,11 | 0,47 |
| *Succisa pratensis* | 0,00 | 0,00 | 0,00 | 0,00 | 0,02 | 0,47 |
| *Tilia* | 0,00 | 0,00 | 0,00 | 0,00 | 0,10 | 1,42 |
| *Trifolium* | 0,08 | 1,82 | 0,00 | 0,00 | 0,09 | 1,42 |
| *Trifolium badium* | 0,01 | 0,30 | 0,00 | 0,00 | 0,02 | 0,47 |
| *Trollius europaeus* | 0,00 | 0,00 | 0,02 | 0,49 | 0,00 | 0,00 |
| *Ulmus glabra* | 0,00 | 0,00 | 2,16 | **9,27** | 0,34 | 2,83 |
| *Urtica dioica* | 0,01 | 0,30 | 0,79 | **5,37** | 0,02 | 0,47 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Vaccinium myrtillus* | 1,65 | **11,23** | 0,16 | 2,44 | 1,01 | **6,13** |
| *Vaccinium uliginosum* | 1,98 | **7,89** | 0,06 | 0,98 | 0,00 | 0,00 |
| *Valeriana* | 0,15 | 1,97 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Veronica* | 0,00 | 0,00 | 0,00 | 0,00 | 0,10 | 2,36 |
| *Veronica beccabunga* | 0,00 | 0,15 | 0,00 | 0,00 | 0,00 | 0,00 |
| *Veronica montana* | 0,00 | 0,00 | 0,00 | 0,00 | 0,01 | 0,47 |
| *Viburnum lantana* | 0,01 | 0,15 | 0,20 | 3,90 | 0,02 | 0,47 |
| *Vicia cracca* | 0,00 | 0,00 | 0,00 | 0,00 | 0,04 | 0,94 |
| *Viola* | 0,00 | 0,15 | 0,00 | 0,00 | 0,19 | 2,83 |

**Appendix 2 – Table A2.** List of plant species used for functional trait measurements. The associated frequences correspond to the relative proportion of the plant in the Armenaz and Charbonnet pastures. The total line gives the number of plant species sampled per season and the sum of the frequencies for each pasture.

| | June | July | September | Armenaz | Charbonnet |
|---|---|---|---|---|---|
| *Abies alba* | x | x | x | 0,00 | 0,01 |
| *Acer pseudoplatanus* | x | | x | 0,00 | 0,28 |
| *Alchemilla alpina* | x | x | x | 0,50 | 0,15 |
| *Alchemilla vulgaris* | x | x | x | 0,68 | 0,24 |
| *Alnus viridis* | x | x | x | 1,87 | 0,11 |
| *Anthyllis vulneraria* | | x | x | 0,03 | 0,09 |
| *Arctostaphylos uva-ursi* | x | x | x | 0,02 | 0,00 |
| *Arnica montana* | x | x | x | 0,00 | 0,00 |
| *Aruncus dioicus* | x | x | | 0,00 | 0,00 |
| *Astrantia major* | | x | x | 0,58 | 0,00 |
| *Bistorta vivipara (Polygonum viviparum)* | | x | | 0,00 | 0,00 |
| *Campanula rotundifolia* | x | | | 0,01 | 0,57 |
| *Cardamine pentaphyllos* | x | | | 0,00 | 0,00 |
| *Carduus defloratus* | x | x | x | 1,56 | 1,09 |
| *Carex sempervirens* | x | x | x | 32,32 | 27,53 |
| *Centaurea uniflora* | | | x | 0,06 | 0,00 |
| *Chaerophyllum hirsutum* | x | x | x | 1,11 | 0,13 |
| *Crocus vernus* | x | | | 0,00 | 0,00 |
| *Dactylis glomerata* | x | x | x | 1,78 | 8,31 |
| *Deschampsia cespitosa* | x | x | x | 4,14 | 3,00 |
| *Epilobium angustifolium* | x | x | x | 0,00 | 0,00 |
| *Fagus sylvatica* | x | | x | 0,00 | 0,00 |
| *Festuca ovina* | x | x | x | 0,59 | 2,40 |
| *Festuca rubra* | x | x | x | 8,14 | 5,41 |
| *Filipendula ulmaria* | x | x | | 0,00 | 0,00 |
| *Fragaria vesca* | x | x | | 0,00 | 0,00 |
| *Fraxinus excelsior* | | | x | 0,00 | 0,00 |
| *Galium odoratum* | x | x | x | 0,19 | 0,19 |
| *Gentiana purpurea* | x | x | x | 3,58 | 1,72 |
| *Geranium sylvaticum* | x | x | x | 1,10 | 1,02 |
| *Geum montanum* | | | x | 0,01 | 0,00 |
| *Globularia nudicaulis* | x | x | x | 0,30 | 0,27 |
| *Hedera helix* | x | | | 0,00 | 0,00 |
| *Helianthemum nummularium* | x | x | x | 0,71 | 0,87 |
| *Heracleum sphondylium* | x | x | x | 1,56 | 5,62 |
| *Hieracium murorum* | | x | x | 0,02 | 0,33 |
| *Hordelymus europaeus* | x | x | x | 0,00 | 0,00 |
| *Hypericum richerii* | x | x | x | 0,19 | 0,11 |
| *Juniperus communis* | x | | x | 1,01 | 0,11 |
| *Knautia arvense* | x | x | | 0,10 | 0,32 |

| | | | | | |
|---|---|---|---|---|---|
| *Laserpitium latifolium* | x | x | x | 1,56 | 5,62 |
| *Lathyrus pratensis* | | x | x | 0,02 | 0,02 |
| *Leontodon hispidus* | | | x | 0,08 | 0,00 |
| *Lonicera xylosteum* | x | x | x | 0,00 | 0,00 |
| *Lotus corniculatus* | x | x | x | 0,02 | 0,02 |
| *Luzula sylvatica* | x | | | 0,14 | 0,16 |
| *Melica uniflora* | x | | | 0,00 | 0,00 |
| *Mercurialis perennis* | x | | x | 0,00 | 0,04 |
| *Nardus stricta* | x | x | x | 6,82 | 0,29 |
| *Onobrychis montana* | x | x | x | 0,00 | 0,08 |
| *Origanum vulgare* | | | x | 0,00 | 0,00 |
| *Phyteuma spicatum* | x | x | | 0,00 | 0,00 |
| *Picea abies* | x | x | | 0,00 | 0,00 |
| *Plantago atrata* | | x | x | 0,02 | 0,03 |
| *Polygala chamaebuxus* | x | | | 0,00 | 0,00 |
| *Potentilla erecta* | | | x | 0,11 | 0,03 |
| *Pulsatilla alpina* | x | x | x | 1,51 | 1,24 |
| *Ranunculus tuberosus* | x | x | x | 0,21 | 0,20 |
| *Rhinanthus alectorolaphus* | | x | | 0,00 | 0,06 |
| *Rhododendron ferrugineum* | x | x | x | 1,03 | 0,38 |
| *Rosa montana* | x | x | x | 0,04 | 0,00 |
| *Rubus fruticosus* | x | x | x | 0,04 | 0,02 |
| *Rumex alpinus* | x | x | x | 0,37 | 0,08 |
| *Rumex arifolius* | x | x | | 0,37 | 0,08 |
| *Salix* | | x | | 0,03 | 0,00 |
| *Salvia pratensis* | | | x | 0,00 | 0,33 |
| *Sambucus nigra* | | | x | 0,00 | 0,00 |
| *Sanguisorba minor* | x | | | 0,00 | 0,00 |
| *Saxifraga rotundifolia* | x | x | | 0,06 | 0,00 |
| *Sempervivum tectorum* | x | x | | 0,00 | 0,00 |
| *Serratula tinctoria* | | x | x | 0,00 | 0,00 |
| *Sesleria caerulea* | x | x | x | 9,43 | 14,21 |
| *Silene dioica* | x | | | 0,38 | 0,32 |
| *Sorbus chamaemespilus* | x | | | 0,00 | 0,07 |
| *Taraxacum officinale* | x | | | 0,00 | 0,00 |
| *Thymus serpyllum* | x | x | x | 0,34 | 0,08 |
| *Trifolium badium* | | x | | 0,17 | 0,00 |
| *Trifolium pratense* | | x | x | 0,17 | 0,00 |
| *Urtica dioica* | | | x | 0,17 | 0,35 |
| *Vaccinium myrtillus* | x | x | x | 1,23 | 0,25 |
| *Vaccinium uliginosum* | x | x | x | 2,66 | 0,02 |
| *Vaccinium vitis-idaea* | x | | x | 0,82 | 0,02 |
| *Valeriana montana* | x | x | x | 1,82 | 1,03 |
| *Veratrum album* | x | x | | 0,28 | 0,61 |
| *Total* | 64 | 59 | 59 | 92,11 | 85,56 |

## Appendix 3

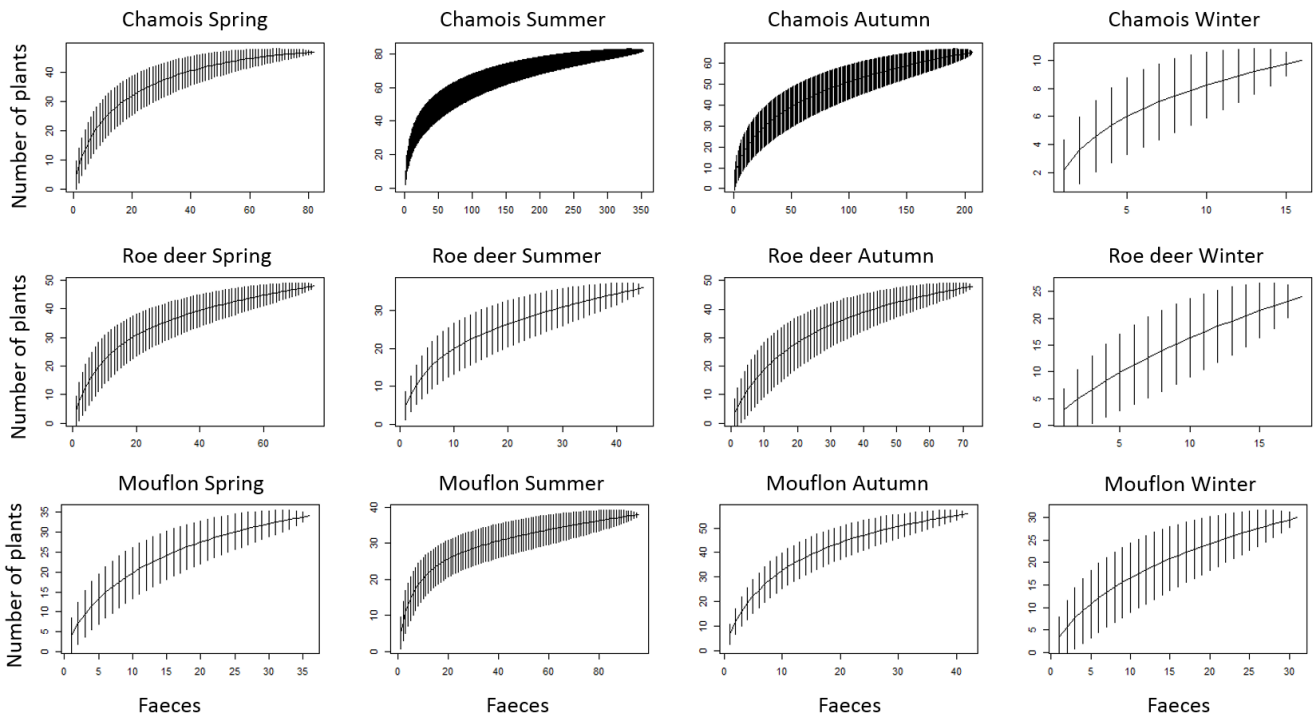


**Figure A1.** Rarefaction curves* for the three species (chamois, roe deer and mouflon) and the four seasons (spring, summer, autumn and winter).

*For each species and season, rarefaction curves are obtained by sub-sampling different subset of faeces samples with different sizes and by calculating the mean number of species found for each of the subset.

## Appendix 4

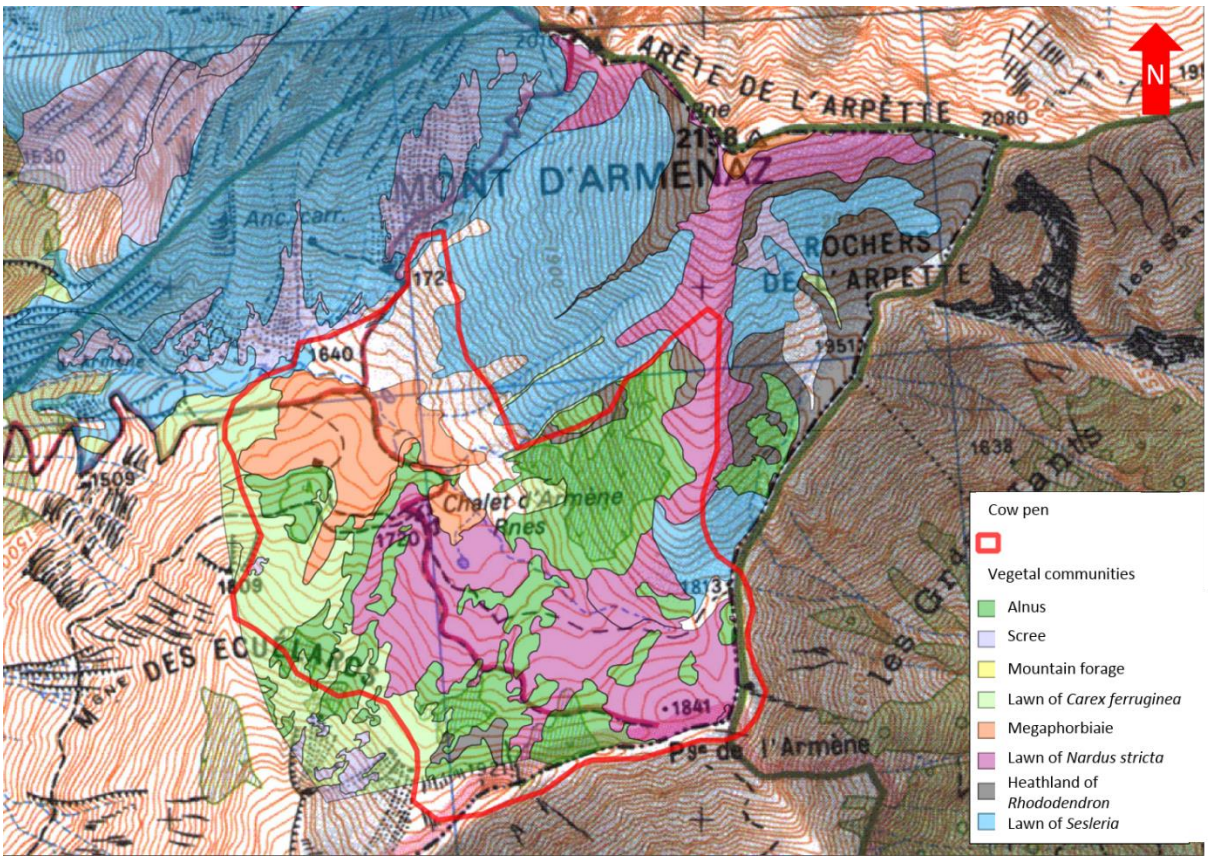


**Figure A2.** Map of vegetal communities in the Armene pasture.

# PAPERS

**Paper I.**

Bison M., Ibanez S., Redjadj C., Boyer F., Coissac E., Miquel C., Rioux D., Saïd S., Maillard D., Taberlet P., Yoccoz N.G., Loison A.

*Upscaling the niche variation hypothesis from the intra- to the inter-specific level*.

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Oecologia
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COMMUNITY ECOLOGY - ORIGINAL RESEARCH

# Upscaling the niche variation hypothesis from the intra- to the inter-specific level

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**Abstract** The "niche variation hypothesis" (NVH) predicts that populations with wider niches should display higher among-individual variability. This prediction originally stated at the intra-specific level may be extended to the inter-specific level: individuals of generalist species may differ to a greater extent than individuals of a specialist species. We tested the NVH at intra- and inter-specific levels based on a large diet database of three large herbivore feces collected in the field and analyzed using DNA metabarcoding. The three herbivores (roe deer *Capreolus capreolus*, chamois *Rupicapra rupicapra* and mouflon *Ovis musimon*) are highly contrasted in terms of sociality (solitary to highly gregarious) and diet. The NVH at the intraspecific level was tested by relating, for the same population, diet breadth and inter-individual variation across the four seasons. Compared to null models, our data supported the NVH both at the intra- and inter-specific levels. Inter-individual variation of the diet of solitary species was not larger than in social species, although social individuals feed together and could therefore have more similar diets. Hence, the NVH better explained diet breadth than other factors such as sociality. The expansion of the population niche of the three species was driven by resource availability, and achieved by an increase in inter-individual variation, and the level of inter-individual variability was larger in the generalist species (mouflon) than in the specialist one (roe deer). This mechanism at the base of the NVH appears at play at different levels of biological organization, from populations to communities.

**Keywords** Individual heterogeneity · Sociality · Large herbivores · DNA metabarcoding · Null models

Communicated by Ilpo Kojola.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00442-015-3390-7) contains supplementary material, which is available to authorized users.

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## Introduction

The Hutchinson's concept (Hutchinson 1957) of the ecological niche is central in community ecology. Niche width, in particular, defines how generalized or specialized species are (Devictor et al. 2009), how they may respond to environmental variation (e.g., species with narrow niche being more vulnerable to extinction) (Clavel et al. 2010), and to what extent coexisting species will compete. These community-level patterns, however, may be affected by a fundament of evolutionary ecology: individuals occupy different portions of the species' niche (Van Valen 1965; Roughgarden 1972). As part of the synthesis between

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evolutionary and community ecology, the question now is how inter-individual variations in niche attributes affect the total niche width of populations within species, and of species within communities.

Based on empirical results on bird populations, Van Valen (1965) proposed that inter-individual variation in terms of resource use should be lower when population niches are narrow than when they are wide, i.e., that the expansion of the population niche is achieved by an increase in inter-individual variation, a proposition thereafter called the "niche variation hypothesis" (NVH). NVH has been mainly tested by comparing populations of the same species in different ecological contexts, such as ecological release (decrease of inter-specific competition) (Knudsen et al. 2007; Costa et al. 2008; Bolnick et al. 2010) or intra-specific competition (Svanbäck and Bolnick 2007; Araújo et al. 2008). Such a positive relationship between niche width and the level of individual heterogeneity is supported in taxa as diverse as gasteropods, fish, amphibians, or reptiles (Bolnick et al. 2007). This relationship could, however, also be extended to the inter-specific level (Araújo et al. 2007, 2009); the level of individual heterogeneity in terms of niche width is expected to be larger in generalist species than in specialist species. Indeed, specialist species may be constrained by cognitive, physiological or morphological adaptations imposing a limit to inter-individual variation (Bolnick et al. 2003). For example, among large herbivores, "browsers" are considered as "obligatory non-grazers" as their morpho-physiological traits perform poorly for eating and digesting grass (Clauss et al. 2003).

At the same time, other mechanisms may be at play to explain inter-specific differences in terms of intra-specific specialization. For instance, the level of sociality of a species, which determines whether foraging is synchronized in time and space among individuals, could directly influence the level of inter-individual variation; in social species, individuals may have more similar diets than individuals of solitary species, as all individuals feed close to each other with the same resource availability. The level of sociality could therefore be negatively correlated with inter-individual variation (Fig. 1b, c), a hypothesis that we hereafter call

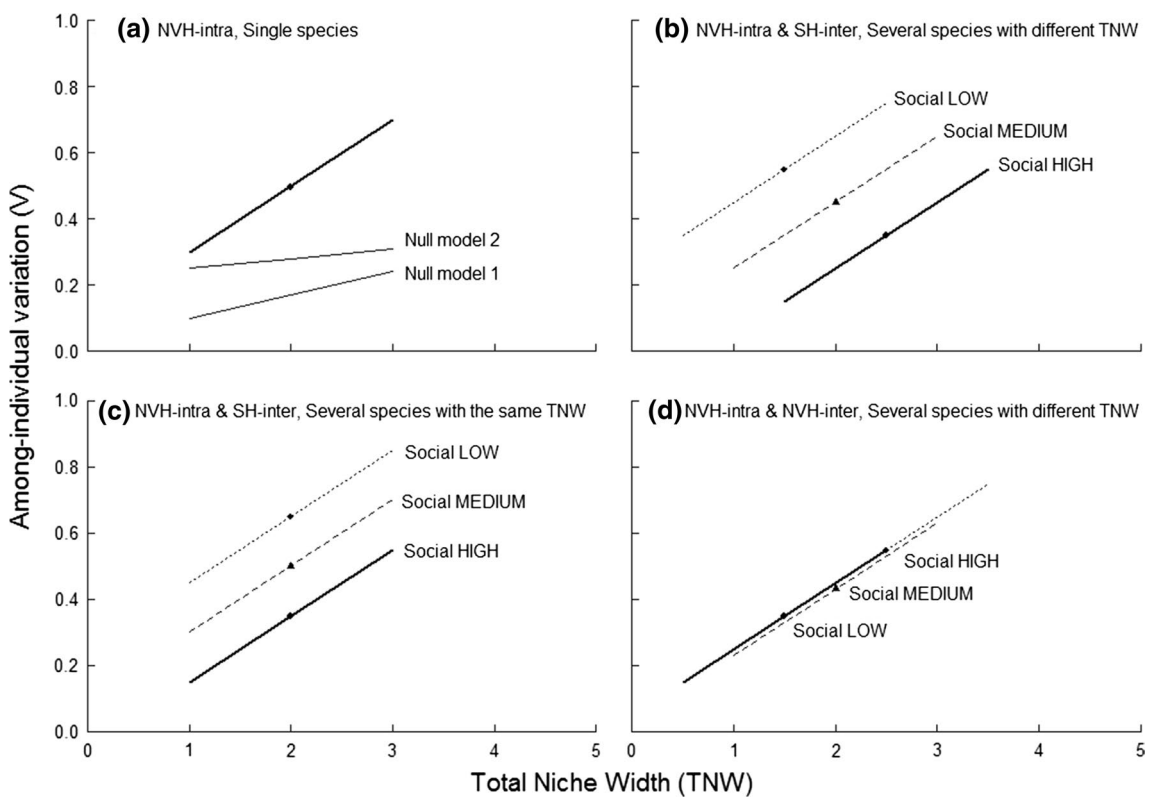


**Fig. 1** Illustration of the correlation hypotheses between the degree of diet variation among individuals (*V*) and total niche width (*TNW*), for the three studied ungulates. **a** niche variation hypothesis applied to a single species, **b** NVH is supported at the intra-specific level for different species differing in their level of sociality, sociality is dependent to TNW and explains the inter-specific differences, **c** NVH is supported at the intra-specific level for different species differing in their level of sociality, sociality is not dependent to TNW but explains the inter-specific differences, **d** NVH is supported at the intra- and inter-specific level for different species whatever their sociality and TNW. For the sake of simplicity, null models are only shown in (**a**), but they can be tested in each case

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the sociality hypothesis (SH). Alternatively, NVH may also hold at the inter-specific level, in which case inter-individual variation is independent of sociality, but increases when species total niche breadth increases (Fig. 1d).

Here, we tested NVH (at the intra- and inter-specific levels) and SH (at the inter-specific level) by collecting data on the diet of three species of large mountain herbivores ranked from solitary to highly gregarious [respectively: roe deer *Capreolus capreolus* (Dzieciolowski 1979), chamois *Rupicapra rupicapra* (Boschi and Nievergelt 2003), and mouflon *Ovis gmelini* (Cransac et al. 1998)] and with contrasting diets (Bertolino et al. 2009; Redjadj et al. 2014). Large herbivores in general feed on a large number of plant items, but until recently, it remained difficult to assess the variety of plant species consumed in the wild (Rayé et al. 2011). We therefore resorted to recently developed DNA metabarcoding methods (Pompanon et al. 2012), which open new avenues to determine diet from easily collected materials (Rayé et al. 2011) and estimate niche width for herbivores (Yoccoz 2012). The strong differences in sociality between the three herbivore species studied here allowed us to test whether the SH (at the inter-specific level) was supported, while the high seasonality observed in mountain environment, which is expected to lead to variation in niche breadth, allowed us to test whether NVH was supported at the intra-specific level. If NVH holds at both intra- and inter-specific levels (Fig. 1d), inter-individual variation should increase with niche width, from the species with the narrower expected niche (roe deer) to the species with the largest expected niche width (mouflon), and from winter to spring and summer. If the level of sociality constrains inter-individual variation, then we should find support for SH, i.e., an overall higher degree of inter-individual variation for the solitary species (roe deer) than for the most social species (mouflon), and an intermediary position of the chamois (Fig. 1b or c). As in our study, the level of sociality is highly correlated with expected TNW, we expect a decrease of inter-individual variation when TNW decreases (Fig. 1d). Comparing large herbivore species and season therefore allowed us to tease apart the best scenario accounting for the covariation between inter-individual variation and niche width.

## Materials and methods

### Study site and organisms

The study was carried out in the Bauges Game and Wildlife Reserve (5170 ha) located in the northern French Alps (45.65°N, 6.23°E). Three herbivore species coexist in the game reserve: chamois, mouflon and roe deer. Their population sizes are, respectively, about 2000, 500, and 250 (Darmon et al. 2012). Chamois are at a high density in subalpine pastures and lower density in forests (Redjadj 2010). Mouflon overlap with chamois in spring and summer in alpine pastures (Darmon et al. 2012) and shift to forest during November when snow comes (Duparc et al. 2012). Roe deer mostly use forests (Redjadj 2010; Redjadj et al. 2014).

### Feces sampling and diet identification

A total of 1243 fresh feces were collected from April to November in 2007 and 2008 either in the field or directly from the animal during captures and during the hunting season. We considered each feces as an approximation of an individual's diet over several days (Castle 1956; Rayé et al. 2011), although an individual diet is likely to be more diversified than the diet assessed from one of its feces. However, we assumed here that the possible underestimation of the individual diet's diversity was similar for all herbivore species. The method used to identify the species and sex of the herbivore that produced the feces is explained in the electronic supplemental material (Appendix 1). A total of 1076 feces could be attributed to one of the three species and were included in the analyses. The dietary composition of feces was investigated using DNA metabarcoding (Taberlet et al. 2007). DNA sequences were obtained and analyzed following Rayé et al. (2011). For each feces, the number of sequences was turned into proportions in order to confer the same weight to each feces (Willerslev et al. 2014), and plants whose proportion was under 2.5 % were removed. Under this threshold, the sequence was considered as a barcoding mistake or as an occasional resource.

We grouped feces according to the vegetation growing seasons: April–May, which corresponds to the start of the growing season ("spring"); June-July–August, around the vegetation peak of biomass ("summer"); September–October, during which the vegetation is senescent ("autumn") and November, when the first snow falls ("winter").

### Measuring the population niche width and degree of diet variation

Following Bolnick et al. (2007), we calculated the Shannon entropy diversity index (Roughgarden 1972) to determine the total niche width (called TNW) per season and species. This index accounts for both abundance (richness) and selectivity (evenness) of the resources found in the diet. A value of 0 corresponds to a diet composed of a single resource.

Testing NVH and SH required that TNW varied among species and season (Bertolino et al. 2009). Given that we had only one TNW value per species and season, we tested for TNW variation among species and season using ANOVA on values obtained by resampling. For each species–season

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level, we randomly sampled with replacement $n$ individuals, where $n$ corresponds to the sample size of the corresponding group. We measured the TNW of the resampled population. We repeated 1000 times this operation.

TNW per species is the average of the four seasons TNWs.

As advised by Bolnick et al. (2007), we used an adaptation of Schoener's proportional similarity index ($PS_i$) (Schoener 1968) to quantify the degree of inter-individual variation, described as the overlap between the diet of an individual and the population diet :

$$PS_i = 1 - 0.5\Sigma \left|p_{ij} - q_j\right| \quad (1)$$

in which $p_{ij}$ is the frequency of resource $j$ in its individual's $i$ diet, and $q_j$ is the frequency of resource $j$ in the population (Bolnick et al. 2002). The $PS_i$ values of all individuals in the population can be calculated and summarized as a population-wide measure of inter-individual variation:

$$V = 1 - \overline{PS_i} \quad (2)$$

Higher $V$ values correspond to a higher inter-individual variation (Bolnick et al. 2007).

We first explored how the degree of diet variation varied among seasons for each species. Hence, we performed an ANOVA with $PS_i$ values as a response variable and species and seasons (factors) as explanatory variables. Then, we tested whether the degree of individual variation was greater than expected under the null hypothesis that each individual ate randomly from the population diet (Bolnick et al. 2002). We used a non-parametric Monte Carlo procedure to generate replicate null diet matrices drawn from the population distribution (Bolnick et al. 2002), from which null values of degree of among-individual variation can be computed. This procedure firstly consists in assigning to each individual of a species-season group, a number of resource items equal to the number of items it was observed eating (Zaccarelli et al. 2013). Then, those $n$ resource items are randomly assigned to the individual's diet via multinomial sampling from the population diet frequencies (Bolnick et al. 2007). The null degree of among-individual variation ($V$) was calculated once all individuals were assigned random diets (Bolnick et al. 2007). We replicated this operation 1000 times for each species–season group in order to evaluate whether the observed degree of among-individual variation was greater than expected by chance, for the 12 species–season groups (Bolnick et al. 2002).

Next, we investigated how $V$ varied with TNW for the different species and seasons in order to test for SH and NVH. Testing for SH is straightforward, as SH predicts the highest intercept for the least social species (roe deer) and the lowest intercept for the most social species (mouflon). In contrast, testing for NVH is less straightforward, as $V$ covaries positively with TNW even under the hypothesis of no inter-individual variation (Bolnick et al. 2007). To get a proper test of NVH therefore requires comparing the observed slope of the $V$ to TNW relationship to the slope estimated under null models. The relationship between among-individual variation and total niche width could be due to just sampling effects, and not to biological mechanisms. We built two null models to disentangle these processes. Our first null model simulated the diet of individuals on the base of the mean diet of the population (Bolnick et al. 2002), as previously described. However, this null model is designed for count data, so that proportion data is first coerced into count data by multiplying proportions by 100. Moreover, this null model did not account for variations of the number of plant species eaten per individual (α-diversity), which can also affect among-individual variation (β-diversity) together with total niche width (γ-diversity) (Wilson and Shmida 1984; Chase et al. 2011). We therefore implemented a second null model which constrained the number of plants eaten by each individual to the observed numbers. For each individual $i$, the observed number $N_i$ of plants eaten was recorded. $N_i$ of plants were then randomly selected, each with probability $q_j$ corresponding to the proportion of plant $j$ in the population diet. This procedure was similar to a null model controlling for α-diversity (Chase et al. 2011). Next, 1000 random samples were simulated for both null models. After estimating the slope expected under the null models, we tested the following models: $V \sim TNW \times species$, $V \sim TNW + species$ (Fig. 1b or c), $V \sim TNW$ (Fig. 1d) to estimate which one best fits our data. Slope of $V$ to TNW relationship was then tested against the slopes estimated with null models.

We used the library RInSP (Zaccarelli et al. 2013) in R (R Development Core Team 2011) for analyses of niche features (total niche width of the population, degree of among-individual variation).

