Universitat Autònoma de Barcelona

Abiotic and biotic factors determining the nutrient stoichiometry of contrasting terrestrial ecosystems

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Abiotic and biotic factors determining the nutrient stoichiometry of contrasting terrestrial ecosystems.

Ph.D. Thesis
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Abstract

Everything on Earth is based on chemistry. This statement has profound implications for ecological interactions. Living organisms generate and control fluxes of energy and matter among the atmosphere, lithosphere and the hydrosphere, shaping the chemistry of the Earth in many different ways. Ecological stoichiometry aims to explore the balance and role of multiple chemical elements in ecological interactions and help us to understand patterns and processes in nature. It represents the link between the biogeochemistry and the ecosystems' function and allows to describe processes across different levels of biological organization, from cellular structures to ecosystems.

In this Thesis I use ecological stoichiometry to describe processes at organism and ecosystem levels in three contrasting terrestrial environment conditions. Autotrophs' stoichiometry is established when these organism use light to fix carbon (C) and simultaneously assimilate nutrients. Plants are able to store nutrients in the cells' vacuole and in different organs, which make them highly flexible (less homeostatic) in terms of their elemental composition. This feature explains the high adaptability of plants to different environments, including soil nutrient limitation conditions. Furthermore, plant-soil interaction could be explored through the foliar stoichiometry, because it has been shown that the foliar N:P is positive correlated with the N:P of soil in all terrestrial ecosystem, suggesting that foliar stoichiometry is a good indicator of the resource availability.

Plant adaptations to soil nutrient limiting conditions are quite common in all terrestrial ecosystems, such as nitrogen fixation, mycorrhiza association, production of phosphatases and nutrient resorption before leave abscission. The species' chemical composition is affected by all these abiotic and biotic interactions, and these exchange of chemical elements between the species and the abiotic part of the system determine the elemental composition of different components of the ecosystems.

In Chapter 2, we explore the biotic effect of the community composition on the species foliar stoichiometry, taken as a proxy of the species' biogeochemical niche. We found that each species has its own biogeochemical niche and is able to readjust its chemical composition in response to different biotic conditions. We conclude that plants can readjust their foliar element composition when they grow in communities with contrasting plant composition through the biogeochemical niche displacement, suggesting a differential use of the resources when the patterns of species coexistence change.

In Chapter 3 we explore the plant-soil stoichiometry changes due the shrub expansion into the subalpine grassland in the Pyrenees. Shrub expansion had a clear impact on the plant-soil stoichiometry spectrum. This expansion represents the transition from pure grassland to shrubland. The grassland is an ecosystem dominated by short-lived species, fast nutrient turnover between the plant-soil compartments, high nitrogen (N), phosphorus (P) and potassium (K) concentrations in the plant-soil system, high productivity but low biomass stocks. The shrubland is an ecosystem characterized by long-lived species with more conservative strategy, slow nutrient turnover (low N and P concentrations in the plant-soil compartments, high C:nutrient ratios in the aboveground biomass) and high stocks of C and nutrients in the plant aboveground biomass. Shrub encroachment increase the acquisition of

N through mycorrhizal associations. The changes in storage and elemental composition of the plant-soil system along the succession from grassland to shrubland suggests that there is a slowdown of the biogeochemical cycle in the subalpine mountain areas where shrub encroachment occurred.

In the Chapter 4, we describe the distribution of C and the most important nutrients for the plant development (N, P, K) in the plant and soil compartments in old-growth tropical forests growing in nutrient-poor soil in French Guiana. We also studied the nutrient resorption from senescent leaves, a poorly explored mechanism that plants use to avoid losing nutrients in this ecosystem. Our results showed that P was the scarcest nutrient in the leaf, leaf-litter and soil. Resorption efficiencies were higher for K and P than for N, and only K resorption efficiency was affected by seasonality. P resorption showed a negative and weak correlation with P in soil (total and available). Relationships between nutrient resorption and species functional characteristics (growth rate, wood density, diameter at breast height and specific leaf area) were weak and varied among the nutrients, and phylogenetic relatedness did not account for the variability in resorption efficiencies. Our results suggest that high K and P resorption from senescent leaves is an adaptive strategy allowing species to cope with soil nutrient scarcity. Furthermore, the level of nutrient immobilization in foliar compounds (N > P > K) seem to significantly determine the resorption process. We conclude that nutrient resorption from senescent leaves is a key process for plants to conserve nutrients in tropical forests of French Guiana, especially for K and P, where soil availabilities are low and depend mainly on soil parent material and leaching process.

To sum up, in this Thesis we have desmostrated how the elemental composition of the plant-soil system reflects ecological interactions and processes, such as intra and inter specific plant interactions (Chapter 2), poorly explored physiological processes such as nutrient resorption (Chapter 4) and the importance of stoichiometry studies for describing changes at ecosystem level and predicting future scenarios (Chapter 3). These studies add new knowledge to the ecological stoichiometry field and highlights the importance of this approach in the ecological studies.

Resumen

Todo en la tierra es química. Esta afirmación tiene profundas implicaciones para las interacciones ecológicas. Los organismos vivos promueven y controlan flujos de materia y energía entre la atmosfera, hidrosfera y litosfera; modificando la composición química de la tierra de muchas maneras diferentes. La estequiometría ecológica estudia el balance y el papel de múltiples elementos químicos en las interacciones ecológicas y nos ayuda a entender patrones y procesos en la naturaleza. Representa el enlace entre la biogeoquímica y el funcionamiento de los ecosistemas, permitiéndonos describir procesos a todos los niveles de organización biológica, desde estructuras sub-celulares a ecosistemas.

En esta Tesis he usado la estequiometría ecológica para describir procesos a nivel de organismo y ecosistema en tres condiciones ambientales terrestres diferentes. La estequiometría de los autótrofos se establece cuando estos usan luz para fijar carbono (C) y simultáneamente asimilan nutrientes. Las plantas son capaces de almacenar nutrientes en la vacuola intracelular y en diferentes órganos, lo que hace que su estequiometría sea muy flexible (baja homeostasis) y se adapten a diferentes ambientes, incluyendo condiciones del suelo limitantes para el desarrollo de las plantas. También, la interacción planta suelo se puede explorar a través de la estequiometría foliar, ya que se ha demostrado en todos los ecosistemas terrestres que el N:P foliar esta correlacionado positivamente con el N:P del suelo, sugiriendo que la estequiometría foliar es un buen indicador de la disponibilidad de nutrientes.

Las adaptaciones de las plantas a condiciones limitantes de nutrientes en el suelo son comunes en todos los ecosistemas terrestres, como es la fijación de nitrógeno, la asociación con micorrizas, producción de fosfatasas o la reabsorción de nutrientes desde las hojas senescentes para el reciclado interno de nutrientes. La composición elemental de las especies es afectada por esas interacciones abióticas y bióticas, y el intercambio de elementos químicos entre las especies y el espacio abiótico determinaran la composición elemental de las diferentes partes del ecosistema.

En el Capítulo 2 exploramos el efecto biótico de la composición de las comunidades sobre la composición química foliar de distintas especies vegetales, a través del nicho biogeoquímico de cada especie. Encontramos que cada especie presenta su propio nicho biogeoquímico y fueron capaces de reajustar su composición química foliar en respuesta a las diferentes condiciones bióticas. Concluimos que las plantas pueden reajustar su composición elemental foliar cuando crecen en comunidades con diferente composición de plantas, a través del desplazamiento del nicho biogeoquímico, sugiriendo un uso diferencial de los recursos cuando los patrones de coexistencia cambian.

En el Capítulo 3 hemos explorado el cambio en la composición química del sistema planta-suelo debido a la expansión de arbustos en los pastizales subalpinos del Pirineo. Esta expansión representa la transición desde pastizales puros a matorrales. Los pastizales son un ecosistema dominado por especies de ciclo de vida corto, rápido intercambio de nutrientes entre los compartimientos planta suelo, altas concentraciones de nitrógeno (N), fósforo (P) y potasio (K) en el sistema planta-suelo, con alta productividad pero capacidad limitada de acumulación de biomasa. En cambio, los matorrales se caracterizan por ser un ecosistema dominado por especies de ciclo

de vida largo, con estrategias más conservativas, con un intercambio de nutrientes más lento (relación de C:nutrientes altos en la biomasa aérea y baja concentración de N y P en el sistema planta suelo) y mayor almacenamiento de nutrientes en la biomasa aérea de las plantas. La matorralización incrementa la dependencia de la adquisición de nutrientes como el N a través de micorrizas desde los pastizales puros a matorrales. Todos los cambios en el almacenamiento y composición elemental del sistema planta-suelo a lo largo de la sucesión desde pastizales a matorrales sugiere una desaceleración del ciclo biogeoquímico en las áreas montañosas donde la expansión de arbustos está presente.

En el Capítulo 4, describimos la distribución de C y los nutrientes más importantes para el desarrollo de las plantas (N, P, K) en el sistema planta-suelo de bosques tropicales maduros en suelos pobres de la Guyana Francesa. También estudiamos el proceso de reabsorción de nutrientes desde las hojas senescentes, un mecanismo de nutrición de las plantas para evitar la perdida de nutrientes poco estudiado en este ecosistema. Nuestros resultados muestran que el P es el elemento más escaso presente en hojas, hojarasca y suelo. Las eficiencias de reabsorción de K y P fueron más altas que la de N y la estacionalidad solo afecto la reabsorción de K. La reabsorción de P fue la única que mostró una correlación, aunque débil, con el P en el suelo (total y disponible). Las relaciones entre la reabsorción de nutrientes y los rasgos funcionales de las especies (tasa de crecimiento, densidad de madera, diámetro a la altura del pecho y el área foliar específica) fueron débiles y variaron dependiendo del nutriente, en tanto que la relación filogenética no explica la variabilidad en las eficiencias de reabsorción de nutrientes de las especies. Nuestros resultados sugieren que la alta reabsorción de K y P desde las hojas senescentes es una estrategia adaptativa de las especies que les permite lidiar con la escasez de estos nutrientes en el suelo. Asimismo, el nivel de inmovilización de los nutrientes en los compuestos foliares (N> P > K) parece determinar significativamente el proceso de reabsorción. Concluimos que la reabsorción de nutrientes desde las hojas senescentes es un proceso clave de las plantas para la conservación de nutrientes en los bosques tropicales de la Guyana Francesa, especialmente para K y P, elementos que presentan una disponibilidad baja en el suelo y esta depende principalmente del material parental y del proceso de lixiviación.

En resumen, en esta Tesis hemos demostrado como la composición elemental del sistema planta suelo refleja procesos e interacciones ecológicas, como son las interacciones intra e inter específica entre plantas (Capítulo 2), procesos fisiológicos poco estudiados en las plantas como la reabsorción de nutrientes (Capítulo 4) y la importancia de los estudios de estequiometría para describir cambios a nivel de ecosistema y predecir escenarios futuros (Capítulo 3). Estos estudios aportan nuevos conocimientos en el campo de la estequiometría ecológica y resaltan la importancia de este enfoque en los estudios ecológicos.

Article references

- **Chapter 2:**
  Urbina, Ifigenia., Sardans, Jordi., Grau, Oriol., Beierkuhnlein, Carl., Jentsch, Anke J., Kreyling, Juergen., & Peñuelas, Josep. (2017). Plant community composition affects the species biogeochemical niche. *Ecosphere* 8(5). doi: e01801. 10.1002/ecs2.1801

- **Chapter 3:**
  Urbina, Ifigenia., Grau, Oriol., Sardans, Jordi., Ninot, Josep., & Peñuelas, Josep. Encroachment of shrubs into subalpine grasslands in the Pyrenees changes the plant-soil stoichiometry. Under review in *Plant and Soil*

- **Chapter 4:**
  Urbina, Ifigenia., Grau, Oriol., Sardans, Jordi., Margalef, Olga., Peguero, Guillerom., Ascencio, Dolores., Llusià, Joan., Ogaya, Romà., Gargallo-Garriga, Albert., Va Langenhove, Leandro., Verryckt, Lore T., Courtis, Elodie A., Stahl, Clément., Soong, Jennifer., Chave, Jerome., Hérault, Bruno., Janssens, Ivan., & Peñuelas, Josep. High foliar K and P resorption efficiencies in old-growth tropical forests growing on nutrient-poor soils. Manuscript in preparation.

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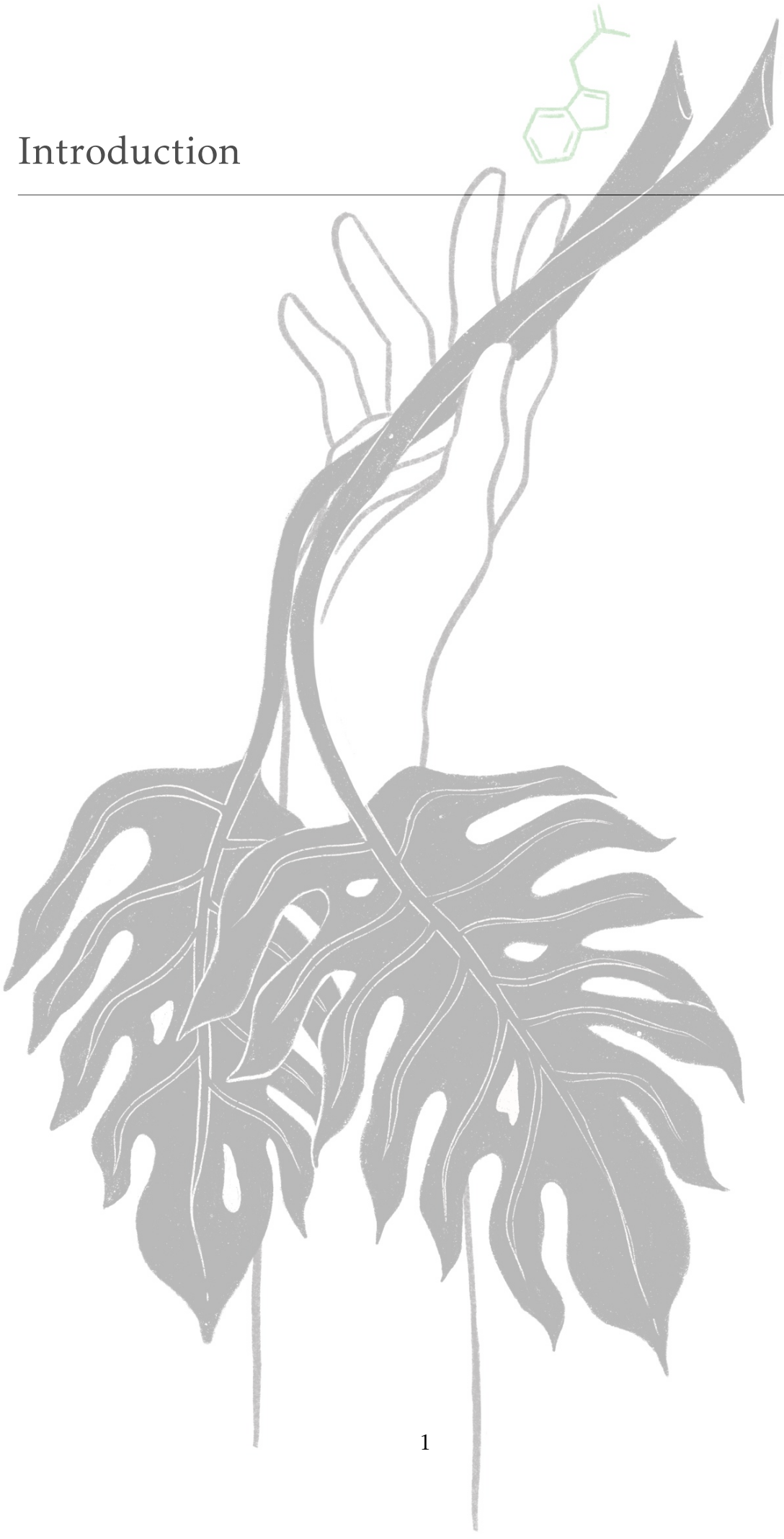
Introduction 1



1.1 Chemestry, life and ecology. A bit of history.

Chemical elements are the essential component of the inert and living matter in the Earth and their transfer and fluxes among the organisms and their environments are determinant for all ecosystems of the planet. The content and transfer fluxes of these chemical elements in organisms and environments constitute an exciting starting point to study the ecology of terrestrial ecosystems. How are chemical elements distributed in the system? What are the biogeochemical feedbacks between the abiotic and biotic parts of the ecosystems, and what are the consequences of their interactions? What is the chemical composition of living organisms?

Eduard Suess, an Austrian geologist, was the first scientist who coined the concept of biosphere in his book The *Origin of the Alps* in 1875. He defined the biosphere as the layer of the Earth where environmental conditions such as temperature, water, pressure, chemical compounds and living organisms can be found. Over half a century later, the Russian geochemist Vladimir I Vernadsky expanded this concept and described the strong relationship between the biosphere and the biogeochemistry of the Earth in his book *The biosphere* (Vernadsky 1926). Vernadsky recognizes that the chemistry of the Earth crust, oceans and the atmosphere is highly affected by the biosphere. He credits the biosphere as the greatest chemical force on earth, and wrote: *'between the living and inert matter of the biosphere, there is a single, continuous material and energetic connection, that appears in form of motion of chemical elements to and from living organism depending on the requirements of life, as feeding, respiration, excretion and reproduction'* (Vernadsky 1938). He also refers to the 'green live organism' as a fundamental part of the biosphere and points to the exchange of gases as the principal evidence of its interaction with the surrounding environment. Indeed, he anticipated the *ecosystem* concept and establish the bases of the ecological biogeochemistry studies. Furthermore, he also highlighted the importance of the human impact on the biogeochemistry of the planet describing it as a *new planetary phenomenon* and coined the concept of *noosphere* as the part of the earth conformed by the humans. He would certainly be surprised by the extent at which humans have changed the chemistry of our planet up to now.

Towards the middle of the last century, biogeochemistry studies mostly focused on the organisms' chemical composition and the ecosystem functioning became more popular. Researchers as G. Evelyn Hutchinson, who wrote the *'The biogeochemistry of the vertebrate excretion'* (Hutchinson 1950) and *'The Paradox of Plankton'* (Hutchinson 1961); and Ramon Margalef publications, especially with his *'On Certain Unifying Principles in Ecology'* paper (Margalef 1963) made fundamental contributions to the understanding of the link between the biogeochemistry and the structure and stability of ecosystems. Later on, in the 1970s, J. E. Lovelock described in more detail the influence of living organism on the composition of the atmosphere (Lovelock 1972). This was previously described by Vernadsky, but Lovelock's main contribution was to describe life's homeostatic capacity on the planetary environment, which finally gave rise to in the Gaia theory (Lovelock 1979).

Simultaneously, Philip J. Gersmehl published 'An alternative biogeography'

where the fluxes of the mineral elements between the abiotic and biotic compartments of the ecosystems were represented in the 'The mineral cycle model' (Gersmehl 1976). This useful scheme was represented for both an ideal 'closed' and a realistic 'open' system, and for different terrestrial ecosystems (Fig.1.1). This mineral circulation model is a powerful tool to understand the biogeochemistry processes of the ecosystems.

In this general framework, this Thesis aims to provide new information about the plant-soil chemical composition and nutrient cycles in three different environmental conditions: a controlled experiment in a botanical garden in Germany, and two field studies: one in the sub-alpine grasslands in the Pyrenees where shrub encroachment takes place, and the other in primary tropical forest growing on nutrient-poor soils in French Guiana. The controlled experiment in a botanical garden (Chapter 2) allows us to better understand how different plant communities with equal substrate conditions affect the foliar stoichiometry of the species. This is a very appropriate approach to evaluate to what extent different competitive conditions (biotic effect) could modify the species foliar stoichiometry. Furthermore, this study provides evidence of the species' Biogeochemical niche (BN) existence (details explained below).