## Results

TNW values varied from 1.12 to 2.98 (Table 1) and significantly differed between seasons for each species ($P$ values <0.05). As expected, TNW was lowest in winter. Highest seasonal values were obtained in summer for chamois, summer and spring for the roe deer, and in autumn for mouflon (see the Supplemental material Appendix 2 Fig. A1 for the mean plant richness per season and species). On

**Table 1** Total niche width values per species and season

| | Spring | Summer | Autumn | Winter | Mean |
|---|---|---|---|---|---|
| Chamois | 2.38 | 2.97 | 1.94 | 1.12 | 2.10 |
| Roe deer | 2.29 | 2.26 | 1.66 | 1.39 | 1.90 |
| Mouflon | 2.18 | 2.17 | 2.98 | 1.65 | 2.25 |

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**Table 2** 95 % Confidence interval of the null $V$ values distribution obtained from null model which constrains the number of plants eaten by each individual to the observed numbers (null model 2)

| | Spring | Summer | Autumn | Winter |
|---|---|---|---|---|
| Chamois | [0.271; 0.275] | [0.426; 0.427] | [0.260; 0.262] | [0.273; 0.280] |
| Roe deer | [0.332; 0.336] | [0.268; 0.272] | [0.251; 0.257] | [0.231; 0.241] |
| Mouflon | [0.310; 0.316] | [0.244; 0.246] | [0.382; 0.385] | [0.284; 0.292] |

average, TNW was largest for mouflon (2.25) and lowest for roe deer (1.9) with chamois in an intermediary position (2.10).

For each species, lowest values of $PS_i$ were obtained in winter. Highest values were found in spring for the roe deer, summer for the chamois and autumn for the mouflon.

For each season and species, observed values of $V$ (confidence intervals provided in the Supplemental material Appendix 3 Table A1) were higher than values expected from the null model 1, showing that there was a significant inter-individual variation in every case (all Monte Carlo simulations $P$ values <0.001). Likewise, observed values of $V$ were not included in the 95 % confidence intervals of the null $V$ values distribution obtained from null model 2 (Table 2), confirming inter-individual variation.

Among the three models fitted to explain variation in V, the best model only included an effect of TNW ($V \sim$ TNW; Figs. 1d, 2). Neither the interaction between TNW and species, nor the main effect of species were significant (interaction TNW and species: $F_{1,6} = 1.20$, $P$ value $= 0.36$; main effect of the species: $F_{1,8} = 0.72$, $P = 0.52$). The lack of significant species effect lead to reject the Sociality Hypothesis (SH). The slope of $V$ against TNW was $b = 0.127 \pm 0.017$ (Table 3) for all species. To test for the NVH, we compared this slope with the slopes estimated under null models. The slopes from both null models were positive (Table 3) and not significantly different from each other (Table 3). The empirical slope was significantly higher than the simulated ones ($P = 0.018$ and $P = 0.034$ for empirical slopes vs. slopes from null model 1 and null model 2, respectively). Inter-individual variation therefore increased with TNW more rapidly than expected under the null models, in agreement with the prediction from NVH.

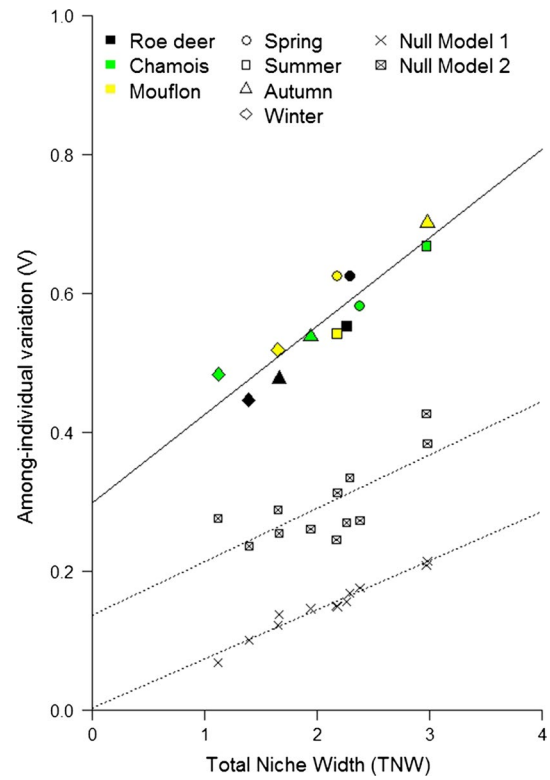


**Fig. 2** Correlation between degree of diet variation and total niche width for three herbivore species. The empirical results are shown with *colored geometric* form. *Crosses* represent the null values of diet variation calculated with the function implemented in the library RInSp where individual diets are reassigned according to the population diet. *Null model 2* is where individual diets are constrained by the number of plants they eat (color figure online)

**Table 3** Results of the empirical and null linear regressions models relating among-individual variation to total niche width

| | Slope | SE | $t$ | $P$ | $r^2$ adj |
|---|---|---|---|---|---|
| Empirical model | 0.128 | 0.017 | 7.39 | <0.001 | 0.84 |
| Null model 1 | 0.071 | 0.005 | 15.78 | <0.001 | 0.96 |
| Null model 2 | 0.077 | 0.021 | 3.62 | <0.01 | 0.52 |

## Discussion

Our analyses of diet niche characteristics at the intra- and interspecific levels led to three main conclusions: (1) inter-individual variation in resource use is found for ungulates with differing feeding regimes, in line with results found in other groups (Araújo et al. 2007; Araújo and Gonzaga 2007; Martins et al. 2008; Tinker et al. 2008; Bolnick et al. 2010); (2) there was no support for the Sociality Hypothesis, and (3) the niche variation hypothesis proposed by Van Valen (1965) held at both the inter- and intra-specific levels. Furthermore, from a methodological perspective, we proposed a new null model of inter-individual variation, complementary to the model of Bolnick et al. (2003).

### Testing for inter-individual variation when diet is measured in terms of proportion

Bolnick et al. (2002) proposed a null model for testing whether inter-individual variation was greater than expected

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under a random choice of items eaten by individuals among the possible items eaten at the population level. However, in the case of large herbivores, diet is more often characterized in terms of proportions of plants consumed. We therefore developed a null model suited for such empirical data. Such an approach is useful for biological systems in which mass and energy flow are more relevant than item numbers. Our model also constrains the number of plants species eaten per individual, so that the total number of plant species eaten by the population is larger than the number eaten by each individual. This approach parallels the distinction between α- and β-diversity (Chase et al. 2011). We recommend that future studies of inter-individual variation also make this distinction when diet is characterized in terms of proportions, in order to disentangle the respective contributions of total niche width and individual niche width on among-individual variation. Note that parametric models fitting the Dirichlet distribution might also be useful in this context (e.g., Ainsworth et al. 2010), in a way similar to the developments of beta regression models to analyze single proportions (Ferrari and Cribari-Neto 2004).

### Significant inter-individual variation whatever total niche width at the inter-specific level

The three herbivores under study all fed on a large number of plant species, though with differing TNW. These differences in TNW were in agreement with previous knowledge about these species' diet and morpho-physiological characteristics (Hofmann 1989; Clauss et al. 2003), which had so far only been studied at the population level. Here, we found in addition that inter-individual variation was significant in all cases. While this result is not surprising for mouflon and chamois, which are able to feed on a large variety of plants, being intermediate feeders, it was less expected for roe deer, which is a browser. Our results indicate that, even for the roe deer, which is sometimes classified as a "obligate non-grazer" and expected to rely on just a few key resources (Redjadj et al. 2014), diets actually differ among individuals at the population level. This is consistent with new studies of roe deer diet variability, such as Abbas et al. (2013), who found that among-individual diet variation in roe deer relates for instance to the type of habitat used by each individual. Along with our results, this exemplifies that the morpho-physiological constraints may not be as strong as previously thought, and that species can exhibit digestive plasticity. This result parallels those found by Araújo et al. (2007) who reported inter-individual variation for species of amphibians, even when their TNW is narrowed by strong interspecific competition. Whatever the mechanisms which may restrain the total niche width of a species, empirical evidence suggests that there is still a significant level of inter-individual variation in diet characteristics. How these inter-individual differences affect individual fitness remains to be investigated empirically (Bolnick et al. 2003).

### Higher inter-individual variation for social species supports NVH instead of SH at inter-specific level

The sociality hypothesis (SH) stated that a more social species should have lower inter-individual variation, assuming that individuals in social species forage at the same time and at the same place on the same pool of plants. In fact, we nearly observed the opposite trend: mouflon, the most social species, had a higher inter-individual variation, especially in autumn and winter. This pattern could result from a stronger intra-specific competition within a group than between individuals in solitary species. Such a mechanism requires a high diversity in plant availability, which is the case in our study site where plant species richness is high (>1500; data from Bauges Massif plants inventory; Lopez, personal communication), allowing a large inter-individual diet variability. Those results are in agreement with some experimental studies performed with fishes (Svanbäck and Bolnick 2007) for which inter-individual variation increased with population density. Abundance also decreased intraspecific overlap in plant–pollinator networks (Tur et al. 2014). In contrast, solitary species such as roe deer could be less prone to intra-specific competition, as each individual should be able to feed on their preferred resources without pressure from congeners in their close vicinity. Given that our herbivore community was composed of three species with only one species in each sociality level category, the lack of support of the Sociality Hypothesis will need to be confirmed on a larger set of species varying both in their sociality level and in their feeding regime. In large herbivores, this would mean acquiring data on total niche width and individual niche width within African communities; for instance, where large herbivore species display a large diversity of sociality levels even within similar type of feeding regimes (Jarman 1974; Fritz and Loison 2006).

### Response of inter-individual variation to seasonal variation in TNW supports NVH at the intra-specific level

At the intra-specific level, niche variation was driven by the seasonal variation in resource availability: TNW declined with resource availability. Some studies (Tinker et al. 2008; Pires et al. 2013) showed that a reduction in resource diversity led to an increase in the total niche width, due to the decline of preferred resources. In such cases, individuals

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may indeed expand their niche width by adding new prey to their diet, or by shifting, independently of each other, to different available resources. For instance, when resource diversity was low, TNW increased both in marsupials (Pires et al. 2013) and sea otters (Tinker et al. 2008), due to an increase of inter-individual variation in diet. We found the contrary: TNW was lowest in winter and highest in summer when the diversity in resource availability was at its maximum. This pattern is in agreement with the concept of ecological opportunity stating that the degree of inter-individual variation increases with higher resource diversity (Roughgarden 1974; Araújo and Gonzaga 2007; Gerardo Herrera et al. 2008). For all three species, high quality resources are much less abundant in winter, and animals must feed on the same available plants, leading to diet homogenization. Highest niche width is achieved in summer for chamois and roe deer as the highest plant diversity occurs during this period. Mouflon reaches the highest niche width in autumn when it shifts from pastures to forest. Accordingly, its diet is then composed of various plants from those two environments. During these periods, several mechanisms may reinforce inter-individual variation in diets, such as local intra-specific competition (in mouflon, for instance), but also individual rank preferences (Araújo et al. 2011), foraging behavior, physiological requirements, or experience (Bolnick et al. 2003).

The contrast between our results at the intra-specific level and those of Tinker et al. (2008) and Pires et al. (2013) on how TNW varies when food availability decreases may be due to (1) differences in how generalist species under study are and (2) the number of food resources available as alternative diet during restricted food periods. In our study, the availability of food resources is particularly low during winter due to snow cover and plant senescence, which may explain why, for example, we obtained opposite results to Tinker et al. (2008) and Pires et al. (2013). More studies on species with differing ranking on the generalist–specialist gradient over a range of restriction in food availability would be needed to understand the temporal dynamics of TNW.

Evolutionary traits such as morpho-physiological constraints (browser-grazer characteristics) and ecological factors such as resource availability appear to constraint niche dynamics at both the intra- and inter-specific level. It needs to be tested further whether NVH holds at these two levels for other species ranking differently on the specialist–generalist gradient, and experiencing different levels of variation in food resources.

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**Author contribution statement** AL, DM and SS originally developed the idea. MB, SI, NGY, AL analyzed the data and wrote the first draft of the manuscript. SI and NGY developed the mathematical model of a new null model. CM, PT, CR, DR developed the DNA metabarcoding methods and applied it on feces. CR performed the feces sampling, laboratory experiments (DNA metabarcoding on feces), database treatment and preliminary analyses. FB and EC performed the statistical analyses and the database treatment of DNA sequences. All authors commented and approved the ms.

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**Paper II.**

Bison M., Dupeyras P.A., Redjadj C., Miquel C., Rioux D., Taberlet P., Maillard D., Yoccoz N.G., Loison A.

*Taxonomic and functional niches differentiation between two large herbivore species.*

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# Taxonomic and functional niches differentiation between two large herbivore species

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**ABSTRACT**

Introduced species set the problem of forced sympatry with native species, especially when they have similar ecological requirements. Chamois (*Rupicapra rupicapra*, native) and mouflon (*Ovis gmelini musimon*, introduced) have co-occured in the Bauges Massif (Alps, France) for 60 years. We analyzed the interspecific trophic interactions between both species in order to determine the mechanisms implied in their coexistence. We used ungulates faeces sampled from May to November in two pastures, one occupied by chamois living in allopatry and one occupied by chamois living in sympatry with mouflon. Firstly, we analyzed faeces plant content with DNA-metabarcoding in order to compare chamois and mouflon taxonomic niche. Secondly, we calculated the functional niche of chamois and mouflon diets using a plant functional traits database (LNC ratio, LPC ratio, LDMC) measured on the main plant species constituting the diet. Taxonomic and functional niches differed between sympatric chamois and mouflons, and their differences varied in intensity with season. No differences between the two chamois subpopulations were seen in spring and autumn. Subtle differences between the two chamois subpopulations were observed in summer but were explained by the presence of shrubs in the sympatric chamois diet. However, the diet quality of the sympatric chamois subpopulation was as good or better than the allopatric one, highlighting that mouflon has no negative impact on chamois. Our results provide the idea that introduced and native species that did not coevolve together can coexist through partitioning their diet. Those results are in line with previous behavioral and spatial studies that concluded to the lack of interference competition in coexisting chamois and mouflon populations.

**Key-words:** coexistence, diet, DNA-metabarcoding, functional traits, niche

## INTRODUCTION

Given the economic, social and cultural values brought by large herbivores (Gordon et al. 2004), they have been introduced in many places. However, as they are a central node in trophic networks, their introductions can have major unexpected abiotic and biotic effects on the components of ecosystems. Their impact has mainly been studied at the vegetation level, as it constitutes one of the main direct effects on ecosystems (Coblentz 1978 for goats introductions on Santa Catalina Island, Chouinard & Filion 2005 for white-tailed deer introductions on Anticosti Island, Martin et al. 2010 for deer introductions on Haida Gwaii islands, Côté et al. 2008). Introduced herbivores can also enhance trophic cascade through vegetation changes on invertebrates (Martin et al. 2010), small mammals (Smit et al. 2001) or birds (Cardinal et al. 2012). Relatively, few studies focused on the impacts of introduced herbivores (wildlife or livestock) on the native herbivore community, which can become strongly disrupted (Forsyth et al. 1998, Voeten and Prins 1999, Vazquez et al. 2002, La Morgia et al. 2009, Acebes et al. 2012, Lovari et al. 2014, Ferretti et al. 2015). Concurrently to the growing herbivore densities (Breitenmoser 1998, Loison et al. 2003), introductions promote stronger interspecific interactions between large herbivores, and set the problem of forced sympatry with native species. According to the differences in physical (e.g. body size, rumen type, Jarman 1974), ecological (e.g. habitat selection, plant organ selection), or behavioral (e.g. sociality) characteristics of animals that are placed in contact, but also according to their density (Richard et al. 2010) and to the availability of food resources (Storms et al. 2008), the direction of the interaction can be positive (facilitation, Arsenault & Owen-Smith 2002), neutral (Darmon et al. 2012, 2014) or negative (competition, Putman & Putman 1996).

Native and introduced herbivore species are potentially subjected to time, space and food resources competition (Pianka 1974). In the absence of top-down controls by predators, food resources appear as one of the main common denominator controlling growth and survival of individuals (Sinclair 1974, White 1978). Consequently, forage resources competition can occur through exploitation or interference (Putman and Putman 1996) at different spatial scales. Herbivores can compete at the patch scale (Weisberg et al. 2006), either directly by repelling the inferior competitor toward lower quality habitat (Chirichella et al. 2013), or indirectly through diversity and composition vegetation changes (Huntly 1991, Murray 2000). Competition can downscale at the plant species or plant part level. By pre-empting the resources of another, the superior competitor can lead native animals to feed on lower quality

resources (Jenkins and Wright 1987), to spend more time searching for food (Kie et al. 1991), or to decrease the quantity of resources ingested (lower food intake rate, Lovari et al. 2014). This could result in changes in individual growth, survival and reproduction (Lindström et al. 1999, Lummaa et al. 2002, Pettorelli et al. 2007, Richard et al. 2010, Ferretti et al. 2015) which in turn could influence the population dynamics of the inferior competitor (Sinclair & Norton-Griffiths 1982, Forsyth & Hickling 1998, Forsyth 2000, Mishra 2004). However, external factors can counterbalance this negative effect. For example, introduced species can be regulated by environmental conditions (snow, Garel et al. 2005) not encountered in their original habitat range, or can be spatially distributed according to different constraints (sociality, landscape of fear, Laundre et al. 2001) reducing the interactions and in turn the impact of the introduced species on the native ones.

Three main limits arise in several studies focusing on interspecific competition. Firstly, as many studies are conducted in single places (Leuthold 1978, Voeten & Prins 1999, Fischer & Gates 2005, Shrestha et al. 2005) and do not compare a species in different ecological contexts, they do not allow the estimation of the strength of the interspecific competition. Secondly, environmental differences between sites when the previous case is achieved (Schmitt & Coyer 1983, Putman et al. 1993, Radtkey et al. 1997, Melville 2002, Corbalàn & Debandi 2014) prevent the separation of the effect of environmental conditions from the pure inter-specific competition. Finally, so far, studies on large herbivores food resource partitioning at the plant species scale are most often performed at the diet taxonomic level (Leuthold 1978, Bodmer 1991, Heroldova 1996, Bertolino et al. 2009). However, looking at the overlap of taxonomic diet is not enough to conclude about the negative effect of a species, as herbivores can feed on different plant parts (Wegge et al. 2006) or at different locations. Moreover, a change in taxonomic diet does not necessarily entail a decrease in diet quality for the herbivore. The few studies which tend to include plant functional traits to describe the diet quality focus on a few number of plant species making up the diet, preventing any robust conclusions on diet comparisons (Lovari et al. 2014). Broader plant functional investigations elaborated from the herbivore point of view are, consequently, relevant to make predictions about the ability of herbivores to reach their energetic requirements (Robbins 1983, Elser et al. 2000, Sterner & Elser 2002) in environments constrained by co-occurring species (Hobbs 1983, Jenkins and Wright 1987). Therefore, as taxonomic niche can influence functional niche, both should be studied jointly (Ibanez 2015 in revision).

In our study, we were interested in the effect of an introduced species (mouflon *Ovis gmelini musimon*) on the trophic taxonomic and functional niche of a native one (chamois *Rupicapra rupicapra*), occurring in a place where no spatial exploitative competition nor behavioral interference by the mouflon are suggested (Darmon et al. 2012, 2014), even if conclusions about its impact on native ungulates are not uniform between studies (Chirichella et al. 2013). Given that species have similar body size (Redjadj et al. 2014), show a great overlap in the diet in Italian Western Alps (81%, Bertolino et al. 2009), and that chamois are known to be highly selective (La Morgia & Bassano 2009, Redjadj et al. 2014), we hypothesize that chamois could be prone to be limited by food resources, and may suffer from exploitative competition by mouflon both taxonomically and functionally. Even if we only have one experimental unit, our study overcomes the problems stated hereinabove, i.e. (1) a study site where two pastures are respectively occupied by a chamois population with and without mouflon, and exhibiting similar environmental conditions (geographically closed and subjected to the same climate and same altitude), (2) precise diet data obtained from DNA-metabarcoding realized on faeces sampled in the two pastures (Taberlet et al. 2007, Yoccoz 2012), (3) a database on locally available plants around faeces, (4) plant functional traits measurements realized in the same area and relevant as proxies for diet quality and animal requirements such as protein (measured as nitrogen content), water, energy (carbohydrates) and macroelements (such as phosphorus) (Mattson 1980, Robbins 1983, Grasman & Hellgren 1993, Elser et al. 2000). This brings robust stands to estimate the potential impact of mouflon on chamois, as well as for the taxonomic and functional niches, over the entire growth period.

As a consequence of competition caused by the presence of mouflon, we expect (1) a low diet similarity and a resource partitioning between chamois and mouflon in the sympatric site, (2) differences between the chamois taxonomic niche living in sympatry with mouflon and the chamois taxonomic niche living in allopatry, and (3) a lower diet quality of the chamois in sympatry because of a focus on less nutritious suboptimal resources not eaten by mouflon.

## MATERIAL AND METHODS

The three herbivore populations (two chamois subpopulations and one mouflon population) have been defined by their taxonomic and functional niches of ingested plant species and also by their taxonomic and functional niches of available plant species in the 48h home range (see fig.1 for a schematic overview of the databases and methods used).

### *Study sites and species*

The study was carried out in the Bauges Game and Wildlife Reserve (5,170 ha). The Bauges mountains constitute a transitional area before the Alps (around 100 km west) and is subjected to a continental climate with an oceanic influence (Loison et al. 1999) causing large precipitations (> 1850mm/year) favorable towards high plant diversity. Mouflon has been introduced in 1950s in the Bauges mountains (Darmon et al. 2007), a place where already occurs four wild native ungulates (chamois (*Rupicapra rupicapra*), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and wild boar (*Sus crofa*)). In the Reserve, chamois and mouflon are the two most abundant species (Darmon et al. 2012) and have no natural predators except for golden eagles that may anecdotally depredate new borns and sick animals.

We focused our study area on two pastures similar in terms of environmental conditions (geographically closed, subjected to the same climate and same altitude, and having similar areal extent, Duparc et al. 2012) but differing in their inhabiting species: the Armene pasture (227.4 ha, 1400 to 2200 m a.s.l) is occupied by an allopatric chamois population whereas the Charbonnet pasture (203.6 ha, 1400 to 2200 m a.s.l) is occupied by both chamois and mouflon populations living in sympatry. In the following, we use the term “subpopulation” to define the three populations studied, i.e. “Chamois in sympatry”, “Chamois in allopatry”, “Mouflon”. Chamois is always situated in pastures, whereas mouflon shifts from forest to pasture in spring (mid-June) and come back in forest in autumn (end of September) when snow comes (Darmon et al. 2012). Animals in pastures do not show any other interactions with other wild ungulates, as roe deer and red deer occupy lower altitudes. However, cows are kept in enclosures on the Armene pasture from June to October, but do not directly interact with chamois as a barrier separate them. Cows do not occupy one entire vegetal community which allows chamois to have an access to all plant communities.

Densities of chamois subpopulations are similar with 57 chamois $km^{-2}$ on the allopatric site and 51 chamois $km^{-2}$ on the sympatric site. Density of mouflon is about 23 mouflon $km^{-2}$ (Darmon 2007a).

Chamois and mouflon are similar in terms of body mass, digestive morphophysiology and diet category (Table 1), possibly leading to feeding competition.

*Calculations of taxonomic niche*

The taxonomic spectrum was determined by the relative abundance of plant species making up the diets. From May to November 2007 and 2008, fresh faeces of chamois and mouflon were collected on the Armene and Charbonnet pastures either in the field or directly on animal during captures and during the hunting season. Each faeces is considered as an approximation of an individual's diet over several days (Castle 1956, Rayé et al. 2011), although an individual diet is likely to be more diversified than the diet assessed from one of its faeces. However, we assumed here that the possible underestimation of the individual diet’s diversity was similar for all herbivore species. The dietary composition of faeces was investigated using DNA metabarcoding (Taberlet et al. 2007). DNA sequences were obtained and analyzed following Rayé et al. (2011). For each faeces, the number of sequences was turned into proportions in order to confer the same weight to each faeces (Willerslev et al. 2014) and plants whose proportion was under 2.5% were removed. Under this threshold, the sequence was considered as a barcoding mistake or as an occasional resource.

20 faeces on 549 have been removed from the dataset because of missing plant trait measurements on occasional plant in diets (low frequency of occurrence) with high (>8%) proportions in faeces (high frequency of sequences).

2007, 2008 (years of faeces sample) and 2013 (year of plant trait measurements) are not similar in terms of heat accumulation over the year (cumulated degree day). Using 2008, 2009 and 2010 phenology measurements on the Armene and Charbonnet pastures, we obtained models predicting phenology against cumulated degree day (Duparc, pers.comm). Thanks to temperatures measured with the Lescheraines weather station (590m altitude), each faeces was associated with a cumulated degree day and hence, to the phenology of the vegetation at the sampling time given previous model prediction. As we did not measure plant traits at each

phenological stage, we matched the phenology range 0 to 1.5 to the spring sampling, 1.5 to 2.5 to the summer sampling, and 2.5 to 4 to the autumn sampling.

The estimation of the relative abundance of plant species around each faeces is described in the appendix 1.

*Plant functional traits and calculations of functional niche*

Plant traits measurements

Using standardized protocols (Cornelissen et al. 2003), plant functional traits (Leaf Nitrogen Content (LNC), Leaf Phosphorus Content (LPC), Leaf Carbon Content (LCC), Leaf Dry Matter Content (LDMC)) have been measured on 50 plant species of the Armene pasture in spring at the start of the growing season (beginning of June), in summer around the vegetation peak of biomass (mid-July) and in autumn when the vegetation is senescent (mid-September), during the year 2013, on the plant species making up around 85% of the total cumulated abundance of the diets, and around 85% of the total cumulated abundance of available plants in the pasture. Each plant species have been sampled in the vegetal community where it was the most abundant (Duparc et al. 2012). We decided to not represent the results of LCC (Leaf Carbon Content) as the coefficient of interspecific variation is small ($CV_{LCC}$=0.06), comparatively to the other traits ($CV_{LPC}$=0.48, $CV_{LNC}$=0.46, $CV_{LDMC}$=0.34). The content of N and P are limiting in plants (Elser et al. 2000). Hence, for the sake of simplicity, we prefer to deal with absolute amount of N and P than C:N and C:P ratios as that are more easily understood, and also because absolute amount such as LNC are used to study large herbivore growth and fecundity (McArt et al. 2009). Moreover, the slopes of the relationship between C:N and N, and C:P and P, are close to 10 and 1000 respectively (different order or magnitude between the nutrients), with high $R^2$, which confirms the strong direct relationships (C:N and N: slope = -10.3, $r^2$ = 0.69; C:P and P: slope = -918, $r^2$ = 0.66), and the use of absolute values.

Because measurements were done at the same altitudinal range (between 1700 and 2000 m) on the same pasture, we considered that intraspecific variability caused by environmental differences was of low contribution to the variability in trait values compared to interspecific differences, and we consequently did not take into account individual variation within species (Albert 2015).

In the diet, frequencies of *Onobrychis montana* has been summed with *Hedysarum hedysaroides*; *Silene latifolia* with *Silene vulgaris; Plantago alpina* with *Plantago; Ranunculus tuberosus* with *Ranunculus;* and *Lathyrus pratensis* with *Lathyrus*. As we only had measurements for the former plant of previous each pair, we assumed that plant functional traits were more similar within a genus than between them and used this value for the new "group".

Four plant species on 50 have not been measured on the field but estimated values have been found in other published or unpublished databases (*Blitum bonus-henricus (*Kerkhoff et al. 2006: LNC ; Thuiller W. pers. comm.: LDMC) ; *Betula pendula*, *Acer pseudoplatanus* and *Pinus sylvestris* (Thuiller W. pers. comm.: LNC, LDMC). Those plant traits, except for *Blitum bonus-henricus*, have been measured in mountain ecosystems during the vegetation peak of biomass (summer period), providing site similarity.

Some plant species were not measured over the three seasons. In order to evaluate a missing value in one season, mean differences between seasons of the corresponding growth form have been added to or removed from the trait value measured.

Leaf phosphorus content missing values (27 out of 183) have been predicted from nitrogen values, through the positive covariation between nitrogen and phosphorus (Owen-Smith & Novellie 1982, Cornelissen et al. 2003, Forsyth et al. 2005).

Functional niche measurements

For the LNC, LPC, and LDMC traits, we calculated the diet functional niche of each individual having produced the faeces based on ingested plants, at the different phenological stages. This measure, hereafter called "Functional Niche", is analogous to the Community Weighted Mean (CWM) metric which is one of the most commonly used indicators of the functional trait structure of communities reflecting the average value of the dominant species (Lavorel et al. 2008, Ibanez et al. 2013, Carmona et al. 2014).

$$Community\ Weighted\ Mean = \sum_{i=1}^{n} p_i \times trait_i \qquad [1]$$

where $n$ is the total number of plant species in the diet or habitat, $p_i$ is the relative abundance of species $i$ in the diet or habitat and $trait_i$ the trait value of species $i$.

In order to determine the quality of the habitat around each faeces, we applied the previous formula ([1]) to the plant species available around each faeces (see above for how relative

plant species abundance have been calculated) and kept the denomination “Community Weighted Mean”.

We assumed that plant functional traits are similar between both pastures, and used the plant trait measurements of the Armène pasture to estimate the diet and habitat functional niche of animals feeding on the Charbonnet pasture.

*Statistical analyses*

Statistical analysis have been realized with the R 2.15.1 Software (R Development Core Team 2014).

First we focused on the taxonomic and functional niches, in order to test for diet differentiation between the three subpopulations. Then, in order to explain the differences that we could observe in taxonomic and functional niches, we constructed models including availability, season and subpopulation as explicative parameters.

Multivariate analysis

Non-symetric correspondence analysis (NSCA) analogous to Simpson diversity index (Pélissier et al. 2003) have been realized on taxonomic niche for each season. In order to test for competition, it requires to evaluate whether chamois and mouflon have different feeding niche positions in the sympatric site, and then whether chamois subpopulations have similar feeding niches.

Hence, we compared the scores on the first and second axis of the NSCA between the three subpopulations with an ANOVA. Tukey’s honestly significant difference (HSD) test have been used to compare the pairs of niche positions between the three subpopulations.

Regression analysis

In order to determine the relative effect of availability, season and population in the variability observed between the three taxonomic and functional diet subpopulations, we constructed for each season a linear model testing the additive effects of availability and subpopulation on the taxonomic of functional diet (Used ~ Available + subpopulation). At the taxonomic level, this model has been applied to the five main plant species explaining the two first axis of the multivariate analysis (*Helianthemum nummularium*, *Onobrychis montana*, *Vaccinium*

*uliginosum, Geranium sylvaticum, Rosodae*). At the functional level, this model has been applied to each functional trait independently (LNC, LPC, LDMC).

We did not account for the interaction between availability and subpopulation as the pattern tend to be the same for the three subpopulations. Moreover, as the range of values for availability differs between the sympatric and allopatric site, a relatively small part overlap and not allow robust conclusions.

## RESULTS

No altitudinal spatial segregation has been observed in the faeces sampling according to the species, allowing the study of interspecific competition.

*Plant species composition of diets among the three subpopulations*

Among the whole DNA fragments detected in all the faeces, evergreen shrub followed by legumes and forbs were the three most abundant growth forms ingested. Feeding patterns with respect to plant families differed between the two ungulate species, but chamois subpopulations were almost similar (Table 2). Chamois populations focused mainly on *Cistaceae* (*Helianthemum nummularium*) and *Fabaceae*, whereas mouflon ate half-time on *Fabaceae*.

*Coexistence at the taxonomic level*

In spring, scores of the three subpopulations on the first and second axis of the NSCA did not differ among them. In summer, taxonomic niche position tend to differ between the two chamois subpopulations in summer ($p=0.08$, Fig.2). This result is explained by the proportion of *Vaccinium uliginosum* in the diet of chamois in allopatry which is positively correlated with the local availability in summer (slope=13.9, $P<0.05$). Removing *Vaccinium uliginosum* from the diets in summer makes decreasing the niche position differences between chamois subpopulations, which are not significantly different anymore on the first axis of the NSCA ($p>0.05$). In addition, after this removal, mouflon population niche is significantly differentiated from both chamois populations niche on the first axis of the ANSC. In autumn, scores of chamois populations do not significantly differ on the first axis of the NSCA, but significantly differ with mouflon. In summer and autumn, mouflon and sympatric chamois do not differ in their score positions on the second axis of the NSCA, contrary to the other pairs (sympatric chamois/allopatric chamois, mouflon/allopatric chamois) that significantly differ.

In spring and summer, no significant relationship are observed between the proportion of plant used and local availability for *Onobrychis montana*, *Geranium sylvaticum* and *Rosodae*.

In summer, the local availability of *Helianthemum nummularium* significantly influences the proportion in the diet (slope=30.3, $P<0.05$). In autumn, plant proportion in the diet is significantly positively related with local availability for the two plant species correlated with

the first axis of the NSCA, i.e. *Helianthemum nummularium* (slope=31.2, P<0.05) and *Onobrychis montana* (slope=161.8, P<0.05). The values of slopes are high as the ratio of proportion in the diet to local availability are high.

*Coexistence at the functional level*

Similar to general expectations (Perez-Harguedinguy 2003), LNC, LPC of diets decreased and LDMC of diets increased during the year along with plant maturity.

Over the year, mouflon tend to have an equal or a better diet quality than chamois in sympatry (equal LDMC in summer and lower LDMC in autumn, equal LNC and LPC in spring and summer, higher LNC and LPC in autumn). In spring, mouflon have significantly higher LDMC functional niche values, but the difference in effect size between mouflon and chamois in sympatry is relatively small (Δ=+0.03). Chamois populations do not differ in diet quality during the main part of the year but when significant differences are noticed the trend goes to a higher diet quality of the sympatric chamois, even if the differences in effect size are also small (significant higher LPC values in spring for chamois in sympatry; lower LDMC, higher LNC and higher LPC values in summer; lower LDMC values in autumn).

Over the year, variation in the availability of LDMC does not account for variation of LDMC in the diet (fig. 3). In spring and summer, for nitrogen and phosphorus, variation in the diet is not explained by the local availability. In autumn, a significant positive relationship relates the functional niche with the local availability (nitrogen: slope = 0.61, P = 0.002 and phosphorus: slope = 0.60, P = 0.002, fig. 3). The similar positive trend observed for nitrogen and phosphorus is in agreement with the strong correlation between the nutrient contents (r=0.97).

## DISCUSSION

In studies of interaction's outcomes between two species, the occurrence of a reference site close by where one of both species is absent is an infrequent opportunity. The existence of two comparable pastures where chamois populations are in contact or not with the mouflon is such an opportunity. Despite the single experimental unit, our study of the taxonomic and functional characteristics of mountain ungulate populations led to two main conclusions: (1) during the main part of the year, sympatric chamois and mouflon differed in their diets in terms of taxonomic and functional niches, (2) sympatric and allopatric chamois subpopulations had similar taxonomic and functional niches leading them to reach at least the same energetic requirements all over the year, supporting that mouflon has no negative impact on chamois diet.

### *Taxonomic and functional niche differentiation between chamois sympatric and mouflon populations*

Although high digestive morphophysiological (Kamler et al. 2001, Clauss et al. 2009), body mass (Redjadj et al. 2014) and diet category similarities (Hofmann 1989, Cransac et al. 1997, Bertolino et al. 2009, Marchand et al. 2013), our analysis revealed variations in the strength of the taxonomic diet dissimilarity between chamois and mouflon. From the vegetative to the beginning of flowering period, no differences were observed between species taxonomic niche position on the sympatric site, in agreement with the highest feeding overlap observed between chamois and mouflon in Bertolino et al. (2009) in spring. A lower richness of developed plant species available in spring could lead the animals to feed on the same first plantlets. In contrast, from the flowering to senescence period (summer and autumn), taxonomic diet differentiation was stressed between species. Even in autumn, when vegetation became senescent and of low quality, mouflon and chamois were able to feed on different plant species, with a focus on the evergreen shrub *Helianthemum nummularium* for chamois and on the leguminous *Onobrychis montana* for mouflon. It confirms the idea that within and between studies, interaction outcomes noticed between species are variable in space and time (Poisot et al. 2012, Maron et al. 2014), i.e. context-dependent according to the local environmental conditions and species states (Poisot et al. 2014).

At the functional level, mouflon population showed an equal or higher diet quality than chamois. This result was consistent with the main plant species mouflon fed on, *Onobrychis montana*, which is a leguminous species known to be nitrogen-rich. In autumn, the positive effect of local availability on the proportion of this plant in the diet, could partly explain its use. Indeed, local availability of *Onobrychis montana* around faeces was higher for mouflon than chamois. Besides, mechanical properties of plants and animal morphological features could play a role in the plant choice. *Helianthemum nummularium*, which discriminated chamois from mouflon population in summer and autumn, had a leaf toughness two third lower than *Onobrychis montana*. As mouflon have a higher masseter mass that chamois (Clauss et al. 2008), we suggest that mouflon are better able to chew plants of high toughness than chamois, explaining the lower proportion of *Onobrychis montana* in the chamois diet. It also provides further evidence to the fact that mouflon cannot always be classified as a grazer species (Marchand et al. 2013, Redjadj et al. 2014), feeding mainly on graminoid species (Bertolino et al. 2009) that are of low digestibility and nutritional intake relatively to forbs. Rather, mouflon tend to have a flexible diet, as the used forage varies within year and across spatial locations (Garcia-Gonzalez & Cuartas 1996, Cransac et al. 1997, Marchand et al. 2013). If highly nutritional food resource is available, mouflon seem to be able to take advantage of it.

The influence of food availability on the diet is a recurrent topic in ecology (Wam et al. 2010). To our knowledge, the studies that reported the importance of resource availability on the field focused on the patch-scale quality or higher scale in order to explain the spatial distribution of animals (Bergman et al. 2001, Fortin et al. 2003, Fryxell et al. 2004, Boone et al. 2006, Van Beest et al. 2010). No studies clearly reported the importance of food availability by taking into account the relative abundance of plants in terms of biomass at the local-scale on the field. Indeed, predictions are not straightforward as many factors, such plant composition, previous diet, or preferences, can influence the selection, as it has been revealed by experimental trials (Parsons 1994). Our analyses revealed that, for chamois and mouflon, the importance in spring and summer was actually given, not to the selectivity degree, but to the relative proportion of plant species in the diet, except for *Helianthemum nummularium* in summer where availability influenced the intake. The positive relationship observed in autumn for *Helianthemum nummularium* and *Onobrychis montana* can be explained by the fact that, as this two species were still eatable at this time of the year, animals had to take advantage of it before winter. As the number of sampled faeces was lower in spring and

summer than in autumn, this could also explain why no significant relationships were observed during these seasons.

Our first hypothesis is partially confirmed as the resource partitioning between both species is partly detected in summer and autumn (fig.3).

Two reasons can determine the cause of the diet partitioning observed: 1) with or without interspecific competition, there is some separation in fundamental niche between species having species-specific requirements, and the separation is not achieved by the presence of each other (Putman & Putman 1996); 2) the diet differentiation observed is due to a shift in the mouflon diet in order to avoid competition with chamois. To answer this question, we would require a comparison with an allopatric mouflon population.

*Is the diet differentiation between chamois and mouflon on the sympatric site caused by a shift in chamois diet?*

From a taxonomic point of view, the main part of the diets of both chamois subpopulations were similar from spring to autumn, when *Vaccinium uliginosum* was removed in summer. The relatively high local availability of *Vaccinium uliginosum* around the chamois allopatric faeces explained the differentiation.

From a functional point of view, the diet quality was only dependent on local availability in autumn for nitrogen and phosphorus. Hence the higher diet quality of sympatric chamois populations in autumn could be partially explained by the higher local habitat quality of N and P. In autumn, as the quality of the vegetation decreased, selection could not compensate for the general decrease in the quality of available plants. As previously suggested, the low number of sampled faeces in spring and summer could also explain the absence of significant relationships during these seasons. However, for the same availability, chamois population on the sympatric site tended to have a higher diet quality than the population on the allopatric site. As chamois subpopulation diets did not differ in terms of main plant species used, we could hypothesize that chamois in sympatry may use suboptimal resources of higher quality than the chamois in allopatry. Several reasons might explain the differences in the intake of secondary resources between chamois subpopulations: 1) the composition of vegetal communities used by both chamois subpopulations differed between pastures, 2) the spatial organization of vegetation differed between pastures making the high-quality resources more

accessible or more attractive as plant neighboring can influence the food intake (Palmer et al. 2003, Bergvall et al. 2006, Bee et al. 2008), and the searching effort reduced on the sympatric site, 3) there was a facilitation effect by mouflon which allowed a better access of higher quality resources for chamois, by reducing grass height or removing stems (Arsenault and Owen-Smith 2002), 4) chamois were more mobile because of the presence of the mouflon on the sympatric site, allowing them to find exclusive resources that they need.

Even though plants contain high undigest carbohydrates levels and low nitrogen-rich protein content (Robbins 1983, Karasov & del Rio 2007), herbivores need to achieve their highly nitrogen-rich protein requirements for growth and reproduction, and to decrease C:N and C:P levels (Elser et al. 2000, Fagan et al. 2002, Raubenheimer et al. 2009). In our case, whatever the site and the presence or absence of a possible competitor, chamois populations reached at least the same nutritional intake, in terms of absolute amounts of LNC and LPC (fig.3). This pattern has also been observed for grasshoppers (Ibanez et al. 2013), where species were able to get similar CWM of plant nutritional quality (leaf economic spectrum, LES) in different habitats. However, we were not able to assess if the quantity of ingested biomass by sympatric chamois was affected, which is in addition to quality, a major factor for animal's development. Indeed, animals could be behaviorally impacted through an increase in vigilance leading to a decrease of foraging, as observed for roe deer in proximity of fallow deer or wild boar (Ferretti et al. 2011).

Even if we are not aware of the quantity of ingested food, our results point out that mouflon does not have negative impact on chamois taxonomic diet and diet quality (fig.3). We consequently reject our second and third hypothesis.

*The absence of negative interaction between chamois and mouflon: a common pattern?*

Even though herbivores can widely overlap in diet, our study show that coexisting species that did not coevolve together can partly partition food and avoid negative interference. Those results are in accordance with previous findings on activity rhythm and habitat selection (Darmon 2012, 2014) for which interactions between chamois and mouflon were marginal. Concurrently to the absence of negative interaction by mouflon on chamois diet, we should not expect a decrease in growth, survival and reproduction of adults and young chamois individuals. Nevertheless, this pattern is not common in all ecosystems having going through species introductions. For example, in Apennines, red deer (reintroduced species) negatively

affected the winter survival of chamois offsprings through modification of the vegetal community leading to a decrease of nutritious plants in the female chamois diet (Lovari et al. 2014, Ferretti et al. 2015). In our study, a large spread of mouflon populations could enhance a reduced access of chamois to high quality forage, and suppose, as observed by Lovari et al. (2014) with the reintroduction of red deers into an area occupied by a threatened chamois populations, a lower fitness of chamois individuals.

Additional long-term studies on population survival and growth rate could be used to confirm the neutral effect of mouflon on chamois populations.

*Conclusion*

Despite a study based on a single experimental item, our results show that at the species-level, a non-native species does not necessarily negatively interfere with the native species, as well as on the taxonomic (ingested plant species) and functional (diet quality) niches. We advise that future studies should take into account jointly taxonomic and functional characteristics of the niche to determine the outcomes of interspecific interactions. Further studies are needed to assess how consistent a coexistence pattern in chamois and mouflon interactions is, and whether facilitation can be a mechanism involved in the coexistence. Downscaling the analysis to the individual level could allow going deeper into the comprehensive mechanisms of coexistence and determining whether competition acts at a finer scale.

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**Fig. 1** Overview of the databases and methods used. The Functional Niche (FN) and Community Weighted Mean (CWM) are calculated from the Community Weighted Mean (CWM) equation: $CWM = \sum_{i=1}^{n} p_i \times trait_i$, where *n* is the total number of plant species in the diet or habitat, $p_i$ is the relative abundance of species *i* in the diet or habitat and $trait_i$ the trait value of species *i*. Questions 1 and 2 are solved with the coupled analysis of diet and habitat data, respectively at the taxonomic and functional level.

**Fig. 2** Non-symetric correspondence analysis (NSCA) on the taxonomic diets of the three ungulate subpopulations for each season, on the top right of each plot are shown the main plant species accounting for the first and second axis of the NSCA, at the bottom of each plot are shown the density plots of the scores of the first axis of the NSCA. *Vaccinium uliginosum* has been removed in summer.

**Fig. 3** Effect of habitat on diet. Functional Niche (FN) in function of Community Weighted Mean (CWM) for (a) LDMC, (b) Leaf Nitrogen Content (LNC) and (c) Leaf Phosphorus Content (LPC) for the three seasons.

**Table 1.** Body mass, digestive morphophysiology, diet category comparisons between chamois and mouflon (from Redjadj et al. 2014 and references therein)

| | Body mass class | Digestive morphophysiology | Diet category |
|---|---|---|---|
| Chamois | Small (~30 kg) | Intermediate | Intermediate |
| Mouflon | Small (~35 kg) | Cattle type/Intermediate | Grazer/Intermediate |

**Table 2.** Estimated eaten percentage of the different plant families by each ungulate subpopulation

| Ungulate subpop. | Percentage eaten | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Cistaceae | Fabaceae | Ericaceae | Geraniaceae | Rosodae | Apiaceae | Poaceae | Others |
| Chamois Armene | 31.03 | 25.68 | 8.36 | 7.94 | 3.53 | 0.53 | 0.18 | 22.75 |
| Chamois Charbonnet | 35.47 | 26.61 | 0.45 | 8.10 | 9.26 | 3.29 | 0.04 | 16.78 |
| Mouflon Charbonnet | 4.15 | 45.01 | 2.24 | 0.66 | 11.58 | 6.74 | 1.37 | 28.25 |

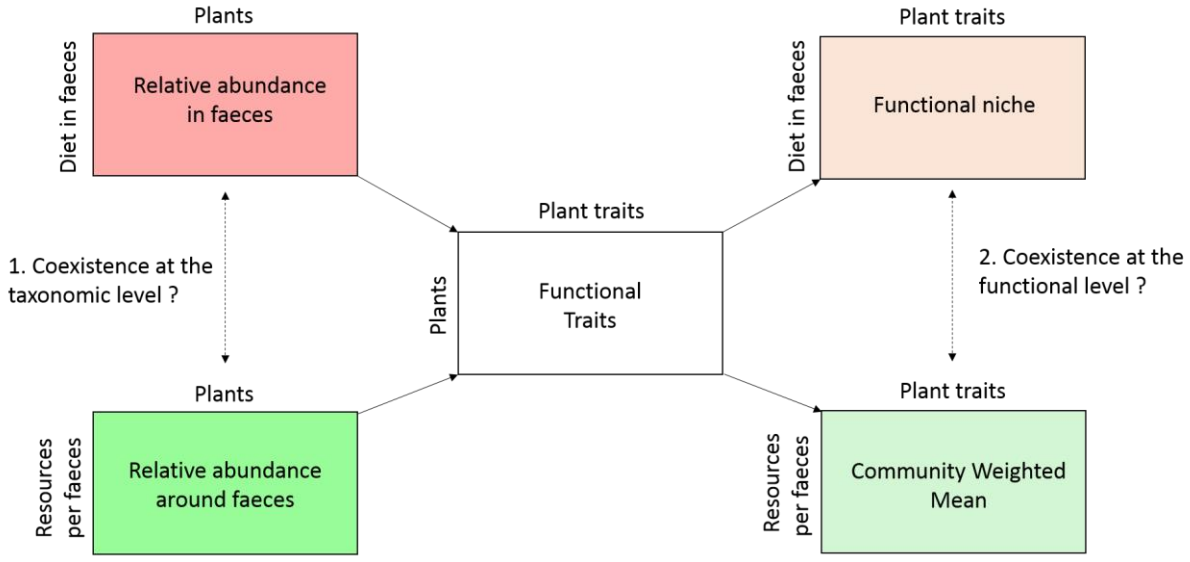
Plants
Diet in faeces
Relative abundance in faeces
Plant traits
Diet in faeces
Functional niche
1. Coexistence at the taxonomic level ?
Plant traits
Plants
Functional Traits
2. Coexistence at the functional level ?
Plants
Resources per faeces
Relative abundance around faeces
Plant traits
Resources per faeces
Community Weighted Mean

**Fig. 1**

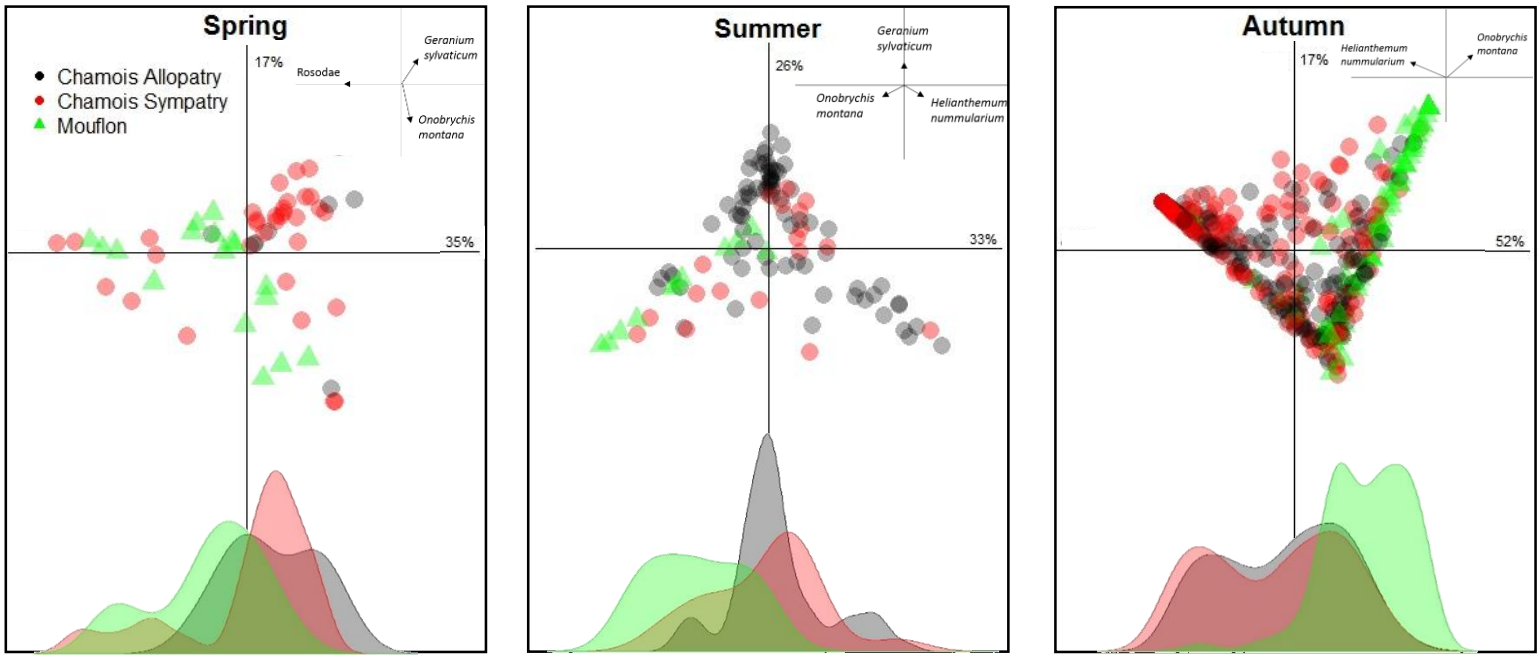
Spring
Chamois Allopatry
Chamois Sympatry
Mouflon
17%
35%
Rosodae
Geranium sylvaticum
Onobrychis montana
Summer
26%
33%
Geranium sylvaticum
Onobrychis montana
Helianthemum nummularium
Autumn
17%
52%
Helianthemum nummularium
Onobrychis montana

**Fig. 2**

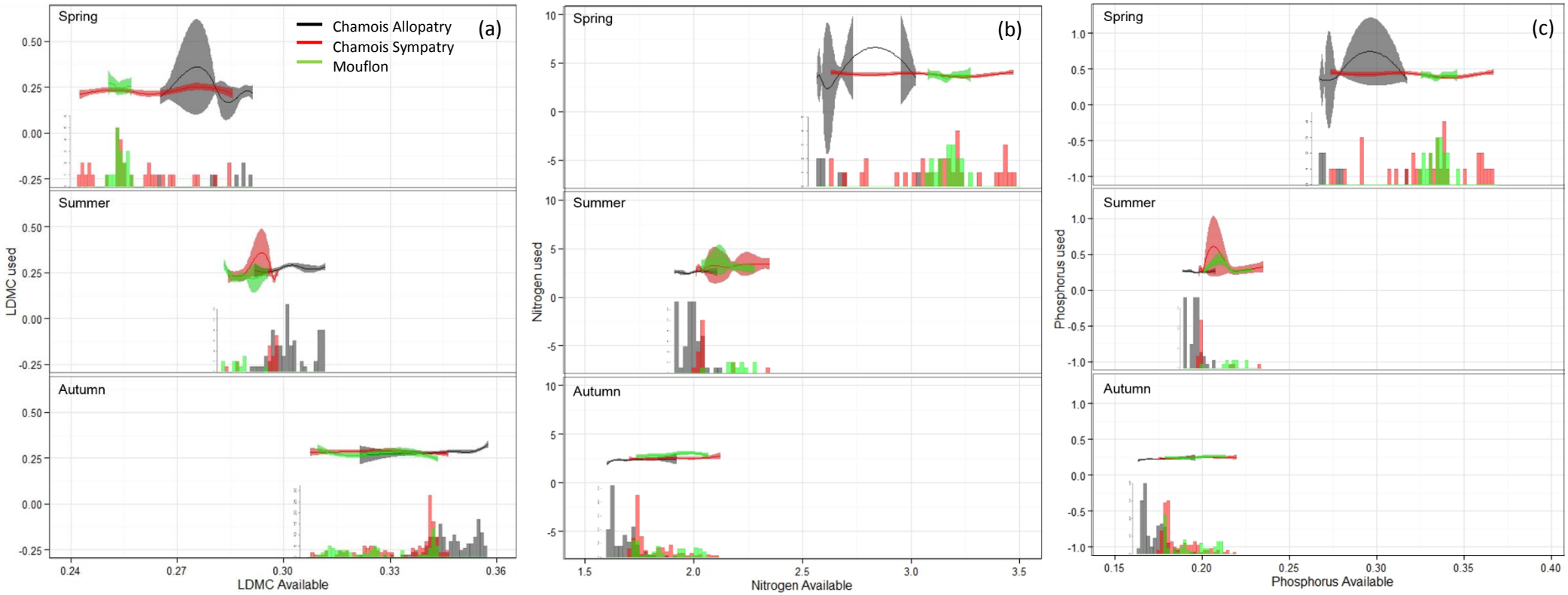
Spring
Summer
Autumn
Chamois Allopatry
Chamois Sympatry
Mouflon
(a)
(b)
(c)
LDMC used
LDMC Available
Nitrogen used
Nitrogen Available
Phosphorus used
Phosphorus Available

**Fig. 3**

## APPENDIX 1

### Calculation of the relative abundance of available plant species within the 48h home-range of each faeces

*Composition of plant communities*

Plant species composition and relative abundance of plant species has been described in July in the sympatric and allopatric pastures sites in the Bauges Game and Wildlife Reserve in 2007 thanks to 149 plots distributed in eight plant communities (lawn of *Sesleria*, Scree, mountain forage, megaphorbiaie, lawn of *Carex ferruginea*, *Alnus*, lawn of *Nardus stricta*, Heathland of *Rhododendron*). The "botanal" method (Tothill et al. 1992, Duparc et al. 2012) has been used in open areas of the pastures to describe the vegetal composition and to estimate biomass of plant species per plot. The "botacub" method ("3D quadrat") (Saïd et al. 2005) has been used in shrubby areas ("*Alnus*") of the pastures to estimate biomass per strata and per plant species. Hence, the relative abundance of available plant species per plant communities, mapped in 2001, has been calculated.

*Estimating the resource available in the 48H home range*

Since our goal was to determine whether plant availability influenced the taxonomic and functional diet, we determined the plant characteristics in the close vicinity of each faeces. Plant found in the faeces are likely summing up several meals depending on the digestibility of plants. We considered (see Rayé et al. 2011, Steuer et al. 2011) that plants eaten during the last 48 hours were most likely to be found in the faeces. Therefore, we estimated plant availability in an area around the faeces which size amounted the average 48 hours home range of chamois and mouflon. The latter was estimated based on GPS-fixed from marked chamois and mouflon.

Given that faeces are not expected to be at the center of the last 48 hours home range, the relative proportion of vegetal community around faeces has been calculated as the mean relative proportion through the bootstrap method with 1000 samplings from 100 buffers randomly located around the faeces GPS location (fig.1). Consequently, each buffer has a centre more or less shifted relatively to the corresponding faeces location. Radiuses of 48h-home range buffers (r) were randomly sampled from the radius value distribution of 48h-home ranges of the summer season.

Then, we estimated the relative proportion of plant species around faeces given the relative proportion of vegetal communities around faeces and the plant proportion within each vegetal community.

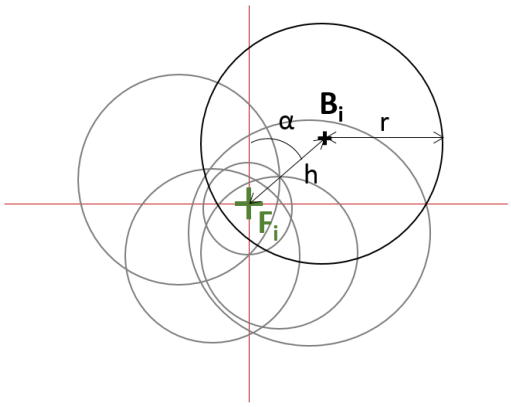


**Fig. 1 –** Visual representation of the method to estimate resource availability at the individual scale. $F_i$ is the localisation of the faeces, $B_i$ is the center of a buffer, with a radius $r$, staggered from a distance $h$ and an angle $\alpha$.

**Paper III.**

Bison M., Ibanez S., Puijalon S., Dehédin A., Langrand E., Redjadj C., Miquel C., Rioux D., Taberlet P., Maillard D., Loison A., Yoccoz N.G.

*The relative contribution of biomechanical and biochemical traits in plant selection by ungulates over the year.*

In prep. for Functional Ecology.

**In preparation, targeted journal:** Functional ecology

# The relative contribution of biomechanical and biochemical traits in plant selection by ungulates over the year

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**ABSTRACT**

1. Functional traits are promising candidates to explain how trophic interaction networks organize themselves. However, the relative importance of correlated functional traits in diet selection criteria has not been investigated, because data is scarce and because of correlations among traits.
2. For this aim, we compiled a unique data base combining (1) the seasonal diet of large co-existing large alpine herbivores (chamois and mouflon) using DNA metabarcoding, (2) the biomass of the plant species available in spring, summer and autumn, and (3) several chemical and biomechanical functional traits of 57 plant species.
3. Our results show that both species have a better diet quality than available, even in autumn when vegetation is senescent. However, correlation among traits obscure this pattern. We therefore used path analyses in order to disentangle the direct and indirect effects of biochemical and biomechanical traits on diet selection. For chamois, the most important characteristic was the toughness whatever the season. For mouflon, instead, there was no selection on the base of the investigated traits in spring, a selection towards nitrogen-rich plants in summer and easily chewed plants in autumn.
4. These contrasting selection criteria among ungulate species can be related to their morphological features (masseter muscles mass, strength of reticulorumen muscle) and can contribute to explain their coexistence. The discovery of diet selection criteria based on traits will also strengthen our ability to predict herbivore effects on the dynamics of plant communities.

**Key-words:** diet selection, seasonal variability, path analysis, DNA metabarcoding, leaf toughness

## INTRODUCTION

The determinants of diet choice and their consequences on resource partitioning between species are central issues in community ecology (Schoener 1974). To which extent spatially co-occurring species from the same trophic level differ in their food choices remains obscure, since many studies investigating the structuring of communities focus on diet taxonomic composition and richness, but not on the factors of diet selection (Beck & Peck 2005, Shreshta et al. 2005, La Morgia & Bassano 2009, Bertolino et al. 2009). Hence, trait-based approaches arises as an encouraging way to better understand trophic interactions (Diaz et al. 2001, McGill et al. 2006, Behmer & Joern 2008, Ibanez et al. 2013a, Green & Côté 2014). Contrary to taxonomic approaches, a functional approach allows to overcome the identity of resources (plant species) and changes in plant community composition. Kartzinel et al. (2015) stress the need to jointly integrate food plants (giving the information of the diet type) with their associated plant traits (giving the information of diet quality) to better understand the primacy of these two factors in herbivores community assemblage, a long-term debate that is carried on. The comprehension of food partitioning among herbivores has been held at the broad scale level, for which herbivores have been classified according to their diet category (Hofman 1989, Isely 1944). For example, the diet of ungulates and grasshoppers were defined according to their proportion of grass and forbs. However, diet composition is more complex than this coarse classification, and ecological and evolutionary interactions between herbivores and food resources happen at the plant species level (Hanley 1997). Moreover, in addition to herbivore physiological and behavioral mechanisms, the quality and quantity of available plants need to be taken into account to understand the food selection and community assemblages of herbivores (Ibanez et al. 2013). Here also, functional approaches are well-suited because taxonomically diverse plant communities can be summarized into their mean trait value (i.e. Community Weighted Mean, Lavorel et al. 2008, Ibanez et al. 2013, Carmona et al. 2014). However, this goal requires to define diets more precisely. The raise of DNA-metabarcoding methods (Taberlet et al. 2007, Pompanon et al. 2012) opens the access to such a precision and to a more accurate description of food choices according to plant functional traits.