The two field studies provide interesting and contrasting frameworks in terms of plant and soil elemental composition. Currently, shrub encroachment into subalpine grassland in the Pyrenees is an expanding phenomenon as it is common in many other regions. This process of shrub expansion could lead to important changes in the biogeochemical cycle of the system with important consequences in the ecosystem functioning. Our main goal in this study (Chapter 3) is to describe the changes in the plant-soil elemental composition and stocks due this succession from grassland to shrubland. Finally, the tropical forest is a paradigmatic case of study regarding the nutrient distribution and cycling. Huge quantity of biomass is supported by extremely poor soils. In Chapter 4 we take advantage of two tropical forests that grow in extremely poor soils to explore the nutrient distribution and the nutrient resorption, an important plant nutrition mechanism that has been poorly explored in this ecosystem.

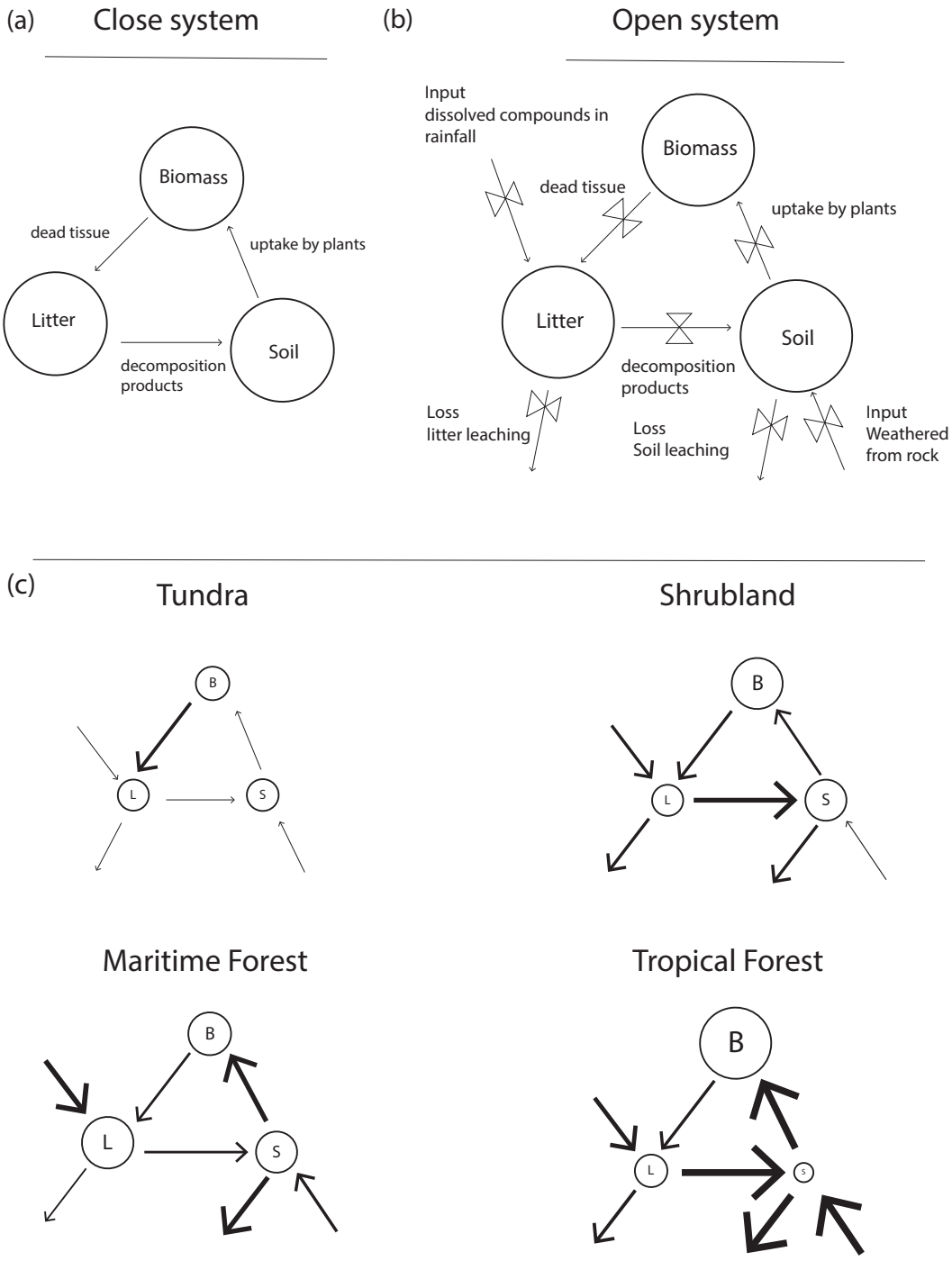


Figure 1.1: The mineral cycle modeled as three compartments (in circles, B: biomass, L: litter and S: soil) in (a) closed and (b) open system with the basic climatic regulators, temperature and precipitation (indicated as valves), controlling the rates of nutrient flows. (c) The nutrient circulation in four idealized terrestrial ecosystem, arrows width indicates the quantity of element flow expressed as a proportion of the amount stored in the source compartment, and circle size denote the amount of nutrient stored in a compartment at steady state. Modified from Gersmehl (1976)

1.2 Elemental composition of photoautotrophs

Carbon (C), Nitrogen (N), Oxygen (O) and Hydrogen (H) are the main elements that make life possible. However, living organisms require several other elements to grow and reproduce successfully, such as phosphorus (P) (essential part of the DNA and RNA), potassium (K) (involved in several physiological functions) and in less quantity calcium (Ca), magnesium (Mg) and iron (Fe). C, O and H are fundamental part of the structure of living organisms, while N, P, K, Ca, Mg are essential nutrients for metabolism and development. Photoautotrophs (autotrophs hereafter) represent the interface between the living and nonliving system in the Earth (Sterner and Elser 2002). Sterner and Elser laid the foundations of the ecological stoichiometry studies (from the Greek root: *'stoicheion'* for element), which focused on the balance of the multiple chemical elements that are crucial for ecological interactions and processes. In another definition, ecological stoichiometry deals with the patterns and processes associated with the chemical content of the species.

The autotrophs elemental composition is established when they use light to fix carbon (CO2) while simultaneously assimilating inorganic nutrients. There are two principal characteristics in the plant cell that mainly determine the plant stoichiometry: the cell wall, rich in C and depleted in P and N; and the presence of the central vacuole with the capacity to store inorganic and organic nutrients not immediately used for growth and reproduction (Sterner and Elser 2002). Also, autotrophs have low homeostasis (the physiological regulation of an organism's internal environment) and present nutrient 'luxury consumption' which is the nutrient uptake above what is required immediately for growth. Because of this, autotroph organisms exhibit a large variation in their stoichiometric composition (C:N:P, and others), low homeostatic capacity/high stoichiometry flexibility, due the uncoupling of carbon fixation and nutrient acquisition together with the variation in the allocation patterns in the different plant organs under different environmental conditions (Sterner and Elser 2002).

The growth rate hypothesis (GRH), developed in aquatic ecosystems (zooplankton), proposes that fast-growing organisms will present low biomass C:P and N:P ratios due the higher demand of P allocation to ribosomal RNA necessary to meet the protein synthesis demands for the faster growth rate (Sterner and Elser 2002). However, the applicability of this hypothesis to terrestrial plants is not so clear as it is in planktonic communities. Plants can assign great amounts of N and P to other functions not directly involved in growth, which makes it difficult to establish a direct relationship between the plant's C:N:P stoichiometry and growth (Peñuelas and Sardans 2009; Yu et al. 2012). The plant nutrient 'luxury consumption' occurs generally when there is another limiting factor as light or other nutrient, and results in an unbalance between the growth and the plant's elemental composition (Sterner and Elser 2002). Moreover, there is an important intra individual stoichiometry variation especially terrestrial plants, where different plant compartments (such as leaves, roots, wood and spurs) present contrasting elemental compositions.

Despite the difficulty to establish the relationship between autotrophs stoichiometry and growth, negative correlation between foliar N:P and maximum

relative growth has been reported for herbaceous and woody species (Güsewell 2004). Furthermore, it is well established for a wide range of plants that foliar N:P is positively correlated with the N:P of the soil, but with great variation within each climatic area (Sterner and Elser 2002; Sardans et al. 2011; Firn et al. 2019). This evidence suggests that the foliar N:P ratio is a good indicator of the resource availability and plant-soil interaction across different ecosystems.

The German botanist Carl Sprengel introduced in the XIX century the first idea about 'law of the minimum' in the field of agriculture chemistry, which establishes that plant growth will be constrained by the scarcer nutrient resource rather than the total amount available. This idea was expanded by German chemist Justus von Liebig, and finally popularized as the Liebig's law of minimum. Nutrient limiting conditions (N, P, K and others) for plant growth are quite common around all terrestrial ecosystems, and plants have adapted to deal with these unfavorable conditions. These adaptations may modify the foliar stoichiometry of the species.

One important strategy present in most terrestrial plants is the fungal mycorrhizas symbiosis association, where fungi provide nutrient to the plant in exchange of C (Bucking et al. 2012a). It has been demonstrated that the exchange of nutrients (widely described for N) between the fungi and plant induce an important change in the foliar N isotopic composition of plants. This allows us to estimate the level of 'mycorrhization' and the type of mycorrhizas associated to plants and make inferences about N cycling of the systems (Craine et al. 2009a). Another important strategy in terrestrial plants is the symbiosis with nitrogen fixing species in the root's nodules. This allows plants to fix atmospheric dinitrogen (N_2), making it more independent from the N soil resources and confers competitive advantages to life on N-poor soils. This association could imply different foliar nutrient concentrations and growth between the fixing and non-fixing species, having an impact in the N and C cycling of the ecosystem (Batterman et al. 2013b). Furthermore, the production of phosphatases enzymes by plants represents another strategy to deal with scarce nutrient conditions (Vance et al. 2003). Phosphatase enzymes release the phosphorus not freely available in soil and make it available for plants in P poor ecosystems, having an important role in plant growth and ecosystem structure (Hofmann et al. 2016; Batterman et al. 2018). Additionally, the nutrient resorption from senescent leaves before the abscission represents an important mechanism that allows plants to be more independent from the external conditions and save the metabolic cost of symbiotic association or enzymes production (Killingbeck 2004; Brant and Chen 2015).

1.3 The Biogeochemical niche concept, a new approach in the Chemical Ecology

Autotrophs can vary their C:N:P ratios greatly under different available resources. As mentioned above, this is due their capacity to store nutrients, which makes them highly flexible in their chemical composition. This can result in different competitive strategies among the diverse autotroph species. Plant foliar elemental composition has been reported to be linked with species life strategies (Sardans

et al. 2012b; Zechmeister-Boltenstern et al. 2015). Plants with higher growth rates have higher foliar nutrient concentrations (N and P) and lower C:N and C:P ratios than plants with lower growth rates (Ågren 2004; Sardans et al. 2012b; Zechmeister-Boltenstern et al. 2015). Moreover, higher N and P concentrations and lower N:P ratios have been observed in leaves during the growing versus non growing seasons, coinciding also with a rise of primary metabolism activity, all of them consistent with the GRH expectations (Rivas-Ubach et al. 2012).

The attempt to introduce a new concept which covers all the variables (biotic and abiotic factors) reflected in the species' chemical composition is not an easy task. The *Biogeochemical niche* concept (BN) represents a new effort to connect the species chemical composition with their evolved genomes and their environmental and competitive conditions. The BN is defined as the multidimensional space of the concentrations of main elements for the organism development (C, N, P, K, Ca, Mg, Fe) in individuals of a given species (Peñuelas et al. 2019). The differences in the biogeochemical niche among species are a function of taxonomy/phylogenetic distance and of the distinct homeostasis' capacity. The evolution of the species in different stress-disturbance gradients and the genetic variability in their population would determine the BN flexibility. Thus the species' BN would also be a function of the available resources and the capacity of changing would present a continuum between high homeostasis/low flexibility and low homeostasis/high flexibility (Peñuelas et al. 2019). Moreover, the elemental compositions should differ more among coexisting (sympatric) than among non-coexisting species to avoid competitive pressure (Peñuelas et al. 2019). This can explain that species of the same genus that recently evolved in different world regions within very distinct communities could have diverged in their elemental composition (Peñuelas et al. 2019). As a result, the biogeochemical niche *'is assumed to be the result of its taxonomical evolutionary determination and its capacity to respond to changes in external conditions'* (Peñuelas et al. 2019). The biogeochemical niche is a promising concept that can be used as a proxy of the organism's performance, which complements the ecological niche theory (Schoener et al. 1986). We will come back to this concept in Chapter 2 of this Thesis and show how the change in the biotic conditions are well reflected in the species' *biogeochemical niche*.

1.4 Carbon and nutrients cycles in terrestrial ecosystems

Plant requirements and nutrient flows will determine the nutrient stocks and availability in the different ecosystems. In this Thesis we report the changes in plant and soil chemical composition (concentration and stocks) reflecting different plant and ecosystem processes. In Chapter 3 we focus our attention in the changes at community level in the plant-soil system along the ecological succession from grassland to shrubland. In contrast, in Chapter 4 we described the nutrient distribution (stocks) in the plant-soil system and explore the plant nutrient internal recycling (nutrient resorption) as important plant nutrition mechanisms in tropical forests growing in poor soils. At the ecosystem level, C,

N, P and K (also O and H, not limiting for plant development) are the elements that represent major fluxes between plants (biotic) and the atmosphere and soil (abiotic) systems. These fundamental elements for plant growth and development have contrasting cycles (Fig.1.2).

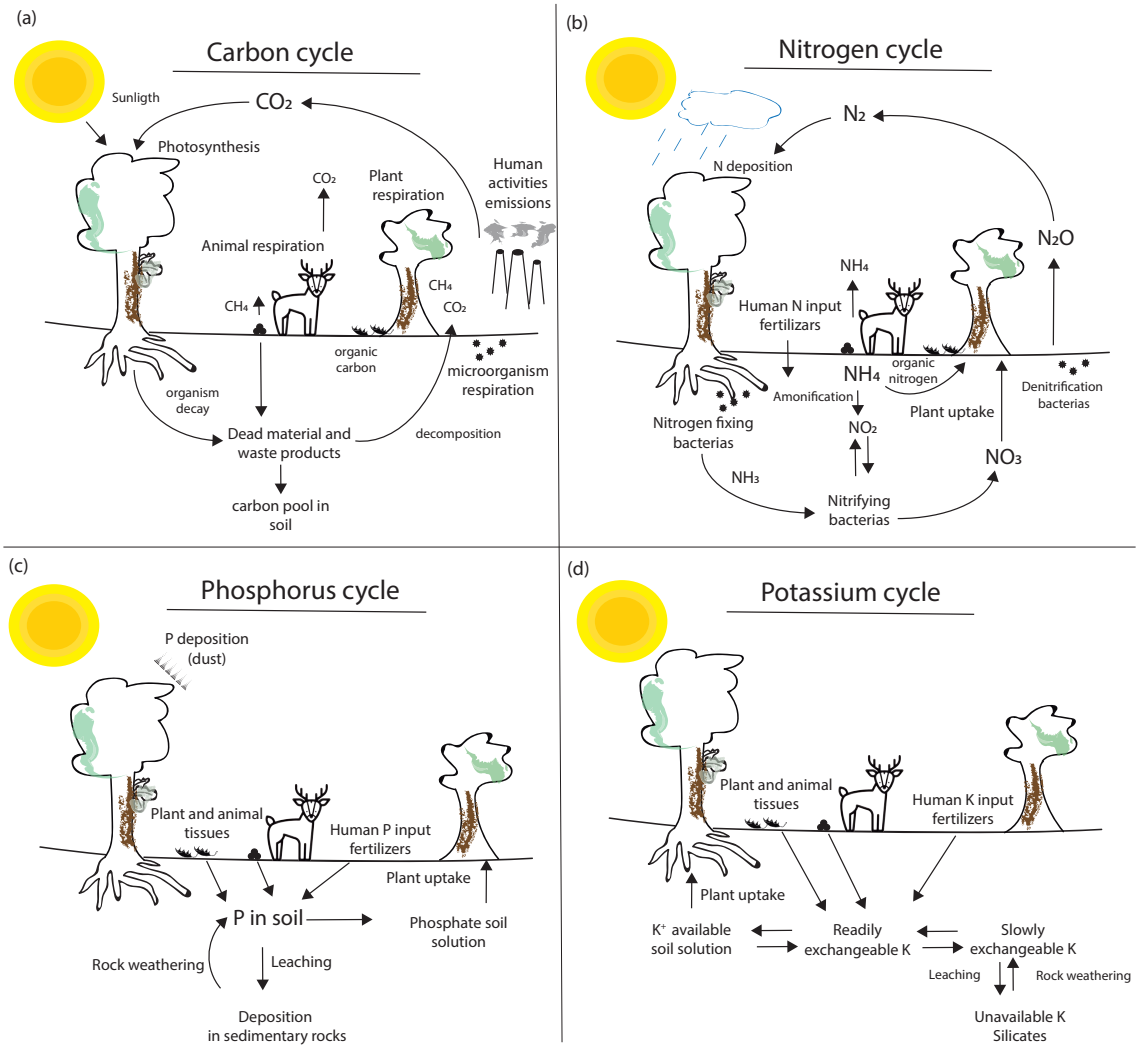


Figure 1.2: (a) The Carbon (b) Nitrogen, (c) Phosphorus and (d) Potassium cycle in terrestrial ecosystems. C and N fluxes between the biotic and abiotic part of the ecosystem are larger than for P and K fluxes. On the other hand, human activities have a higher impact in the C and N cycle than in the P and K cycles at global scale. All this will determine specific changes in the plant-soil stoichiometry composition.

Carbon is the plant structural component and represent approximately 50% of the plants dry weight. It is incorporated into plant biomass from the atmosphere through photosynthesis and returns to the environment through respiration and plant, animal and microbial organic matter inputs. It is widely present in the soil in the parent material (Fig.1.2a). N represents around 2-4% of plant biomass, it is highly abundant in the atmosphere in form of dinitrogen (N_2) but almost absent from parent material, which make young ecosystems poor in N (Turner and Condron 2013). N is absorbed by plants through biological fixation (by symbiotic

bacteria association in root nodes), from atmospheric N_2 and directly from soil in organic and inorganic forms via root uptake. It is returned to the system through plant and animal organic matter which promotes N accumulation and availability in soil (Chapin 1980) (Fig.1.2b). In contrast to N, P is highly sequestered in the parent material and is only available for plants by rock weathering (Walker and Syers 1976). Also, atmospheric dust deposition could represent and important P input in poor soil tropical forests (Gross et al. 2016) (Fig.1.2c). Similarly, K is mainly present in the parent material in the form of silicates and is provided to plants from by rock weathering and the decomposition of the organic matter (Fig.1.2d). All this implies, more opened cycles for C and N and more closed cycles for P and K between the plant-soil.

As discussed above, all these fluxes (C, N, P, and K) between the biotic and biotic parts of the ecosystem are reflected in the species stoichiometry. For example, at global scale N:P ratios in leaves and soil tend to decrease from higher latitudes towards the tropics, reflecting more N limitation in the cold ecosystems and more P limitation in the tropical ecosystems. (Sardans et al. 2011). Also, high foliar C:P ratios in the tropical tree are consistent with the P scarcity in the old weathered tropical soils (Sterner and Elser 2002). Patterns in plant and soil C:N:P ratios along successional gradients have predictable consequences on the biogeochemical cycles and productivity of the system (Sterner and Elser 2002). Furthermore, the availability of nutrients is a main regulator of important processes and properties from individual to ecosystem level, such as growth (Chapin 1980; Elser et al. 2000), primary productivity (Terrer et al. 2017; Vicca et al. 2018), forest diversity (John et al. 2007; Hillebrand et al. 2014) and the ecosystem structure and dynamics (Sayer and Banin 2016; Grau et al. 2017). However, there are still uncertainties regarding the nutrient role in the ecosystem function and processes, especially in a context of climate change, where the chemistry of the Earth has changed drastically during the last years.

1.5 Aim of the Thesis

This Thesis aims to show how the ecological stoichiometry allows us to study processes from the organism to the ecosystem level, through the evaluation of the chemical composition of the plant's organs and soil in different terrestrial ecosystem. This general objective poses novel and challenging questions such as: 'are the interactions between plant and soil and the species evolutionary history reflected in the species *Biogeochemical niche*?' and 'what does the chemical composition of the plant-soil system tell us about ecosystem functions and processes?' that I have tackled in this thesis. The specific questions that I have addressed in the different chapters are:

- **Chapter two:** How is the plant community composition reflected in the species' foliar chemical composition? A test of the *Biogeochemical niche* concept.

  In this chapter we conducted a controlled experiment to better understand

the biotic effect on the species foliar chemical composition. To do so, we explored the relationships between the plant community composition (number of species co-habiting in the same plot) on different species' biogeochemical niche.

- **Chapter three:** How is the succession from grassland to shrubland in a sub-alpine ecosystem reflected in the plant-soil chemical composition?

  In this chapter we explored the stoichiometry changes along the succession from a grassland to a shrubland in the subalpine grassland of the Central Pyrenees, and how these chemical changes may shape the C and nutrient cycle of this ecosystem.

- **Chapter four:** How do plants growing in extremely nutrient-poor soils use the resorption process as a mechanism to deal with nutrient limitation?

  In this chapter we explored the cycling of nutrients, the resorption process, how these processes are controlled, and their importance in old-growth tropical forests growing on nutrient-poor soils.

Plant community composition affects the species biogeochemical niche 2

Abstract

Nutrients are essential for plant development and their availability and stoichiometric ratios can influence the composition of plant communities. We investigated the possibility of the reverse influence: whether the conditions of contrasting species coexistence determine foliar element concentrations and plant stoichiometry, i.e. species biogeochemical niche (BN). The experiment was conducted at the Ecological-Botanical Garden of the University of Bayreuth, Germany. We analyzed foliar element concentrations of two dwarf shrubs (*Calluna vulgaris* and *Vaccinium myrtillus*) and two grasses (*Holcus lanatus* and *Arrhenatherum elatius*) growing in different community compositions (monocultures and various mixed stands). Foliar nutrient concentrations and stoichiometry (taken as a proxy of species BN) were species-specific; each species showed its own BN in all communities. Furthermore, *Vaccinium myrtillus* and *Holcus lanatus* species shifted their BN in response to changes in their community, accomplishing the *'biogeochemical niche displacement'* hypothesis. We conclude that plants can readjust their foliar element concentration if they grow in communities with contrasting plant composition, suggesting a differential use of element resources when the patterns of species coexistence change. These results also support the complementary niche hypothesis.

- **Key words:** biogeochemical niche; community composition; grass; homeostasis; shrub; stoichiometry.

2.1 Introduction

In recent decades plant nutrient stoichiometry, defined as the relative proportion of C and nutrients in plant tissues (leaves, roots, shoots and wood), has gained significant importance in ecological studies (Elser and Urabe 1999, Sterner and Elser 2002, Moe et al. 2005, Cross et al. 2005, Elser and Hamilton 2007; Sardans et al. 2012a). It has been found that plant nutrient stoichiometry influences several ecological processes that are pivotal to ecosystem functions and dynamics, such as plant-herbivore interactions (Daufresne and Loreau 2001, Millett et al. 2005, Newingham et al. 2007), the structure and function of arbuscular mycorrhizae (Johnson 2010) and population dynamics (Andersen et al. 2004). Recent studies have also found a strong relationship between changes in foliar C:N:K ratios and changes in foliar metabolites in response to varying environmental conditions (Peñuelas and Sardans 2009, Rivas-Ubach et al. 2012). However, the link between foliar nutrient concentration and community composition has proven to be challenging and remains poorly explored. This is understandable because the composition of plant communities depends of several ecological processes and functions such as productivity, resource use, nutrient cycling, biotic interactions (Grime 1974, Tilman et al. 1996, Roem 2000), while plant stoichiometry is influenced by several factors such as soil fertility, source and quantity of water supply, phylogenetic affiliation and climatic conditions (Sterner and Elser 2002). Additionally, soil layer interactions and competition with microbial communities make

it difficult to establish a direct link between soil resources and plant diversity (Hooper and Vitousek 1997), increasing even further the difficulties associated with studying the link between plant stoichiometry and community composition.

In this context, (Peñuelas et al. 2008, 2019) shed light into the relationship between plant-stoichiometry and plant coexistence. The authors proposed the 'biogeochemical niche hypothesis' where species have a species-specific stoichiometry in a multivariate space generated by the contents of macro and micronutrients in plant tissues. This hypothesis is based on coexisting plant species tending to use the main nutrients N, P and K (and other essential nutrients such as Ca, Mg and S) in differing proportions (Peñuelas et al. 2008 and 2010). Since different plant structures and metabolic processes have distinct and divergent requirements for each of the essential nutrients, the species-specific biogeochemical niches should be the result of species specialization to particular abiotic and biotic conditions. BN should reflect the different species-specific strategies of growth and uptake of resources and the differences in soil space and time occupation reducing direct competition among sympatric species and optimizing the nutrient use by the overall community. BN also hypothesized that the existence if stoichiometric flexibility (the capacity to change the biogeochemical niche) of a species is an important trait, as it is related to the quality of plants tissues and their capacity to cope with changes in environmental conditions. Building further on this hypothesis, Yu et al. (2010) argued that species exhibit stoichiometric flexibility in response to environmental changes (including ontogenic and seasonal changes) and competitive situations, probably under a tradeoff between the stoichiometry flexibility and homeostatic regulation (the maintenance of constant internal conditions in the face of externally imposed variation). Recent studies in Spanish and European forests have shown that elemental stoichiometries of different forest species were strongly determined genetically which is consistent with their long-term adaptation to specific abiotic and biotic environments leading to optimized metabolic and physiological functions and morphological structures determining the specific use of various nutrients (Sardans et al. 2015, 2016). These studies have also shown that current climate is also an important driver of uptake and nutrient use and that BN should reflect trade-offs among several functions such as plant growth, resource storage and/or anti-stress mechanisms for maximizing plant fitness in each of particular climate. More differences in foliar composition stoichiometry among sympatric species than among allopatric species have been reported (Sardans et al. 2015, 2016). The BN hypothesis thus claim that all species are adapted, to some extent, to different combinations of environmental factors implying a trade-off between different basic functions such as growth rates, reproduction and stress tolerance. The different BN can also be taken as a final result of an optimum adaptation to environmental gradients and can be compared to the proposed ecological strategies as for example the Grime R-C-S model (1977) where different species can be placed in a specific 'space' in the gradient among these different ecological strategies.

Following Peñuelas et al. (2008, 2019) BN hypothesis, we here investigated the inverse relationship between the changes in species coexistence and the BN of species. We aimed to determine if the composition of plant communities affects the BN of individual species. We included as many elements as possible in the analysis

because of the well known importance of several elements like K, Mg, S and Ca in plant physiology in water stress avoiding strategies, photosynthetic machinery, regulation of ion balance (homeostasis) in chloroplast and vacuoles, transport of sugar into the phloem and secondary metabolism (Sawhney Zelitch 1969; Knight et al. 1991; Romeis et al. 2001; Shaul 2002; Cakmak 2005; Franceschi Nakata 2005; Rennenberg et al. 2007; Gill Tuteja 2011; Sardans Peñuelas 2015). Our main research question was: Are plants able to modify their foliar stoichiometry (species BN) in response to varying species coexistence?

To address this question we conducted a comparative experiment in which different species were grown in different plant communities with the same substracte supply in order to test if the species BN changed in response to their neighboring co-occurring plants. We hypothesized that stoichiometric flexibility could occur in three different ways (see Fig.2.1): (1) BN *expansion*, which would represent an increase in the stoichiometry spectrum of a species as a consequence of the competition with neighboring individual plants; (2) BN *contraction*, where species specialize in absorbing nutrients in a more narrow and concrete proportion as a strategy to reduce competition; and (3) BN *displacement*, where certain physiological functions (e.g. more woody biomass production or lower growth rate) are re-adjusted as a result of the shift of competition scenarios.

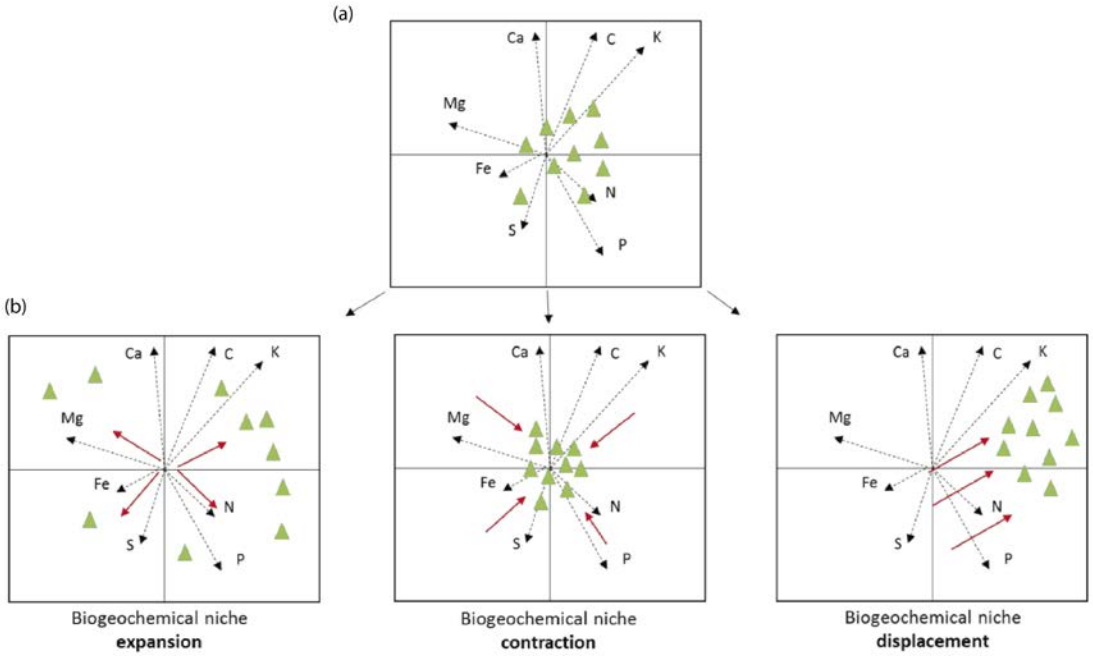


Figure 2.1: Hypothetical stoichiometry spectrum (in the PCA) based on the foliar elemental concentrations and the possible hypothetical shifts proposed in this article. (a) Species growing in a monoculture and (b) The possible hypothetical shifts (expansion, contraction, displacement) in the stoichiometry spectrum in response to different community composition. Filled triangles show individuals (replicas) of a same species.

2.2 Material & Methods

Experimental setup

The experiment was conducted at the Ecological-Botanical Garden of the University of Bayreuth, Germany (49°5519N, 11°3555E; 365 m a.s.l.) as part of the EVENT I experiment (Jentsch et al. 2007, 2011). Mean annual precipitation at the site is 709 mm, with a major peak in June/July and a second peak in December/-January. The communities were established in April 2005 by planting pre-grown individuals of all species in 2x2m plots. All species were harvested in May 2011, after 6 years of pre-grown phase. Four different types of plant communities were studied (monocultures, heath, and grass, Table2.1) and a total of seven community compositions were sampled: monocultures (MCs) of two dwarf shrubs (*Calluna vulgaris* and *Vaccinium myrtillus*) and of two grass species (*Holcus lanatus* and *Arrhenatherum elatius*); two heath communities, one composed of the two shrub species used in the monocultures (H2); and the other one (H4) with the same two shrub species and two naturally co-occurring grass species (*Agrostis stolonifera* and *Deschampsia flexuosa*); and one grass community (G4) with the two grass species (*H. lanatus* and *A. elatius*) combined with a naturally co-occurring herb (*Plantago lanceolata*) and legume (*Lotus corniculatus*). All community compositions were selected because they represent the most prevailing and widespread species in Europe and have significant landscape and ecological importance. The original species composition of each community was maintained throughout the experiment by removing invading species at least twice per growing season.

Each plot was divided using a 20x20cm grid, with one individual was planted in each grid square (for a total of 100 individuals per plot). Initial species density for plot in the monocultures resulted in 1 dwarf shrub individual or one grass individual per grid square. In H2 individual were planted alternating species within the grid, resulting in 50 individuals of each species (half density of species in the monocultures). Similarly, in the four-species communities (H4, G4) individual species were planted alternatively within the grid resulting in 25 individuals of each species (one-quarter density of species in the monocultures). All the communities (four monocultures and three mixed stands) had five replicates, for a total of 35 plots. In one H4 replicate one species (*V. myrtillus*) was lost at the time of sampling.

All plots were established on 60 cm of homogenized sandy soil overlaid with 20 cm of topsoil. The topsoil had a total carbon content of 2% and a pH of 4.5 (measured in 1 M KCl), and the lower sandy layer had a total carbon content of 0.2% and a pH of 6.2. A gravel bed and drainage tubes below 80 cm averted any influence from irregular groundwater. The soil texture was loamy sand (82% sand, 13% silt, 5% clay). Soils were collected from a nearby sand quarry (the topsoil of the quarry) where all study plants are found. Four soil samples were collected in three replicas of each community (MC, H2, H4, G4) with soil cores of 28 mm in diameter to a depth of 100 mm at the same time of harvest event in May 2011. The four soil samples of each community were bulked into one sample, for a 3 bulked samples for each community and a total of 21 soil samples (3 replicas x 7 communities) that were used in stoichiometric analyses. The stoichiometry of

Table 2.1: Characterization of the plant communities of the EVENT I experiment.

Community	Description	Species
MC	Monocultures of four target species	***Calluna vulgaris***, ***Vaccinium myrtillus***, ***Holcus lanatus***, ***Arrhenatherum elatius***
H2	Two species, one functional group (dwarfshrub)	***Calluna vulgaris*** and ***Vaccinium myrtillus***
H4	Four species, two functional groups (dwarf shrubs and grass)	***Calluna vulgaris***, ***Vaccinium myrtillus***, *Agrostis stolonifera*, *Deschampsia flexuosa*
G4	Four species, three functional groups (grass, herb and legum)	***Holcus lanatus***, ***Arrhenatherum elatius***, *Plantago lanceolata*, *Lotus corniculatus*

the soil nutrients did not differ significantly among the communities; the total element concentrations are presented in the supporting information section (Table A.2.1).

Sampling, chemical analysis and biomass calculation

All species were harvested in May 2011 for chemical analyses. An equal number of top and bottom leaves were collected from mature, reproductive individuals. We collected a total of 15 foliar samples from each species of dwarf shrub and a total of 10 foliar samples from each grass species. Sampling was conducted in two differing ways depending on the occurrence or not of regeneration within the plots. When no regeneration occurred (true for the both dwarf shrubs *Calluna vulgaris* and *Vaccinium myrtillus*) 20x40 cm sampling frames were centered in two dwarf shrubs for H2 and H4, and a 20x20 sampling frame centered in each shrub for the monocultures. When regeneration occurred (G4 and grasses monocultures) the 20x40 sampling frames were placed randomly in every community composition because it was not possible to locate originally planted individuals. The biomass per area was calculated in 20x40cm ($0.08 m^2$) of sampling area. In the dwarf shrubs monoculture, because the sampling was carried out with 20x20 frame, the value was corrected by multiplying by two, resulting in the same area.

The collected leaves were oven-dried at 70 °C for 72 h, pulverized, re-dried at 70 °C for 48 h and stored in desiccators until analyzed (<15 days). C and N concentrations were determined for 0.7-0.9 mg of pulverized dried samples by combustion coupled to gas chromatography with an Elemental Analyzer CHNS Eurovector 3011 Thermo Electron Gas Chromatograph, model NA 2100 (CE Instruments/Thermo Electron, Milan, Italy). The concentrations of the other elements (P, K, Ca, Mg, S and Fe) were determined for 0.25 g of pulverized dried sample dissolved with an acidic mixture of HNO_3 (60% w/v) and H_2O_2 (30% w/v) and digested in a MARSXpress microwave system (CEM GmbH, Kamp-Lintfort, Germany). The digests were then diluted to a final volume of 50 mL with ultrapure water and 1% HNO3. Blank solutions (5 mL of HNO_3 and 2 mL of H_2O_2 with

no sample) were regularly analyzed. The concentrations of Ca, K, Mg, S, P and Fe after digestion were analyzed by ICP-OES inductively coupled plasma optical emission spectrometry (Optima 4300 DV, Perkin-Elmer, Waltham, USA). The accuracy of the biomass digestions and analytical procedures were assessed with a certified biomass NIST 1573a (tomato leaf, NIST, Gaitherburg, USA) standards.

Statistical analysis

Principal component analyses (PCAs) were conducted based on the foliar stoichiometric data of each species in order to explore the possible different groups formed by the community factor. A complementary PERMANOVA analysis was conducted to test the statistical significance of the groups. One-way ANOVAs were conducted using element concentrations and C:N, C:P, C:K, N:P, N:K and P:K ratios as dependent variables and community as a categorical factor for each species in order to evaluate the differences in elements concentrations within the different communities. ANOVA was also applied to test differences in species biomass per area in the different communities. We used Tukey's HSD test and the pairwise test with Bonferroni corrections to differentiate community groups. Prior to the statistical analyses, we tested for normality and homogeneity of variances of element concentrations using the Shapiro-Wilk normality test. Also, the residuals versus the expected plots and the normal qq-plots of the linear models were examined. All statistical analyses were conducted with R packages in R 3.2.1 (RCoreTeam 2017). FactoMineR package (Le et al. 2008) was used for the PCA analysis, Vegan package (Oksanen et al. 2017) for the ANOVAs, Permute package for the PERMANOVA analysis and ggplot2 for graphics (Wickham 2009).

2.3 Results

Monoculture communities

The PCA of the four monocultures showed the separation of the dwarf-shrub and grass functional groups on the first axis ($p< 0.0001$), which explained 56.4% of the variability (Fig.2.2). Furthermore, the grass species were separated along the PC1 axis ($p< 0.0001$), while the shrubs were not separated.