Herbivores have to face the paradox of living in a green world without having the morpho-physiological characteristics enabling them to feed on all available plant species (Hofmann 1989, Polis 1999, Clauss 2003). They are consequently exposed to exert a trade-off between nutrient requirements and digestive constraints, in order for them to meet the nutritional

demands required for growth, survival and reproduction (Raubenheimer & Simpson 1993, Hanley 1997). If all plant species were equally profitable or if herbivores were not selective, they should consume plant species proportionally to their abundance in the environment and diet would depend on plant species composition (Bernays & Chapman 1970, Singer & Stireman 2001). However, many herbivores are known to be selective in their food choices and to forage on higher quality plants than the average available vegetation (Waldbauer & Friedman 1991, Prache 1998, Zweifel-Schielly 2012). In the case of ungulates, several studies have tried to demonstrate, experimentally or in the field, the factors influencing the herbivore choices such as for red deer (Forsyth et al. 2005, Lloyd et al. 2010, Zweifel-Schielly et al. 2012), white-tailed deer (Berteaux et al. 1998, Sauvé & Côté 2005, Dostaler et al. 2011), roe deer (Pollock et al. 2007) or fallow deer (Bergvall & Leimar 2005). Often, previous works focused on either chemical or physical plant traits, e.g. protein and carbohydrates (Chapin 1980, Berteaux et al. 1998), silica (McNaughton et al. 1985, Massey 2009), spines and thorns (Cooper & Owen-Smith 1986) or plant secondary compounds (Bergvall & Laimar 2005). In other cases, a higher number of traits were combined, but they were analyzed separately in order to determine which ones were positively or negatively correlated with the plant eaten (Forsyth et al. 2005, Zweifel-Schielly 2012). Plant functional traits measured are generally correlated, which might confound the relationship of an independent variable on the response variable (Ray-Mukherjee et al. 2014). In order to identify the direct and indirect effects of plant functional traits in the process of diet selection, our first objective is to test the validity of causal models relating multiple plant functional traits and herbivore diet using path analysis (Wright 1921).

Alpine environments offer a strong heterogeneity in the spatial distribution of vegetation and a strong seasonality leading to a limited period of time for herbivores to track nutritious forage (Zweifel-Schielly et al. 2012). As the quality and quantity of forage fluctuates over the year (Duncan 2005), the temporal variability in diet selection is an important aspect of ecological interactions (Wam et al. 2010). In addition to habitat changes, herbivores are also constrained by their annual cycles and their concurrent requirements (Kaske & Growth 1997, Parker et al. 2009) during fetus gestation by female, lactation, growth of young or fat storage before winter, and these factors can influence the diet selection criteria. Hence, our second objective is to analyze the temporal variability of trait selection.

As different ungulate species co-occur in mountains environments, they can potentially compete for food resources. Exploring whether diet choices differ between the species can

help to go deeper in the understanding of the alpine ungulate assemblages. Our third objective is to identify the functional niche partitioning of co-occurring ungulates (Behmer & Joern 2008).

To achieve these goals, we studied the diet selection criteria of chamois (*Rupicapra rupicapra*) and mouflon (*Ovis gmelini musimon*) from spring to autumn. These species have to develop efficient strategies of diet selection in poor-quality pastures to fulfill their high nutritional demands caused by a high-metabolic rate as a function of low body mass (Demment & Van Soest 1985, White & Seymour 2003). We focused our analysis on chemical and biomechanical plant traits. Nitrogen content, as a proxy of dietary protein content is directly implied in the animal's growth (Robbins 1983, Parker et al. 2009, Dostaler et al. 2011) and is known to account for selection (Raubenheimer & Simpson 2004, Dostaler et al. 2011). Biomechanical traits have been sparsely used for large herbivore diet selection studies (Wright et al. 1996, Lloyd et al. 2010) but widely studied in resistance to herbivorous insect foraging (Coley 1983, Choong 1996, Perez-Harguindeguy et al. 2003, Clissold 2007, Ibanez et al. 2013b). Indeed, plant toughness plays a major role in the choice as it acts as a deterrent: it increases the metabolic costs of chewing and of digestion to access nutrients (Choong 1996, Clissold 2007). Because of the strong masseter muscle of studied ungulates (Clauss et al. 2008), we hypothesize that the biochemical traits will be more important in the diet selection that biomechanical traits, but this selection could be affected by the ungulate species and the season. The combination of DNA-metabarcoding and plant functional trait measurements on a large number of plant species over three seasons offers the opportunity to identify (1) the direct and indirect effects of plant traits on diet selection, (2) the seasonal variability of plant choices, (3) a species-effect and its consequence on coexistence.

## MATERIAL & METHODS

*Diet composition of chamois and mouflon from faeces*

We studied diet composition of chamois and mouflon populations living in two pastures (Armène and Charbonnet) situated in the Bauges Game and Wildlife Reserve (5,170 ha) with elevation comprised between 1400 and 2200 m. Chamois are usually classified as intermediate feeder whereas mouflon are defined as intermediate feeder/grazer (Hofmann 1989, Clauss et al. 2003, Marchand et al. 2013, Redjajd et al. 2014). Both pastures are similar in terms of environmental conditions: geographically closed, subjected to the same climate and same altitude, and having similar surfaces (Duparc et al. 2012). Chamois are in sympatry with mouflon population in a pasture, however not negatively impacting the chamois diet (papier 2 in prep.). The other pasture is only occupied by chamois. Faeces have been sampled in the two pastures from May to November 2007-2008, either in the field or directly on animal during captures and during hunting season (Bison et al. 2015). We determined diet composition by DNA-metabarcoding (Taberlet et al. 2007, Bison et al. 2015). DNA sequences were obtained and analyzed following Rayé et al. (2011). After having removed plants whose proportions were under 2.5% (Willerslev et al. 2014, Bison et al. 2015), sequences were turned into presence/absence data in order to remove the bias of digestibility. Faeces have been grouped into three seasons: spring, summer and autumn as in Bison et al. (in prep.).

*Plant functional traits*

Using standardized protocols (Cornelissen et al. 2003), four plant functional traits (Leaf Nitrogen Content (LNC), Leaf Dry Matter Content (LDMC), Leaf thickness (LT), Leaf Punch Toughness (LPT, see Ibanez et al. 2013b for device details) have been measured over the three seasons: in spring at the start of the growing season (beginning of June), in summer around the vegetation peak of biomass (mid-July) and in autumn when the vegetation is senescent (mid-September), during the year 2013. The 57 measured plant species made up around 85% of the total cumulated abundance of the diets, and around 85% of the total cumulated abundance of available plants in the pastures.

We calculated for each trait, the “Functional niche” of each faeces. This measure is analogous to the Community Weighted Mean (CWM) metric which is one of the most commonly used

indicators of the functional trait structure of communities reflecting the average value of the dominant species (Lavorel et al. 2008, Carmona et al. 2014).

$$Community\ Weighted\ Mean = \sum_{i=1}^{n} p_i \times trait_i \qquad [1]$$

where $n$ is the total number of plant species, $p_i$ is the relative abundance of species $i$ and $trait_i$ the trait value of species $i$. For diet calculations, $p_i$ is a proportion and is the same for each plant species $i$ in the faeces (if a faeces is composed of 10 plants, then each plant will have a proportion of 1/10).

In order to compare the functional niche with the community weighted mean trait values of the habitat, we estimated the composition of available plant species around each faeces (see appendix 1).

An overview of databases used in the study is presented in fig.1.

*Statistical analyses*

Prior to analyses, continuous traits were log(x+1) transformed to correct for right-skewed distribution. We used the free R statistical software (R Development Core Team 2014).

First, we performed a principal component analysis (PCA) to identify the pattern of covariation among plant functional traits (LNC, LDMC, LPT, LT).

Testing diet selection

In order to test if chamois and mouflon are selective in both pastures, we compared for each trait the functional niche and the habitat CWM using Tukey's honest significant difference (HSD).

Testing causal models

In order to detect the direct and indirect effects of the traits involved in diet selection of chamois and mouflon, and to elucidate their relative importance, we tested for each season, two causal models using path analysis. Each model is representative of two different strategies: (1) a diet selection based on biochemical traits as hypothesized in the introduction, and (2) an alternative hypothesis suggesting a diet selection based on biomechanical traits. As both models are tested for each season, a total of six models are tested (fig.3). We used the d-separation approach, and calculated Fisher's C composite probability statistic for each causal

model, which was tested using the $\chi^2$ probability distribution (Shipley 2002). A causal model was not rejected when its p-value was larger than 0.1 (Shipley 2002). As in Pillar et al. 2013, if both model were valid, we selected the one with the highest p-values for C. We centered and standardized the variables in order to be able to compare the path coefficients (β) across models. Generalized linear mixed models, with faeces and plant species as random factors, were used to determine the path coefficients and the corresponding probability of each causal link.

## RESULTS

*Main plants in the diet and in the field*

*Carex sempervirens* and *Sesleria caerulea* are the two dominant plant species available on the field. Diets of chamois are mainly characterized in frequency of occurrence by *Rosodae*, *Helianthemum nummularium* and *Lotus corniculatus* in spring; and *Lotus corniculatus*, *Geranium sylvaticum*, *Helianthemum nummularium* and *Onobrychis montana* in summer and autumn. Diets of mouflon are mainly characterized by *Rosodae*, *Onobrychis montana* and *Lathyrus* in spring, *Onobrychis montana*, *Lotus corniculatus* and *Lathyrus* in summer, and *Onobrychis montana* and *Apiaceae* in autumn.

*Correlations among plant functional traits*

The first axis of the PCA done on the plant functional traits (LNC, LDMC, LPT, LT) explained 57% of the total variation in spring, 49% in summer and 53% in autumn. LNC was positively correlated with the first axis (scores: 0.78 in spring and autumn, 0.74 in summer), whereas LT (scores: -0.67 in spring, -0.69 in summer and -0.59 in autumn), LPT (scores: -0.86 in spring and autumn, -0.90 in summer) and LDMC (scores: -0.67 in spring, -0.32 in summer and -0.65 in autumn) were negatively correlated. The leaves with low values on the first axis were thick, hard to punch, poor in nitrogen and poor in water.

*Diet selection*

A strong selection for diets with high nitrogen content, low leaf dry matter content and low punch toughness relatively to what is available in the field is observed (fig.2, all TukeyHSD test with a $P < 0.05$) for chamois. The same results are observed for mouflon, except the absence of selection toward LDMC in spring (Tukey HSD with $P=0.99$). Selection for thin leaves is noticeable in spring, but not in summer and autumn (TukeyHSD with $P < 0.01$ in spring, 0.26 in summer and 0.52 in autumn) for chamois. For mouflon, selection for thin leaves is only observed in autumn (TukeyHSD with $P=0.99$ in spring, 0.90 in summer and $<0.01$ in autumn).

*Causal models*

For chamois and for each season, the highest p-value was found for the models involving biomechanical trait as the main direct criteria of diet selection (P = 0.5 in spring, 0.34 in summer and 0.43 in autumn, table 1). Further, the path coefficient for LPT → Diet in this causal model was negative and significant for the three seasons. This indicates that this hypothesized model represents plausible causal relationships. The models involving the direct effects of LDMC and LNC were rejected or presented lower p-values indicating that representations were less plausible. The path models selected for mouflon were different over the three seasons. In spring and autumn, the model taking into account the effect of biomechanical trait had higher p-values than the model including the direct effects of chemical content (table 1). The path coefficient for LPT → Diet in this causal model was negative in spring and autumn, but not significant in spring. In summer the first path model LNC + LDMC → Diet presented the highest p-value (table 1). The path coefficient for LNC → Diet was positive and significant, while the one for LDMC → Diet was not significant.

## DISCUSSION

*Herbivore selectivity*

Chamois are selective ungulates (La Morgia & Bassano 2009, Redjadj et al. 2014) that invest energy in search for plant quality, as the quality of the diets are higher than the average vegetation offers throughout the year even in harsh environments marked by a strong seasonality. Mouflons are also highly selective, which contradicts previous statements defining mouflon as a grazer feeding on unpalatable plant species (Hofmann 1989, Clauss et al. 2003), but which confirms the idea that mouflon can also have diet rich in dicotyledonous species (Marchand et al. 2013). Both ungulate species avoid the two dominant grass species making up the pasture (*Carex sempervirens* and *Sesleria caerulea*) and preferentially feed on subordinate shrubs and leguminous plant species (*Helianthemum nummularium*, *Lotus corniculatus*, *Onobrychis montana*, *Rosa montana*). Our results support the notion that large herbivores living in Alpine conditions focus on forage of high quality relatively to what is available in all seasons.

*The plant traits involved in diet choice*

We found different patterns of trait selection by chamois and mouflon, but for both species biomechanical leaf traits were causally involved at least once in the year. Lloyd et al. (2010) also supported the importance of the biomechanical aspect (tensile strength) of plants in diet selection by sheep, right after specific leaf area, in cafeteria experiments. Plant toughness was also noticed as an important diet selection criteria of sheep in field studies, even though the relationship between punch toughness and sheep selectivity was positive, but not significant, for values below 5 $N.mm^{-1}$ (Cingolani et al. 2004), a threshold not exceeded by our data. This pattern could be explained by high amounts of chemical defenses in the softer leaves of the Argentina flora (Cingolani et al. 2004).

Two processes might explain the predominant role of biomechanical traits over biochemical traits: (1) the biomechanical trait in itself is implied in the food choice, (2) some unmeasured correlated traits are relevant for the choice. Specifically, more attention should be directed towards the plant fiber content (cellulose, hemicellulose, and lignin) which are correlated with punch toughness (Choong et al. 1992, Onoda et al. 2011). Even though fiber content is also correlated with LDMC (Al Haj Khaled et al. 2006, $r = 0.63$, $p<0.05$ for grass species), we

hypothesize that fiber content could have a direct effect on diet selection, in addition to an indirect effect through LDMC. Indeed, fiber content is supported to be a strong diet selection criteria for red deer (Forsyth 2005, Zweifel-Schielly et al. 2012), white-tailed deer (Sauvé & Côté 2005) and eland (Watson & Owen-Smith 2002) as fibers reduce the digestibility of food to ruminants (Van Soest 1994) and makes nutrient access more difficult (Clissold 2007). Further estimations of fiber contents should be realized to disentangle the factors related to the biomechanical trait and how they impact diet selection.

*Temporal and species-specific variability of diet selection criteria*

For chamois, the feeding niche was well predicted by leaf punch toughness throughout the year. This result is contrary to what was expected but is line with other studies on intermediate feeders (Watson & Owen-Smith 2002, Zweifel-Schielly et al. 2012), where diet selection did not indicate a direct significant quest for high content of crude proteins (regarded as nitrogen content) during the growing season. The selection process is slightly different for the mouflon. Indeed, in spring, none of the measured traits are responsible of the diet composition, while LNC was preferred in summer and leaf toughness was avoided in autumn. In spring, when the mean leaf nitrogen content of plants is high in average and variability is low (Robbins 1983), selectiveness is likely to be relatively less important than in other seasons (Weckerly & Kennedy 1992). Still, the most abundant plant species did not dominate the diet, and ungulates selected plants on the basis of the four traits considered (Figure 2). Unmeasured traits might drive the selection criteria, or the too low sample size might prevent any significant pattern for the path Leaf Punch Toughness → Diet in spring. In summer, mouflon would select the plants with high nitrogen content in order to support nutritional demands, particularly for yearling growth (Crête & Huot 1993, Parker et al. 2009, Dostaler et al. 2011), horn development and body mass maintenance (Asleson et al. 1997), but also for lactating females that have to meet the protein demand for milk production (Reese & Robbins 1994) and to replenish their body condition before a new breeding cycle and winter (Crete & Huot 1993, Gerhart et al. 1997). Finally, in autumn, as plant quality decreases, mouflon focused on easily chewed plants.

*Morphology might explain the difference in the species-specific temporal variability of diet selection*

Morphological traits of ungulate might be involved in the differences observed in diet selection criteria. Mouflon have more muscular rumen and stronger masseter muscles than chamois (Clauss et al. 2008). Given these features, mouflon would have a higher ability to overcome the forage resistance both orally (mouth) and internally (rumen). Hence, in summer, when plants are still easy to chew or digest for the mouflon, they prefer to focus on the nitrogen content than on the leaf toughness. On the contrary, as masseter muscle mass of chamois are twice lower than mouflon (Clauss et al. 2008), we hypothesize that the work required to chew or digest is the most important criteria for the choice over the year. For example, leaf punch toughness and nitrogen content are lower for *Helianthemum nummularium* (preferentially selected by chamois) than *Onobrychis montana* (preferentially selected by mouflon). Each of these plants build up a large proportion of chamois and mouflon diets respectively and play a role in taxonomic diet differentiation, especially in summer and autumn (Bison et al. in prep).

Even if our results showed significant effects of some plant traits on diet selection (leaf toughness and nitrogen content), we hypothesize that the absence of significant relationships for other traits could be related to (1) the absence of effect for all the individuals, or (2) a strong inter-individual variation hiding a general pattern at the species level (Bison et al. 2015). Indeed, path analyses took into account the inter-individual variability but did not quantitatively measure it. Additional analysis on functional β-diversity (Swenson et al. 2010, Meynard et al. 2011, Villéger et al. 2013) could give insights about the among-individual variation in diet functional niches. This could help to determine if some individuals benefit from a higher resource quality intake over others, which could in turn have consequences on population dynamics.

*Niche partitioning*

Inter-specific interactions study on chamois and mouflon (Bison et al. in prep.) showed that ungulate species were able to partly partition resources during the main part of the year, and even in autumn when the quality of the vegetation began to decrease. We propose that this partial taxonomic niche partitioning can partly by explained by differential diet selection criteria, as suggested for grasshoppers niche partitioning (Ibanez et al. 2013). In autumn, as the selection of both herbivore species is based on plant toughness while the diets do not

largely overlap (paper 2), we hypothesize that (1) herbivores exploit different range of values depending on their needs (Behmer & Joern 2008), or (2) additional plant functional traits not included in our analysis are be involved in the food selection. For example, other components and characteristics, such as secondary compounds like anti-feedant condensed tannins (Bergvall & Leimar 2005, Sauvé & Coté 2007), toxins, micronutrients such as minerals (Watson & Owen-Smith 2002, Joern et al. 2012) or vitamins of plants (Robbins 1983) could exert significant additional pressure on diet selection and especially explain some isolated cases of ingested plants that do not match the general pattern of selection.

*Conclusion*

Previous studies were unable to discriminate the direct and indirect effects of multiple planr functional traits on diet selection by herbivores, because of correlations among them. In three seasons for chamois, and in autumn for mouflon, our results demonstrate that biomechanical traits have a direct effect on diet selection whereas chemical traits have an indirect effect through their correlation with leaf toughness. In spring, mouflon are less selective with regard to traits, and in summer they choose leaves with a high nitrogen content, which is consistent with the phenology of plant traits and with the morpho-physiological characteristics of mouflon. This functional niche partitioning explains for example the preference of chamois for *Helianthemum nummularium* and the preference of *Onobrychis montana* by mouflon, and this could contribute to the coexistence between both species.

Our results could be included in the models predicting the effects of herbivores on the structure of plant communities (McGill et al. 2006). Up to now, the models predicting vegetation dynamics include climate and land use variables (Boulangeat et al. 2014). The traits preferred by large wild herbivores could also be incorporated in the models as herbivores are known to impact strongly the vegetation in some places (Olff & Ritchie 1998, Wardle et al. 2001, Garibaldi et al. 2007). Moreover, in a community where different herbivore species coexist, their effects on vegetal communities could be complex if the plant functional trait preferences vary across ungulates (Lloyd et al. 2010).

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**Table 1** Fisher's C statistic value, degrees of freedom (d.f.) and p-value (P) associated to both tested causal models within the three seasons and for both species

| | | **Chamois** | | **Mouflon** | |
|---|---|---|---|---|---|
| | | **Chemical causal model** | **Biomechanical causal model** | **Chemical causal model** | **Biomechanical causal model** |
| **Spring** | Fisher's C | 8.21 | 5.34 | 5.77 | 4.61 |
| | d.f. | 4 | 6 | 4 | 6 |
| | P | 0.084 | 0.5 | 0.22 | 0.6 |
| **Summer** | Fisher's C | 9.44 | 6.83 | 2.23 | 8.12 |
| | d.f. | 4 | 6 | 4 | 6 |
| | P | 0.05 | 0.34 | 0.69 | 0.23 |
| **Autumn** | Fisher's C | 9.67 | 5.90 | 3.90 | 5.29 |
| | d.f. | 4 | 6 | 4 | 6 |
| | P | 0.046 | 0.43 | 0.42 | 0.51 |

**Figure 1** Overview of the databases used and questions asked. The Diet Weighted Mean (DWM) and Community Weighted Mean (CWM) are calculated from the equation: $CWM = \sum_{i=1}^{n} p_i \times trait_i$, where *n* is the total number of plant species in the diet or habitat, $p_i$ is a proportion and is the same for each plant species *i* in the faeces (if a faeces is composed on 10 plants, then each plant will have a proportion of 1/10),or $p_i$ is the relative abundance of plant species *i* in the habitat and $trait_i$ the trait value of plant species *i*.

**Figure 2** Relationships between functional niche and community weighted mean for the four traits considered across the three seasons (spring, summer, autumn). Letter "C" and "M" correspond respectively to chamois and mouflon

**Figure 3** Causal models representative of two different strategies. Diet is a binomial variable (0/1), all other variables are continuous. Solid lines represent the relationship between two variables, the one at the beginning of the arrow is the independent variable and the one at the end of the arrow is the dependant variable. Dotted lines symbolize correlations. In bold are the significant relationships. For the sake of simplicity, significant correlations are not in bold

**Figure 4** Plausible causal models for the three seasons (spring, summer, autumn) and the two species (chamois and mouflon)

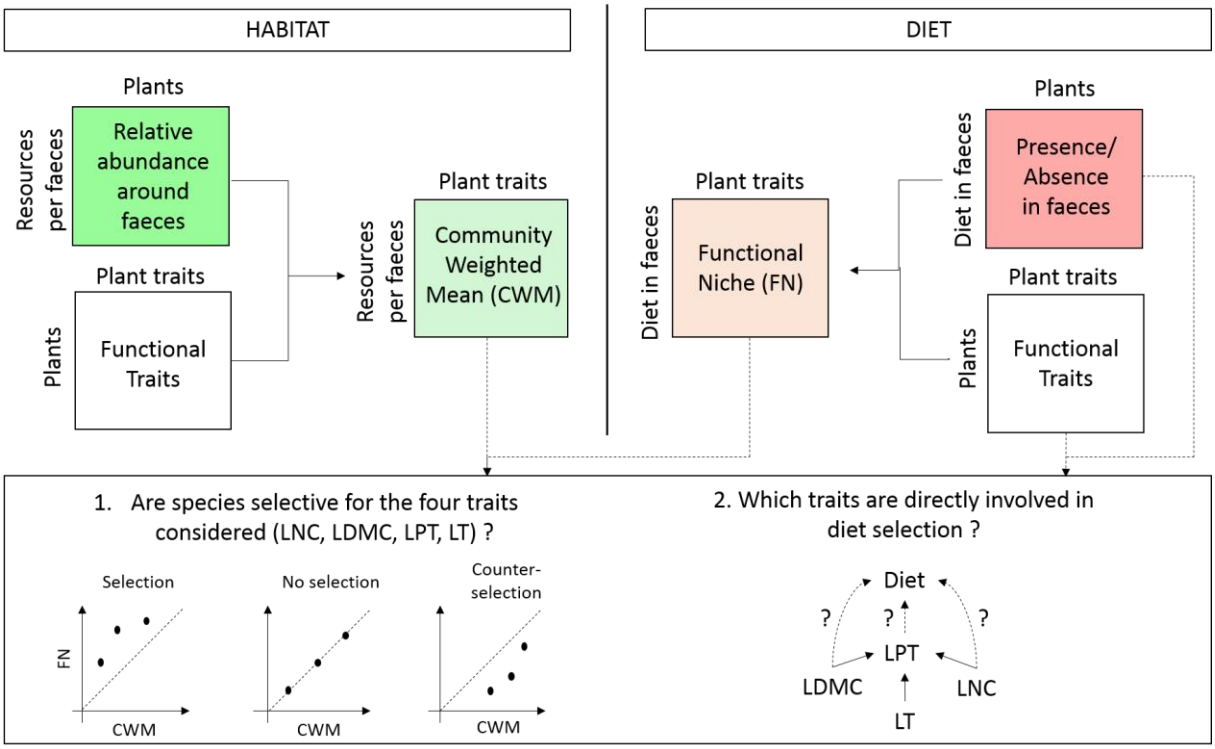

HABITAT
DIET
Plants
Resources per faeces
Relative abundance around faeces
Plant traits
Plants
Functional Traits
Resources per faeces
Plant traits
Community Weighted Mean (CWM)
Plant traits
Diet in faeces
Functional Niche (FN)
Plants
Diet in faeces
Presence/ Absence in faeces
Plant traits
Plants
Functional Traits
1. Are species selective for the four traits considered (LNC, LDMC, LPT, LT) ?
Selection
No selection
Counter-selection
FN
CWM
CWM
CWM
2. Which traits are directly involved in diet selection ?
Diet
?
?
?
LPT
LDMC
LNC
LT

**Fig. 1**

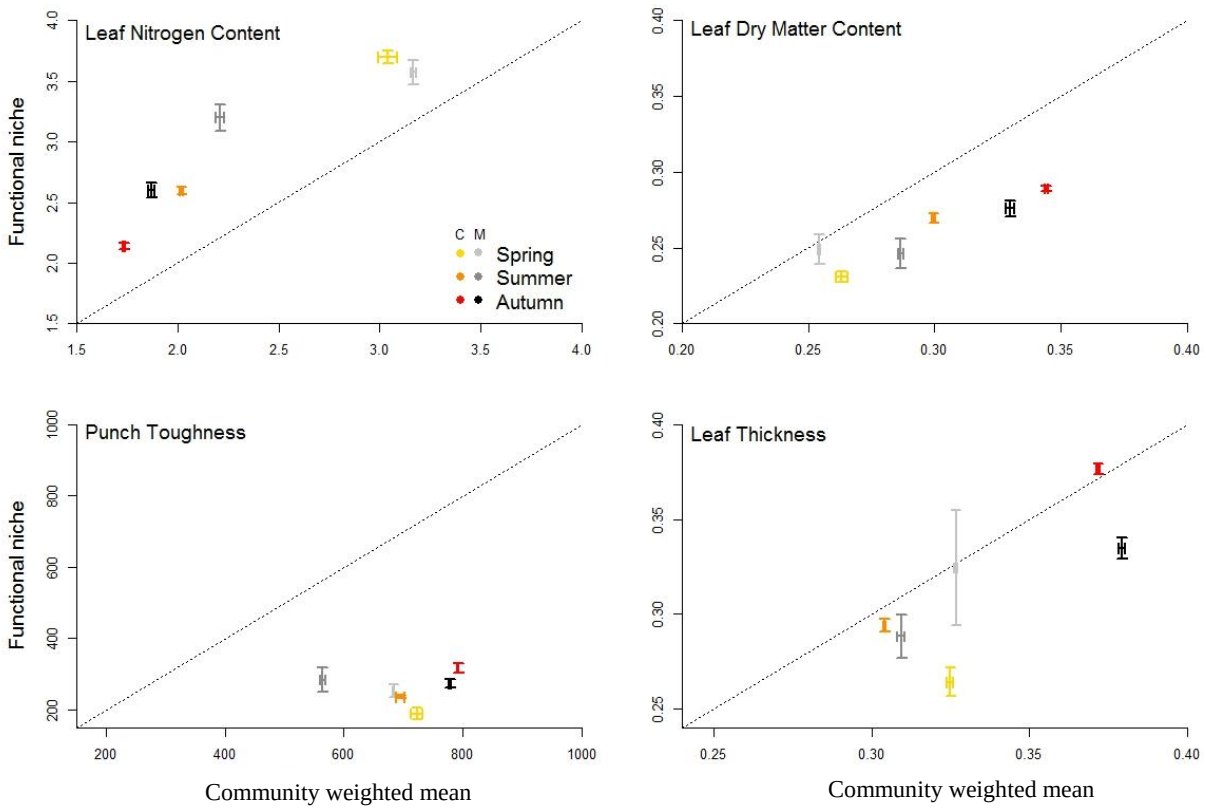
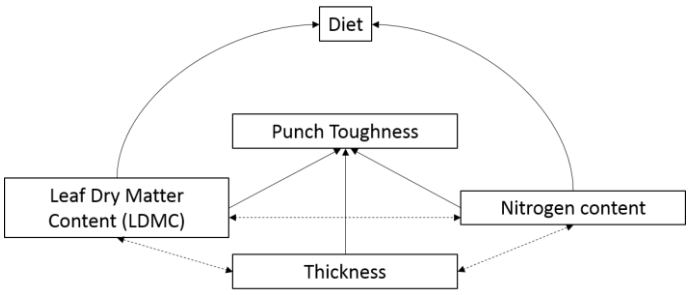

Leaf Nitrogen Content
Leaf Dry Matter Content
Punch Toughness
Leaf Thickness
Functional niche
Functional niche
C M
Spring
Summer
Autumn
Community weighted mean
Community weighted mean