Heath communities (H2 vs H4 vs MCs)

The PERMANOVA for *V. myrtillus* discriminated among the different communities ($P< 0.001$) (Table A.2.2). The BNs of *V. myrtillus* individuals in the different communities were separated in the first two PCA axes, PC1 ($p< 0.01$) and PC2 ($p< 0.0001$) (Fig.2.3a,b). H2 was separated from H4 and MC on the PC1 axis and MC from H4 and H2 on the PC2 axis. These two PC axes explained 37.9 and 25.5% of the variability, respectively. *C. vulgaris* individuals were separated marginally (Table A.2.2). H4 and the MC individuals were separated (p=0.05) along PC1, which explained 41.4% of the variability (Fig.2.3c,d)

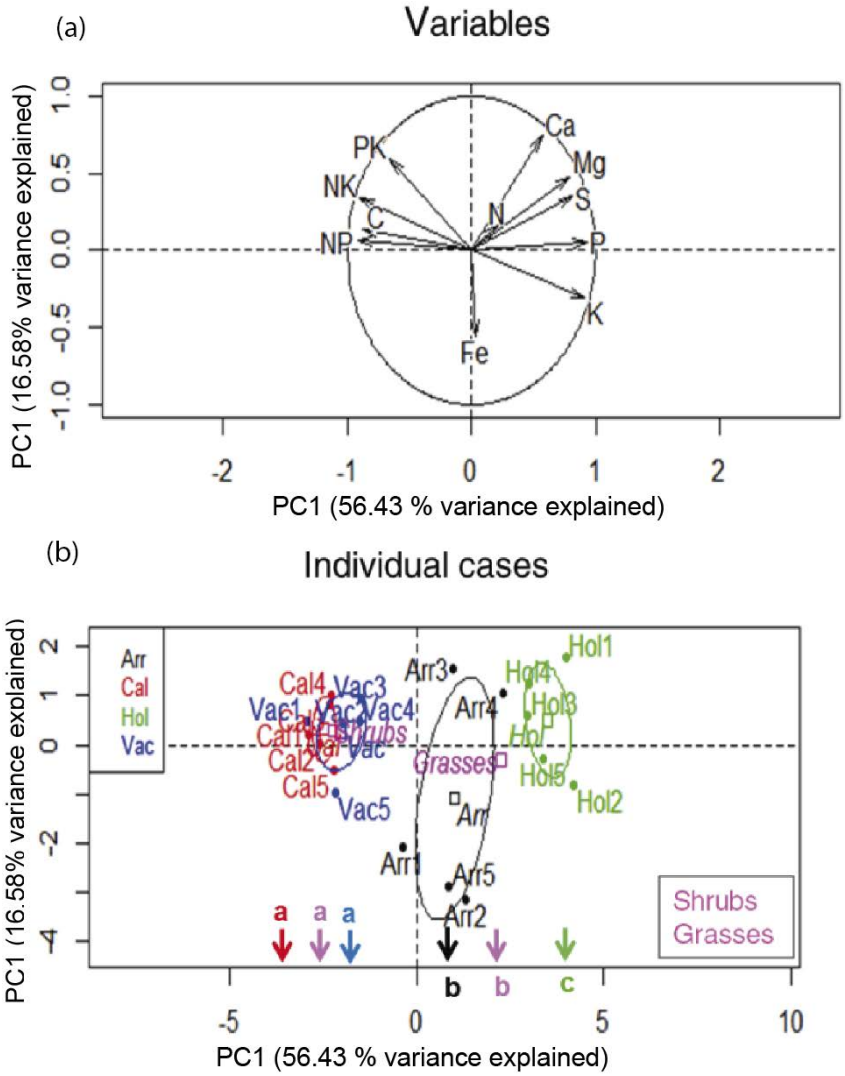


Figure 2.2: PCAs of foliar C, N, P, K, Ca, S, Mg and Fe concentrations and N:P, N:K and P:K ratios. (a) PC1 versus PC2 loadings for the included variables. (b) Estimate site scores for the monocultures of *Calluna vulgaris* (Cal), *Vaccinium myrtillus* (Vac), *Holcus lanatus* (Hol) and *Arrhenatherum elatius* (Arr). Numbers display right to the species acronym indicate the replica of each species. Shrubs and grass functional groups were separated significantly in PC1. Between the four species, *H. lanatus* was separated significantly from *A. elatius*. *C. vulgaris* and *V. myrtillus* were not separated. Differently colored arrows and letters indicate significant differences.

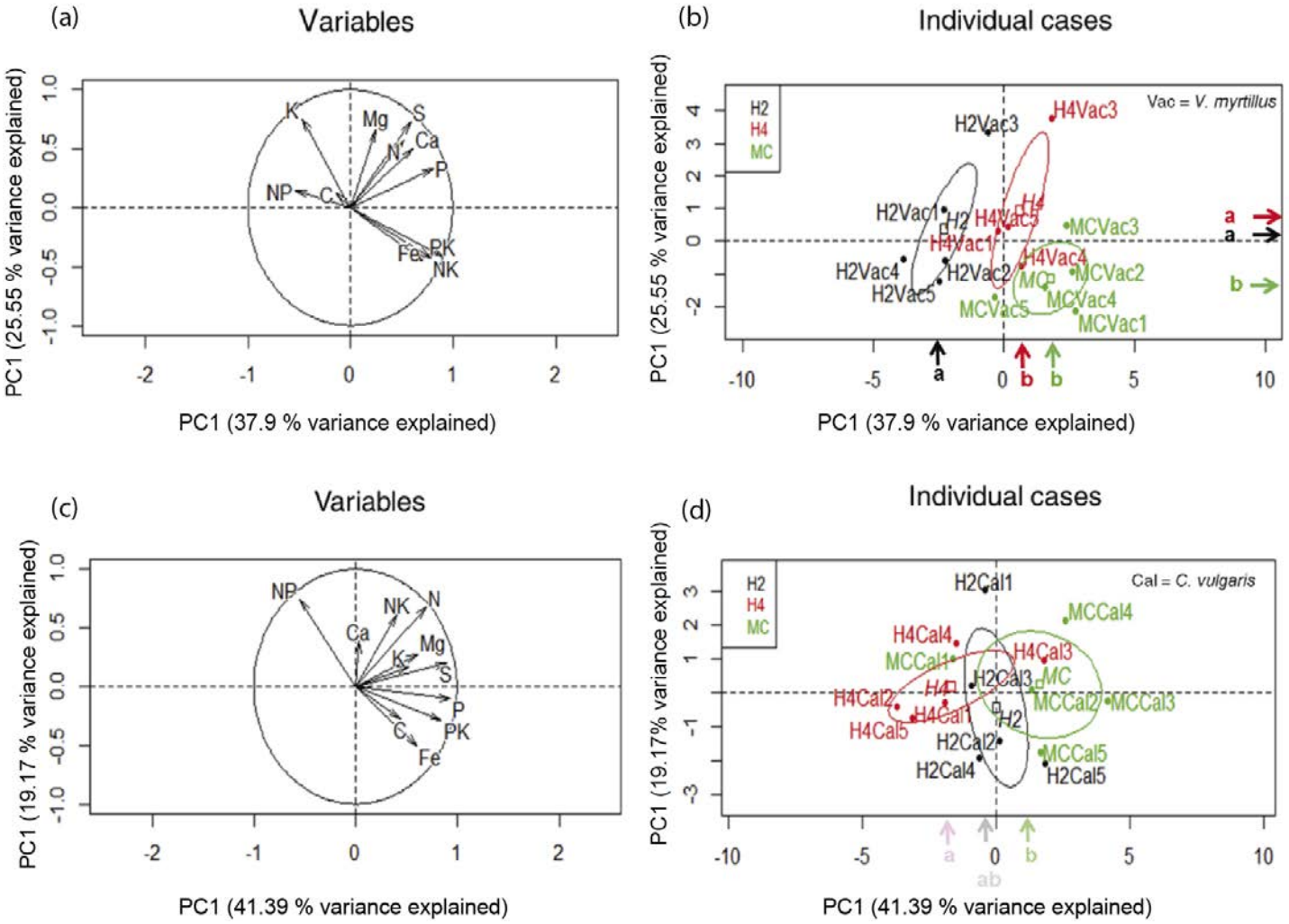


FIGURE 2.3: PCAs of foliar C, N, P, K, Ca, S, Mg and Fe concentrations and N:P, N:K and P:K ratios for the shrubs species. (a, c) PC1 versus PC2 loadings for the included variables. (b) Estimate site scores for the *Vaccinium myrtillus* (Vac) and (d) *Calluna vulgaris* (Cal) in different communities. Community richness are indicated with MC for the monocultures, H2 for the two species community and H4 for the four species community. Numbers display right to the species acronym indicate the replica of each specie. Differently colored arrows and letters indicate significant differences. Lightly colored arrows and letters indicate marginally significance differences.

K and Fe concentrations of *V. myrtillus*, as well as the C:K, N:K, P:K ratios, varied when grown in different communities (Fig.2.4, ANOVA and post-hoc results summarized in Tables A.2.4, A.2.5 and A.2.6). K concentration was significantly higher in H2, followed by H4, and lowest in MC. Fe concentration was lowest in H2, and C:K, N:K, P:K ratios were higher in the monoculture. Mg concentration was marginally significantly higher in H4. For *C. vulgaris* we found only marginal differences. P concentration was lower in H4 relative to H2 and the monoculture, and the C:P ratio was higher in H4 relative to the monoculture (Fig.2.5, ANOVA and post-hoc results summarized in Tables A.2.4, A.2.5, A.2.7).

The PERMANOVA for both species together discriminated groups by species ($p< 0.0001$) by community ($p< 0.0001$) and the interaction between them ($p< 0.0001$) (Table A.2.3). The BN of *C. vulgaris* and *V. myrtillus* occupied different PC spaces on the first two PCA axes (PC1 and PC2 $p< 0.0001$), which explained 36.3 and 21.6% of the variability respectively (Fig.2.6a,b). Also, *V. mytillus* in MC was separated on the PC2 ($p< 0.001$) axis from the H2 and H4 communities. The ANOVA reflected differences in element concentrations by species and differences in P and C:P, N:P, and N:K ratios by community (Table2.2 and 2.3).

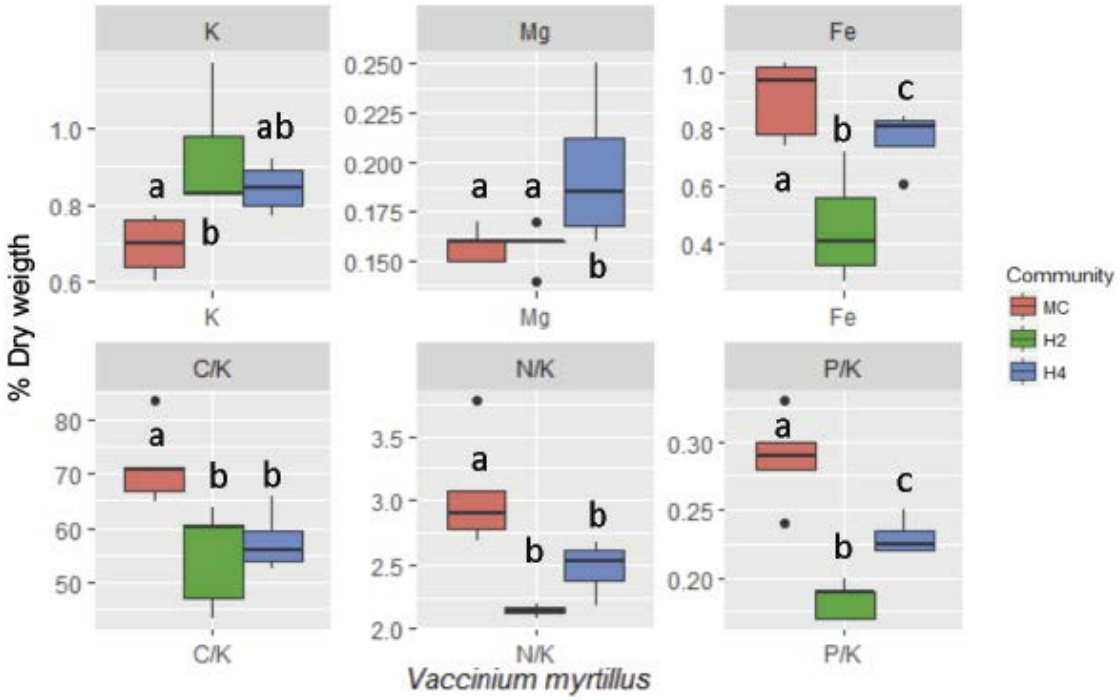


Figure 2.4: Foliar elemental concentrations and ratios (mean % dry weight ±SE) of *Vaccinium myrtillus* for the different communities. Black line inside the box show the data median and the bars indicate the smallest (down) and largest (top) non-outlier value. Different letters indicate significant differences ($p< 0.05$) between communities.

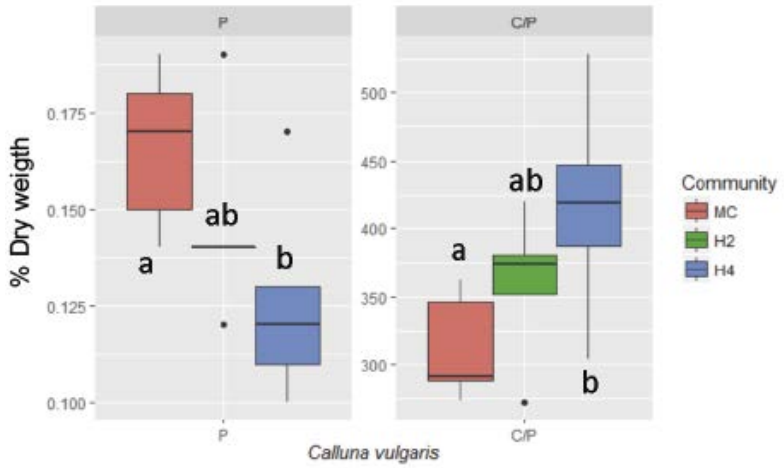


FIGURE 2.5: Foliar elemental concentrations and ratios (mean % dry weight ±SE) of *Calluna vulgaris* for the different communities. Black line inside the box show the data median and the bars indicate the smallest (down) and largest (top) non-outlier value. Different letters indicate significant differences ($p < 0.05$) between communities.

Table 2.2: ANOVA results for the element concentration (C, N, P, K, Ca, Mg, S, and Fe) for the different functional groups studied and the effect of the species and community factor.

Factor	C	N	P	K	Ca	Mg	S	Fe
Shrubs								
Species (Sp.)	< 0.01	< 0.0001	< 0.0001	< 0.0001	n.s	n.s	=0.05	< 0.001
Community	n.s	n.s	< 0.01	=0.05	=0.05	n.s	n.s	< 0.01
Sp. x Community	n.s	n.s	n.s	< 0.001	n.s	< 0.01	n.s	< 0.001
Grasses								
Species (Sp.)	< 0.001	=0.05	=0.05	n.s	< 0.01	n.s	< 0.0001	< 0.01
Community	n.s	n.s	< 0.01	n.s	n.s	n.s	< 0.01	< 0.01
Sp. x Community	n.s	=0.05	< 0.001	n.s	n.s	n.s	< 0.001	n.s

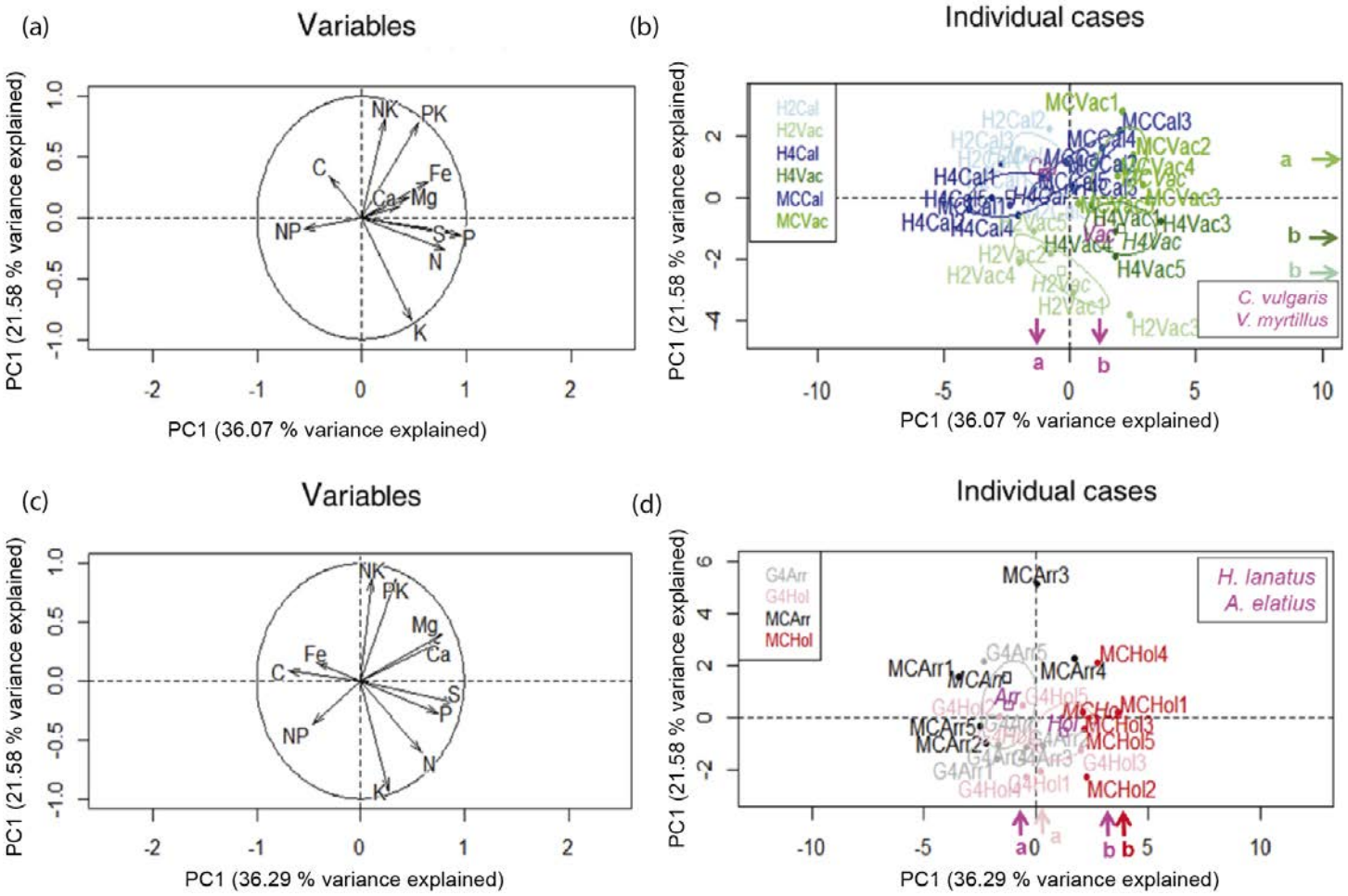


FIGURE 2.6: PCAs of foliar C, N, P, K, Ca, S, Mg and Fe concentrations and N:P, N:K and P:K ratios for the shrubs and grass species.(a, c) PC1 versus PC2 loadings for the included variables. Estimate site scores for *Calluna vulgaris* (Cal) and *Vaccinium myrtillus* (Vac) (b) and *Holcus lanatus* (Hol) and *Arrhenatherum elatius* (Arr) (d) in different communities. Community richness are indicated with MC for the monocultures, H2 for the two species community and H4 for the four species community. Numbers displayed right to the species acronym indicate the replica of each species. Community and species were significantly separated in both analyses. The different species are shown in magenta. Blue color intensity show the different communities for *C. vulgaris*, and green color intensity show different communities for *V. myrtillus*. Red color intensity show the different communities for *H. lanatus* and grey color intensity show different communities for *A. elatius*.

Table 2.3: ANOVA results for the elements ratios (C:N, C:P, C:K, N:P, N:K and P:K) for the different functional groups studied and the effect of the species and community factor.

Factor	C:N	C:P	C:K	N:P	N:K	P:K
Shrubs						
Species (Sp.)	< 0.0001	< 0.0001	< 0.0001	n.s	n.s	n.s
Community	=0.05	< 0.01	n.s	n.s	< 0.001	< 0.0001
Sp. x Community	n.s	=0.05	< 0.001	n.s	< 0.01	< 0.001
Grasses						
Species (Sp.)	=0.05	=0.05	=0.05			< 0.01
Community	n.s	n.s	n.s	< 0.01	< 0.01	< 0.001
Sp. x Community	n.s	< 0.01	n.s	< 0.01	n.s	n.s

Grassland communities (G4 vs monocultures)

For *H. lanatus*, the PERMANOVA discriminated between the different communities ($p< 0.01$) (Table A.2.2). The BNs of *H. lanatus* in G4 were separated ($p< 0.001$) from the monoculture on the PC1, which explained 43.6% of the total variance (Fig.2.7). In contrast, the PERMANOVA for *A. elatius* found no differences between groups, and the PCA analysis did not separate the species when growing in monoculture or in G4 (Table A.2.2).

The P, S and Fe concentrations of *H. lanatus* were higher and the C:P and N:P ratios were lower in the monoculture than in G4 (Fig.2.8,ANOVA and post-hoc results summarized in Table A.2.4, A.2.5). Concentrations of N and Mg, and the C:N ratio showed only marginal differences. For *A. elatius*, only the N:K ratio was higher in the monoculture compared to the G4 community (Fig.2.9, ANOVA and post-hoc result summarized in Table A.2.5).

The PERMANOVA for *H. lanatus* and*A. elatius* together discriminated by species ($p< 0.001$) and by community ($p< 0.01$), but the interaction between them was not significant (Table A.2.3). The PCA separated the BNs of the two species on PC1, which explained 36.2% of the variability and *H. lanatus* individuals from G4 were separated from monoculture individuals on PC1 (Fig.2.6c,d). The two grass species showed differences in their element concentrations due the species and community factor (Table2.2).

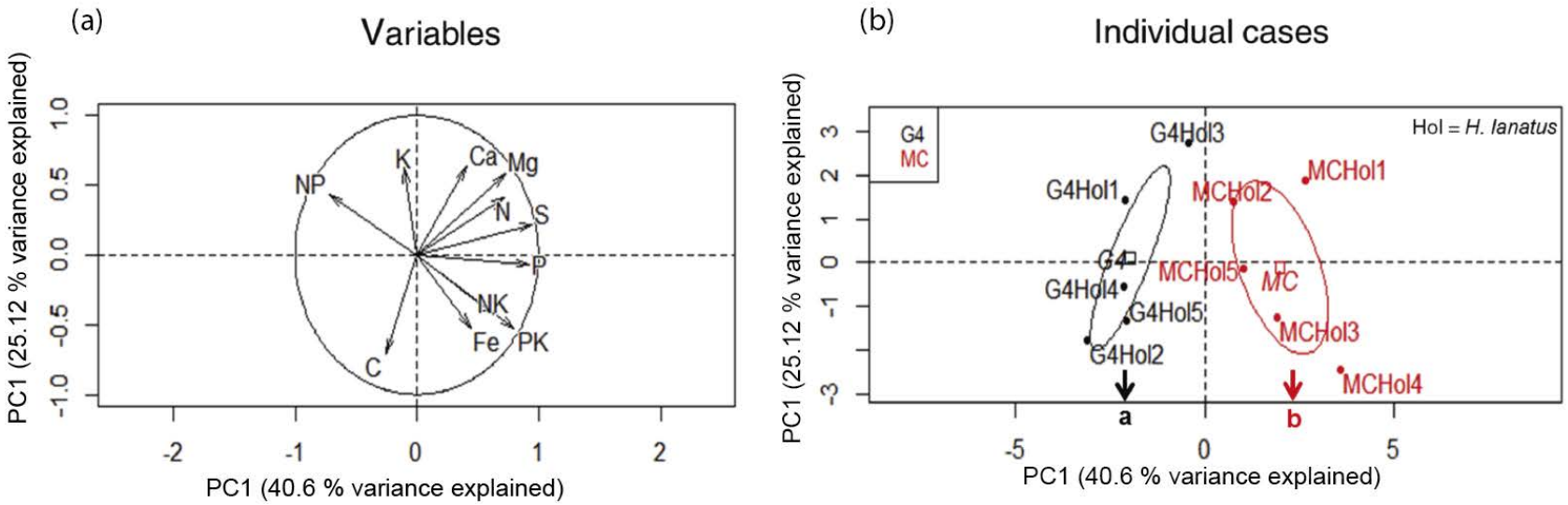


Figure 2.7: PCAs of foliar C, N, P, K, Ca, S, Mg and Fe concentrations and N:P, N:K and P:K ratios (a) PC1 versus PC2 loadings for the included variables. (b) Estimate site scores for *Holcus lanatus* (Hol) in different communities. Community richness are indicated with MC for the monocultures, H2 for the two species community and H4 for the four species community. Numbers displayed right to the species acronym indicate the replica and arrows indicate significant differences.

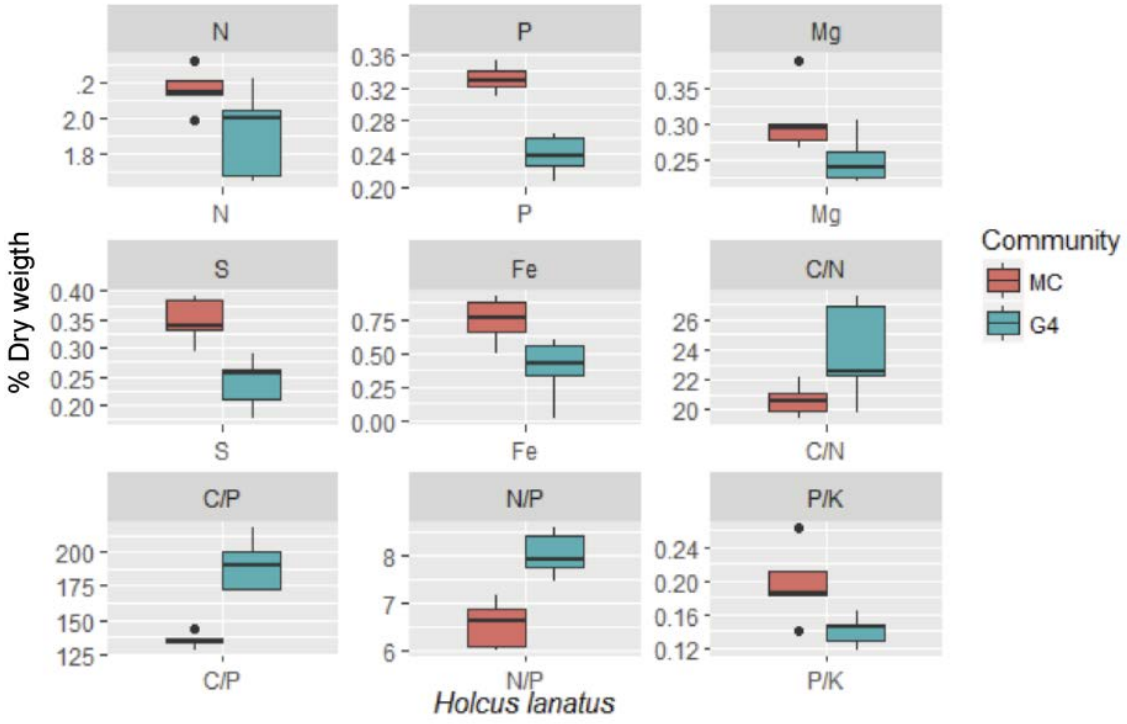


Figure 2.8: Foliar elemental concentrations and ratios (mean % dry weight ±SE) of *Holcus lanatus* for the different communities. Black line inside the box show the data median and the bars indicate the smallest (down) and largest (top) non-outlier value. Different letters indicate significant differences ($p < 0.05$) between communities.

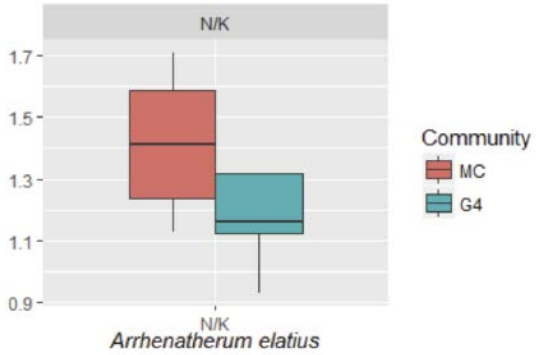


Figure 2.9: Foliar elemental concentrations and ratios (mean % dry weight ±SE) of *Arrenatherum elatius* for the different communities. Black line inside the box show the data median and the bars indicate the smallest (down) and largest (top) non-outlier value. Different letters indicate significant differences ($p < 0.05$) between communities.

Biomass per unit area

The ANOVA showed no differences in biomass per unit area (g m^{-2}) for any species in all the different communities. In other words, the biomass per unit area (g m^{-2}) of each species was similar in every community.

2.4 Discussion

We hypothesized that BNs would be species-specific and could change in three ways: 1) BN expansion, increasing the variability among individuals without changing the mean foliar stoichiometric spectrum, 2) BN contraction, decreasing the stoichiometric spectrum among individuals or 3) BN displacement, shifting the mean foliar stoichiometric spectrum of all individuals. The four monocultures occupied different BNs in the PCA space and all the BN changes observed occurred though *biogeochemical niche displacement*, supporting the third possibility that we postulated.

The anatomical differences and life cycles of grass and shrub species may lead to important differences in the nutrient contents of leaves (Reich and Oleksyn 2004). In our study, the BN spectra of the two functional groups were clearly discriminated (Fig.2.2). However, BN did not differ between the two shrub species even though they have different life strategies (evergreen and deciduous). In contrast, the foliar BN of the two grass species *H. lanatus* and *A. elatius* was different, probably because of differences in the life spans of their leaves. Both species grew under the same climatic and soil conditions (soil nutrients did not differ significantly between the communities), so these results can be interpreted as different resource exploitation strategies. The different soil exploitation strategies is a logical explanation for our results and is in line with the complementary niche hypothesis, which claims that different species occupy different ecological niches based on resource acquisition (Loreau and Hector 2001).

The community composition produced a BN displacement in three of the four species. The BNs of *V. myrtillus* and *H. lanatus* shifted when growing in different communities, the BN of *C. vulgaris* also shifted although only marginally significantly, whereas the BN of *A. elatius* did not shift. The displacement of the BNs indicates differences in the exploitation of soil resources depending on each community composition. This is in accordance with what Sistla and Schimel 2012 expose as stoichiometric flexibility ecosystem mechanism, where an organism can alter its allometric patterns or tissue chemistry to adjust its overall stoichiometry, which can differ among and within organisms and across trophic groups.

Another reasonable explanation could be that stoichiometry changes reflect changes in plant function (e.g. growth, root length, flowering, LAI and above ground net primary production) in response to the different abiotic resources available in different communities (Kreyling et al. 2008a, 2008b, Mirzaei et al. 2008, Jentsch et al. 2009). Consistent with the changes in foliar stoichiometry of species depending on community species-specific composition observed in this experiment some previous studies have observed that species richness and functional group richness affect plant community C:N (Guiz et al. 2016) and C:P and N:P ratios (Abbas et al. 2013). Despite these two latter studies were at community and not species level, such as the present study, these results show the link between different species composition and different overall function and the proportional use of different elements.

V. myrtillus foliar K concentration was highest in the community where it was competing with the other dwarf shrub (H2). High foliar K concentration is related to drought-resistance strategies, such as high insolation rate and stomatal control

(Sawhney and Zelitch 1969; Cakmak 2005; Sardans et al. 2012a; Sardans and Peñuelas 2015a). Thus, high foliar K concentration in *V. myrtillus* growing in the H2 community suggest that the coexistence with *C. vulgaris* increased the water stress on this species more than when it grew with grasses, or on its own in the monoculture. This could have happened because *C. vulgaris* has higher individual biomass than the grass species and therefore demands more water.

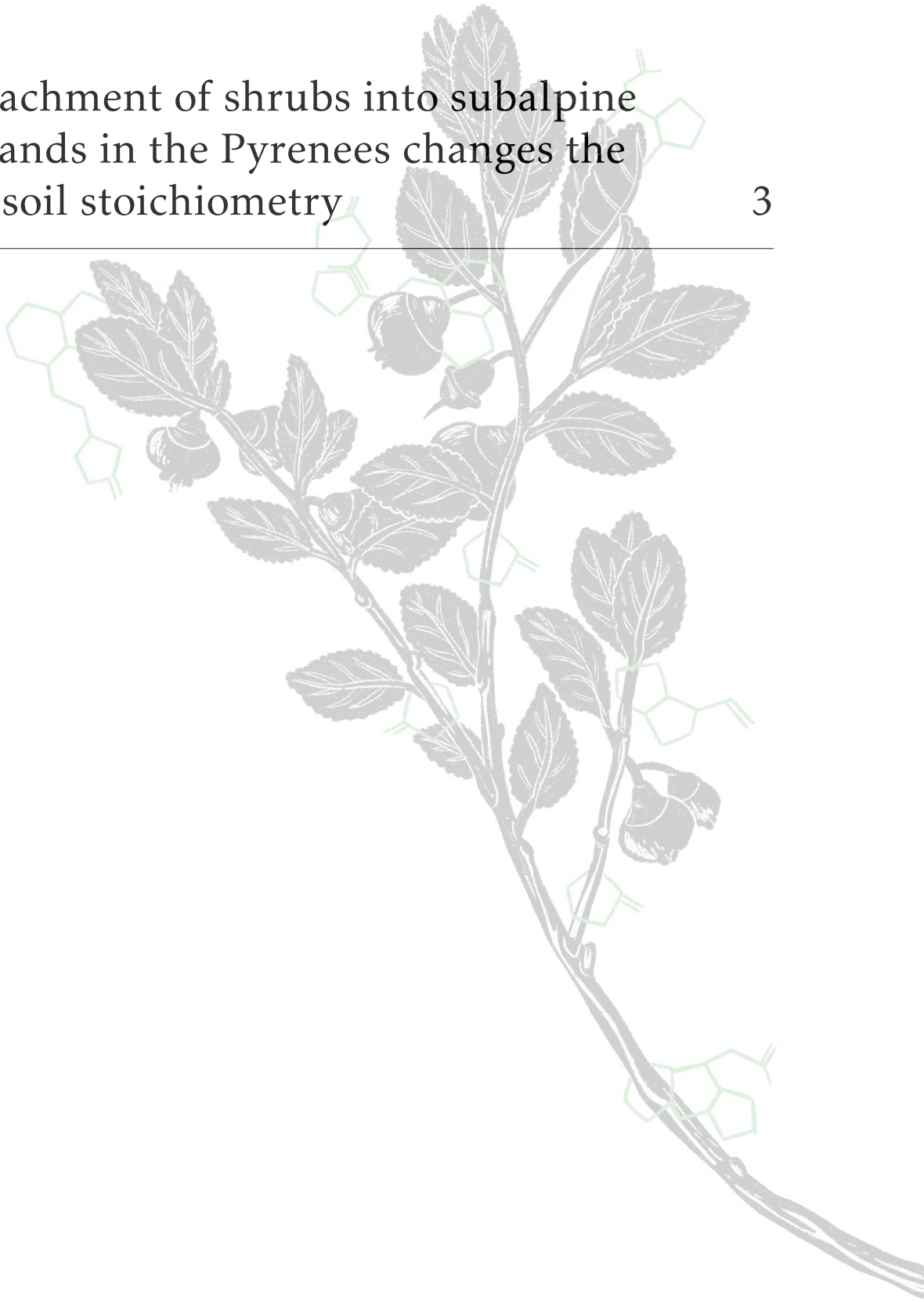
H. lanatus nutrient concentrations were higher in the monoculture than in the mixed stands, suggesting that interspecific competition was detrimental to its nutrient uptake. Foliar element concentrations for *A. elatius* differed very little among the communities. The lack of stoichiometric flexibility in species that did not change their BN indicate a more homeostatic stoichiometry, i.e. the ability to maintain an specific elemental composition despite changes in the availability of elements in the surrounding environment (Elser et al. 2010; Yu et al. 2010). This type of stoichiometry could have important implications on the stability and prevalence of some species in changing environments.

Finally, our results provide good examples of the possible stoichiometric responses of several species in different community conditions (displacement of their BN). The compromise between stoichiometric flexibility and homeostasis determined the differential responses of the species. However, this study lacked physiological data that could explain the physiological mechanisms of these shifts. New and more thorough studies on foliar stoichiometry and community complexity, with a larger number of coexisting species and physiological measurements will guaranteed enhance our knowledge about the stoichiometry of terrestrial system.

2.5 Conclusion

The study provides evidence that plant stoichiometry responds to changes in plant community composition. The BNs of the species changed in response to the community composition in which they grew, and these changes occurred by displacement of their stoichiometry spectrum. Nevertheless, foliar composition was more homeostatic in *C. vulgaris* and especially in *A. elatius*. Regardless of the underlying mechanisms ruling foliar stoichiometry changes, our results suggest that changes in plant composition affect the species foliar stoichiometry. Our results are also a good example of a possible complementary BN hypothesis, and highlight the importance of plant stoichiometric flexibility in the ecosystem. We encourage further and deeper studies that contribute to better understanding the relationship between community composition and plant stoichiometry.

Encroachment of shrubs into subalpine grasslands in the Pyrenees changes the plant-soil stoichiometry 3



Abstract

Shrub encroachment has been reported over a large proportion of the subalpine grasslands across Europe and is expected to have an important impact on the functioning and the biogeochemical cycle of these ecosystems. Shrubs and herbaceous species differ in their growth forms, resource acquisitions and allocation strategies, so the succession from grassland to shrubland is expected to cause major changes in the plant-soil stoichiometry and cycling of nutrients in the ecosystem. We investigated the stoichiometric changes in the soil and in the plant aboveground compartments of the main herbaceous species and shrubs involved in this succession at two sites with contrasting abiotic and biotic conditions in the Central Pyrenees.We analyzed the chemical composition (C, N, ^{15}N, P, K, Ca, Mg and Fe) in the soil and in the plant compartments (leaves, leaf-litter and stems) of the main herbaceous species and shrubs at three contrasting stages of the succession from grassland to shrubland: grassland, young shrubland and mature shrubland. The plant-soil stoichiometry spectrum differed between the successional stages. Shrub encroachment generally increased the C and Ca concentration and the C:N ratio and often decreased the N, P and K concentrations in the leaves and leaf-litter, while the concentrations of several soil nutrients (N, P, K Ca and Mg) mostly decreased. The stocks of C, N, P, Ca, and Mg in the total aboveground biomass compartments increased with the advance of the encroachment. The stock of nutrients in the upper 10 cm in the soil was 100-fold larger than in the biomass in all successional stages at North face and 100-fold larger in the grassland and young shrubland and 10-fold lager in the mature shrubland at South face. Soil nutrient stocks mostly decreased along the succession at South face but not at North face.Shrub expansion into subalpine grassland has a clear impact on the plant-soil stoichiometry spectrum. The pure grassland is an ecosystem dominated by hemicryoptophyte species with fast turnover of nutrients between the plant compartments and the soil; the plant-soil system generally shows high concentrations of N, P and K. The productivity of the grassland is high, but the stock of biomass is low. The expansion of shrubs, though, favored the dominance of long-lived species, with a more conservative strategy, with low concentrations of N and P in the plant-soil compartments and high C:nutrient ratios in the aboveground biomass. The total stocks of C and nutrients in the aboveground biomass were nevertheless high because the biomass of the mature shrubland was very high compared to the grassland. We thus highlight the role of shrubs in the sequestration of C and nutrients, through the allocation to the aboveground biomass. Moreover, shrub encroachment alters the strategy with which N is acquired, possibly through an increased uptake of N through ericoid or ectomycorrhizae. The changes in plant-soil elemental composition and stocks suggest a slowdown of the biogeochemical cycles in the subalpine mountain areas where shrub encroachment occurred.

- **Key words:** shrub encroachment, plant-soil stoichiometry, nutrient stocks, subalpine grassland succession, plant strategy.

3.1 Introduction

The encroachment of shrub species into grasslands causes important changes in many grass-dominated ecosystems at landscape and regional scales from low to high latitudes (Van Auken 2009, Myers-Smith et al. 2011, Naito and Cairns 2011, Komac et al. 2013, Formica et al. 2014). The expansion of shrubs is mostly caused by changes in climatic conditions and land use (Eldridge et al. 2011). For example, the ongoing increase in mean annual temperature and the thinning of permafrost at high latitudes are promoting the expansion of shrubs across the Arctic and subarctic tundra (Tape et al. 2006; Hallinger et al. 2010; Myers-Smith et al. 2011). Shrub expansion has also been observed in most mountain ranges and massifs across Europe at lower latitudes, where humans have used the subalpine and alpine grasslands for traditional activities such as extensive livestock herding (Roura-Pascual et al. 2005; Anthelme et al. 2007; Hallinger et al. 2010; Targetti et al. 2010; Ferré et al. 2013) for many centuries or even millennia (Gassiot and Jiménez 2006; Pèlachs et al. 2007; Gassiot et al. 2016). The progressive abandonment of these practices in recent decades has favored the encroachment of shrubs into subalpine and alpine grasslands (MacDonald et al. 2000; Dullinger et al. 2003; Komac et al. 2013; Ameztegui et al. 2016). Climatic warming is also expected to promote the expansion of woody species in subalpine and alpine regions in the Pyrenees (Grau et al. 2013; Peñuelas et al. 2016; Angulo et al. 2019) and across Europe in the coming decades (Sanz-Elorza et al. 2003; Körner and Paulsen 2004; Wookey et al. 2009; IPCC 2013). The abandonment of subalpine and alpine grasslands is very apparent in the Pyrenees (northeastern Iberian Peninsula), where traditional extensive livestock grazing and the frequency of intentional fires to create or maintain pasture has substantially decreased in the last century, mainly due to socio-economic changes in this area (Lasanta et al. 2000; MacDonald et al. 2000; Serrano et al. 2000; Roura-Pascual et al. 2005; Jiménez and Pujol 2010; Barrio et al. 2013).