**Fig. 2**

(a) Biochemical causal model




(b) Biomechanical causal model

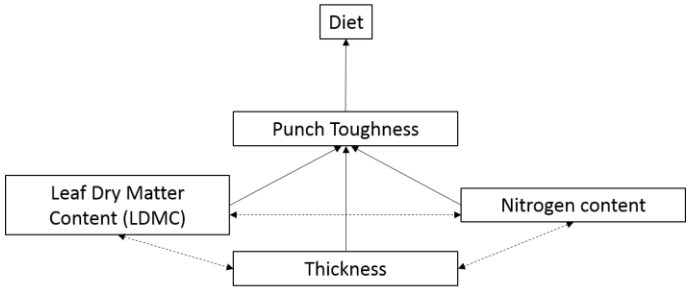



**Fig. 3**

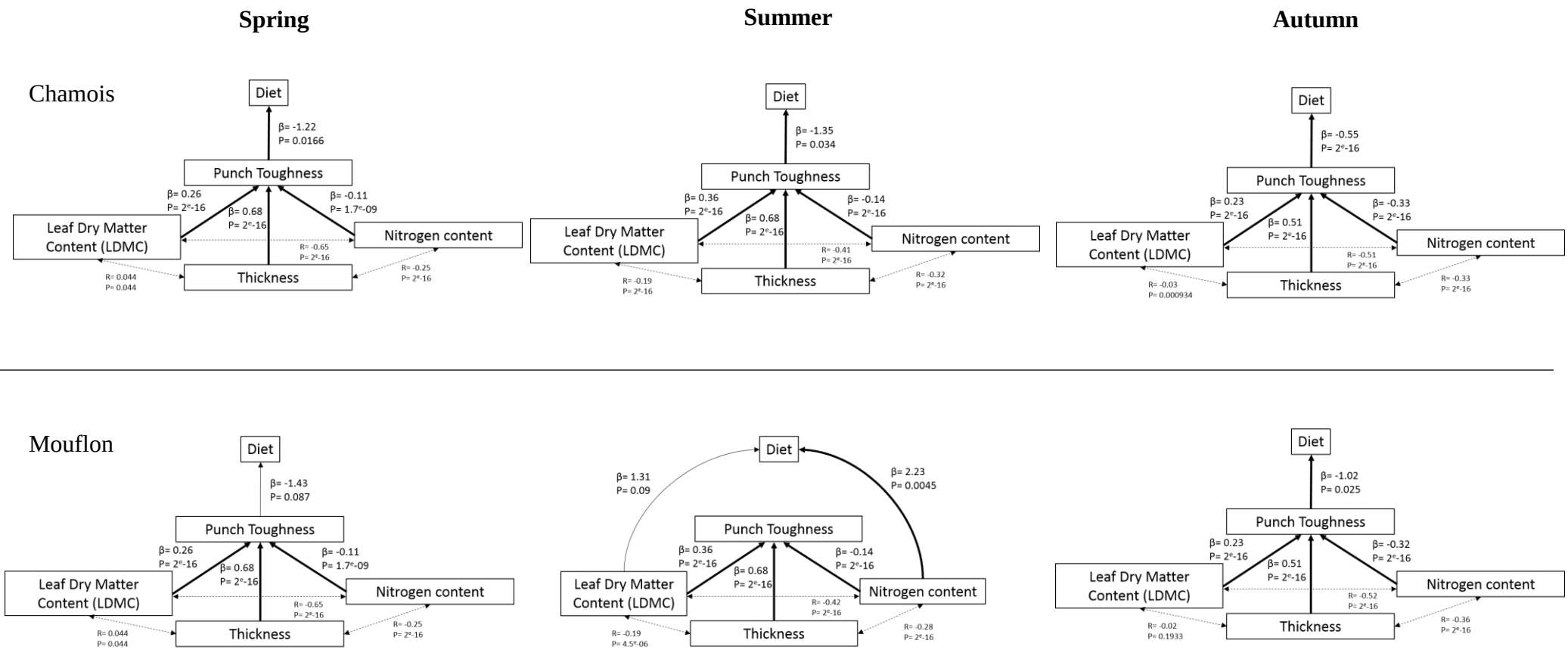

Spring
Summer
Autumn
Chamois
Diet
β= -1.22
P= 0.0166
Punch Toughness
β= 0.26
P= 2e-16
β= 0.68
P= 2e-16
β= -0.11
P= 1.7e-09
Leaf Dry Matter Content (LDMC)
Nitrogen content
R= -0.65
P= 2e-16
R= 0.044
P= 0.044
Thickness
R= -0.25
P= 2e-16
Diet
β= -1.35
P= 0.034
Punch Toughness
β= 0.36
P= 2e-16
β= 0.68
P= 2e-16
β= -0.14
P= 2e-16
Leaf Dry Matter Content (LDMC)
Nitrogen content
R= -0.41
P= 2e-16
R= -0.19
P= 2e-16
Thickness
R= -0.32
P= 2e-16
Diet
β= -0.55
P= 2e-16
Punch Toughness
β= 0.23
P= 2e-16
β= 0.51
P= 2e-16
β= -0.33
P= 2e-16
Leaf Dry Matter Content (LDMC)
Nitrogen content
R= -0.51
P= 2e-16
R= -0.03
P= 0.000934
Thickness
R= -0.33
P= 2e-16
Mouflon
Diet
β= -1.43
P= 0.087
Punch Toughness
β= 0.26
P= 2e-16
β= 0.68
P= 2e-16
β= -0.11
P= 1.7e-09
Leaf Dry Matter Content (LDMC)
Nitrogen content
R= -0.65
P= 2e-16
R= 0.044
P= 0.044
Thickness
R= -0.25
P= 2e-16
Diet
β= 1.31
P= 0.09
β= 2.23
P= 0.0045
Punch Toughness
β= 0.36
P= 2e-16
β= 0.68
P= 2e-16
β= -0.14
P= 2e-16
Leaf Dry Matter Content (LDMC)
Nitrogen content
R= -0.42
P= 2e-16
R= -0.19
P= 4.5e-06
Thickness
R= -0.28
P= 2e-16
Diet
β= -1.02
P= 0.025
Punch Toughness
β= 0.23
P= 2e-16
β= 0.51
P= 2e-16
β= -0.32
P= 2e-16
Leaf Dry Matter Content (LDMC)
Nitrogen content
R= -0.52
P= 2e-16
R= -0.02
P= 0.1933
Thickness
R= -0.36
P= 2e-16

**Fig. 4**

## APPENDIX 1

### Calculation of the relative abundance of available plant species within the 48h home-range of each faeces

*Composition of plant communities*

Plant species composition and relative abundance of plant species has been described in July in the sympatric and allopatric pastures sites in the Bauges Game and Wildlife Reserve in 2007 thanks to 149 plots distributed in eight plant communities (lawn of *Sesleria*, Scree, mountain forage, megaphorbiaie, lawn of *Carex ferruginea*, *Alnus*, lawn of *Nardus stricta*, Heathland of *Rhododendron*). The "botanal" method (Tothill et al. 1992, Duparc et al. 2012) has been used in open areas of the pastures to describe the vegetal composition and to estimate biomass of plant species per plot. The "botacub" method ("3D quadrat") (Saïd et al. 2005) has been used in shrubby areas ("*Alnus*") of the pastures to estimate biomass per strata and per plant species. Hence, the relative abundance of available plant species per plant communities, mapped in 2001, has been calculated.

*Estimating the resource available in the 48H home range*

Since our goal was to identify selection criteria at a fine scale, we determined the plant characteristics in the close vicinity of each faeces. Plant found in the faeces are likely summing up several meals depending on the digestibility of plants. We considered (see Rayé et al. 2011, Steuer et al. 2011) that plants eaten during the last 48 hours were most likely to be found in the faeces. Therefore, we estimated plant availability in an area around the faeces which size amounted the average 48 hours home range of chamois and mouflon. The latter was estimated based on GPS-fixed from marked chamois and mouflon.

Given that faeces are not expected to be at the center of the last 48 hours home range, the relative proportion of vegetal community around faeces has been calculated as the mean relative proportion through the bootstrap method with 1000 samplings from 100 buffers randomly located around the faeces GPS location (fig.1). Consequently, each buffer has a centre more or less shifted relatively to the corresponding faeces location. Radiuses of 48h-home range buffers (r) were randomly sampled from the radius value distribution of 48h-home ranges of the summer season (fig.1).

Then, we estimated the relative proportion of plant species around faeces given the relative proportion of vegetal communities around faeces and the plant proportion within each vegetal community.

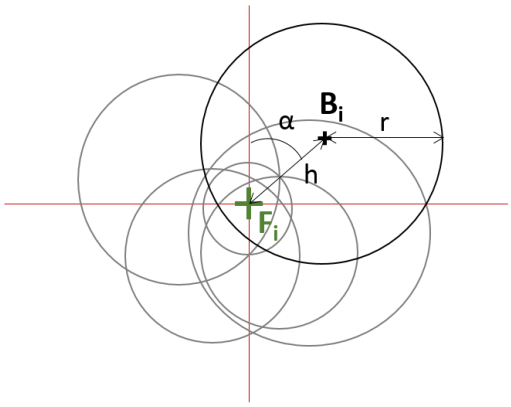



**Fig. 1 –** Visual representation of the method to estimate resource availability at the individual scale. $F_i$ is the localisation of the faeces, $B_i$ is the center of a buffer, with a radius $r$, staggered from a distance $h$ and an angle $\alpha$.

**Note.**

Bison M., Redjadj C., Saïd S., Miquel C., Rioux D., Taberlet P., Maillard D., Yoccoz N.G., Loison A.

*Comparison between two methods for estimating diet quality in large herbivores: NIRS-derived fecal nitrogen vs dietary nitrogen from ingested plants.*

# Comparison between two methods for estimating diet quality in large herbivores: NIRS-derived fecal nitrogen vs dietary nitrogen from ingested plants

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**ABSTRACT**

The estimation of diet quality is a useful tool to study the status of wildlife populations. Fecal nitrogen (FN) content has been widely used a proxy of dietary nitrogen (DN). However, the reliability and accuracy of FN as a good estimator of DN has been questioned, mainly because of the effect of secondary compounds on the nitrogen release in the faeces. Our objective is to determine to which extent and at which temporal scale we can rely on FN to estimate DN in two ungulate species (chamois and mouflon). We also tested whether the comparison of FN between species is feasible. Finally, we analyzed whether the feeding type could explain the different slopes relating FN and DN for different species by reviewing the relationships found in the literature on ungulates. Our results show that the equations relating FN and DN of chamois and mouflons are not predictive enough to quantitatively study the variation of diet quality from fecal indices in our study area at the intra- and inter-seasonal level. Also, the use of FN values is not suitable to compare the diet quality of ungulates differing in their diet and morpho-physiology. Finally, the feeding type cannot explain the different relationships observed between FN and DN for different ungulate species, and we suggest that other parameters should be included to explain the slope variations. To conclude, we advise to use FN estimations to predict diet quality in qualitative way at a large time scale and for a specific species and location, as the diet composition and secondary associated compounds could influence the FN release in a significant manner.

**Key-words:** Fecal nitrogen, diet quality, ungulates, feeding type, DNA-metabarcoding

## INTRODUCTION

The quality of the diet allows to describe and understand the status of wildlife populations (Leslie et al. 2008) as it's an important determinant of animal body condition (White 1983). In order to maintain a population positive growth rate, individuals have to reach their nutritional demands for growth (Christianson and Creel 2009), reproduction and survival. When the access of high quality vegetation decreases, for example when competition modifies the diet of a targeted species or when environmental changes impact the composition of plant species communities, the energy and protein intakes can be reduced, which influences physical condition of animals and in turn, population dynamics through fitness-related traits (Mysterud et al. 2001). Consequently, in order to complete the population indices for monitoring; such as indicators of populations abundance, quality and performance of individuals in the population, habitat use and habitat impact (Morellet et al. 2007); determining in a reliable and precise way the quality of diets could be useful to predict how animal respond to their environment (Blanchard et al. 2003). It could also bring complementary information, for example, to determine the relative importance of diet quality on body size variation relatively to other factors such as density-dependance (Mysterud et al. 2001, Bonenfant et al. 2009), level of stress (cortisol concentration), sex (Mysterud et al. 2001), age (Mysterud et al. 2001) or abiotic components (temperature, Douhard et al. 2013).

The determination of diet quality requires a huge amount of work as the diet of animals and the plant quality should be precisely defined. Hence, quality of diets is usually determined by the fecal nitrogen content (FN) to evaluate the diet protein intake (Mould et al. 1981, Leslie & Starkey 1985, Nunez-Hernandez et al. 1992, Irwin et al. 1993, Ueno et al. 2007, Redjadj et al. 2014), by the fecal 2,6-diaminopimelic acid (FDAPA) to control the diet energetic content (Hodgman 1996, Osborn & Ginnett 2001), and more rarely by phosphorus (Leslie et al. 1985, Wrench et al. 1997) and fiber content (Jhala et al. 1997, Wrench et al. 1997, Osborn & Ginnett 2001). These contents can be easily determined with classical chemical methods applied on faeces or with the NIRS method.

Authors agree to support the fact that nitrogen found in faeces is actually the remained nitrogen from the diet not absorbed by the animal, added to the undigested microbial N and endogenous N (Robbins 1983). Despite these multiple fecal nitrogen origins, many scientists consider fecal nitrogen content as a good indicator of the quality of ingested plant species (Leslie and Starkey 1985, Caughley and Sinclair 2002). If correctly used (Leslie and Starkey 1985, Hobbs 1987) – and it has not been the case in all studies (Leslie et al. 2008) –, it allows

to explore the diet quality variation in time (seasonal or annual variations, Osborn & Jenks 1998, Redjadj et al. 2014), in space (habitat, Osborn & Jenks 1998), according to biotic environnement (interspecific and intraspecific interactions, density) (Asada et al. 1999, Blanchard et al. 2003, Hernandez et al. 2005) and animal characteristics (sex, status of female as lactating or non-lactating, Blanchard et al. 2003, Monteith et al. 2014). However, some others authors are wondering about the reliability and accuracy of this estimator and advice to not use it in ecological studies (Robbins 1983, Robbins et al. 1987, Hobbs 1987, Servello et al. 2005). Their reluctance comes from the fact that phenolic compounds such as tannins can make the fecal content misevaluated and not reliable (Robbins et al. 1987). Tannins occur in many dicotyledonous species and bind with proteins during the chewing and digestive process (Verheyden et al. 2011). As the new complex tannin-protein are not digestible at rumen pH, they cannot be assimilated and directly go into the faeces, which in turn increases the fecal nitrogen content (Osborn & Ginnett 2001), with a greater effect in animals having a nitrogen-rich diet (Osborn & Ginnett 2001). Moreover, Hobbs (1987) support the fact that it is only possible to differentiate the different level of nitrogen content in the diet when the fecal nitrogen content have a large range of values. Indeed, while reanalyzing the data of Leslie et al. (1985) where DN depend significantly on FN at the inter-seasonal level, no more significant relationships are observed at the intra-seasonal level (Hobbs 1987).

As previously stated, the use of fecal nitrogen can be useful tool to predict diet quality. However, as the relationships between dietary (DN) and fecal nitrogen (FN) is specific to a species and to a location (Hobbs 1987), we need to know to which extent we can rely on the FN data to predict DN for our specific study site. Hence, our first goal is to question again the predictive ability of FN measurement to estimate DN, and at which temporal scale the estimation can be reliable. Hence, we analyzed this relationship at the inter- and intra-seasonal levels for two species, chamois and mouflon, for which it has never been tested. Secondly, as approached by Wrench et al. (1997), we tried to evaluate to which extent the inclusion of other fecal indices (lignine, hemicellulosis, cellulosis) to determine DN is useful.

Finally, it has been hypothesized that the slope value of the relationship between DN and FN would depend on the feeding type of the animal (Arman et al. 1975, Leslie et al. 1985, Wrench et al. 1997). Browsers are supposed to feed on plants rich in tannins which would increase the FN, unlike grazers that are supposed to have a diet rich in grass and low-tannin concentrated (Hanley et al. 1992). Moreover, as the digestive tract of grazer is longer than the one of browser, the turn over digesta would be slower and they would experience lower levels of fecal nitrogen loss than smaller animals (Demment and Van Soest 1985). Here we are

interested in testing this hypothesis stating that the slope of the relationships between DN and FN is higher for grazers than browsers, with intermediate feeders at an intermediate slope (Arman et al. 1975, Leslie et al. 1985, Wrench et al. 1997). As chamois and mouflon differ morpho-physiologically (chamois as intermediate feeder, mouflon as intermediate feeder/grazer, Hofmann 1989, Clauss et al. 2003, Redjadj et al. 2014), they could experience different strength of slope's relationships between DN and FN. In order to include our results in a more general framework, we reviewed the papers that clearly describe the relationship between DN and FN obtained with field and experimental data and compared the slopes to test the hypothesis. We focused our analysis on ruminant species, as this taxon offers a wide gradient of digestive capabilities and as the question of their diet quality has been widely studied (Leslie et al. 2008).

## MATERIAL AND METHODS

*Estimating fecal indices and dietary nitrogen of chamois and mouflon*

Chamois and mouflon faeces have been sampled in the Bauges Massif during the years 2007 and 2008 on two pastures (see Bison et al. 2015 for details on faeces sampling and study site, see table 2 for sample size). They have been dried at 60°C for 72h, grinded and analyzed with near-infrared spectroscopy (NIRS) to estimate the fecal nitrogen (N), hemicellulosis (neutral detergent fiber, NDF), cellulosis (acid detergent fiber, ADF) and lignin (acid detergent lignin, ADL) content. In order to calibrate the NIRS, fecal chemical composition have been measured previously with the Kjeldahl method for nitrogen and Van Soest method for fiber (Redjadj 2010).

Concurrently with fecal indice measurements, DNA-metabarcoding realized on faeces (see Bison et al. 2015) gave access to the relative proportion of plants in the diet. Leaf nitrogen content estimations on the main ingested plants in spring, summer and autumn 2013 have been weighted by the relative proportion of plants in the diet to obtain the dietary nitrogen content.

*Review of the published articles relating DN and FN*

We collected the published articles relating DN and FN that were cited in the review of Leslie et al. (2008). Some older papers (Arman et al. 1975, Mould et al. 1981) were included in the list (table 2). One paper published after 2008 was found (Verheyden et al. 2011) but was not included as no equation relating DN and FN were given. We included our results from wild chamois and mouflon populations but not hold them in the statistical analysis. We get the linear equations that were significant and specified in each case for which ruminant species they were obtained, the associated feeding type and if the values came from experiment or field (table 2). We did not include the relationships from Leslie & Starkey (1985) recalculated by Hobbs (1987) that were not significant.

*Statistical analyses*

Statistical analyses were performed with the R software (R Development Core Team, 2011). First, in order to test the relationship between DN and FN at the inter- and intra-seasonal level, we built four linear models for each ungulate species: one global model including the values of the three seasons and one per season. Each model was defined with DN as

dependent variable and FN as independent variable (DN ~ FN). For significant models, we tested for slope differences between the two species. We used DN as dependent variable, and the interaction between FN and species as independent variables (DN ~ FN*species).
Secondly, in order to test to which extent other fecal indices (lignin and fiber content) improve the estimation of DN, we built two full models (one for each species) including DN as dependent variable, and FN, ADL and NDF as additive independent variables (DN ~ FN+ADL+NDF). We did not include ADF, as this variable is strongly correlated with ADL and NDF (respectively r=0.78 and 0.83). Then, the best models ($\Delta$AIC<2 from the best model) were averaged ("full" averaged). This has only been performed for the global model (all seasons mixed).
Thirdly, in order to test the hypothesis that slopes differ according to the feeding type, we focused on the field data and realized a t-test to compare the slopes of the grazers and intermediate feeders (no field data for browsers). We realized an ANOVA to compare the slopes of three feeding type obtained from experiments.

## RESULTS

*Simple regression equations to predict dietary nitrogen from fecal nitrogen at the inter- and intra-seasonal level*

When taking into account the three seasons, 38% and 14%, respectively for chamois and mouflon, of the variation in dietary nitrogen content could be explained by fecal nitrogen content (table 2, $DN_{chamois}=0.07+0.81*FN_{chamois}$ ; $DN_{mouflon}=1.92+0.4*FN_{mouflon}$).

None of intra-seasonal relationships were significant (table 2).

Slopes of chamois and mouflon of global models significantly differed (interaction between FN and species, $F_{1,176} = 6.35$, $P = 0.01$, fig. 1), with a higher slope for chamois. For the chamois population, FN is significantly higher than DN in summer and autumn ($p<0.05$). For the mouflon, FN is significantly lower than DN in spring ($p<0.05$). No differences are observed during the other seasons.

*Multiple regression to predict dietary nitrogen*

The inclusion of other fecal indices (NDF and ADL) improved the proportion of the variation of dietary nitrogen explained by +7% for chamois and +12% for mouflon. NDF in chamois and ADL in mouflon are both significantly negatively related to the dietary nitrogen content.

*Test for slope differences between different feeding types*

The t-test comparing the slopes of the relationships relating DN and FN of grazers and intermediate feeders, obtained from field data, do not reveal any significant differences between the two feeding types ($F_{1,8} = 0.93$, $P = 0.36$). No significant differences were neither observed between grazer, browser and intermediate feeder from experimental trial ($F_{2,15} = 0.12$, $P = 0.89$).

## DISCUSSION

*DN and FN relationships of chamois and mouflon*

We found seasonal variation in the fecal nitrogen content of chamois and mouflon, with higher concentrations in spring during the growth period of plants decreasing throughout the year (Verheyden et al. 2011).

Contrary to the previous studies listed below (table 1), fecal nitrogen content cannot be used to precisely predict the dietary nitrogen content over the year (38% and 14% of variation of DN explained by FN for chamois and mouflon respectively) although the relationship is significant. Even if fiber (NDF) for chamois and lignin (ADL) content for mouflon are helpful to better estimate DN, the multiple regressions proposed here are also insufficiently accurate to correctly estimate diet quality, for both species. At the intra-seasonal level, the relationships are not significant anymore (table 1). Our results are in line with the statement of Hobbs (1987), Verheyden et al. (2011) for browsers and Massey et al. (1994) who are not confident with the use of FN to evaluate diet quality. Indeed, a given value of FN is associated to a large variation of DN, which precludes any satisfactory prediction. For example, based on our data, a fecal sample from chamois in autumn equal to 3.43% may result from diets ranging from 2.67 to 3.64% N. Hence, it is only possible to detect DN variation when FN content are spread along a large range of values (Hobbs 1987). However, as the large range of values is obtained from different seasons in our case, it is also possible that FN would be biased by confounding effects of diet and plant chemical composition. For example, if no changes are seen in the diet composition between seasons, but the secondary compounds of the plants such as tannins are increasing throughout the year (Frutos et al. 2004), the loss of fecal nitrogen could be overestimated because of the role of tannins in protein digestion. Consequently a high level of FN in autumn could be due to a higher tannin concentration rather than a diet with high nitrogen level. Besides, this statement has been specified by Hobbs (1987) and Leslie and Starkey (1987) in their cautions on how to viably use FN index, i.e. to evaluate “relative changes in inter-seasonal diet quality of a single population, assuming no radical changes in the consumption of secondary compounds”. FN could only be used to qualitatively describe the large tendencies of diet quality variation (Hobbs 1987).

As slopes and intercepts of the relationships between DN and FN are significantly different between both species, comparison of diet quality estimated with FN should be excluded. Moreover, chamois and mouflon display different ways of excluding nitrogen. Indeed, when significant differences are observed, FN is higher than DN for chamois and lower than DN for

mouflon. For a same value of dietary nitrogen content, chamois is more prone to release a higher nitrogen concentration than what it has been ingested than mouflon. As stated above, tannins are indirectly implied in the increase of loss nitrogen and in turn in the increase of fecal nitrogen content. It has been shown that intermediate feeder and browser produce tannin-binding salivary proteins (TBSPs) with higher affinity to tannins than grazers (Clauss et al. 2005). Consequently, chamois should be more efficient to cope with tannins, and loose less nitrogen than mouflon. However, Shimada et al. (2006) reviewed that TBSPs were also present in the salivary of mouflon *Ovis orientalis*, suggesting that our mouflon *Ovis gmelini musimon* may also hold them, and be able to deal with tannins. In our study area, in autumn, chamois largely focus its diet on *Helianthemum nummularium*, an evergreen shrub, whereas mouflon feed on a large proportion of *Onobrychis montana*, a forb. Those shrub and forb are both supposed to be moderately rich in tannins (Barroso et al. 2001, Min et al. 2003). Therefore, chamois and mouflon should be both able to overcome a tannic diet. Different explanations could be at play to interpret the differences observed. First, as diets of chamois and mouflon differ (paper 2), the complementary plants included in the diets of chamois could be richer in tannins than the mouflon diet which may lead to a larger release of undigested proteins by the chamois. Secondly, Clauss et al. (2005) reported the ability of an ungulate (Black rhinoceros) to induce TBSPs production in response of tannin supplementation in the diet. We could conceive that our mouflon individuals that have been introduced in the pasture in 1950's have also been able to induce TBSPs to adapt to their new environment, allowing them to lose less nitrogen. Thirdly, the microbial rumen community could differ between both species, and produce more nitrogen in the chamois individuals that we would find in the faeces. Finally, as the intake energy content (Brown et al. 1995 for white-tailed deer) and especially the digestible carbohydrates (Hawlena et al. 2010 for grasshoppers) may influences the fecal nitrogen content, the diet composition difference between chamois and mouflon associated with different levels of carbohydrates could explain the differences in fecal nitrogen content for a same dietary nitrogen content.

Nevertheless, the main shortcoming of our results is that our method to estimate ingested nitrogen from diet composition obtained from barcoding, combined with plant nitrogen, is not yet validated as a reliable method to estimate true nitrogen intake. Experimental trials controlling the initial quality or resource ingested should be used to determine the factors and the methodological bias affecting the diet quality measurements.

*Effect of feeding type on slope values*

Our result are not in agreement with hypothesis stated in the introduction postulating that the slope of the relationship between DN and FN would be smaller for browsers than for grazers with intermediate feeders at intermediate position. Indeed, chamois (intermediate feeder) show a higher slope than mouflon (intermediate feeder/grazer). As the slopes comparisons extracted from previous studies do not show any significant differences between feeding types with experimental or field data, we do not support the hypothesis. The low effective of studies in each feeding type could explain the absence of significant differences. As experimental and field data do not follow the same trend, other factors than only the feeding type should be taken into account to explain the slopes differences between species. A larger experiment including contrasted feeding types for which the proposed diet should be rigorously and clearly described in terms of botanical and chemical compositions, in addition to a good knowledge about the digestive and assimilation process of nutrients, would bring more robust conclusions.

*Conclusion*

The equations of chamois and mouflons are not predictive enough to quantitatively study the dietary nitrogen content variation in our study area, and this could be related to the amount and type of tannins in the diet (Verheyden et al. 2011). Qualitative estimations are better suited in this case. Moreover, we do not advice to use the FN values to compare the diet quality of species differing in their diet and morpho-physiology, such as chamois and mouflon. The ability to cope with protein-precipitating metabolites of plants may differ and leads to different relationships between DN and FN. Consequently, estimations of diet quality of chamois and mouflon are possible over a broad range of values, should be qualitative, species-specific and location-specific. The use of FN as a predictor of individual and population short-term response to environmental changes would not be possible. However, as demonstrated by Blanchard et al. (2003) and confirmed by Verheyden et al. (2011), nitrogen fecal index could be reliable for species-specific population long-term monitoring, if large changes are identified.

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**Table 1.** Studies for which significant relationships between DN and FN have been demonstrated

| Author | Species | Scientific name | Feeding type | Experiment or field | Season | Range of fecal nitrogen * | Range of dietary nitrogen * | Equation | $R^2$ |
|---|---|---|---|---|---|---|---|---|---|
| Raymond et al. 1948 | Domestic sheep | | Grazer | Experiment | - | 1.7 to 4% | 1.2 to 3% | DN=-0.140+0.795*FN | - |
| Hebert et al. 1973 | Bighorn sheep | Ovis Canadensis A | Grazer | Experiment | - | 0.9 to 3% | 0.6 to 3% | DN=-0.15+1.034*FN | 0.82 |
| | Bighorn sheep | Ovis Canadensis B | Grazer | Experiment | - | 1 to 2% | 0.4 to 2.5% | DN=-1.118+1.79*FN | 0.92 |
| Arman et al. 1975 | Eland | Taurotagus oryx | Intermediate feeder | Experiment | - | - | - | DN=-2.88+3.472*FN | - |
| | Hartebeest | Alcelaphus buselaphus | Grazer | Experiment | - | - | - | DN=-5.49+4.464*DN | - |
| | Thomson's gazelle | Gazella thomsonii | Intermediate feeder | Experiment | - | - | - | DN=-9.35+3.745*FN | - |
| | Duiker | Sylvicapra grimmia | Browser | Experiment | - | - | - | DN=-11.74+3.952*FN | - |
| | Corriedale sheep | | Grazer | Experiment | - | - | - | DN=-2.92+2.725*FN | - |
| | Fat-tailed sheep | | Grazer | Experiment | - | - | - | DN=-3.23+2.933*FN | - |
| | Boran zebus | Bos indicus | Grazer | Experiment | - | - | - | DN=-1.93+2.525*FN | - |
| | Friesians | Bos taurus | Grazer | Experiment | - | - | - | DN=-19.3+2.079*FN | - |
| Mould et al. 1981 | Elk | Cervus elaphus nelsoni | Intermediate feeder | Field | Spring, summer, autumn | 0.8 to 2.7% | 0 to 5% | DN=-1.57+2.041*FN | 0.97 |
| Holechek et al. 1982 | Domestic cattle | | Grazer | Experiment | - | 1.5 to 3.3% | 1 to 2.3% | DN=-0.281+0.851*FN | 0.81 |
| Leslie et al. 1985 | Roosevelt elk | Cervus elaphus nelsoni | Intermediate feeder | Field | 4 seasons | 1 to 4% | 1 to 3.5% | DN=0.17+0.843*FN | 0.77 |
| | Black-tailed deer | Odocoilus hemionus columbianus | Intermediate feeder | Field | 4 seasons | 1.5 to 4.5% | 1.5 to 4.5% | DN=-0.722+1.025*FN | 0.95 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Nunez-Hernandez et al. 1992 | Cattle | | Grazer | Experiment | Summer, autumn | 0.9 to 1.7% | 0.6 to 2% | DN=-0.009+14.41*FN | 0.52 |
| | Goat | | Intermediate feeder | Experiment | Summer, autumn | 0.9 to 1.7% | 0.6 to 2% | DN=0.010+10.17*FN | 0.62 |
| Irwin et al. 1993 | Bighorn sheep | Ovis canadensis | Grazer | Experiment | Winter | 0.9 to 1.6% | 0.7 to 1.4% | DN=-0.36+1.31*FN | 0.82 |
| | Bighorn sheep | Ovis canadensis | Grazer | Field | Winter | 0.9 to 1.6% | 0.7 to 1.4% | DN=-0.31+1.04*FN | 0.87 |
| Jhala et al. 1997 | Blackbuck | Antilope cervicapra | Grazer | Field | 3 seasons according to precipitations | 1.1 to 1.5% ** | 0.5 to 1.7 % ** | DN=-3.7+3.891*FN | - |
| Wrench et al. 1997 | Impala | Aepyceros melampus | Intermediate feeder | Experiment | - | - | 0.9 to 2.1% | DN=-6.9+1.24*FN | - |
| | Impala | Aepyceros melampus | Intermediate feeder | Field | - | 1.1 to 2.3% | 0.9 to 2.2% | DN=3.8+0.83*FN | - |
| | Blue wildebeest | Connochaetes taurinus | Grazer | Field | - | 0.7 to 1.5% | 0.4 to 1.3% | DN=-0.97+0.84*FN | - |
| | Zebra | Equus burchelli | Grazer | Field | - | 0.5 to 1.2% | 0.4 to 1.3% | DN=-0.4+0.95*FN | - |
| | Cattle | | Grazer | Field | - | 1.2 to 2.3% | 0.9 to 2% | DN=-1.6+0.9*FN | - |
| Osborn & Ginnett 2001 | White-tailed deer | Odocoilus virginianus | Browser | Experiment | - | 1.7 to 2.9% | 1.2 to 4.2% | DN=-3.88+3*FN | 0.81 |
| Ueno et al. 2007 | Sika A | Cervus Nippon | Intermediate feeder | Field | 4 seasons | 0.3 to 3.5% | 0.3 to 4.3% | DN=0.675+1.023*FN | 0.58 |
| | Sika B | Cervus Nippon | Intermediate feeder | Field | 4 seasons | 0.8 to 3.1% | 0.6 to 3.5% | DN=0.217+1.027*FN | 0.61 |
| Bison, personal data *** | Chamois | Rupicapra rupicapra | Intermediate feeder | Field | 4 seasons | 2.1 to 4.6% | 1.3 to 4.3% | DN=0.073+0.81*FN | 0.38 |
| | Mouflon | Ovis gmelini musimon | Grazer | Field | 4 seasons | 1.4 to 3.9% | 1.8 to 4.2% | DN=1.91+0.4*FN | 0.13 |

* if not precised in the papers, they have been crudely evaluated from the graphs shown in the papers
** results were given in % of crude protein in the respective papers. As CP=6.25*N, we used this equation to estimate FN and CN
*** not included in the analysis of slopes comparisons
“-“ means that information was not found in the paper

**Table 2.** Simple regression equations to predict dietary nitrogen from fecal nitrogen at the inter- and intra-seasonal level, for wild chamois and mouflon

| Species | Season | Intercept | Slope | SE of intercept | SE of slope | n | $r^2$ | P |
|---|---|---|---|---|---|---|---|---|
| Chamois | All seasons | 0.07 | 0.81* | 0.33 | 0.10 | 115 | 0.38 | <0.05 |
| | Spring | 4.17 | -0.09 | 0.81 | 0.21 | 21 | 0.01 | 0.67 |
| | Summer | 4.78 | -0.47 | 0.87 | 0.23 | 13 | 0.27 | 0.07 |
| | Autumn | 3.14 | -0.015 | 0.35 | 0.01 | 81 | 0.04 | 0.06 |
| Mouflon | All seasons | 1.92 | 0.40* | 0.36 | 0.12 | 65 | 0.14 | 0.002 |
| | Spring | 3.28 | 0.13 | 0.84 | 0.27 | 14 | 0.02 | 0.65 |
| | Summer | 2.22 | 0.27 | 1.34 | 0.43 | 11 | 0.04 | 0.54 |
| | Autumn | 2.54 | 0.01 | 0.57 | 0.01 | 40 | 0.01 | 0.58 |

SE=standard error ; * significantly different from 0

**Table 3.** Multiple regressions to predict dietary nitrogen from multiple fecal indices obtained from model averaging on the global models (all seasons included)

| Species | Intercept | N | NDF | ADL | $r^2$ |
|---|---|---|---|---|---|
| Chamois | 2.03 | 0.60* | -0.03* | -0.01 | 0.45 |
| Mouflon | 2.02 | 0.55* | 0.01 | -0.06* | 0.26 |

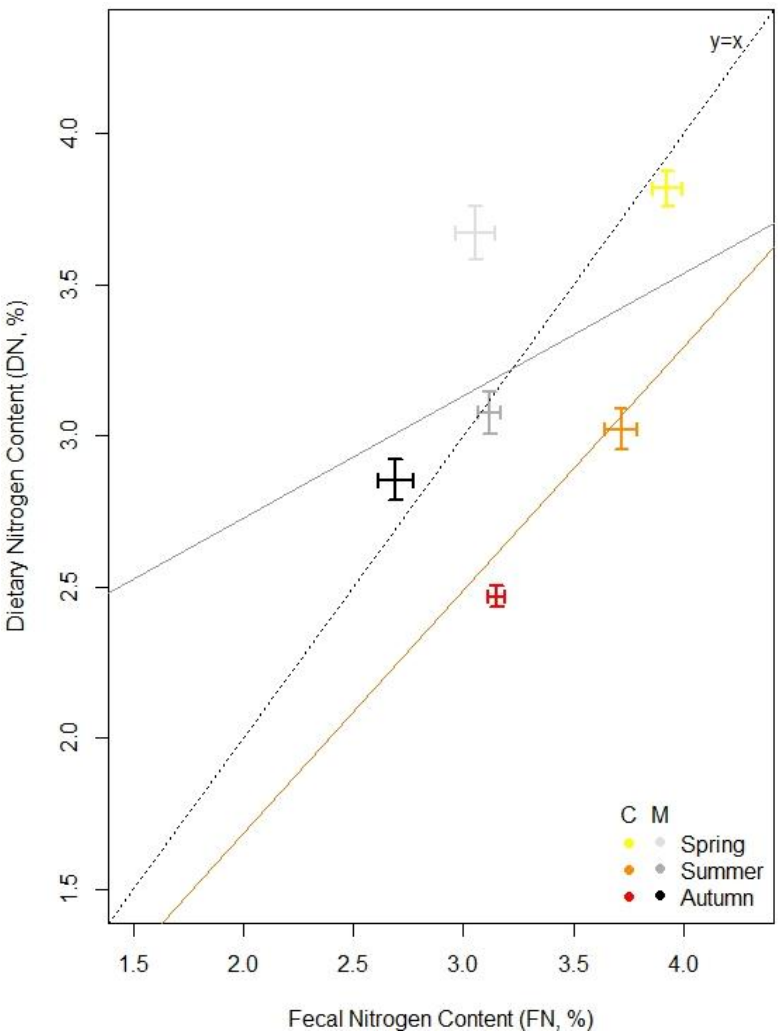


**Fig.1** Correlation of dietary and fecal nitrogen for chamois (C) and mouflon (M). For the sake of simplicity, we only presented the mean ± standard errors for each season, instead of all the data points. Dotted line represents the correlation when DN equals FN. Orange and grey lines represent the equations of the linear models of DN on FN on all the data points, respectively for mouflon and chamois (mouflon: DN = 1.92 + 0.40*FN, chamois: DN = 0.07 + 0.81*FN).

Version française de la thèse

# Introduction

## **Chapitre I :** Comment être enthousiasmé par l'écologie

### 1.1 Image du monde actuel

Selon le prix nobel Paul Crutzen, nous sommes actuellement au cœur de l'ère de l'Anthropocène, qui a commencé à l'aube de la révolution industrielle (fin du 18ème siècle) et qui a laissé derrière elle l'ère de l'Holocène. Même si ce concept est toujours débattu par la communauté scientifique (Lewis and Maslin 2015), l'origine de l'idée est claire : l'activité humaine est global et a de fortes implications sur les changements environnementaux, des cycles biogéochimiques à l'évolution de la vie (Lewis and Maslin 2015).
Etant donné que le mot « naturel » ne peut être utilisé sans incorporer l'action des humains, la relation et la question de l'interaction avec la Nature est essentielle, mais varie selon la culture et la société. Par le début de cette phrase, mes pensées sont déjà contraintes par l'idée d'une différence d'essence entre l'Humanité et la Nature. Cette vision de notre science moderne, initiée durant le 17ème siècle par Galilée, a mené à l'idée de l'indépendance de la Nature face aux humains (Larrère & Larrère 1997). Même si les humains font partie de la Nature, nous – personnes ayant grandi au sein de la société occidentale moderne – nous sentons hors de ce système. Nous en venons à penser que nous sommes capables de décrire la Nature avec des lois mathématiques et l'utilisons comme un outil pour lequel nous pouvons définir une valeur. La diversité dans la nature est ainsi considérée en termes de bénéfices apporté pour les populations humaines (services écosystémiques, Maris 2011). Ce point de vue est ancré dans notre société occidentale, mais n'existe pas partout. Par exemple, pour les peuples amazoniens (indiens Kayapos), l'humanité n'est pas restreinte aux hommes, mais inclue l'air que nous respirons, les plantes que nous mangeons, les animaux que nous chassons, etc (Klein 2010).
Etant donné que l'action de l'homme cause des changements globaux menant à une perte de la biodiversité qui pourrait affecter la dynamique et le fonctionnement des écosystèmes (Halpern et al. 2008, Cardinale et al. 2012, Newbold et al. 2015, McGill 2015), la « société moderne » essaie de maintenir un environnement aussi stable que possible en atteignant un compromis avec les développements économiques et industriels. Ainsi, nous réalisons que la protection/gestion de la nature existe seulement avec l'émergence de sociétés industrielles (Larrère 2013). Les philosophes en viennent à la conclusion que ce n'est pas la nature qui dominent les humains, ni que les humains dominent la nature, mais qu'une nouvelle interaction apparaît et que nous ne savons pas comment définir (Larrère & Larrère 1997, Maris 2007). Ainsi il apparaît nécessaire d'intégrer une nouvelle vision de la transaction avec l'environnement, dans le but de proposer des solutions pour le changement écosystémiques à venir.
La première étape de clairement identifier les causes des changements ecosystémiques pour mieux comprendre le fonctionnement des écoles système et leur réponse aux fluctuations environnementales. Le terme de fonctionnement des écosystèmes inclut les propriétés des écosystèmes les biens et les services ecosystémiques (Christensen et al. 1996). Dans la suite du document, nous nous réfèrerons aux propriétés des écosystèmes quand nous ferons référence au fonctionnement des écosystèmes. Cela inclut la taille des compartiments (par exemple les pools de carbone de matière organique) et les processus impliquant les flux de biomasse et d'énergie entre les niveaux trophiques et l'environnement (Hooper et al. 2005). Comme évoqué par Naeem en 1999, « fonctionnement » veut dire « montrant une activité ». Quand un écosystème est altéré, par exemple en termes d'identité des espèces, de composition des communautés ou de diversité des communautés, mais aussi en termes de conditions abiotiques,

son taux de production des plantes, son taux de décomposition, le stockage de carbone ou les cycles de nutriments sont des processus qui peuvent être détériorés (Naeem et al. 1999). La stabilité des écosystèmes peut par conséquent être modifiée.

Le climat et le changement d'usage des terres sont les facteurs qui ont le plus d'influence sur les changements de la biodiversité dans les régions tempérées (Boulangeat et al. 2014). La distribution des espèces et la diversité sont affectés par les changements climatiques (Parmesan 2006, Lenoir et al. 2010, Gottfried et al. 2012). Le changement d'usage des terres ont aussi été montrés comme ayant une forte influence sur la structure de la diversité de la végétation (Foley et al. 2005) à travers de scénarios : l'abandon dans des cultures menant à la fermeture des aires ouvertes (Gehrig-Fasel et al. 2007) ; et l'intensification de l'agriculture menant à l'extinction d'espèces (Hodgson et al. 2005). L'usage des terres est aussi associé à la fragmentation de l'habitat (Wallis De Vries et al. 1998) et à sa destruction (Wright & Muller-Landau 2006), tel que la conversion des forêts en agriculture, la canalisation des rivières et les installations routières. Quatre autres facteurs peuvent affecter la biodiversité, et peuvent agir à une plus courte échelle de temps que le changement climatique : la pollution comme les dépôts d'azote (Thuiller 2007, Zvereva et al. 2008) ou les contaminants chimiques (Bickham et al. 2000, Ricciardi et al. 2009) ; les invasions biologiques, les introductions et réintroduction d'espèces (Wilcove et al. 1998, Thuiller 2007, Hahn & Orrock 2014), l'augmentation des niveaux de dioxyde de carbone dans l'atmosphère (Thuiller 2007) ; et la surexploitation menant à l'épuisement des ressources (Lotze et al. 2006). Tous ces impacts et ses interactions contribuent à affecter l'environnement biotique et abiotique d'un certain nombre d'espèces (Wilcove et al. 1998, Hahn 2014). Alors que les conséquences majeures du climat et du changement d'usage des terres sur la biodiversité sont généralement associés avec une perte des espèces, l'augmentation de taille de certaines populations est tout aussi importante (Buntgen et al. 2014). En effet, certaines espèces tirent un avantage des nouvelles conditions environnementales associées à l'augmentation de température, au changement d'usage des terres, à la diminution des prédateurs, à la limitation des quotas de chasse, aux introductions dans des environnements où elles sont libérés de leurs prédateurs (« enemy release hypothesis », Maron & Vilà 2001). Ainsi, les augmentations de densité de population sont aussi une menace majeure pour la biodiversité, phénomènes habituellement omis.

Plusieurs auteurs sont d'accord pour dire que nous sommes sur la voie d'une crise environnementale (Ehrlich & Mooney 1983, Ceballos et al. 2002, Pereira et al. 2010), confirmée par un récent article de Ceballos et al. (2015) stipulant que les taux d'extinction actuels sont beaucoup plus élevés que la moyenne naturelle observée durant l'histoire de la Terre. Ces érosions ne sont pas limitées à une forte décroissance et à une homogénéisation (Clavel et al. 2010) de la distribution des espèces (extinction des espèces endémiques et introduction des espèces exotiques, Mack et al. 2000, Mooney & Hobbs 2000) mais mènent aussi à une dégradation du fonctionnement des écosystèmes dans leur ensemble (tels que la dynamique des feux, la séquestration du carbone, les cycles biogéochimiques, Estes et al. 2011). Ceci peut, en retour, affecter les services apportés par la nature à l'humanité, tels que l'utilisation de la biodiversité pour des objectifs médicaux, les réserves de ressources alimentaires et non-alimentaires ou la production d'énergie ; les valeurs esthétique, éthique ou spirituelle de la nature qui sont des aspects importants pour motiver les humains à protéger la nature ; les valeurs écologiques de la biodiversité. Les écosystèmes peuvent devenir déséquilibrer et fonctionnellement altérés (tel qu'un mauvais recyclage des nutriments, une mauvaise résilience) (Schröter et al. 2005), à différents degrés dépendant du type écosystème (Thuiller 2007) et de sa sensibilité. Dans ce contexte, les scientifiques essaient de comprendre à quelle échelle les changements se passent, ses origines, son maintien et essaient de prédire l'évolution des systèmes en prenant en compte le passé et les états actuels, notamment pour

apporter des faits, des idées et des éléments de décision pour les processus de décision politique (Thuiller 2007).

## 1.2 De l'écologie des communautés à l'écologie fonctionnelle

### a) Décrire les communautés

Les premiers pas de l'écologie ont été caractérisés par des tâches descriptives et de classification. Qu'est-ce qu'une espèce ? Comment les classifier ? Combien d'espèces vivent dans un même endroit ?
[voir détails et suite en anglais dans la thèse]

### b) Caractériser l'assemblage d'espèces

Les questions suivantes concernent l'assemblage des espèces dans les communautés. Pourquoi est-ce qu'il y a plus d'espèces à certains endroits que dans d'autres ? Comment est-ce que les espèces interagissent et coexistent dans une communauté ? Est-ce que l'une facilite le développement d'une autre ? Est-ce qu'il y a de la compétition ? Quelles sont les influences relatives de la température et des caractéristiques du sol dans la complexité des communautés végétales ? Comment est-ce que la composition évolue dans le temps ?
Les buts de l'écologie des communautés en tant que discipline scientifique sont de répondre à ces questions et d'élucider les processus en jeu dans les patrons de composition des communautés. Dans ce contexte, les approches se focalisent principalement sur les interactions écologiques parmi les organismes et avec leur environnement abiotique. Les interactions parmi les organismes (compétition, facilitation, prédation, parasitisme, symbiose, mutualisme, neutralisme, commensalisme) se réalisent sur une ou plusieurs axes de la niche écologique (boîte 1) tels que la nourriture, le temps ou l'espace.
[voir détails et suite en anglais dans la thèse]

**Boîte 1** – La niche écologique
La niche écologique est définie comme un hypervolume à n-dimensions (Hutchinson 1957) décrit les conditions environnementales qui permettent à une espèce d'avoir un taux de croissance positif (Grinnell 1917). Cet hypervolum est appelé la « niche fondamentale », cependant il ne peut pas être estimé étant donné qu'il n'est pas possible de tester toutes les conditions où l'espèce pourrait persister (Panzacchi et al. 2014). La niche que nous observons est la « niche réalisée » et est déterminée par les interactions biotiques et les limitations de la dispersion. Ainsi, la niche réalisée peut fluctuer avec les changements de l'environnement. D'autres concepts sont liés à la niche écologique. La « niche grinnellienne » (Grinnell 1917) se réfère aux besoins essentiels d'une espèce pour sa survie, alors que la « niche eltonnienne » (Elton 1927) se réfère à la façon dont une espèce impacte son environnement local (la « fonction »). Dans les deux cas, le concept de ressources est central, quelle que soit la définition de la niche.

### c) Trouver des règles générales dans la structuration des communautés

Des questions plus récentes ont émergées pour comprendre comment marche l'assemblage des espèces. Quelles sont les fonctions de l'espèce dans la communauté ? Comment un certain assemblage d'espèces influence les processus écosystémiques ? Comment peut-on généraliser les conclusions locales à des patrons plus généraux à large échelle ?
La vision taxonomique ne reflète pas la fonction des organismes et ne permet pas de donner des principes généraux à propos de l'assemblage des communautés (Cornwell & Ackerly 2009, Spasojevic & Suding 2012, Mason et al. 2012), ni de prédire l'abondance des espèces (Shipley

et al. 2006, Laughlin et al. 2012), ni de comprendre l'influence des organismes sur le fonctionnement des écosystèmes (Diaz & Cabido 2001, Lavorel & Garnier 2002). Il y a environ 25 ans, l'approche fonctionnelle des communautés a été introduite (Lavorel & Garnier 2002, Cornelissen et al. 2003, Violle et al. 2007) dans un contexte où les écologues se demandaient si des lois universelles pouvaient gouverner l'écologie (Lawton 1999, McGill et al. 2006).
Basée sur les individus, l'écologie fonctionnelle décrit les organismes selon leur caractéristiques (par exemple, hauteur végétative, aire des feuilles, densité des racines) et leur fonction (par exemple, interception de la lumière, apport des ressources, des nutriments et absorption d'eau) dans leur environnement au lieu de les décrire avec leur identité taxonomique (Calow 1987). Suivant la revue de définition des « traits » donnée par Violle et al. (2007), qui détaille comment la signification du terme « trait » varie selon les espèces, nous avons utilisé la définition des traits au niveau individuel tel que Garnier & Navas (2013), c'est-à-dire « un trait est une caractéristique morphologique, physiologique ou phénologique mesurable au niveau de l'individu seulement, de la cellule à tout l'organisme, sans référence à un environnement particulier ou à un autre niveau d'organisation ». Selon cette définition, un trait n'est pas influencé par des facteurs environnementaux ou par tout autre niveau d'organisation (Violle et al. 2007). Des espèces qui sont taxonomiquement différentes peuvent en fait être similaires en terme de fonctions et de caractéristiques biologiques (morphologiques, chimiques, phénologiques, biomécaniques). Ces caractéristiques qui ont un impact direct sur la fitness (survie, croissance ou reproduction) sont appelés les traits fonctionnels (Lavorel et al. 1997, Violle et al. 2007). La valeur d'un trait fonctionnel est le résultat de compromis parmi les différentes fonctions des plantes (Diaz & Cabido 1997). L'utilisation de traits fonctionnels indépendants permet de décrire les stratégies fonctionnelles générales des plantes utiles pour la compréhension du fonctionnement des écosystèmes (Lavorel et al. 1997). Par exemple, le contenu en matière sèche des feuilles (LDMC) est négativement corrélé à la surface foliaire (SLA) : les espèces avec un fort LDMC (faible SLA) sont composées de tissus foliaires de faible densité, ont un faible taux photosynthétique ne permettant pas un fort apport en ressources mais ont une forte capacité de conservation, et par conséquent un faible taux de croissance. Ces espèces sont appelées des espèces à stratégie conservative. L'opposé concerne les espèces à stratégie exploitatrice et sont dominantes dans les environnements fertiles (Grime et al. 1997, Reich et al. 1999).
[voir détails et suite en anglais dans la thèse]

## 1.3 Descendre d'une échelle et étudier l'écologie au niveau intra-spécifique dans le but de mieux comprendre la dynamique des communautés et le fonctionnement des écosystèmes

La plupart des études sur la niche en écologie des communautés se focalisent sur les traits moyens des espèces, suggérant que les individus se comportent de la même façon (Layman et al. 2015). Cependant, comme établi par Darwin (1859), les individus diffèrent à cause de leur plasticité phénotypique et de la diversité génétique (Byars et al. 2007), ou parce que les interactions biotiques altèrent les valeurs de traits (par exemple la compétition, Gross et al. 2009). Par exemple, dans le cas d'espèces de plantes, l'inclusion de la variabilité intra-spécifique peut aider à distinguer les populations qui diffèrent dans leur valeur de traits le long de gradients environnementaux (Albert et al. 2010). Résumer les données avec les valeurs moyennes des espèces surestime la contribution des espèces rares (Paine et al. 2011). Ainsi, l'omission de la variabilité intra-spécifique peut mener à une mauvaise interprétation du fonctionnement des communautés (Jung et al. 2010). Par exemple, la productivité nette primaire aérienne (ANPP) augmente avec la diversité génotypique des plantes à cause d'une plus grande

complémentarité de niche, dans une communauté expérimentalement peu diversifiée (Crutsinger et al. 2006). Si l'objectif de l'étude est de déterminer les facteurs influençant l'ANPP, alors l'omission de la variabilité intra-spécifique pourrait empêcher une interprétation correcte des résultats. Dans l'étude de La Morgia & Bassano (2009), si la mesure d'overlap avait été mesurée entre toutes les paires d'individus au lieu de la moyenne des régimes de chamois et de moutons, ils auraient pu être capables de déterminer si seulement un petit nombre d'individus chamois sont affectés par les moutons. Cette information peut être particulièrement importante pour résoudre des problèmes de gestion et changer notre vision de la conservation des espèces (Bolnick et al. 2003). En effet, protéger l'habitat d'une espèce en se basant sur les préférences moyennes de l'habitat alors que l'espèce montre une forte variabilité inter-individuelle n'est pas adapté.

Parce que la sélection naturelle, et ainsi l'adaptation, agit à un niveau individuel, étudier la variabilité intra-spécifique est essentiel pour comprendre comment les populations s'adaptent à son environnement et pour comprendre l'évolution de leurs niches réalisées (Tinker et al. 2008, Pires et al. 2013, Salvidio et al. 2014). Quand les ressources en nourriture diminuent en automne, les populations de salamandre augmentent la largeur de niche de leurs populations grâce à une plus forte spécialisation des individus sur des ressources alternatives (Salvidio et al. 2014). Sans cette approche multi-niveaux, les auteurs n'auraient pas été capables d'expliquer les mécanismes responsables de l'augmentation de largeur de niche de la population. Ces améliorations pourraient aider à mieux prédire comment une population ou une espèce répondrait numériquement, spatialement et de manière comportementale aux changements environnementaux (Bolnick et al. 2011). Par exemple, si une des ressources utilisées par une population d'herbivores avec une forte variabilité inter-individuelle décroît, seulement une partie des individus seraient affectés. Sans mesures de variation inter-individuelle, les prédictions sur l'évolution des populations mènerait à suggérer que tous les individus souffrent de la diminution des ressources, et nous aurions prédit une diminution de la fitness de tous les individus au lieu de quelques individus seulement. Ainsi, inclure la variabilité intra-spécifique dans les modèles décrivant la dynamique des populations pourrait aider à améliorer le pouvoir prédictif de l'étude (Bolnick et al. 2003).

Les interactions intra-spécifiques peuvent aussi affecter les interactions écologiques et en retour l'assemblage des communautés (Hughes et al. 2008, Bolnick et al. 2011), à la fois dans des communautés à faible diversité (Crutsinger et al. 2006, Hughes et al. 2008) et dans des systèmes plus diversifiés (Cornwell & Ackerly 2009, Jung et al. 2010, Paine et al. 2011). Par exemple, l'augmentation de la diversité des ressources disponibles aux herbivores augmentait avec la richesse en arthropodes grâce à l'association entre certains herbivores et certains génotypes hôtes-plantes, ainsi favorisant le nombre d'interactions écologiques entre les herbivores et les plantes (Crutsinger et al. 2006). Dans un contexte d'augmentation du chevauchement spatial entre espèces dû à une augmentation de la taille des populations, une plus forte compétition inter-spécifique pourrait impacter de manière différente les individus d'une espèce cible selon son degré de variabilité inter-individuelle. En effet, c'est seulement une partie des individus ou tous les individus qui seraient affectés par une espèce chevauchante (voir fig.1 p8). Ainsi, la variabilité inter-individuelle promeut la coexistence entre les espèces en limitant la similarité avec une espèce compétitive et en ajustant les valeurs de traits des individus et des espèces à leurs besoins abiotiques (filtres environnementaux, Jung et al. 2010). Les données au niveau individuel apparaissent comme un indicateur plus sensible à la différentiation des niches et aux filtres environnementaux que les données moyennes d'espèces (Jung et al. 2010).

Etant donné l'importance de la variabilité intra-spécifique dans la réponse des communautés aux changements environnementaux, dans l'assemblage de la biodiversité ou dans le fonctionnement des écosystèmes (Dupont et al. 2014, Willmer & Finlayson 2014, Tur et al. 2015), un nombre de plus en plus grand de chercheurs essaient de la prendre en compte dans

leurs études. Par exemple, Tur et al. (2014) ont étudié les réseaux plantes-pollinisateurs au niveau intra-spécifique et ont montré que les différents paramètres (densité des liens, connectivité, imbrication, diversité des interactions) décrivant la structure des réseaux changeaient significativement en réponse à un haut degré de spécialisation individuelle.

Inclure la variabilité inter-individuelle dans les études en écologie n'est pas seulement utile pour expliquer les variations spatiales ou temporelles de la composition des communautés, mais aussi pour explorer les processus évolutifs à une plus large échelle de temps. En effet, dans une perspective d'évolution, étant donné que les filtres environnementaux agissent à un niveau individuel, différents temps de survie, taux de croissance et de reproduction peuvent être observés parmi les individus, menant à des changements dans la fréquence des allèles, ce qui peut en retour modifier l'évolution des populations à travers la sélection naturelle (Bolnick et al. 2003).

# **Chapitre II :** Contexte théorique

## 2.1 Les grands herbivores comme bon modèle d'étude

a) Leur place centrale dans les écosystèmes

Les grands herbivores sont d'une importance économique, sociale et culturelle (Gordon et al. 2004) et leur étude et gestion est ainsi d'un intérêt particulier. Dans une perspective économique, les herbivores sont aussi d'un intérêt cynégétique pour le sport et les trophées de chasse et génèrent des bénéfices économiques (Palazy et al. 2012). La diversité et les aspects emblématiques de certains herbivores favorisent aussi le tourisme (Cederna & Lovari 1985). Aussi, les grands herbivores apportent une composante culturelle importante aux services écosystémiques par la maintenance de certaines cultures (par exemple, les peuples Sami de Scandinavie dépendent la domestication du renne).

Etant donné leur position intermédiaire, les densités de grands herbivores sont contrôlées par la disponibilité des ressources (effet « bottom-up »), par les prédateurs (prédation naturelle et humaine) (effet « top-down »), les maladies (effet « top down ») et les compétiteurs (effet « transversal »). Les grands herbivores qui requièrent une plus grande abondance de plantes sont plus à même d'être limités par la disponibilité en nourriture, alors que les petits herbivores qui requièrent un plus fort contenu en nutriments seraient plus sensibles à la prédation (Olff et al. 2002, Hopcraft 2010). Dans les environnements libérés des prédateurs, la stochasticité environnementale (Saether 1997) et la densité-dépendant (Skogland 1985, Coulson et al. 2000) agissent en partie à travers la limitation des ressources, mais aussi les soins maternaux (Loison et al. 2004) et l'effet de cohorte (Gaillard et al. 2003), jouent un rôle important dans la dynamique complexe des populations d'herbivores.

En plus d'être régulés par ces facteurs, les herbivores peuvent fortement les impacter (disponibilité des ressources, prédateurs et compétiteurs) à travers des effets en retour.
Comme les herbivores dans leur ensemble consomment environ 20% de la productivité nette primaire (Agrawal 2011 et références cités, différents taux de dommage par les grands herbivores dans les forêts pour les arbres spécifiques dans Danell et al. 2006) et ont un fort impact sur la biomasse des producteurs primaires dans les écosystèmes terrestres et marins (Poore et al. 2012), ils jouent un rôle majeur dans la régulation de la diversité des plantes et dans le fonctionnement des écosystèmes (Olff & Ritchi 1998, Belovsky & Slade 2000). Cependant, prédire les conséquences des herbivores sur la diversité des plantes est difficile étant donné que les études mènent à des résultats contradictoires, allant d'effets positifs (Belsky 1992, Collins et al. 1998) à négatifs (Milchunas et al. 1998, Wardle et al. 2001) et neutres (Stohlgren et al. 1999, Adler et al. 2005). En effet, différents paramètres sont impliqués dans l'impact des grands herbivores : type alimentaire (gradient « grazer/browser »), intensité de la pression d'herbivorie et type de perturbation physique (par exemple, piétinement, recherche de racines, frottement des bois, Latham 1999). De plus, les caractéristiques des sites telles que les différences de productivité parmi les localisations (Bakker et al. 2006) et l'histoire évolutive de pâturage (Milchunas et al. 1998) modulent aussi la réponse. Par exemple, les herbivores « grazer », supposés être moins sélectifs, devraient impacter les espèces dominantes (Milchunas & Lauenroth 1993) et augmenter la diversité des plantes à une forte productivité mais diminuer la diversité à de faibles productivités (Bakker et al. 2006). C'est le cas du bison (*Bos bison*), qui maintient la diversité des plantes dans les systèmes productifs des prairies d'Amérique du Nord (Collins et al. 1998). De plus, les prédictions ne sont pas simples étant donné que la relation entre la pression d'herbivorie et la diversité des plantes est non-linéaire. Une pression d'herbivorie modérée a été montrée comme favorisant une plus forte diversité de plantes qu'un

fort et faible pâturage (Hobbs & Huenneke 1996, Rooney & Waller 2003, Boulangeat et al. 2014, Ganjurjav et al. 2015), ce qui correspond à l'hypothèse de perturbation intermédiaire expliquant la diversité des plantes (Connell 1978) même si la base théorique de cette hypothèse a été questionnée récemment (Violle et al. 2010).

Par leur action de pâturage et de broutage, les herbivores sont un outil utile pour la gestion des paysages. Ils peuvent accélérer ou ralentir les processus de succession, par exemple en maintenant des habitats ouverts et en empêchant l'établissement de la forêt (Menoni et al. 2008), ou en favorisant l'implantation de la forêt en promouvant le pâturage sélectif sur certaines espèces de plantes (Prins 1998). Ainsi, les troupeaux domestiques peuvent être utilisés pour restaurer les prairies (Garnier & Navas 2013) et éviter la fermeture des aires ouvertes. Par exemple, dans le massif des Bauges (notre site d'étude), des vaches sont tenues dans des enclos sur l'alpage d'Armène en été pour empêcher le développement de l'aulne vert. Une bonne adéquation entre la connaissance de la structure fonctionnelle de la végétation et le régime de pâturage, est important pour le succès des introductions d'herbivores (Dulphy 1995, Garnier & Navas 2013). En effet, l'introduction d'une espèce d'herbivore n'est pas possible partout, particulièrement parce qu'elle peut avoir des effets négatifs sur la diversité des plantes dans une zone non productive (Bakker et al. 2006) ou dans des zones qui ne sont pas habituées à une forte pression de pâturage (Milchunas et al. 1998).

En plus des flux directs d'énergie et de matériel entre les plantes et les herbivores ou les prédateurs et les herbivores, les herbivores génèrent aussi des cascades trophiques à travers des changements de végétation sur les invertébrés (Martin et al. 2010), les petits mammifères (Smit et al. 2001) ou les oiseaux (Cardinal et al. 2012). Par exemple, les changements de densité ou de couverture forestière à travers le broutage intensif par le cerf de virginie ont mené à une homogénéisation des communautés d'oiseaux (Cardinal et al. 2012). Les herbivores influencent aussi indirectement les processus écosystémiques tels que les cycles de nutriments (Wardle et al. 2004, Garibaldi et al. 2007), la productivité nette primaire (Wardle et al. 2004), modifient les perturbations abiotiques tels que les feux (Hobbs 1996), promeuvent la disponibilité en lumière au-dessus du sol dans les écosystèmes prairiaux à travers le retrait de la biomasse des compétiteurs supérieures (les plantes les plus hautes) (Borer et al. 2014).