Shrub expansion is expected to cause several changes in ecosystem functioning, but few studies have focused on the impacts of expansion on the functioning of subalpine or alpine grasslands in the Pyrenees (Vitousek 1984; Montané et al. 2007, 2010; Barrio et al. 2013; Catalan et al. 2017; Grau et al. 2019) and across Europe (MacDonald et al. 2000; Wookey et al. 2009). Some studies have reported that shrub encroachment has increased the carbon (C) and nitrogen (N) concentrations in the soil and decreased soil pH (Knapp et al. 2008; Eldridge et al. 2011), but such changes may strongly depend on shrub traits and ecosystem features. Shrub and grass species have contrasting growth forms and differ in many functional traits of their adaptive and reproductive capacities and their strategies of resource acquisition and allocation (Chapin and Körner 1994). Different co-occurring shrub species may also have contrasting traits and strategies (Illa et al. 2017), so the identity of shrubs is also crucial to understand the changes in ecosystem functioning when encroachment occurs (Grau et al. 2019). Succession from grassland to shrubland is thus expected to cause important changes in the distribution, cycling and stoichiometry of chemical elements in the plant-soil system. Furthermore, the increase in woody biomass during the succession adds complexity to the persistence and cycling of C and nutrients in the ecosystem.

Wood is highly persistent and stores C and nutrients such as N, phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) (Chave et al. 2009; Sardans and Peñuelas 2015b). The nutrient stocks stored in wood, however, depend mainly on wood density and may vary greatly among species (Sardans and Peñuelas 2013; 2015).

Most of the research on the impacts of shrub encroachment into grasslands has focused on soil C and N balances (Hibbard et al. 2001; Jackson et al. 2002; Hood et al. 2003; Throop and Archer 2008; Van-Auken 2009), including studies conducted in the Pyrenees (Garcia-Pausas et al. 2007; Montané et al. 2007; Garcia-Pausas 2010). However, changes in the chemical composition of both plants and soils, including key elements such as P, K, Ca, Mg and iron (Fe), along the succession from grassland to shrubland, have not yet been investigated in detail. We studied two common and contrasting landscapes where shrubs encroachment typically occurs in the Pyrenees, one facing north (hereafter North face) and one facing south (hereafter South face). The two sites differ greatly in their abiotic conditions (macro and microclimate, bedrock lithology, topography) and in the functional characteristics of their vegetation (biotic conditions); the dominant shrubs are: *Calluna vulgaris* (L.) Hull, *Rhododendron ferrugineum* L. and *Vaccinium myrtillus* L. at North face and *Arctostaphylos uva-ursi* (L.) Spreng, *Juniperus communis* L. and *Juniperus sabina* L. at South face. Each study site represents an independent case study because the grassland and shrubland species differ between the two sites. In each of the two sites, we established a replicated sampling design in three contrasting stages along the succession: a) the pure grassland (initial stage of succession, where shrubs are not yet present), b) the mixed, young shrubland (intermediate stage of succession, where grassland has small patches of shrubs) and c) the mature shrubland (advanced stage of succession, where the dominant shrub species form large, monospecific patches). The main aim was to analyze the changes in the chemical composition (concentrations and stocks) of the aboveground-biomass compartments (leaves and leaf-litter in herbaceous species; leaves, leaf-litter and stems in shrubs) and the soil along the succession from grassland to shrubland in the Central Pyrenees.

A previous study conducted in the same study sites in the Pyrenees highlighted that the concentrations of some soil nutrients (e.g. N, P or K) generally decreased along the succession from grassland to shrubland (Grau et al. 2019), although there were differences between sites and shrub species. It was hypothesized that shrubs may store nutrients in the biomass to take control of nutrients of the ecosystem and promote their further expansion along the succession. In this current study, we test this hypothesis and investigate whether the decrease in the concentrations of soil nutrients along the succession was coupled with changes in concentrations and/or stocks of nutrients in the aboveground biomass and in the soil. Moreover, shrub expansion into grassland is expected to shape the plant-soil stoichiometry spectrum and the biogeochemical cycle through changes in the abiotic and biotic conditions along the succession from grassland to shrubland. We thus hypothesized that each successional stage would show a contrasting plant-soil stoichiometric spectrum, resulting from changes in vegetation structure and allocation strategies in each stage, and that C:nutrient ratios in the aboveground biomass and soil would increase over the succession due to the lignification of

plant compartments.

3.2 Material & Methods

Study area, species and sampling design

The two study sites were located in the Central Pyrenees (NE Iberian peninsula), one site on the North face (Bargadèra, Val d'Aran, 42°3953.9N, 00°5007.8E, with Cambro-Ordovician schists, sandstones and quartzites) and one on the South face (Fogueruix, Pallars Sobirà, 42°3532.9N, 01°0428.8E, with a Devonian slaty limestone), both on the periphery of the 'Aigüestortes i Estany de Sant Maurici' National Park (Fig.A.3.1). The two sites are separated by only 22 km but are characterized by contrasting macro and microclimates; the site in Val d'Aran has a strong Atlantic influence, whereas the site in Pallars Sobirà is more continental. The mean annual number of days with precipitation or fog and the relative air humidity are therefore higher at North face than South face (Catalan Meteorological Service, http://meteo.cat/wpweb/climatologia, accessed on March 2018). The North face is a smooth north-facing slope at an altitude of 1800-1900 m a.s.l. Since 2015, annual precipitation at the nearest meteorological station to North face (Bagergue, 1400 m a.s.l.) has ranged from 800 to 900 mm, mean annual temperature has ranged from 8 to 9 °C and minimum and maximum absolute temperatures were -14 and 30 °C, respectively. The South face site is a steep south-facing slope at an altitude of 2000-2100 m a.s.l. Since 2015, annual precipitation at the nearest meteorological station to South face (Planes de Son, 1540 m a.s.l.) has ranged from 600 to 800 mm, mean annual temperature has ranged from 8 to 10 °C and minimum and maximum absolute temperatures were -15 and 30 °C, respectively. Both meteorological stations are at altitudes (ca. 400 m) lower than the study sites, so precipitation is expected to be higher and temperatures are expected to be lower (average and range) than the reported values at both sites. Both sites are in areas that have been intensively used for livestock grazing for many centuries, but socio-economic changes in the Pyrenees since the 1950s decreased the density of livestock, and some summer pastures have been abandoned (MacDonald et al. 2000; Jiménez and Pujol 2010), which have favored the expansion of shrubs into these former grasslands.

The grassland at North face is classified as a mesophilic, dense subalpine grassland *'Nardion'*(Galvánek and Janák 2008), dominated mostly by grasses (*Festuca eskia* Ramond ex DC. and *Festuca nigrescens* Lam.) with scarcer forbs (*Trifolium alpinum* L., and *Cerastium arvense* L.). The succession is mostly driven by the dwarf shrubs *Calluna vulgaris*, *Rhododendron ferrugineum* and *Vaccinium myrtillus*. The grassland at South face is classified as a xerophilic, open montane grassland '*Xerobromion*' (Carreras et al. 1983; EAE 2019), dominated by grasses (*Festuca ovina* L. and *Festuca gautieri* (Hack.) K.Richt) that co-occur with small forbs (such as *Hieracium pilosella* L., *Achillea millefolium* L. and *Potentilla neumanniana* Rchb.). The succession from grassland to shrubland is mostly driven by the dwarf shrubs *Arctostaphylos uva-ursi*, *Juniperus communis* and *Juniperus sabina* L. and to a much lesser extent by *Helianthemum nummularium* (L.) Mill. or *Thymus pulegioides* L.

Soils at the North face site are built from Cambro-Ordovician schists, sandstone and quartzite, and, given the moderate slope of the area, they are moderately deep, rich in organic matter, and acidic, corresponding in general to Humudepts. Those at the South face site result mainly from Silurian pelite and also from Devonian lime-rich slate. Being this site steeper, soils are shallower and irregular, moderately acidic and humic, broadly corresponding to lithic Humudepts (Boixadera et al. 2014). Moreover, soils at the South face experience summer drought episodes resulting into apparent drying off of the shallow soil level, which does not occur at the North face. The soil pH in the North face tend be more acidic (pH around 4.7-5) than in South face (pH 5.7-6.3) (Grau et al. 2019). The percentage of soil organic matter measured in the same study sites were higher at North face (herbaceous species: 21.2%, *Calluna vulgaris*: 37.8%, *Rhododendron ferrugineum*: 40.12% and *Vaccinium myrtillus* 40.6%) than South face (herbaceous species: 16.5%, *Arctostaphylos uva-ursi*: 16.2%, *Juniperus communis*:13.2% and *Juniperus sabina*: 13.4%) (Grau et al. 2019).

We selected three stages at each site along the succession from grassland to shrubland: a) pure grassland, which is still regularly grazed by domestic animals (Oriol Grau, pers. observation), is dominated by herbaceous species and has no shrubs, b) young shrubland, composed of a mosaic of herbaceous species and shrub patches of ca. 1-2 m2 and c) mature shrubland, where the dominant shrub species form large monospecific patches (at least 10 × 10 m). We selected the most common shrubs at each site (*C. vulgaris*, *R. ferrugineum* and *V. myrtillus* at North face and *A. uva-ursi*, *J. communis* and *J. sabina* at South face (see suplementary Table A.3.1, A.3.2 and A.3.3 for the ecological and functional characterization of the shrub species studied). The herbaceous species and each of the shrubs occurring along the succession in each site will hereafter be referred to as 'vegetation types'.

The replication of the successional stages was done in each site separately; the two sites were not treated as replicates in the analyses but as two independent study cases. Sampling plots were established to reproduce the extant structure of the vegetation in each successional stage. We established four replicate 2 × 2 m plots (separated by a minimum of 10 m) in the pure grassland, and in each of three mature shrublands at each site. In the mixed shrubland, though, the plots for each vegetation type (grass, shrub 1, shrub 2, shrub 3) were grouped because all vegetation types co-occurred; in this intermediate stage, we established four groups of four plots (Fig.A.3.2 and Grau et al. 2019 for further details). In total we sampled 32 plots per site along the succession from pure grassland to mature shrubland. The distance between the successional stages or among the mature shrublands of each shrub species was >100 m. The plots in the pure grassland were placed in areas that represented the mixture of grass species that co-occurred in this successional stage, whereas the plots in the mature shrubland were placed in large patches dominated by each of the shrub species.

We collected plant and soil samples towards the end of the growing season (September 2015) for the analysis of their elemental compositions. The litter layer was removed prior to the soil sampling; root samples could not be collected and analyzed in this study. We collected a total of 144 shrub samples: 2 sites × 2 successional stages where shrubs were present (young shrubland and mature

shrubland) × 3 shrub species × 4 replicates × 3 plant compartments (leaves, leaf-litter -dead leaves still attached to the plant- and stems). We collected a total of 32 samples of herbaceous species in the pure grassland and the young shrubland: 2 sites × 2 successional stages where these were present (pure grassland and young shrubland) × 4 replicates × 2 plant compartments (leaves and leaf-litter). We also collected soil samples to a depth of 10 cm next to each plant sample with a 5-cm diameter soil corer, 48 samples for shrubs (2 sites x 2 successional stages x 3 shrubs species x 4 replicas) and 16 samples for herbaceous species (2 sites x 2 successional stages x 1 grassland x 4 replicas), for a total of 64 soil samples. The concentrations of C, N, P, K, Mg, Ca and Fe (see details of the chemical analyses in the next section) and ^{15}N were measured in leaves, leaf-litter, lignified stems and soils. The samples were transported to the laboratory in paper envelopes, the soil samples were sieved (2 mm) and all samples were oven-dried at 60 °C for 48 h. They were then ground with a ball mill (Retsch, model MM400. RestchGmbH. Haan, Germany), weighed with an AB204 Mettler Toledo (Mettler Toledo, Barcelona, Spain) and analyzed in the chemistry laboratories at Servei d'Anàlisi Química, Autonomous University of Barcelona (Barcelona, Spain), where the percentages of P, K, Mg, Ca and Fe in dry weight were determined, and at the University of California Davis Isotope Facility (Davis, USA), where the isotopic compositions ($\delta^{15}N$) and percentages of C and N in dry weight were determined.

We revisited the same sampling areas in September 2016 and collected more samples to characterize the mean biomass of each plant compartment and the bulk density of the soil, which we used to estimate the stocks of C and nutrients in the aboveground compartments (g m^{-2}) of the vegetation types and the soil. We assumed that the aboveground biomass of the herbaceous species was similar between the two years, because their aboveground structures are entirely or nearly newly produced each year and the climatic conditions (mean annual temperature and precipitation) did not vary much (http://meteo.cat/wpweb/climatologia). The changes in biomass of shrubs are very limited in this ecosystem (Ninot et al. 2010a), so we do not expect large significant variations between two consecutive years with similar climatic conditions. We collected the aboveground vegetation within 25 × 25 cm quadrats in the young shrubland and 50 × 50 cm quadrats in the pure grassland and mature shrubland. The biomass was then transported in plastic bags to the laboratory and sorted manually into leaves and woody stems (only for shrubs). We collected a total of 48 foliar samples and 48 stem samples from shrubs (2 sites × 2 successional stages where shrubs were present × 3 shrub species × 4 replicates) and 16 foliar samples from herbaceous species (2 sites × 2 successional stages where herbaceous species were present × 4 replicates). The plant material was sorted and oven-dried at 60 °C to a constant weight, and the dry weight was measured. We collected soil samples with a bulk-density corer (9 cm diameter to a depth of 10 cm) to estimate the bulk density for each vegetation type. The quantity of soil (g cm^{-2}) was calculated as the product of the bulk density multiplied by the core depth. We also calculated the annual leaf-litter production based on estimates of foliar persistence conducted in nearby locations (Ninot et al. unpublished data). Leaf-litter productivity was used to estimate the annual input of nutrients from the leaf-litter for each vegetation type.

Chemical analyses

The concentrations of C, N (dry weight (dw)/dry weight) and ^{15}N isotope were determined by gas chromatography. The amount of sample used was based on the percentages of C and N in each type of sample. For leaves, leaf-litter and stems, 4.5 mg of dry sample were weighed and encapsulated in tin capsules. For soils, 8.6 mg of each sample were used. The samples were then analyzed for C and N with an Elementar Cube system (Elementar Analyzen system GmbH, Hanau, Germany). ^{15}N isotope was analyzed by an Elementar Vario EL Cube or Micro Cube elemental analyzer connected to a PDZ Europa 20-20 isotope-ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The plant samples were combusted at 1000°C in a reactor packed with chromium oxide and silvered copper oxide and soils were combusted at 1080 °C in a reactor packed with copper oxide and tungsten (VI) oxide. The oxides were then removed in a reduction reactor (reduced copper at 650 °C). The samples were interspersed during the analysis with several replicates of at least two laboratory standards. These standards, which were selected for their compositional similarity to the samples, had been previously calibrated against National Institute of Standards and Technology (NIST) Standard Reference Materials (IAEA-N1, IAEA-N2, IAEA-N3, USGS-40 and USGS-41). Preliminary isotopic ratios were measured relative to the reference gases analyzed for each sample. These preliminary ratios were refined by correcting for the entire batch based on the known ratios of the laboratory standards. The long-term standard deviation was 0.3‰ for $\delta^{15}N$.

The concentrations of P, Mg, K, Ca and Fe in the leaves, leaf-litter, stems and soil were determined by inductively coupled plasma mass spectrometry (ICP-MS) after digestion. For leaves, leaf-litter and stems, 0.25g of dry material were diluted in 5 ml of concentrated HNO_3 and digested in a MARSXpress microwave system (CEM GmbH, Kamp-Lintfort, Germany). The solution generated was analyzed by ICP-MS to determine the elemental concentrations. The accuracy of the biomass digestions and analytical procedures was assessed using certified biomass NIST 1573a (tomato leaf, NIST, Gaitherburg, USA) standards and regularly analyzing blank solutions with no sample (5 mL of HNO_3 and 2 mL of H_2O_2 with no sample). Dry soil subsamples of 0.1 g were dissolved in an acidic mixture of HNO_3, HCl and HF and digested as described for the plant samples. The solutions were diluted with 1% HNO_3 (v/v) before injection into the spectrometer. Blank solutions (5 mL of HNO_3 and 2 mL of H_2O_2 with no sample) were regularly analyzed. Total stocks of the elements (g m^{-2}) for each plant compartment and the soil were calculated as the biomass (leaves, leaf-litter or stems) and soil weights multiplied by the concentration of each element analyzed. The C:N, C:P and N:P ratios were calculated on a mass basis.

Statistical analyses

We conducted principal component analyses (PCAs) of the elements to visualize the overall differences in the chemical composition of the plant and soil samples collected for each vegetation type (shrub and herbaceous species) and successional stage. We standaridzed the variables before the analyses. We per-

formed a PCA for the elements of all compartments (leaves, leaf-litter and soil) for each site separately to assess the differences in the chemical compositions among the successional stages and vegetation types within each site. If two variables were highly positively correlated (Pearson's product moment correlation coefficient >0.6), we excluded one of the two in the PCA to avoid the overfitting of variables. We also conducted PCAs of each plant compartment (leaves, leaf-litter, and stems) and soil using data from both study sites to identify differences between vegetation types and sites. To identify links between the functional traits of the shrubs and the foliar elements concentrations (C, N, P, K, Mg, Ca and Fe), we conducted PCAs of the foliar elemental concentrations and some key functional measurements, as seed weight, specific leaf area (SLA), wood density, biomass per unit area, height and annual leaf-litter production. All PCAs were conducted with the 'ggbiplot' (Wickham 2009) and 'FactoMineR' (Le et al. 2008) packages in R. The significance of the differences between vegetation types and successional stages in each PCA were tested by permutational multivariate analyses of variance (PERMANOVAs) with the R 'vegan' package (Oksanen et al. 2017). β dispersion homogeneity tests were conducted prior to PERMANOVA analyses. We analysed the differences in elemental concentrations (dry weight (dw) of nutrient/dw of sample × 100) and stocks (g m^{-2}) in the plant and soil and between vegetation types (herbaceous species and shrub 1, shrub 2, and shrub 3) and successional stages at each site. Although in our sampling design we maximised the independence of samples as much as possible given the extant structure of vegetation along the succession, it was not possible to find a completely independent, random distribution of combinations of successional stage × vegetation type in the field sites. We therefore firstly checked if there were any underlying patterns of spatial aggregation in our data with autocorrelation semivariograms ('nlme' package, (Pinheiro et al. 2017)), that are used for measuring the degree of spatial dependence between observations as a function of distance. As in Grau et al. (2019) the initial visual interpretation of the semivariograms indicated that in most cases the data was not autocorrelated despite the plots were not completely randomized. Yet, we preferred to statistically check if autocorrelation should be accounted for in the models. To do so we built two types of models: 1) Generalised least square (GLS) models that did not account for spatial autocorrelation, 2) GLS models that included the 'corSpatial' R function to account for potential spatial autocorrelation. For this second type of GLS models, the coordinates (longitude and latitude) of each plot were specified and we fitted five different models using a different autocorrelation structure each time (e.g. exponential, linear, gaussian, rational quadratic or spherical). After running all the models (one without and five with autocorrelation structure) for the element concentrations and stocks in each soil or plant compartment, we compared and selected the best model using scores of the Akaike Information Criterion (AIC). We used maximised log-likelihood in all cases and checked the normal distribution of the data before run the models. The pure grassland was the initial stage of the succession from grassland to shrubland, and we considered it as the reference level in all models. The two study sites were analyzed as two independent study cases, so 'site' was not included in the models as a factor. All analyses were performed with R 3.2.4 (RCoreTeam 2017).

3.3 Results

Significant differences in the elemental concentrations and ratios in the plant compartments (leaves and leaf-litter and stems (only for shrubs)) and soil for all the vegetation types along the encroachment succession in both study sites are summarized in Tables 3.1, 3.2 and 3.3. Means and sd values are summarized in Table A.3.4.

Patterns of plant-soil stoichiometry along the succession at North face

The plant-soil stoichiometry spectrum at North face varied along the two axes of the PCA (Fig. 3.1a). The first PC axis explained 32.6% of the variance and the second axis explained 23%. Such variation was explained by differences between successional stages and also between vegetation types (PERMANOVA tests $p<0.001$ in both cases). Because the β-dispersion homogeneity test showed significant differences between groups (successional stages or vegetation types), the significance of these PERMANOVA tests may thus result from differences in the position of the centroids as well as from differences in the dispersion of the data around the centroids.

Leaves and leaf-litter of grass herbaceous spp. patches in the young shrubland had a higher C:N ratio and lower [N], [K] and [Fe] than the herbaceous spp. in the pure grassland; the soil [P] was lower in herbaceous spp. patches in the young shrubland than in the pure grassland (Table 3.1). Generally, shrub leaves in the young and mature shrubland had higher [C], C:N ratio and lower [N], [K], [Fe] and N:P than herbaceous spp. in the pure grassland (Table 3.1 and Table 3.2); leaf-litter [C] was higher in shrubs than in the pure grassland. Foliar [Ca] and [Mg] in the mature shrubland were higher than in the pure grassland. Regarding the soil, generally vegetation types in young shrubland had higher soil C:N ratio and lower [P] and [Mg] than in pure grassland. Soil N was also lower in the young shrubland of *R. ferrugineum* and *V. myrtillus* and soil K, Ca and Fe were lower in the mature shrubland of *C. vulgaris* compared to the pure grassland. Foliar δ^{15}N and [N] were lower for all the vegetation types in the young and the mature shrubland than herbaceous spp. in pure grassland (Fig. A.3.3).

We found stoichiometric differences within shrub species between the young and mature shrublands (Table 3.3 and Table A.3.4). In the mature shrubland, *C. vulgaris* had higher [K] and C:N ratio and lower [Mg] in the leaf-litter, higher C:N ratio and lower [N] and [K] in the stems. *R. ferrugineum* had higher foliar N:P, higher [N], [K] and lower C:N ratio in leaf-litter, higher stem [N] and N:P and lower C:N and higher soil [Mg], N:P and C:P ratio in the mature than the young shrubland. *V. myrtillus* had higher foliar [C] and [Fe], lower leaf-litter N:P and C:P ratios and higher soil [Mg] in the mature than the young shrubland (Table 3.3 and A.3.4).

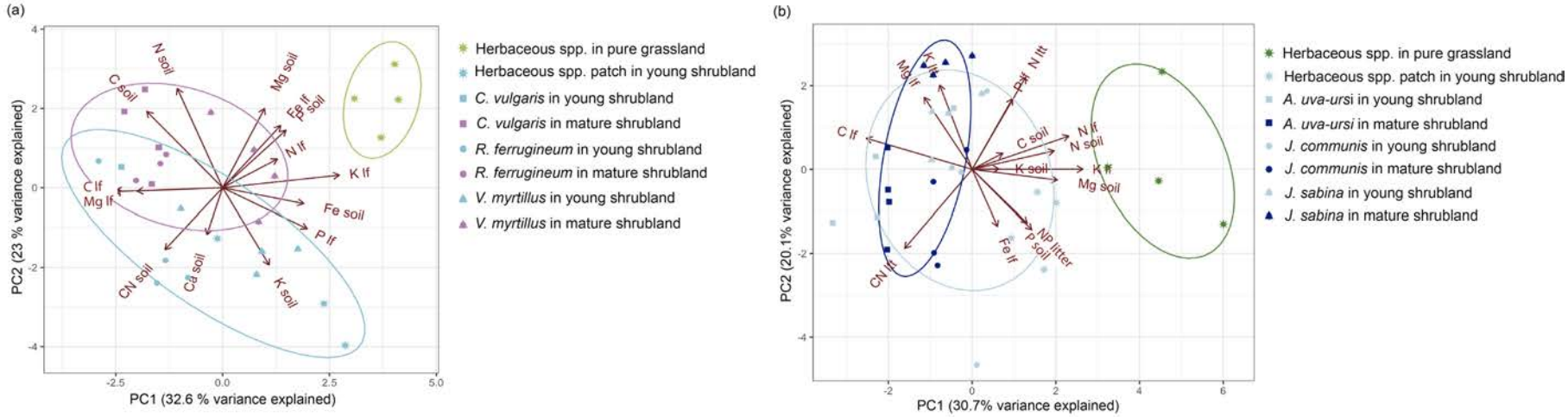


Figure 3.1: Principal component analysis (PCA) based on the chemical elements (C, N, P, K, Ca, Mg and Fe) in leaves (lf), leaf-litter (ltt) and soil for the successional stages and vegetation types at (a) North face and (b) South face. Only variables that were poorly correlated with each other (Pearson's coefficients < 0.6) are included in the analyses. Different colors indicate the successional stages and different shapes indicate the vegetation types. The ellipses represent the dispersion around the centroid for each successional stage with a normal probability of 0.85.

The stocks of nutrients were generally much larger in the soil (generally 100-fold in all successional stages) than in the biomass. The stocks of N (9.4 g m^{-2}), P (0.71 g m^{-2}), K (8.5 g m^{-2}) and Fe (0.06 g m^{-2}) in herbaceous spp. leaves in the pure grassland were significantly larger than in the leaves of all vegetation types in the young and mature shrublands (Fig. 3.2. Table A.3.4). Nevertheless, shrub stems generally stored larger amounts of C and nutrients compared to leaves, so that the stocks of C and nutrients in the aboveground biomass in the young and mature shrubland were generally much larger than in the grassland (except for K). *C. vulgaris* and *R. ferrugineum* in the mature shrubland had higher stocks of soil C (8953.5 g m^{-2}, 7388.7 g m^{-2}, respectively) and soil N (507.5 g m^{-2}, only *C. vulgaris*) than the pure grassland (C: 3645.7 g m^{-2}, N: 294.7 g m^{-2}). The stocks of soil P and K stocks in *C. vulgaris* in the mature shrubland tend to increase but not significantly. Herbaceous spp. in pure grassland showed the highest annual leaf-litter production (509.4 g m^{-2} $year^{-1}$) (Fig. A.3.4 and Table A.3.4); also, the annual leaf-litter production in *V. myrtillus* was higher in the mature (99.8 g m^{-2} $year^{-1}$) than the young shrubland (53.8 g m^{-2} $year^{-1}$) (Fig. A.3.4 and Table A.3.4), implying higher inputs of C (49.5 g m^{-2} $year^{-1}$), N (1.1 g m^{-2} $year^{-1}$), P (0.12 g m^{-2} $year^{-1}$), K (0.68 g m^{-2} $year^{-1}$), Mg (0.2 g m^{-2} $year^{-1}$), Ca (0.7 g m^{-2} $year^{-1}$) and Fe (0.007g m^{-2} $year^{-1}$) in the mature shrubland (Fig. A.3.4 and Table A.3.4). Leaf-litter production did not differ significantly for the other vegetation types.

Patterns of plant-soil stoichiometry along the succession at South face

The plant-soil stoichiometry spectrum at South face varied greatly along the two axes of the PCA based on the chemical composition of leaves, leaf-litter and soil (Fig. 3.1b). The first PC axis explained 30.7% of the variance, and the second PC axis explained 20.1%. We found significant differences between successional stages (PERMANOVA $p<0.001$) and vegetation types (PERMANOVA $p<0.004$). In general, herbaceous spp. in the young shrubland had lower [N] and [K] in leaves, higher [C], [Fe], N:P and C:N ratio and lower [N] and [P] in the leaf-litter and higher [Fe] and lower [N], [Ca] and [Mg] in soil than the pure grassland (Tables 3.1 and A.3.4). Shrub leaves in the young shrubland generally had higher [C] and C:N ratios and lower [N], [K] and N:P ratio than herbaceous spp. in pure grassland, and shrub leaf-litter generally had higher [C] and lower [N] than herbaceous spp. in pure grassland (Table 3.2). The soil for all vegetation types in young shrubland had lower [N], [Ca], [Mg] and N:P ratio than the soil in herbaceous spp. in pure grassland. Leaves in the mature shrubland often had higher [Ca] and [Mg] and lower [N], [K] and N:P ratio, and leaf-litter with higher [C] and lower [Fe] than the pure grassland. Soil [N], [Ca], [Mg] were always lower in the young shrubland than the pure grassland (Table 3.1); soil [P] was also lower in the mature shrubland of *A. uva-ursi* and *J. sabina.* (Table 3.2). Foliar $\delta^{15}N$ and [N] were also lower for all vegetation types in the young and the mature shrubland than herbaceous spp. in pure grassland (Fig. A.3.3), as observed at North face.

Table 3.1: Significant changes in the element concentrations for the different vegetation types in the **young shrubland** compared to the herbaceous spp. in the **pure grassland**. Changes increases (↑) or decreases (↓) identified by generalized least square models (GLS) based on the elemental concentrations (C, N, P, K, Ca, Mg and Fe) (dw/dw) and the C:N, C:P and N:P ratios. Means and sd are summarized in Table A.3.4

Site	Vegetation types	Leaves		Leaf-litter		Soil	
		↑	↓	↑	↓	↑	↓
North face	Herbaceous spp.	C:N	C, N, K, Fe	C:N	N, K, Mg,Fe	Ca, C:N	P
North face	*Calluna vulgaris*	C, Ca, Mg, N:P, C:N, C:P	N, P, K	C, N, Ca, Mg	Ca,N:P, C:N, C:P	P, Mg	
North face	*Rhododendrum ferrugineum*	C, Ca, Mg, C:N	N, K, Fe	C, Ca, Mg, C:N, C:P	N, K, Fe	Ca, K,C:N	N, P, Mg
North face	*Vaccinium myrtillus*	C, Ca, Mg	N, K, Fe	C, Ca, K, Mg	n.s	Fe	N, Ca, Mg, N:P
South face	Herbaceous spp.	n.s	N, K	C, Fe, N:P, C:N	N, P	Fe	N, Ca, Mg
South face	*Arctostaphylos uva-ursi*	C, C:N, C:P	N, K, N:P	C, K, Mg,C:N	N, Fe, N:P	Fe, C:N	N, K, Ca, Mg, N:P
South face	*Juniperus communis*	C, Ca, C:N, C:P	N, K,N:P	C, Ca,C:P	N, P, Fe		N, Ca, Mg, Fe N:P
South face	*Juniperus sabina*	C, Ca, Mg, C:N	N, K, N:P	C, K, Ca, Mg	n.s	Fe	N, Ca, Mg, N:P

Table 3.2: Significant changes in the element concentrations for the different vegetation types in the **mature shrubland** compared to the herbaceous spp. in the **pure grassland**. Changes increases (↑) or decreases (↓) identified by generalized least square models (GLS) based on the elemental concentrations (C, N, P, K, Ca, Mg and Fe) (dw/dw) and the C:N, C:P and N:P ratios. Means and sd are summarized in Table A.3.4

Site	Vegetation types	Leaves		Leaf-litter		Soil	
		↑	↓	↑	↓	↑	↓
North face	*Calluna vulgaris*	C, Ca, Mg, N:P, C:N, C:P	N, P, K, Fe	C, Ca, Mg	n.s	C:N, C:P	K, Ca, Fe
North face	*Rhododendrum ferrugineum*	C, Ca, Mg, C:N, C:P	N, K, Fe	C, Ca, Mg, C:N, C:P	N, P, K, Fe	C, Ca, N:P, C:N, C:P	Mg
North face	*Vaccinium myrtillus*	C, Ca, Mg, C:N	N, K, Fe, N:P	C, P, K, Ca, Mg	Fe	Ca, C:N	n.s
South face	*Arctostaphylos uva-ursi*	C, Ca, Mg	N, P, K, N:P, C:N, C:P	C, Mg, C:N, C:P	N, P, Fe, N:P	C:N	N, P, K, Ca, Mg
South face	*Juniperus communis*	C, Ca, C:N	N, K, N:P	C, Ca, C:N, C:P	N, P, Fe	n.s	N, Ca, Mg N:P
South face	*Juniperus sabina*	C, Ca, Mg, C:N	N, K, N:P	C, P, K, Mg, Ca	Fe, N:P	n.s	N, P, Ca, Mg

Table 3.3: Significant changes in the element concentrations for the different vegetation types in the **mature shrubland** compared to the **young shrubland**. Changes increases (↑) or decreases (↓) identified by generalized least square models (GLS) based on the elemental concentrations (C, N, P, K, Ca, Mg and Fe) (dw/dw) and the C:N, C:P and N:P ratios. Means and sd are summarized in Table A.3.4

Site	Vegetation types	Leaves		Leaf-litter		Stems		Soil	
		↑	↓	↑	↓	↑	↓	↑	↓
North face	*Calluna vulgaris*	n.s	n.s	K, C:N	Mg	C:N	N,K	n.s	n.s
North face	*Rhododendrum ferrugineum*	N:P	n.s	N,K	C:N	N, N:P	C:N	Mg, N:P, C:P	n.s
North face	*Vaccinium myrtillus*	C, Fe	n.s	n.s	N:P, C:P	n.s	n.s	Mg	n.s
South face	*Arctostaphylos uva-ursi*	C:P	P	N:P	n.s	n.s	n.s	n.s	n.s
South face	*Juniperus communis*	n.s	n.s	n.s	Ca, C:N	n.s	Ca	n.s	n.s
South face	*Juniperus sabina*	C	n.s	C, N, P	Ca, C:N, C:P	Mg	C:	n.s	n.s

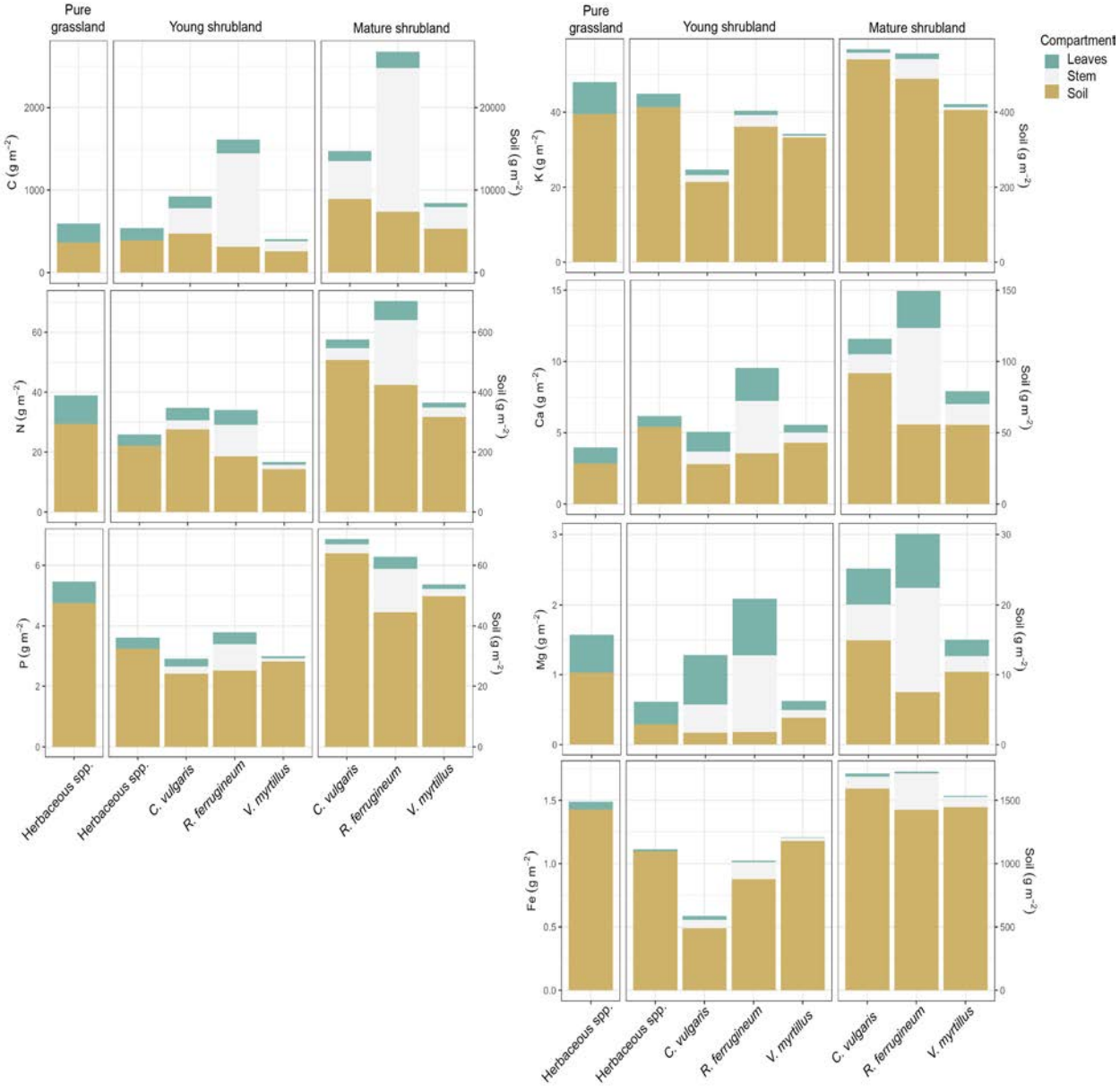


Figure 3.2: Stocks of chemical elements (C, N, P, K, Ca, Mg and Fe) in leaves, stems and soil (g m^{-2}) for each successional stage and vegetation type at the North face. The left column in each figure represents the pure grassland, the columns in the middle represent the vegetation types in the young shrubland, and the columns on the right represent the vegetation types in the mature shrubland. Leaf-litter data are not included because the data available for leaf-litter could only be used to estimate the productivity (g m^{-2}), not the stocks. See Fig. A.3.4 for the leaf-litter production per year

We also found some stoichiometric differences within shrub species between the young and mature stages (Table 3.3). *A. uva-ursi* had lower foliar [P] and higher C:P ratio and higher leaf-litter N:P ratio in the mature than the young shrubland, *J. communis* had lower [Ca] and C:N ratio in the leaf-litter and lower [Ca] in the stems in the mature than the young shrubland and *J. sabina* had higher foliar [C], leaf-litter [C], [N] and [P] and lower [Ca], C:N and C:P ratios and higher stem [Mg] and lower C:N ratio in the mature than the young shrubland (Tables

3.3 and A.3.4). Soil stoichiometry did not differ significantly between the young and mature shrublands.

The stocks of nutrients were generally much larger in the soil (100-fold in pure grassland and young shrubland and 10-fold for mature shrubland) than in the biomass. The stocks of C, N, P, Mg and Ca in the leaves of J. *communis* and *J. sabina* in the young and mature shrubland were higher than in the leaves of the herbaceous spp. in the pure grassland (C: 191.1 g m^{-2}, N: 8.2 g m^{-2}, P: 0.56 g m^{-2}, K: 6.7 g m^{-2}, Mg: 0.45 g m^{-2} Ca: 1.7 g m^{-2}), and the leaves of *A. uva-ursi* had lower K stocks (Fig. 3.3 and, Table A.3.4). As observed at North face, shrub stems generally stored larger amounts of C and nutrients compared to leaves, so that the overall stocks of C and nutrients in the aboveground biomass were generally much larger than in the grassland. The soil for most shrubs had smaller stocks of Mg and Ca in the young and mature shrublands than in the pure grassland (Mg 77.1 g m^{-2} and Ca: 431.1 g m^{-2}). The soil for *A. uva-ursi* (348.19 g m^{-2}) and *J.communis* (315.76 g m^{-2}) in the mature shrubland had lower N stocks than in young shrubland. P tended to decrease along the succession, but not significantly (Fig. 3.3). Annual leaf-litter production by *J. sabina* was higher in the mature (938.9 g m^{-2} $year^{-1}$) than the young shrubland (566.2 g m^{-2} $year^{-1}$) (Fig. A.3.4 and Table A.3.4). Leaf-litter production did not differ significantly for the other vegetation types.