Ces effets peuvent agir à différentes échelles spatiales et temporelles (Hobbs 1996) et ont en retour différentes conséquences. Par exemple, les herbivores peuvent favoriser le développement d'espèces de plantes résistantes au pâturage ou évitées ce qui, par conséquent, peut réduire la diversité des communautés végétales à l'échelle du paysage, alors que la libération en nutriments par les faeces devrait accélérer le cycle des nutriments et augmenter la diversité à des échelles locales (Crawley 1997).

b) Les grands herbivores en Europe

Les augmentations rapides de densité de grands herbivores (Putman et al. 1996, Loison et al. 2003, Milner et al. 2006, Maillard et al. 2010, liste rouge de l'IUCN) observées durant les 30 dernières années en Europe ont mené à une forte augmentation des études sur les relations plantes-ongulés (Coulson 1999, Côté et al. 2004, Ward 2005, Shelton et al. 2014). Dans le but de faire un parallèle avec le début de l'introduction, nous allons détailler les facteurs impliqués dans ces variations de densité de population. D'abord, les environnements alpins et nordiques sont spécifiquement affectés par de plus faibles précipitations neigeuses, par un déneigement plus précoce et par une diminution de la productivité végétale (Hamel et al. 2009). Ces hivers plus chauds mènent à une diminution de la mortalité hivernale alors des printemps plus précoces réduisent la période critique de recherche de nourriture (Mysterud et al. 2007, Pettorelli et al.

2007). Cependant, ces conséquences doivent être interprétées avec précaution, étant donné qu'une même cause peut avoir de multiples, et quelques fois opposées, conséquences. Des printemps précoces ne sont pas nécessairement bénéfiques pour les animaux à cause de l'asynchronie entre le pic de biomasse de végétation et la période pour faire des réserves de gras (Pettorelli et al. 2007, Post et al. 2008). Cette différence de synchronisation a été déjà montrée chez des herbivores mammifères et des plantes (Inouye et al. 2000), des parasites et leurs hôtes chez les insectes (Van Nouhuys & Lei 2004), ou chez les insectes pollinisateurs avec la date de floraison des plantes (Visser et al. 2005, Harrington et al. 1999). Le déplacement altitudinal de la limite des arbres vers des altitudes plus élevées, causées par le réchauffement climatique et par les changements d'usage des terres durant le 20ème siècle (Gehrig-Fasel et al. 2007), augmente la disponibilité de biomasse végétale de haute qualité, ce qui peut être bénéfique pour maintenir les besoins énergétiques et améliorer la fitness des individus. De plus, la diminution des densités de prédateurs et les changements dans la façon dont les populations sont gérées (Apollonio et al. 2010) à travers des plans de chasse ou les introductions d'espèces, contribuent largement à augmenter les populations d'ongulés en France et en Europe. Ceci contraste avec le déclin général des grands herbivores à une échelle globale (Hopcraft et al. 2010, Ripple et al. 2015). Luo et al. (2014) ont montré que les modifications des composantes environnementales utilisées par les ongulés, tel que la disponibilité des plantes, la qualité des ressources ou des autres paramètres d'habitat, ont dramatiquement altérés la distribution spatiale des espèces sur le plateau tibétain. Les résultats de modélisation montrent que certaines espèces pourraient perdre plus de 50% de la surface de leur domaine vital mais que le pourcentage varie avec les espèces et les sites (18% de contraction du domaine vital chez les mammifères africains, Thuiller et al. 2006), alors que certaines autres pourraient occuper de nouvelles aires de distribution à travers la dispersion (Luo et al. 2014).

c) Les motivations qui nous ont menées à initier ce travail

Suite à l'augmentation de l'aire de distribution des grands herbivores en Europe (Loison et al. 2003, Milner et al. 2006, Maillard et al. 2010, liste rouge de l'IUCN), les chevauchements spatiaux entre espèces – incluant les troupeaux domestiques – augmentent et peuvent mener à des changements dans les interactions intra- et inter-spécifiques. Si la compétition apparaît ou se renforce entre les individus d'une espèce et entre les espèces, les individus pourraient souffrir d'une diminution de la fitness qui pourrait mener à des changements dans les dynamiques de population. Par exemple, ceci pourrait être problématique pour les espèces natives souffrant des conséquences de l'augmentation de densités d'espèces introduites (Forsyth & Hickling 1998, Lovari et al. 2014, Ferretti et al. 2015). En parallèle, leurs augmentations pourraient devenir un problème à cause d'un plus fort impact sur la composition, structure et dynamique des communautés végétales (Hobbs & Huenneke 1996), et peuvent aussi mener à des dommages agriculturaux et forestiers. Dans le but de mieux comprendre la structuration des communautés d'herbivores, l'impact des herbivores sur les écosystèmes et donner des clés pour la gestion des populations et de l'habitat, il apparaissait nécessaire de déterminer comment des herbivores coexistant spatialement sélectionnent et partagent les ressources alimentaires aux niveaux intra- et inter-spécifiques. Pour atteindre ces objectifs, nous nous sommes focalisés sur la communauté d'herbivores du massif des Bauges où quatre ongulés peuvent être trouvés (chamois, chevreuil, mouflon et cerf) incluant une espèce introduite (le mouflon dans les années 1950, Darmon et al. 2007).

## 2.2 Le régime comme lien entre deux niveaux trophiques

a) Une question d'échelle spatio-temporelle

Le régime des animaux est la dernière étape d'un processus de sélection spatio-temporel (Johnson 1980, Senft et al. 1987). La sélection par un animal requiert que la ressource soit sur- ou sous-consommée relativement à sa disponibilité dans l'environnement.
Les espèces ou les populations se répartissent au sein d'une aire de distribution (1$^{er}$ ordre de sélection), où les organismes définissent leur domaine vital (2$^{ème}$ ordre de sélection) et sélectionnent des stations de nourrissage (communautés végétales) (3$^{ème}$ ordre de sélection) au sein desquelles ils sélectionnent leurs items alimentaires (4$^{ème}$ ordre de sélection). La sélection à chaque échelle spatiale impacte la sélection aux échelles sous-jacentes.

Prédire le comportement alimentaire des animaux a été et est toujours un des buts majeurs en écologie (Pyke et al. 1977). Une des théories les plus connues est la « théorie de l'approvisionnement optimal » (OFT), d'abord développée par McArthur & Pianka (1966) puis approchée par différents auteurs (Pyke et al. 1977, Stephens & Krebs 1986). D'abord détaillée chez les carnivores, elle prédit que les individus devraient maximiser leur apport en énergie par unité de temps tout en minimisant les coûts associés à la recherche, à la manipulation et aux processus digestifs. Les ruminants doivent prendre des décisions sur un gradient opposant deux options comportementales : maximiser le temps de recherche pour maximiser l'énergie gagnée ou bien minimiser le temps de recherche et être moins sélectif pour le fourrage dans le but d'économiser du temps pour les autres tâches (Schoener 1971, Bergman et al. 2001, Fortin et al. 2003). Avec cette vision, des compromis sont par conséquent requis entre choisir un régime optimal (en terme de quantité et de qualité), et perdre autant d'énergie que possible avec les déplacements et la manipulation du fourrage, et allouer le bon temps aux patchs (Searle et al. 2005). Ainsi, la théorie inclue différentes échelles de sélection et différentes contraintes reliées aux caractéristiques des animaux (temporelle, énergétique, cognitive). Cette théorie de l'approvisionnement optimal a été décrite dans un environnement où la seule contrainte est la distribution hétérogène des ressources. Cependant, les contraintes biotiques et abiotiques telles que les interactions inter- et intra-spécifiques, la prédation (Grignolio et al. 2007, McArthur et al. 2014) et les perturbations sont connues comme jouant un rôle majeur dans les décisions animales. De plus, ce principe peut ne pas être spécifique à l'énergie mais aussi à d'autres caractéristiques biomécaniques-physiques-chimiques (Chapin et al. 1980, Massey et al. 2009, Dostaler et al. 2011, Zweifel-Schielly et al. 2012). Ainsi, ces facteurs additionnels devraient être inclus dans les analyses pour mieux correspondre à l'environnement « réel » rencontré par les espèces.

La variabilité temporelle de la sélection alimentaire est aussi un aspect important des interactions écologiques (Wam et al. 2010), étant donné que la qualité et la quantité du fourrage fluctue au cours de l'année (Duncan 2005). En plus des changements de l'habitat, les herbivores sont aussi contraints par leur cycle annuel et leurs besoins (Kaske & Growth 1997) durant la gestation, lactation, croissance des jeunes ou stockage de gras avant l'hiver. Par exemple, certaines études suggèrent que les herbivores devraient sélectionner des protéines en été pour la croissance et des carbohydrates digestibles en hiver quand la thermorégulation est élevée (Berteaux et al. 1998, Dostaler et al. 2011).

Dans ce travail, nous nous sommes principalement focalisé à l'échelle de l'item, et nous avons aussi utilisé l'information des ressources disponibles à l'échelle des communautés végétales quand nous avons analysé l'échelle de sélection alimentaire. La sélection d'habitat à une plus large échelle était le sujet de thèse de Gaëlle Darmon (Darmon 2007) sur le même site d'étude,

et est actuellement poursuivi par Antoine Duparc pour son doctorat. Alors d'autres facteurs tels que les caractéristiques des animaux (morphologiques, physiologiques, cognitifs, sociaux), la présence permanente des prédateurs, la connaissance que les animaux ont gagné à partir de leur régime précédent (Provenza 1995) et la distribution spatiale précise, l'organisation et l'accessibilité (Dumont & Petit 1998) des plantes sont importantes dans les décisions des individus pour choisir où se nourrir, nous nous sommes focalisé sur les décisions locales et sur les caractéristiques locales des abondances de plantes et des traits qui influencent ces décisions. Nous avons aussi incorporé la dynamique temporelle dans nos analyses.

b) Les facteurs influençant le régime des animaux

Les facteurs influençant le régime des animaux sont détaillés dans le manuscrit : les caractéristiques morpho-physiologiques et les besoins en nutriments, la disponibilité des ressources (l'abondance relative en termes de biomasse), les caractéristiques fonctionnelles des plantes, les interactions inter-spécifiques, les interactions intra-spécifiques.
[voir détails en anglais dans la thèse]

## 2.3 Objectifs

Les objectifs de ces trois ans de travail étaient d'améliorer notre compréhension de la structure et du fonctionnement des écosystèmes à travers l'étude des interactions entre grands herbivores et communautés végétales aux niveaux intra- et inter-spécifiques. Au niveau intra-spécifique, nous nous sommes demandé si la socialité affectait le comportement alimentaire des individus (papier I). Au niveau inter-spécifique, nous avons testé si des espèces introduites pouvaient modifier la communauté native d'herbivores à travers des interactions trophiques (papier II), et nous avons essayé d'identifier les critères de sélection alimentaire des grands herbivores à une échelle fine (papier III). Finallement, nous avons exploré l'utilité de la méthode NIRS dans les études en écologie (papier IV et note).
[voir détails en anglais dans la thèse]

Les parties « Matériel et méthodes » et « Résultats » sont détaillées en anglais dans la thèse.

# Synthèse, Perspectives et Directions

Tout d'abord, je détaillerai certaines considérations méthodologiques qui doivent être prises en compte dans l'interprétation des résultats. Ensuite, je discuterai des interactions plantes-herbivores à la lumière des résultats obtenus dans cette thèse. Enfin, j'aimerais montrer que nos résultats, en plus d'apporter certaines clarifications à propos des interactions trophiques entre les ongulés et les communautés végétales, pourraient être utiles dans les études qui tentent de prédire la distribution des espèces en réponse au changement climatique.

## 7.1 Considérations méthodologiques

a) Questionner la fiabilité des estimations de régime à partir des données de faeces

Habituellement évalué à partir d'échantillons de rumens ou de faeces, de relevés de broutage ou d'observations directes (Pompanon et al. 2012), la composition des régimes peut être mal évaluée. Dans le cas d'échantillons fécaux, le régime réel est habituellement biaisé à cause d'une différence de digestibilité entre les plantes (Verne & Ullrey 1984). En effet, comme les plantes avec un plus faible contenu en fibres sont plus facilement digérées que les plantes avec un plus haut contenu en fibres, elles sont difficiles à identifier et tendent à être sous-estimées (Skinner & Telfer 1974). Dans ce travail, les espèces de graminées qui décomposent lentement étaient faiblement représentées dans les faeces, ce qui renforce nos conclusions que les espèces de graminées sont mangées en faibles proportions par le chamois et le mouflon. Cependant, les régimes décrits pour le chamois et le mouflon n'étaient pas toujours en accord avec les autres études, comme détaillé dans le « Summary of Results (6.2 dans la thèse) ». Différentes hypothèses expliquant ces différences sont plausibles. Tout d'abord, les espèces de graminées étaient sous-représentées dans les crottes même quand une forte proportion (entre 20 et 50% du régime) était trouvée dans le rumen des chamois et mouflons (Redjadj et al. 2014). Cependant, la comparaison doit être faite avec précaution étant donné que Redjadj et al. (2014) s'étaient focalisés sur la période de Septembre à Janvier, période qui a été peu étudiée dans notre travail car un faible nombre de faeces avait été échantillonné en hiver. Deuxièmement, les régimes obtenus à partir des autres études (La Morgia & Bassano 2009, Bertolino et al. 2009, Garcia-Gonzalez et al. 1996) ont été déterminé par micro-histologie, une méthode ou l'identification des fragments hautement digestibles est très difficile et qui tend à surestimer les graminées. Il serait nécessaire d'analyser les faeces à la fois par microhistologie et par ADN-metabarcoding pour proprement comparer les différences entre les deux méthodes. Enfin, selon la revue de Marchand (2013), les régimes des mouflons ne sont pas restreints aux graminées, et l'environnement influence les choix alimentaires. Ainsi, il est concevable que les mouflons du massif des Bauges préfèrent un régime riche en dicotylédones. Comme les chamois sont classés comme ayant un type alimentaire « intermédiaire », il n'était pas surprenant que leur régime ne contienne que peu de graminées.

b) L'expérience de NIRS

Un des sujets stimulant dans les études en écologie est l'identification de processus à large échelle dirigeant la structure et la dynamique des communautés (Sutherland et al. 2013). Nous avons ainsi un besoin urgent de méthodes d'échantillonnage et d'analyses adaptées pour de grosses bases de données, comme les traits fonctionnels chimiques des plantes par exemple. Les appareils comme l'analyseur CHN utilisé pour des analyses classiques de chimie, par exemple pour estimer le contenu en azote des plantes, sont chronophages, coûteux et destructeurs. L'utilisation de la méthodologie NIRS est une approche prometteuse en ce qui concerne les problèmes de temps, de coût et de destruction d'échantillons. Plusieurs études,

parmi celles présentées dans la section 6.5.b de la thèse, ont démontré que la NIRS était un outil utile pour prédire le contenu en azote des plantes (Petisco et al. 2005, Moron & Cozzolino 2002, de Aldena et al. 1995, Meuret et al. 1993). Des mesures additionnelles doivent être réalisées pour déterminer pourquoi la NIRS n'est pas aussi précise pour estimer les contenus en carbone et en phosphore. De plus, les résultats ont montré qu'une seule calibration pour des échantillons d'écosystèmes sub-arctic et tempérés donnaient des résultats similaires que des modèles spécifiques à chaque site pour les trois nutriments étudiés (C, N et P). Ceci ouvre de nouvelles voies pour l'échantillonnage intensif tout en sauvant du temps et de l'argent : au lieu d'avoir à calibrer un modèle pour chaque site, un seul modèle global pourrait donner des résultats similaires.

c) Les indices fécaux, un indicateur peu fiable de la qualité des régimes

La NIRS a été utilisée sur des échantillons de faeces pour évaluer la qualité du régime des animaux qui ont produit la crotte. Ici, la fiabilité de la NIRS n'a pas été testée en comparant les résultats aux résultats obtenus avec des méthodes d'analyses chimiques traditionnelles, mais nous avons indirectement évalué sa pertinence pour détecter la qualité du régime en comparant les résultats aux moyennes des contenus en azote des plantes ingérées (déterminées par la méthode d'ADN meta-barcoding). La relation entre les deux estimateurs de qualité de régime était faible, suggérant que l'estimateur d'azote à partir de la NIRS était un mauvais proxy de la qualité du régime aux niveaux intra- et inter-saisonniers. Les indices fécaux pourraient être moins efficaces à cause de l'effet des tanins sur la libération d'azote. Cependant, le principal problème vient du fait que notre méthode d'estimation de l'azote à partir de la composition du régime n'est pas encore validée comme étant une méthode fiable pour estimer le contenu en azote ingéré. Des designs expérimentaux contrôlant la qualité initiale des ressources ingérées devraient être utilisés pour déterminer les facteurs et les biais méthodologiques affectant les mesures de qualité de régime.

## 7.2 Les interactions plantes-herbivores

Les écosystèmes européens de montagne sont affectés par le réchauffement climatique, simultanément avec l'augmentation de densité des grands herbivores et des changements dans la dynamique de la végétation. Dans le but de prédire les dynamiques de populations et les interactions entre les ongulés, les caractéristiques des ressources importantes pour les herbivores doivent être déterminées. Ceci implique l'identification des facteurs expliquant la sélection alimentaire et la coexistence entre les herbivores aux niveaux intra- et inter-spécifiques.

a) Réflexions à propos de la sélection alimentaire

Les résultats de ce travail suggèrent que les régimes des ongulés vivant dans des alpages alpins (chamois et mouflons) étaient affectés par la qualité de la nourriture plutôt que par la quantité (papiers II & III), et que la sélection des ressources se réalisait à l'échelle fine du domaine vital 48h plutôt qu'à l'échelle large du domaine vital saisonnier. A cette large échelle, la sélection était dirigée vers des communautés végétales de forte biomasse (et de relativement faible qualité) (Duparc A., communication personnelle), et vers des zones où les ressources préférées étaient localement rares. Ces résultats contredisent d'autres études où les ongulés sélectionnent des patchs de haute-qualité au sein de leur domaine vital saisonnier dans le but d'atteindre leurs besoins en énergie pour la lactation ou la croissance (cerf en hiver dans Zweifel-Schielly et al. 2009, élan en été dans van Beest et al. 2010). Cela suggère que, dans notre étude, d'autres

facteurs tels que la socialité, la peur (recherche de sites refuges contre les prédateurs, Ruckstuhl & Neuhaus 2002) ou la physiologie (la température joue un rôle dans la sélection d'habitat, Marchand et al. 2015) motivaient les choix de communautés végétales. Cependant, au sein des communautés végétales choisies, les ongulés étaient hautement sélectifs et capables de composer un régime de meilleure qualité que la moyenne de la végétation disponible (papier III).
Alors que nous avons montré que la position de la niche fonctionnelle des espèces (calculé comme le CWM) était influencée par les préférences alimentaires pour le chamois et le mouflon, nous avons aussi démontré que la variation de diversité fonctionnelle de chaque trait fonctionnel (estimée comme la dispersion fonctionnelle, Laliberté & Legendre 2010) ne dépendait pas de la diversité de l'habitat. Le niveau de généralisation du régime de ces ongulés semblait être spécifique à l'espèce, contrairement aux criquets où les largeurs de niche des espèces dépendaient de la diversité fonctionnelle des habitats (Ibanez et al. 2013b). Même si des espèces coexistantes diffèrent dans leur position de niche, tel que le chamois et le mouflon dans le papier II, des niches fonctionnelles larges et chevauchantes pourraient limiter le partitionnement des niches (Ibanez et al. 2013b).

Nous résultats (papier III) montraient que les traits fonctionnels des plantes pourraient être utilisés comme déterminant de la sélection alimentaire et que différents critères de choix parmi les ongulés peuvent agir comme des mécanismes à l'origine du partitionnement des niches. De plus, nos analyses ont souligné l'importance des traits biomécaniques dans la sélection alimentaire, comme cela avait déjà été observé chez les insectes (Gomez et al. 2008, Perez-Harguindeguy et al. 2003, Ibanez et al. 2013a). Les propriétés biomécaniques des plantes ont ainsi le potentiel pour agir en tant que défenses anti-herbivores (Sanson et al. 2001), même contre les grands herbivores. De plus, nos résultats allaient plus loin car ils discriminaient les effets directs et indirects des traits fonctionnels corrélés impliqués dans la sélection alimentaire, et montraient que l'idée habituelle que les herbivores focaliseraient leur sélection vers l'ingestion de protéines (Berteaux et al. 1998, Dostaler et al. 2011) était vraie mais pas complète : ce paramètre n'avait pas toujours un effet direct mais un effet indirect sur la sélection alimentaire à travers le trait biomécanique.

- Nous avons mesuré les traits fonctionnels des plantes, mais qu'en est-il de leur correspondance avec les traits fonctionnels des herbivores ?

Etant donné que les grands herbivores sont capables de faire un choix basé sur les caractéristiques biomécaniques des plantes, cela suggère que leur morphologie contraint l'utilisation des ressources, et/ou qu'ils sont morphologiquement capable de se nourrir sur toutes les ressources mais ont des préférences pour les plantes tendres. Les plantes tendres requièrent en effet moins d'énergie pour être digérées. Dans les deux cas, cela implique des capacités innées ou apprises afin que l'animal réalise la sélection appropriée (Provenza 1995, Tixier et al. 1998). L'importance des associations morphologiques entre les ressources et les consommateurs a été mise en avant dans les études de réseaux plantes-herbivores (Ibanez et al. 2013a), les interactions prédateurs-proies (Song & Kim 2014, Spitz et al. 2014), ou les systèmes plantes-pollinisateurs (Temeles et al. 1996, Maglianesi et al. 2014). Par exemple, Ibanez et al. (2013) a montré que les traits des mandibules reflétaient les préférences alimentaires des espèces de criquets, étant donné que les criquets avec des hautes forces mandibulaires étaient capables de consommer des plantes plus dures. Dans les interactions colibri-fleur, les oiseaux avec des becs courbés atteignaient mieux le nectar des fleurs courbées que les colibris à bec droit (Maglianesi et al. 2014). Ces résultats indiquent que la morphologie peut fortement influencer l'utilisation des ressources, ce qui permet le partitionnement des niches au sein des assemblages d'espèces, et réduit la compétition. Différentes études ont démontré le lien entre

la stratégie d'alimentation des grands herbivores (browser-grazer) et les caractéristiques de l'animal (tel que le type de réticulo-rumen : Clauss et al. 2003b, Clauss et al. 2009 ; forme du museau : Gordon & Illius 1988 ; l'activité des protéines salivaires : Clauss et al. 2005 ; la taille des dents : Williams & Kay 2001, Mendoza et al. 2002, la force des muscles de mâchoires : Clauss et al. 2008a). Par exemple, Clauss et al. (2003) a montré que les grazers étaient plus à même d'avoir un régime composé de graminées que les browsers, étant donné que leur forte musculature et leur plus grand volume de réticulo-rumen permet la digestion des plantes fibreuses en relativement grande quantité. Ainsi, les grands herbivores avec des muscles forts de mâchoires et de rumen devraient être plus capables de se nourrir sur des plantes dures que les herbivores avec de muscles de mâchoires et de rumen moins forts par exemple. Ces associations sont suggérées dans le papier III. La confirmation quantitative de telles associations serait en accord la théorie d'approvisionnement optimal prédisant qu'une forte correspondance entre les traits des ressources et des consommateurs favoriserait l'efficacité d'utilisation des ressources (Pyke et al. 1977). Ainsi, une des étapes suivantes dans les interactions plantes-herbivores serait d'explorer plus loin les relations quantitatives entre les caractéristiques des plantes mangées, c'est-à-dire pas seulement leur proportion dans leur régime, et les caractéristiques intrinsèques des animaux pour déterminer jusqu'à quel point les traits fonctionnels des producteurs primaires et des consommateurs sont reliés, et pour expliquer de manière mécanique les réseaux d'interactions trophiques (Clauss et al. 2008b). Ce type d'analyses devrait être réalisé sur une grande communauté d'ongulés, tel que trouvé en Afrique, pour obtenir un gradient de caractéristiques d'herbivores. Cependant, nous sommes conscients que les traits biomécaniques ne sont pas les seuls traits impliqués dans la sélection alimentaire, étant donné que le régime résulte de multiples contraintes. D'autres traits biochimiques pourraient exercer une pression de sélection additionnelle sur la sélection alimentaire (Daering et al. 2005, Iason et al. 2005) et mener certains animaux ou espèces à se nourrir sur des plantes non-optimales en termes de traits biomécaniques (Ibanez et al. 2013a). Par exemple, les grands herbivores avec une faible force de mâchoires et de rumen pourraient se nourrir sur des plantes dures pour éviter les effets négatifs des composés métaboliques secondaires.
De plus, étant donné les plantes sélectionnées et l'anatomie des herbivores, le taux d'ingestion et de mastication serait affecté. Comme les graminées sont plus dures que les dicotylédones (plus haut contenu en fibres, plus haut contenu en matière sèche et plus faible contenu en azote), les processus de mastication et de digestion prendront plus de temps (Robbins 1983, Choong et al. 1992, Wright & Illius 1995), particulièrement si les animaux ont un réticulo-rumen moins adapté à la digestion des plantes fibreuses (Clauss et al. 2003b). Cependant, le gain en énergie que les animaux gagneraient avec un taux de passage de la nourriture plus rapide permis par une plus grande digestibilité, serait potentiellement compensé par la perte en énergie requise pour la recherche des ressources de haute qualité mais moins visibles (Pyke 1977, « process 1 » dans Spalinger & Hobbs 1992).

- Inclure la distribution spatiale de la végétation à fine échelle dans les études de sélection alimentaire

Même si nos résultats montrent l'importance de la qualité de la nourriture plutôt que la quantité dans la sélection alimentaire et un choix indépendant des fréquences de plantes dans l'environnement, nous avons tout de même besoin de plus précisément évaluer l'influence de la disponibilité des plantes dans les choix alimentaires. Une analyse spatiale plus précise de la végétation disponible serait utile pour démêler la relation entre l'hétérogénéité spatiale de la végétation (Laca et al. 2008), la sélection des ressources et la variabilité inter-individuelle. Par exemple, il a été montré que les plantes voisines peuvent influencer l'apport de nourriture (Palmer et al. 2003, Bergvall et al. 2006, Bee et al. 2008). Les études de terrain ont montré que le cerf était moins capable de brouter des plantes quand elles étaient entourées par des plantes

de moins bonne qualité car les herbivores évitaient les patchs de faible qualité, en accord avec l' « hypothèse des plantes repoussantes » (Bee et al. 2008). Au contraire, l' « hypothèse de protection grâce à l'attractivité des plantes voisines » prédit qu'une plante croissant parmi des plantes de haute qualité gagnerait une protection face aux herbivores, étant donné que les herbivores se nourriraient sur les autres plantes de bonne qualité, mais perdrait leur protection si elles étaient entourées de plantes de moins bonne qualité (Bee et al. 2008, Bergvall et al. 2006). Comme dans notre étude, les chamois et mouflon sélectionnaient des patchs de forte biomasse où les ressources préférées étaient rares, nous suggérons que le nourrissage sur des plantes préférées était accentué par les plantes voisines de faible qualité, comme prédit par l' « hypothèse de protection grâce à l'attractivité des plantes voisines ». Ici, nous aimerions déterminer si de tels processus influencent les choix alimentaires des chamois et mouflons dans la nature. Pour cela, les positions des stations d'alimentation et les régimes associés, en plus de cartes précises de la végétation décrivant l'abondance, la distribution spatiale et la qualité des plantes à une échelle fine seraient nécessaires, ce qui représente une énorme quantité de travail de terrain. Ceci pourrait aider à apporter des conclusions à propos de l'importance relative de la qualité, de la quantité et de l'hétérogénéité spatiale des plantes dans le processus de sélection alimentaire individuel par des grands herbivores sauvages.

- Problèmes

Différents problèmes ont surgi suite à l'étude des critères de sélection alimentaire. Comme nous avons utilisé des modèles mixtes généralisés dans les analyses de piste, nous n'étions pas capables de déterminer le pourcentage de variation du régime expliqué par les variables incluses dans le modèle. Comme le processus de sélection n'est pas contrôlé par un seul trait, mais par un jeu complexe de caractéristiques, il est probable que d'autres facteurs ou traits fonctionnels de plantes non inclus dans le modèle expliquent une autre large part de la variation que nous ne mesurons pas. Inclure d'autres variables dans les analyses de piste requiert de savoir comment les traits co-varient. Nous avons déjà une bonne connaissance des relations entre certains traits chimiques et biomécaniques (LNC, LCC, LDMC et dureté des feuilles) et de structure (surface foliaire, hauteur), mais nous avons peu de connaissances à propos des inflorescences de traits et leurs liens avec les traits des plantes décrits précédemment par exemple. En effet, nous pourrions facilement concevoir que la taille ou la couleur des inflorescences joue un rôle dans la sélection alimentaire (Gomez et al. 2003) en été étant donné que nous avons observé un grand nombre de plantes étêtées sur le terrain. Comme la concentration en nutriments varie entre les organes de plantes et influence l'apport de nutriments pour un herbivore (Bailey et al. 1996), l'information à propos des parties de plantes préférées par les herbivores pourrait améliorer les connaissances sur le partitionnement des ressources entre les espèces, mais ceci nécessiterait de réaliser de la microhistologie sur les faeces (Pompanon et al. 2012), des observations directes ou bien filmer des animaux en train de se nourrir à partir de caméras posées sur la tête ou le cou des animaux.

De plus, même si l'importance des traits biomécaniques, relativement aux traits de défenses chimiques tels que les composés secondaires, a été soulignée par certains chercheurs travaillant sur les insectes herbivoes (Coley 1983, Lowman & Box 1983n Ohmart & Edwards 1991, Steinbauer et al. 1998) et les mammifères (Wright & Vincent 1996), inclure des données de composés secondaires des plantes et les capacités des ongulés à les gérer pourrait aider à améliorer notre compréhension de la sélection de certaines espèces de plantes.

b) Réflexions à propos des règles d'assemblage des communautés d'ongulés

Dans notre site d'étude, la compétition n'était pas responsable de la sélection d'habitat du chamois et du mouflon à l'échelle de la communauté végétale (Darmon et al. 2012). Bien qu'il n'y avait pas d'effet négatif du mouflon sur l'axe spatial de la niche écologique du chamois, le

fort chevauchement spatial observé à une plus large échelle nous a mené à nous questionner à propos de la compétition pour les ressources alimentaires. Notre travail ne démontrait pas d'impact négatif du mouflon sur les populations de chamois sur l'axe alimentaire taxonomique et fonctionnel de la niche écologique du chamois (papier II). Les chamois et mouflons étaient en partie capables de partitionner les ressources durant les principales périodes de l'année, et même en automne quand la qualité de la végétation commençait à décroître. Ce partitionnement taxonomique et fonctionnel partiel semblait en partie permis par des différences de critères de sélection alimentaire entre le chamois et le mouflon au cours de l'année (papier III), en partie expliqué par les caractéristiques morpho-physiologiques des deux espèces (papier III). Cela maintient l'idée que des ongulés différant dans leur morpho-physiologie occupent des niches alimentaires séparées (Murray & Illius 2000). Ainsi, une espèce introduite avec une masse corporelle et des besoins écologiques similaires à une espèce native du même niveau trophique n'influence pas nécessairement les relations entre l'espèce native et ses ressources négativement, ce qui contredit l'habituelle compétition observée entre les espèces introduites et natives (Forsyth 2000, Lovari et al. 2014, Ferretti et al. 2015). Même si la coexistence a été observée sur les axes spatiaux, temporels et alimentaires de la niche écologique, la question de la compétition entre les ongulés doit continuer à être explorée dans le contexte d'augmentation des ongulés, de densité-dépendance (Garel et al. 2011), et au regard des conclusions contrastées quant à la co-occurrence du chamois et mouflon (Bertolino et al. 2009, Chirichella et al. 2013). La fiabilité du patron de coexistence dans les interactions chamois/mouflons devrait être précisée à partir d'autres études sur d'autres paires de sites en sympatrie/allopatrie par exemple. De plus, passer les analyses du partitionnement de la ressource au niveau individuel pourrait aider à déterminer si la compétition pourrait agir à une plus fine échelle et impacter seulement quelques individus.

Alors que la compétition entre espèces est principalement vue comme une force structurante des communautés, la facilitation pourrait aussi être impliquée dans la coexistence (Gordon 1988, Arsenault & Owen-Smith 2002, Waldram et al. 2008). Dans notre étude (papier II), nous avons observé que la population de chamois en sympatrie avait un régime égal ou de meilleure qualité qu'en allopatrie. Nous avons suggéré différentes hypothèses pour cela (voir discussion du papier II), et l'une d'elle était un effet de facilitation des populations de mouflon. En effet, les mouflons pourraient permettre un meilleur accès à des ressources sous-optimales de bonne qualité pour le chamois en sympatrie, en réduisant la hauteur des graminées ou en retirant les tiges (Arsenault & Owen-Smith 2002). Cet effet pourrait être prédominant durant la saison de végétation (Arsenault & Owen-Smith 2002). Cependant, la compétition devrait dominer la facilitation durant les périodes de faible disponibilité de biomasse (Arsenault & Owen-Smith 2002). A cause d'un trop faible effectif et parce que le mouflon migrait vers les habitats forestiers en hiver, nous n'avons pas été capables d'évaluer les interactions entre les chamois et les mouflons durant une période de faible disponibilité des ressources (en automne, la disponibilité des ressources décroît mais pas assez pour entraîner de la compétition, papier II). Cependant, en hiver, les mouflons sont sujets à des chevauchements avec les autres ongulés (chevreuils et cerfs, Redjadj 2010), et comme les individus devraient être restreints à de petites zones refuges où la végétation est encore disponible, la densité de population dans les zones de nourrissage devrait augmenter et en retour, augmenter la compétition inter- et intra-spécifique. Cependant, cela reste à tester, comme réalisé dans le papier II, en comparant des situations en sympatrie et en allopatrie.

c) Pourquoi les espèces diffèrent dans leur variabilité inter-individuelle ? Une question non résolue

Une des questions qui reste sans réponse à la fin du papier I était la détermination des causes de la variabilité intra-spécifique et pourquoi le degré variait entre les espèces. Nos résultats démontraient que l'hypothèse de variation de niche (NVH, Van Valen 1965) était supportée aux niveaux intra- et inter-spécifiques : plus la niche était large, plus la variation inter-individuelle était grande. Notre hypothèse que la socialité (SH) puisse jouer un rôle dans la variabilité inter-individuelle a été rejetée. Au contraire, l'espèce la plus sociale avait le plus haut degré de variabilité inter-individuelle et la plus large niche de population. La socialité pourrait en fait être vue, non pas comme un facteur de similarité de régime due aux chevauchements spatiaux d'individus dans un groupe, mais comme un facteur de dissimilarité à cause d'une plus forte compétition intra-spécifique dans un groupe social (l'agressivité dans des grands groupes d'élans a été notée par Molvar & Bowyer 1994) menant à une plus forte différentiation de régime. Ces résultats sont en accord avec des études expérimentales réalisées avec des poissons (Svanbäck & Bolnick 2007) pour lesquels la variabilité inter-individuelle augmentait avec les densités de population. Au contraire, les espèces solitaires comme le chevreuil devraient être moins sujettes à la compétition intra-spécifique à une échelle spatiale fine, comme chaque individu devrait être capable de se nourrir sur ses ressources préférées sans pression par les congénères dans leur voisinage proche. De plus, comme les chevreuils sont supposés avoir une plus faible plasticité digestive que le mouflon, nous supposions que les caractéristiques morpho-physiologiques contraindraient le degré de variabilité inter-individuelle.

Des analyses plus poussées devraient être réalisées pour aller plus loin dans la compréhension des causes de la spécialisation individuelle et pourquoi cela varie entre espèces. Ceci nécessiterait une plus grande diversité d'herbivores, comme trouvée en Afrique, qui retirerait les effets confondants entre la socialité, la masse corporelle et le type alimentaire (par exemple, différents niveaux de socialité pour un seul type alimentaire, Jarman 1974, Fritz et Loison 2006).

Au niveau intra-spécifique au sein d'une saison, différents facteurs tels que le sexe, l'âge, l'expérience, le statut social, les besoins physiologiques ou les préférences (Araùjo et al. 2011) peuvent promouvoir la variabilité inter-individuelle. Par exemple, les statuts des individus au sein d'un groupe peuvent favoriser les différences de régime : des individus subordonnés pourraient être forcés à se nourrir sur des ressources suboptimales (Araùjo et al. 2009). De plus, le sexe pourrait partiellement expliquer la variation inter-individuelle, et le degré de variation inter-individuelle pourrait varier entre les sexes (Nifong et al. 2015, Smith et al. 2015). Des analyses supplémentaires avec nos données seraient nécessaires pour estimer les effets du sexe sur la variabilité inter-individuelle. Des facteurs environnementaux pourraient aussi entrer en jeu dans la spécialisation individuelle. En effet, des différences fines dans la distribution spatiale des ressources (Durell 2000) pourraient accentuer la variabilité inter-individuelle.

Finalement, dans ce travail, une faeces n'était pas associée à un individu et nous n'étions pas ainsi au courant de la variation de régime sur plusieurs jours ou mois. Même si les faeces sont une approximation du régime d'un individu sur plusieurs jours (Castle 1956, Rayé et al. 2011), le régime d'un individu tend à être plus diversifié que le régime évalué à partir d'une de ses crottes. Ainsi, une autre exploration idéale serait de mener des études longitudinales dans le but de suivre des individus sur une longue période de temps, à travers l'identification génétique des faeces, et étudier la variation intra-individuelle des régimes.

d) Lier la β-diversité taxonomique et fonctionnelle

Au sein d'une espèce, il a été démontré que les individus domestiques et sauvages (moutons, chèvres, daims) montrent différentes préférences pour les ratios protéines/énergie pour atteindre leurs besoins (Atwood et al. 2001), mais aussi qu'ils peuvent répondre différemment aux composés secondaires des plantes (Provenza et al. 2003, Bergvall 2009). Ainsi, des différences

individuelles dans les critères de sélection alimentaire pourraient être observées chez le chamois et le mouflon comme il a été noté une relativement forte variabilité inter-individuelle au niveau taxonomique. Nos analyses de critères de sélection alimentaire (papier III) ont pris en compte tous les individus et pas la moyenne des régimes de la population. Cependant, la variabilité inter-individuelle n'a pas été quantitativement mesurée. Même si nos résultats ont montré des effets significatifs de certains traits des plantes sur la sélection alimentaire (dureté des feuilles et contenu en azote, papier III), nous faisons l'hypothèse que l'absence de relation significative pour les autres traits pourrait être reliée à (1) l'absence d'effets pour tous les individus, (2) une forte variabilité inter-individuelle cachant un patron général au niveau de l'espèce. Des analyses supplémentaires sur la β-diversité fonctionnelle (Swenson et al. 2010, Meynard et al. 2011, Villéger et al. 2013) pourraient donner des informations sur la variation inter-individuelle des niches des régimes fonctionnels. Ceci pourrait aider à déterminer si certains individus bénéficient d'une meilleure qualité de ressources que d'autres. Par exemple, une population peut montrer une forte variabilité inter-individuelle au niveau fonctionnel, suggérant que les individus évitent la compétition intra-spécifique en se nourrissant sur différentes espèces de plantes, mais aussi évitent des apports différents en nutriments entre individus en se nourrissant tous sur des plantes de bonne qualité. Au contraire, une plus haute β-diversité fonctionnelle suggérerait un déséquilibre dans l'accès aux nutriments entre individus, et ainsi, des différences dans le développement, la croissance et la reproduction. Ces approches taxonomique et fonctionnelle de la variabilité inter-individuelle des régimes pourraient aider à comprendre la variation de fitness entre individus, et par conséquent les conséquences sur la dynamique des populations.

e) Réflexions à propos de l'effet des densités croissantes de populations sur la variation inter-individuelle, et comment cela pourrait affecter les dynamiques de populations

En plus de l'émergence de l'écologie fonctionnelle, l'intégration de la variabilité intra-spécifique est une approche prometteuse pour comprendre la dynamique des communautés (voir l'introduction de la thèse). Nos résultats sur les ongulés (papier I) ont aussi confirmé que la variabilité inter-individuelle était la règle plutôt que l'exception et que les contraintes morpho-physiologiques n'étaient pas aussi fortes que précédemment pensé, étant donné que les trois espèces (et même le chevreuil, quelques fois classifié comme « non-grazer obligatoire » et supposé se nourrir sur quelques ressources, Abbas et al. 2011, 2013, Redjadj et al. 2014) montraient une plasticité digestive. En effet, la variabilité inter-individuelle au niveau de la niche alimentaire est un phénomène ubiquiste rencontré chez les vertébrés et les invertébrés comme les gastéropodes, les poissons, les amphibiens ou les reptiles (Bolnick et al. 2007).

Comme nous sommes dans un contexte d'augmentation des densités de populations en Europe (Milner et al. 2006, Maillard et al. 2010, liste rouge de l'IUCN : augmentation des tendances pour le chevreuil, le cerf, l'isard et le bouquetin/tendances inconnues pour le chamois et le sanglier), nous sommes particulièrement intéressés à la façon dont les individus répondraient à des augmentations de densités conspécifiques et allospécifiques. Si la densité de population augmente, est-ce que les individus vont plus se spécialiser dans le but de réduire la compétition ? Ou vont-ils augmenter leur largeur de niche pour inclure des ressources suboptimales comme les préférées vont décroître, et ainsi homogénéiser la population ?

Dans la fig.39 (voir thèse en anglais), nous suggérons différentes évolutions possibles de la variabilité inter-individuelle comme une conséquence de l'augmentation des densités de populations (due à une plus forte abondance des populations ou une agrégation d'individus dans des zones spécifiques), à travers la compétition intra-spécifique (fig.39 a, b), et la compétition inter-spécifique (fig.39 c, d). Ces prédictions ne sont pas exhaustives et nous avons vu dans

l'introduction que, en plus du type de préférence de l'espèce focale, plusieurs autres facteurs peuvent influencer la direction de la spécialisation individuelle, tel que la plasticité ou la disponibilité des ressources (voir section 2.2.d, Araùjo et al. 2011). Pour ces prédictions (fig. 39), nous nous sommes focalisés sur des cas où les ressources préférées diminuaient. Cependant, la compétition intra-spécifique peut aussi faire diminuer l'abondance des ressources suboptimales de l'espèce focale. Nous avons supposé que cela impacterait la position ou la largeur de niche des individus et des espèces focales, et nous ne l'avons pas exploré.

A cause de l'augmentation des densités de populations d'ongulés en Europe (Milner et al. 2006, Maillard et al. 2010, liste rouge de l'IUCN : augmentation des tendances pour le chevreuil, le cerf, l'isard et le bouquetin/tendances inconnues pour le chamois et le sanglier), la disponibilité des ressources préférées pourrait décroître. Ainsi, la compétition intra- et inter-spécifique serait renforcée et influencerait la variabilité inter-individuelle avec les espèces compétitrices.
Malgré les multiples sorties de la variabilité inter-individuelle, nous avons vu dans l'introduction que différentes études sur différents organismes tirent des conclusions similaires, soit (1) la compétition intra-spécifique induit une variation inter-individuelle à travers des individus qui utilisent différents types de ressources (Araùjo et al. 2008, Svanbäck et al. 2004, 2007, fig.40), (2) la compétition inter-spécifique réduit la largeur de niche à travers la diminution de variation dans l'utilisation des ressources (inverse de la « libération écologique ») (Van Valen 1965, Knudsen et al. 2007, Costa et al. 2008, Bolnick et al. 2010, fig.40). En retour, les changements de variabilité inter-individuelle causés par le changement de position de certains individus sur des ressources suboptimales mèneraient à une diminution de la qualité des régimes. Comme la qualité des régimes affecte les conditions corporelles (Prins 1996, WallisDeVries 1998, Stewart et al. 2005), cela impacterait la survie, la croissance et la reproduction des individus (fitness) (Kie 2003, Cook et al. 2004, fig.40). Par conséquent, la densité de populations diminuerait en retour (fig.40). Ce patron serait en accord avec les phénomènes de densité-dépendance (Saether 1997, Coulson et al. 2000, Stewart et al. 2005, Bonenfant et al. 2009, et références citées) et de compétition inter-spécifique (Forsyth & Hickling 1998, Latham 1999, Richard et al. 2010, Lovari et al. 2014, Ferretti et al. 2015) régulant les densités de populations dans un environnement libéré des prédateurs.

Comment la densité affecte la variabilité inter-individuelle n'est pas bien comprise. En effet, suivre l'évolution de la variation inter-individuelle comme une fonction de la densité de population requiert que des échantillonnages de faeces extensifs à long terme et intensifs, en plus de comptages de populations, soient faits pour des espèces co-existantes au sein d'une communauté (Nicholson et al. 2006). De plus, comme les caractéristiques de l'habitat (composition de la végétation et structure) peuvent fluctuer en réponse à la pression d'herbivorie ou aux changements abiotiques environnementaux (température, précipitation), cela peut mener à des réponses variables des animaux pas seulement dépendantes de la densité (Kie et al. 2003).
Sinon, nous pourrions suivre l'effet de la densité sur la variabilité inter-individuelle en comparant des sites différant dans leurs densités de populations. Cependant, cela nécessiterait que les sites aient les mêmes capacités d'accueil pour que la qualité de l'habitat ne soit pas un effet confondant de la densité-dépendance (Kie et al. 2003).

Les études essayant de déterminer ou de prédire comme les densités de populations affectent la dynamique de population à travers la densité-dépendance (Stewart et al. 2005, Bonenfant et al. 2009 et références citées) ou la compétition inter-spécifique (Forsyth & Hickling 1998, Richard et al. 2010, Lovari et al. 2014, Ferretti et al. 2015), se focalisent habituellement sur une espèce impactée. Dans le but de prédire comment différentes populations sont régulées en considérant

les interactions intra- et inter-spécifiques, il est nécessaire d'utiliser une approche basée sur les réseaux incluant de multiples espèces d'une communauté (Montoya et al. 2006n Ings et al. 2009, Blüthgen et al. 2010). En effet, l'approche en réseau serait utile pour mieux comprendre comment les grands herbivores se structurent en fonction des ressources alimentaires, mais aussi comment la diversité et la densité des ongulés et des ressources affectent la stabilité des systèmes (Thebault et al. 2005, 2010, Narwani & Mazumder 2012). Nous avons vu dans cette thèse que les approches basées sur les individus pourraient améliorer notre compréhension de la structuration des communautés. Ainsi, étudier les réseaux d'interactions trophiques au niveau individuel (Ings et al. 2009, Dupont et al. 2011, Tur et al. 2014, Dupont et al. 2014) serait nécessaire pour explorer l'évolution de la stabilité des réseaux dans le temps en réponse aux changements de densité des populations d'ongulés ou aux compositions et abondances des communautés de plantes.

### 7.3. Intégrer les interactions fonctionnelles entre grands herbivores et communautés végétales dans des modèles de distribution d'espèces

Face aux changements globaux, les modèles de distribution d'espèces cherchent à développer des modèles quantitatifs capables de prédire à quel point une espèce s'adaptera dans le futur (Thuillet et al. 2013, Wisz et al. 2013). Cependant, ces modèles n'intègrent pas souvent les interactions biotiques entre espèces (tels que la compétition, la prédation ou la facilitation) qui varient dans le temps et dans l'espace (Poisot et al. 2012) et qui influencent les patrons de distribution des espèces (Guisan & Thuiller 2005, Van der Putten et al. 2010, Thuiller et al. 2013, Wisz et al. 2013). Ainsi, la combinaison des réseaux trophiques et des modèles de distribution des espèces pourrait être particulièrement utile pour prédire les variations spatiales et temporelles de la composition des communautés (Pellissier et al. 2013).
Dans les paragraphes suivants, nous voulons montrer comment nos résultats sur les interactions fonctionnelles entre grands herbivores et communautés végétales pourraient être utilisés dans les modèles de distribution d'espèces. Etant donné nos résultats, les modèles de distribution d'espèces pourraient concerner les grands herbivores ou les communautés végétales. Cependant, comme ils sont liés par des interactions trophiques, ils peuvent mutuellement s'affecter, ce qui rend les modèles compliqués. Dans le premier cas où les espèces de plantes limitent les distributions d'herbivores, nous supposerons que les fluctuations de la composition de la végétation et les abondances relatives dépendent d'autres facteurs que la sélection par les herbivores, tels que le climat et le changement d'usage des terres. Dans le deuxième cas où les herbivores limitent les distributions d'espèces de plantes, nous supposerons que la pression d'herbivorie est constante sur les mêmes traits fonctionnels et n'est pas influencée par les changements de végétation.

a) Les espèces de plantes peuvent limiter la distribution des herbivores

Comme les herbivores doivent faire face aux changements environnementaux où la disponibilité des ressources alimentaires fluctue en termes de composition et d'abondance relative, prédire si une population d'ongulés pourrait persister dans un tel environnement fluctuant pourrait être utile aux programmes de conservation ou à la gestion des populations.
Dans cette thèse, nous avons montré que la sélection alimentaire est en partie dépendante des traits fonctionnels de plantes tels que la dureté et le contenu en azote des feuilles, suggérant que les traits fonctionnels des plantes peuvent déterminer la distribution des herbivores. En effet, si la sélection alimentaire des herbivores est connue en termes de traits fonctionnels, il serait ainsi possible d'ignorer la composition locale des communautés végétales et de prédire le régime des grands herbivores dans un nouvel environnement à partir de la connaissance des traits fonctionnels des plantes et des préférences des herbivores. Même si nous savons que les

dynamiques de populations d'ongulés se sont pas seulement dirigées par des contrôles « bottom-up » (ressources alimentaires) mais aussi par des effets « top-down » ou « transversaux », la connaissance de la qualité de leur régime dans un environnement changeant pourrait en partie aider à prédire leur persistance.
Cependant, cette approche requerrait d'avoir des informations à la fois sur les traits des plantes, la distribution et la proportion relative des espèces de plantes dans le paysage étudié et leur évolution dans le temps, dans le but de déterminer des cartes fonctionnelles précises de la végétation. De plus, la connaissance des limites physiologiques des ongulés, tels que leur limite inférieure de besoins énergétiques requis pour survivre et se reproduire ou comment ils répondent à des augmentations de température (Marchand et al. 2015), devrait être évaluées pour modéliser leur distribution spatiale dans des environnements changeants (Thuiller et al. 2013).
Finalement, pour avoir confiance dans nos résultats sur les critères de sélection alimentaire et déterminer s'ils peuvent être généralisés et utilisés pour d'autres études telles que la modélisation des distributions spatiales d'espèces, des études supplémentaires dans d'autres zones géographiques différant dans la composition d'espèces de plantes devraient d'abord être conduites.

b) Les herbivores peuvent limiter la distribution des espèces de plantes

Le climat et le changement d'usage des terres (intensification ou abandon du pâturage domestique) sont deux facteurs supposés influencer fortement la végétation des écosystèmes tempérés (Boulangeat et al. 2014). Leurs impacts simultanés sur la biodiversité à des échelles régionales dans le parc des écrins ont été testés (Boulangeat et al. 2014). Nous pourrions supposer que les grands herbivores pourraient aussi modifier ou accentuer ces précédents effets. En effet, comme les densités de grands herbivores sont en augmentation dans certaines régions du monde depuis des dizaines d'années (Côté et al. 2004, Milner et al. 2006, Maillard et al. 2010, liste rouge de l'IUCN), ils peuvent avoir des impacts significatifs sur la végétation et sur les communautés aériennes et souterraines, menant à des problèmes de conservation (Rooney 2001, Mysterud 2006). Ainsi, une nouvelle approche prenant en compte les effets triples du climat, du changement d'usage des terres et des herbivores sauvages, pourrait être utilisée pour prédire plus spécifiquement la dynamique des communautés végétales. Le modèle pourrait prendre en compte les préférences taxonomique et fonctionnelle des herbivores, leur sélection d'habitat et leur intensité d'utilisation de la ressource.

En 1983, à la fin du premier chapitre de son livre « Wildlife feeding and nutrition », Robbins disait que « l'application de la plupart des données de nutrition de la faune sauvage pour la gestion est à la fois un art et une science à cause du manque de connaissance adéquate des principaux mécanismes de contrôle déterminant les conséquences des manipulations ». Enfin, il conclut que beaucoup de défis sont à relever, particulièrement à propos des questions de compétition, de nutrition hivernale, de choix de régime alimentaire, de l'effet de la manipulation d'habitat, des interactions prédateurs-proie, etc. Quarante-deux ans plus tard, je me sens plus optimiste à propos de la fiabilité des données que nous obtenons à propos de l'écologie alimentaire de la faune sauvage. La littérature citée et le travail réalisé ici montrent que des progrès ont été faits sur la compréhension des interactions entre individus et entre espèces, sur le régime alimentaire des animaux lors des périodes de faible disponibilité en ressources, sur les facteurs impliqués dans les choix alimentaires mais aussi sur l'amélioration de la

méthodologie qui permet notamment d'avoir des données beaucoup plus précises sur les régimes alimentaires (ADN-metabarcoding). Une grande part des importantes bases de données utilisées dans cette thèse reste à explorer (par exemple, les régimes des populations forestières, l'influence des traits fonctionnels foliaires sur le choix alimentaire, les ratios de sélection) et devraient apporter de nouvelles clés de compréhension sur le fonctionnement des écosystèmes. J'espère finalement que je vous ai convaincu que les approches taxonomiques et fonctionnelles, et les études des interactions intra- et inter-spécifiques, peuvent apporter des visions complémentaires utiles à notre compréhension des processus écologiques, et que certains de nos résultats peuvent être pertinents pour des questions de conservation et de gestion.

# Résumés des différents articles insérés dans la thèse

## 1. L'hypothèse de variation de niche étendue de l'intra- à l'inter-spécifique

Bison M., Ibanez S., Redjadj C., Boyer F., Coissac E., Miquel C., Rioux D., Saïd S., Maillard D., Taberlet P., Yoccoz N.G., Loison A.

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Résumé
L'hypothèse de variation de niche (NVH) prédit que les populations avec des niches plus larges devraient avoir une plus forte variabilité inter-individuelle. Cette prédiction avait été développée à l'origine au niveau intra-spécifique. Nous émettons ici l'hypothèse qu'elle pourrait être étendue au niveau inter-spécifique : les individus d'une espèce généraliste devraient être plus différents entre eux que les individus d'une espèce spécialiste. Nous avons ainsi testé l'hypothèse de variation de niche à l'intra- et à l'inter-spécifique en se basant sur une large base de données de régime alimentaire de trois grands herbivores sauvages, déterminée à partir de faeces collectées sur le terrain et analysés avec la méthode d'ADN meta-barcoding. Les trois grands herbivores (chevreuil *Capreolus capreolus*, chamois *Rupicapra rupicapra* et mouflon *Ovis gmelini musimon*) sont hautement contrastés en terme de socialité (solitaire à grégaire) et de régime. L'hypothèse de variation de niche au niveau intra-spécifique a été testée en reliant, pour la même population, la largeur de niche et la variabilité inter-individuelle, pour quatre saisons. Comparés aux modèles nuls, nos données supportent l'hypothèse de variation de niche aux niveaux intra- et inter-spécifiques. La variabilité inter-individuelle des régimes des espèces solitaires n'était pas plus grande que chez les espèces sociales, bien que les individus sociaux se nourrissent ensemble et pourraient avoir des régimes similaires. Ainsi, l'hypothèse de variation de niche expliquait mieux la largeur de niche que d'autres facteurs comme la socialité. L'expansion de la niche des populations des trois espèces était dirigée par la disponibilité en ressources, et obtenue par une augmentation de la variabilité inter-individuelle, et le niveau de variabilité inter-individuelle était plus grand chez les espèces généralistes (mouflon) que chez les espèces spécialistes (chevreuil). Ce mécanisme à la base de l'hypothèse de variation de niche apparaît entrer en jeu à différents niveaux de l'organisation biologique, des populations aux communautés.

## 2. Différentiation des niches taxonomique et fonctionnelle deux espèces de grands herbivores

Bison M., Dupeyras P.A., Redjadj C., Miquel C., Rioux D., Taberlet P., Maillard D., Yoccoz N.G., Loison A.

En préparation pour Journal of Ecology

Résumé
Les espèces introduites posent le problème de sympatrie forcée avec des espèces natives, plus particulièrement quand elles ont des besoins écologiques similaires. Les chamois (*Rupicapra rupicapra*, natif) et mouflons (*Ovis gmelini musimon*, introduit) ont coexisté dans le massif des Bauges (Alpes, France) depuis 60 ans. Nous avons analysé les interactions trophiques inter-spécifiques entre les deux espèces dans le but de déterminer les mécanismes impliqués dans leur coexistence. Nous avons utilisé des faeces d'ongulés échantillonnées de mai à novembre dans deux alpages, l'un occupé par des chamois vivant en allopatrie et l'autre occupé par des chamois vivant en sympatrie avec le mouflon. Tout d'abord, nous avons analysé les plantes contenues dans les faeces avec la méthode d'ADN meta-barcoding dans le but de comparer les niches taxonomiques des chamois et des mouflons. Deuxièmement, nous avons calculé les niches fonctionnelles des régimes de chamois et mouflons en utilisant une base de données de traits fonctionnels de plantes (LNC, LPC, LMDC) mesurés sur les plantes composant principalement le régime. Les niches taxonomiques et fonctionnelles diffèrent entre les chamois en sympatrie et les mouflons, et leurs différences varient en intensité avec la saison. Aucune différence n'est observée entre les deux sous-populations de chamois au printemps et en automne. Des petites différences entre les deux sous-populations de chamois sont observées en été, mais sont expliquées par la présence de buissons dans le régime du chamois en sympatrie. Cependant, la qualité du régime de la sous-population de chamois en sympatrie est toujours égale ou supérieure à la population en allopatrie, soulignant que le mouflon n'a pas d'impact négatif sur la population de chamois. Nos résultats montrent que des espèces introduites et natives n'ayant pas co-évolué ensemble peuvent coexister en partitionnant leur régime. Ces résultats sont en lien avec de précédentes études comportementales et spatiales qui concluaient sur l'absence de compétition par interférence chez les mêmes populations coexistantes de chamois et de mouflon.

## 3. La contribution relative des traits biomécaniques et biochimiques dans la sélection des plantes par des ongulés au cours de l'année

Bison M., Ibanez S., Puijalon S., Dehédin A., Dupeyras P.A., Langrand E., Redjadj C., Miquel C., Rioux D., Taberlet P., Maillard D., Loison A., Yoccoz N.G.

En préparation pour Functional Ecology

Résumé

1. Les traits fonctionnels sont des candidats prometteurs pour expliquer comment les réseaux d'interactions trophiques s'organisent. Cependant, l'importance relative des traits fonctionnels corrélés dans la sélection alimentaire n'a pas été étudiée, car les données sont rares et à cause de la corrélation entre les traits.
2. Pour répondre à cet objectif, nous avons compilé une base de données unique combinant (1) le régime saisonnier de grands herbivores alpins coexistant dans le massif des Bauges (chamois et mouflons) obtenu à partir de la méthode d'ADN meta-barcoding réalisée sur des crottes échantillonnées toute l'année, (2) la biomasse des plantes disponibles au printemps, en été et en automne, et (3) différents traits fonctionnels biomécaniques et biochimiques mesurés sur 57 espèces de plantes.
3. Nos résultats montrent que les deux espèces ont un régime de meilleure qualité que ce qui est disponible, même en automne où la végétation est sénescente. Cependant, la corrélation entre les traits obscurcit ce patron. Nous avons ainsi utilisé des analyses de pistes dans le but de démêler les effets biomécaniques et biochimiques directs et indirects sur la sélection alimentaire. Pour le chamois, la caractéristique la plus importante était la dureté quelle que soit la saison. Pour le mouflon, au contraire, il n'y avait pas de sélection pour les traits mesurés au printemps, une sélection pour les plantes riche en azote en été, et pour les plantes facilement mastiquables en automne.
4. Ces critères de sélection contrastés entre les espèces d'ongulés peuvent être reliés à leur caractéristiques morpho-physiologiques (masse des muscles de mâchoires, force des muscles du reticulo-rumen) et peuvent contribuer à expliquer leur coexistence. La découverte des critères de sélection alimentaire basés sur les traits pourrait aussi renforcer notre capacité à prédire l'effet des herbivores sur la dynamique des communautés de plantes.

## 4. Comparaison de deux méthodes pour estimer la qualité du régime des grands herbivores : mesure du contenu en azote fécal dérivé du NIRS vs mesure du contenu en azote du régime à partir des plantes inégérées.

Bison M., Redjadj C., Saïd S., Miquel C., Rioux D., Taberlet P., Maillard D., Yoccoz N.G., Loison A.

Résumé
L'estimation de la qualité du régime est un outil utile pour étudier le statut des populations naturelles. Le contenu en azote fécal (FN) a été largement utilisé comme proxy de l'azote ingéré (DN). Cependant, la fiabilité et la précision du FN comme estimateur du DN a été questionnée, principalement à cause de l'effet des composés métaboliques secondaires sur la libération de l'azote dans les faeces. Notre objectif est de déterminer jusqu'à quel point et jusqu'à quelle échelle temporelle nous pouvons compter sur le FN pour estimer le DN chez deux espèces d'ongulés (chamois et mouflon). Nous avons aussi testé si la comparaison du FN entre espèces était faisable. Enfin, nous avons analysé si le type alimentaire de l'ongulé pouvait expliquer les pentes reliant le FN au DN pour différentes espèces en examinant les relations trouvées dans la littérature sur les ongulés. Nos résultats montrent que les équations reliant le FN et le DN des chamois et mouflons ne sont pas assez prédictives pour étudier quantitativement la qualité du régime à partir des indices fécaux dans notre étude, aux niveaux intra- et inter-saisonniers. Aussi, l'utilisation des valeurs de FN n'est pas adaptée pour comparer la qualité du régime d'ongulés différant dans leur régime et dans leur morpho-physiologie. Enfin, le type alimentaire ne peut pas expliquer les différentes relations observées entre le FN et le DN pour différentes espèces d'ongulés, et nous suggérons que d'autres paramètres pourraient être inclus dans les modèles pour expliquer les variations de pente. Pour conclure, nous conseillons d'utiliser les estimations de FN pour prédire la qualité du régime de manière qualitative à une large échelle de temps et pour des espèces et des sites spécifiques, étant donné que la composition du régime et les composés secondaires associés pourraient influencer la libération du FN de manière significative.