General patterns in the plant-soil stoichiometry spectrum at North and South face

The PCA of the functional traits and foliar elemental composition of the shrubs species from both study sites (Fig. A.3.5) showed that the shrub species at North face differed from those at South face (PERMANOVA $p<0.01$) along the PC2 (31.2% of variance explained). Moreover, differences between shrub species within each site were also discriminated (PERMANOVA $p<0.01$) along the PC 1 (39.1% of variance explained).

The PCA based on the foliar stoichiometry including both sites showed significant differences between shrubs and herbaceous spp. (PERMANOVA $p<0.05$), mostly along the first axis (39.4% of variance explained, Fig. A.3.6a). The chemical composition of leaf-litter showed a similar pattern (PERMANOVA $p<0.001$) but varying mostly along the second axis (24% of variance explained, Fig. A.3.6b). The chemical composition of the soil, however, differed more between the two sites (PERMANOVA $p<0.001$) (41.4% of variance explained along the first axis,Fig. A.3.6c) than between herbaceous spp. and shrubs. In this case, though, because the β-dispersion homogeneity test in Fig. A.3.6c was significant, the significance of the PERMANOVA test may result not only from differences in the position of the centroids but also from differences in the dispersion of the data around the centroids. Finally, the chemical composition of the stems differed mostly between sites (PERMANOVA $p<0.001$) (39.3% of the variance explained along the first axis, Fig. A.3.6d).

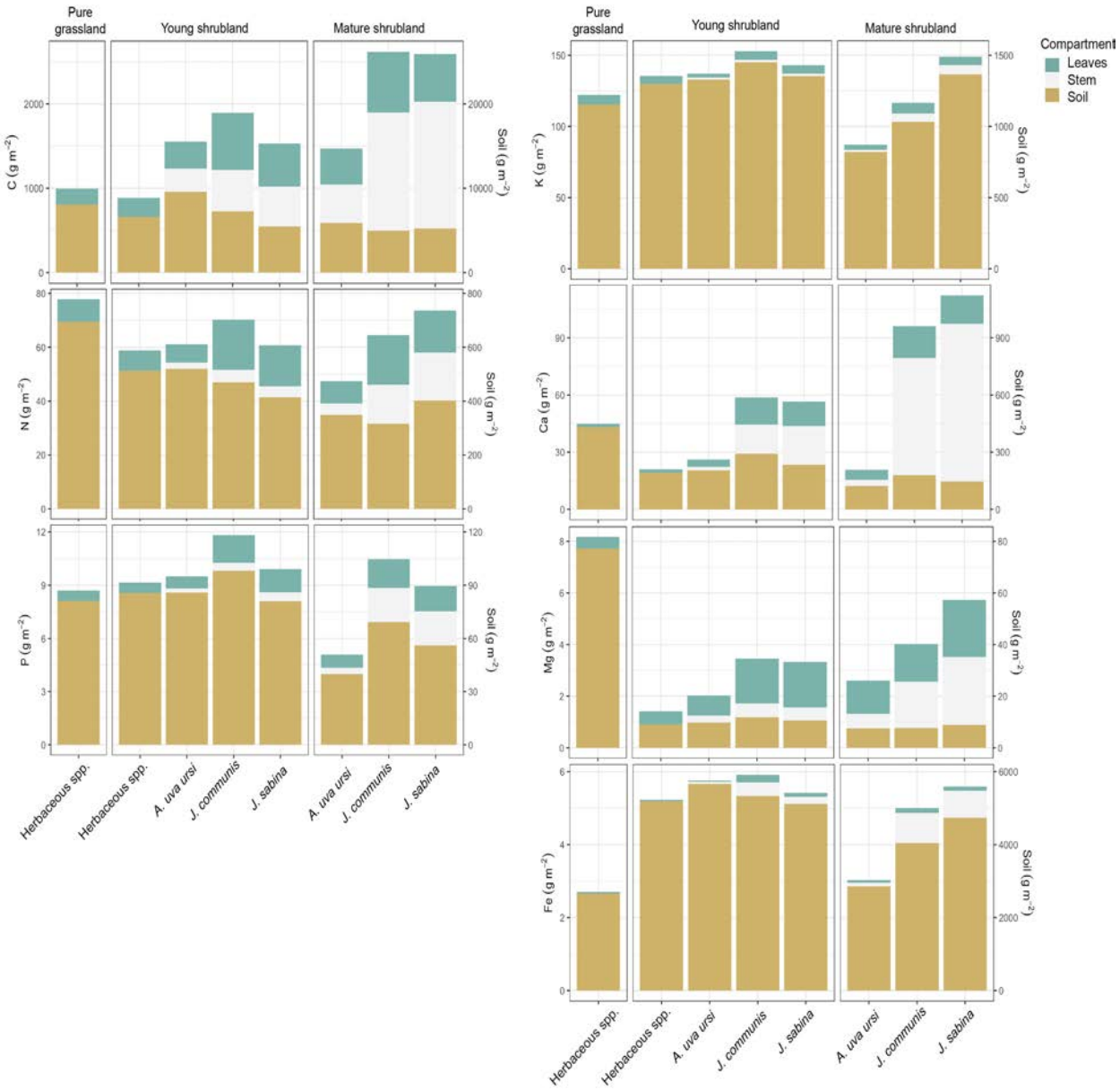


Figure 3.3: Stocks of chemical elements (C, N, P, K, Ca, Mg and Fe) in leaves, stems and soil (g m^{-2}) for each successional stage and vegetation type at the South face. The left column in each figure represents the pure grassland, the columns in the middle represent the vegetation types in the young shrubland, and the columns on the right represent the vegetation types in the mature shrubland. Leaf-litter data are not included because the data available for leaf-litter could only be used to estimate the productivity (g m^{-2} y^{-1}), not the stocks. See Fig. A.3.4 for the leaf-litter production per year

3.4 Discussion

The plant-soil stoichiometry spectrum differed greatly along the succession from pure grassland to mature shrubland (Fig. 3.1), as initially hypothesised. The shift in plant-soil stoichiometry spectrum was more apparent and consistent between the pure grassland and the young shrubland than between the young and mature shrubland (Tables 3.1 and 3.3), suggesting that changes in growth forms (from grass-dominated to shrub-dominated) play a greater role in shaping the plant-soil stoichiometry spectrum than differences in successional stage as such. In fact, the plant-soil stoichiometry spectrum is expected to be primarily shaped by changes in abiotic and/or biotic factors, such as changes in vegetation along the succession. This is evidenced by the shift between herbaceous spp. and shrubs, from high foliar [N] and [K] for herbaceous spp. to high foliar [C], [Ca], [Mg], C:N and C:P ratios and low [N], [P] or [K] for shrubs (Table 3.1 and A.3.4); or from low to high [C], C:N and C:P ratios in litter, and from high to low [N], [P], [Mg] and [Ca] in soil. Such changes in stoichiometry may have major consequences on the functioning of ecosystems (Eldridge et al. 2011). For example, the high [C] and C:N and C:P ratios in the shrub leaves and leaf-litter promote the formation of recalcitrant organic matter accumulation with slow decomposition rates in the top soil of shrubland compared to the grassland (Ninot et al. 2010b; Garcia-Pausas et al. 2017).

The changes in foliar chemical spectrum enabled us to detect shifts in the biogeochemical niche (Urbina et al. 2017; Peñuelas et al. 2019)(Fig. A.3.6). We found that shrubs and herbaceous spp. differed significantly in their biogeochemical niche, as expected from these two contrasting growth forms. For example, herbaceous spp. in the pure grassland had higher foliar nutrient concentrations (N, K), characteristic of plants with fast growth rates; whereas shrubs had higher C concentration and C:N and C:P ratios in leaves, characteristic of plants with slow growth rates (Ågren 2004; Sardans et al. 2012b; Zechmeister-Boltenstern et al. 2015).

The stoichiometry changes in the plant aboveground compartments along the succession were coupled with a decrease of the soil concentrations of some essential nutrients needed for plant development. Grau et al. (2019) suggested that this decrease in nutrient concentrations in the soil was possibly due to the decline in nutrient inputs from excrements of domestic herbivores and to the transfer of nutrients from the soil to the biomass of the shrubs (Horton et al. 2009). The allocation of nutrients to the biomass of shrubs could be a mechanism of nutrient accumulation by which shrubs control nutrients in an ecosystem through a positive feed-back (Chapin et al. 1997) to outcompete herbaceous spp. and expand into the grassland (Grau et al. 2019). In our study we found that the concentrations of N, P and K often decreased in the aboveground biomass along the succession in both study sites (Tables 3.1 and 3.2); at the same time, the concentrations of soil N, P or K often decreased in the mature shrubland of several shrubs. The stocks of N and P in the aboveground biomass (leaves + stems), though, increased for several shrubs along the succession, particularly in *R. ferrugineum*, *J. communis* and *J. sabina* (Figs. 3.2 and 3.3). This indicates that the dilution of these nutrients in the aboveground biomass of shrubs was

overbalanced by their increase in aboveground biomass. This evidences that shrubs may act as net reservoirs of essential nutrients in the biomass even if the concentration in the biomass and soil decrease along the succession.

The amount of C and nutrient stocks in aboveground biomass differed notably amongst shrub species, which implied differences in the final stocks in the mature shrubland within each site. Shrub identity is therefore crucial to understand the variability in concentrations and stocks of nutrients in the vegetation and in the soil within each site. However, we also found differences between the North and South face. Shrubs were generally smaller and less sclerophyllous at North face (Fig. A.3.5), where solar radiation and evapotranspiration are lower, the slope is smoother, and the soil is deeper than at South face (Ninot et al. 2010a). The increases in the C and nutrient stocks (g m^{-2}) in the aboveground plant compartments along the succession from grassland to shrubland were less evident at North face than at South face. In contrast, shrubs developed more woody tissue, were taller and had higher wood density and more sclerophyllous leaves at South face. This is probably because shrubs are adapted to harsher conditions, with more solar radiation, higher evapotranspiration, less precipitation, a steeper slope and shallower soil with more rocky outcrops than at North face (Ninot et al. 2010a).

Finally, herbaceous spp. in young shrubland and shrubs in young and mature shrubland showed lower foliar δ^{15}N in leaves and [N] than herbaceous spp. in pure grassland (Fig. A.3.3). This was often coupled with a decrease in soil [N] in the young and/or mature shrubland for several shrub species (Table 3.1). These results evidence that nitrogen may become limiting in cold ecosystems (Fay et al. 2015), such as subalpine ecosystems with high shrub cover (Angulo et al. 2019). Lower δ^{15}N generally indicates more N uptake by ectomycorrhizal or ericoid mycorrhizal fungi compared to arbuscular mycorrhizal plants such as herbaceous spp. (Michelsen et al. 1998), with more recycled N leading to lower N losses from the ecosystem (Garten 1993; Robinson 2001; Craine et al. 2009b; Anadon-Rosell et al. 2016). All shrubs at North face are ericaceous and have ericoid mycorrhizae; in the South face, *A. uva-ursi* hosts both ericoid and ectomycorrhizal fungi. Thus, the lower ^{15}N in young and mature shrubland possibly indicates that more N had been uptake by ericaceous or ectomycorrhizal fungi in a relatively closed N cycle (Angulo et al. 2019). The N uptake by ectomycorrhizal or ericoid mycorrhizal fungi possibly promotes the uptake of organic N (e.g. from litter) by shrubs (Akhmetzhanova et al. 2012) as N becomes more limiting in the soil. This suggests that the advance of the succession changed the mechanisms by which plants uptake N to overcome N limitation.

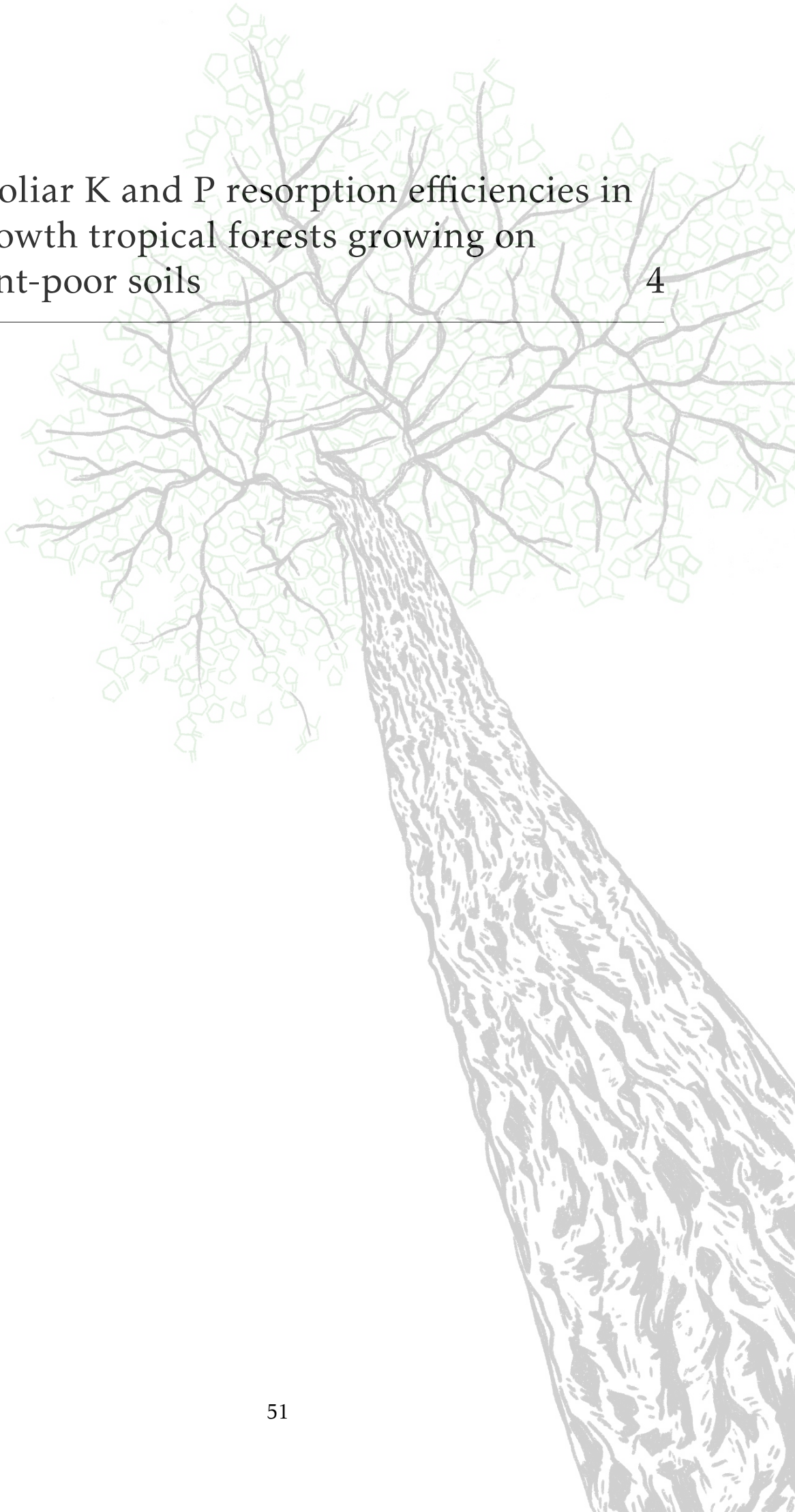
3.5 Conclusion

Shrub expansion have an important impact in the plant-soil elemental composition of the grassland ecosystems in the Pyrenes. Grassland ecosystem are dominated by species with faster nutrients turn-over between the plant-soil compartments, high N, P and K concentrations in the plant aboveground and soil biomass but limited biomass accumulation capacity. The expansion of shrub, though, favored the dominance of long live species, with more conservative strategy, high C:

nutrient ratios and low N, P and K concentration in the aboveground biomass and low nutrient concentrations in soils. The total stocks of C and nutrients in the aboveground biomass were nevertheless high because the biomass of the mature shrubland was very high compared to the grassland. We thus highlight the role of shrubs in the sequestration of C and nutrients, through the allocation to the aboveground biomass. Moreover, shrub encroachment alters the strategy with which N is acquired, possibly through an increased uptake of N through ericoid or ectomycorrhizae. Our results suggested that shrubs play an important role in the C and nutrient sequestration in the aboveground biomass (through the allocation into the plant compartments) along the succession, where the woody tissues play a main role as storage compartment. The changes in plant-soil elemental composition and stocks suggest a slowdown of the biogeochemical cycles in the subalpine mountain areas where shrub encroachment occurred.

High foliar K and P resorption efficiencies in old-growth tropical forests growing on nutrient-poor soils

4



Abstract

Tropical forests in the Guiana Shield store large amounts of aboveground biomass, even though they grow on old, weathered, nutrient-poor soils. Nutrient resorption from leaves before abscission may be very advantageous for trees to avoid nutrient leaching but remains poorly explored in tropical forests. We investigated foliar resorption from the senescent leaves and nutrient stocks in soil, leaves and leaf-litter in two tropical forests in French Guiana, a tropical region with particularly low total and available phosphorus (P) in the soil. P was the scarcer nutrient stored in the leaves (4.6 and 6.5 kg ha^{-1}), leaf-litter (2.2 and 1.8 kg ha$m$1) and soil (205 and 158.7 kg ha^{-1} in the 0-15 cm layer and 205 and 142 kg ha^{-1} in the 15-30 cm layer) in Nouragues and Paracou respectively. Resorption efficiencies were higher for K and P (total means of 44.6 and 35.9%, respectively) than N (total mean of 10.3%). K resorption was higher in the wet (70.2%) than in the dry (41.7%) season. P resorption was weakly negatively correlated with total soil P but not with available P, and neither N nor K resorption were correlated with soil N or K concentrations. Relationships between nutrient resorption and species functional characteristics (growth rate, wood density, diameter at breast height and specific leaf area) were weak and varied among the nutrients. Phylogenetic relatedness did not account for the variability in resorption efficiencies. Instead, the level of nutrient immobilization in foliar compounds probably determine significantly the resorption process. Our results suggest that high K and P resorption efficiencies are an adaptive strategy allowing species to cope with soil nutrient scarcity. We conclude that nutrient resorption from senescent leaves is a key process for plants to conserve nutrients in these tropical forests in French Guiana, especially for K and P, which are difficult to acquire in these old, weathered soils.

- **Key words:** nutrient, resorption, nitrogen, phosphorus, potassium, recycling, tropical forest, stocks, soil.

4.1 Introduction

Tropical forests store large amounts of aboveground biomass, even though they grow on old, highly weathered and nutrient-poor soils (Gersmehl 1976; Vitousek and Sanford 1986). This issue has attracted the attention of the ecological community, and several mechanisms have been proposed to explain this apparent paradox (Vitousek and Sanford 1986; Bond 2010; Sayer and Banin 2016; Turner et al. 2018). One proposed essential mechanism is high efficiency of nutrient recycling on tropical forests growing on nutrient-poor soil. In fact, higher internal nutrient recycling by plants may be a key mechanism to avoid nutrient losses from aboveground biomass in tropical forests with low soil nutrient concentrations (Vitousek 1984; Brant and Chen 2015; Grau et al. 2017).

The resorption of nutrients from leaves (also called 'backmigration', 'retranslocation', 'remobilization' or 'reabsorption') is the active withdrawal of nutrients before abscission (Fries 1952; Hill 1980; Killingbeck 1996). This process begins with senescence, which leads to a series of metabolic changes associated with a decrease in auxin levels, protein breakdown, chlorophyll degradation and eventually

death (Fuente and Leopold 1968). The resorption of foliar nutrients is activated by kinetin signals, which promote nutrient mobilization through the phloem from old senescent leaves, as the source, to other plant organs like stems, roots or new leaves, as the sink (Hill 1980). This process of internal nutrient recycling plays an important role in plant nutrition and survival, allowing plants to be more independent from external conditions (Aerts 1996; Killingbeck 1996; van Heerwaarden et al. 2002; Reed et al. 2012; Brant and Chen 2015). Nutrient resorption can be estimated by resorption efficiency, defined as the percent reduction of a nutrient between green and senesced leaves (Killingbeck 2004). The resorption of nutrients within a plant can vary greatly from year to year depending on the environmental conditions, such as soil water availability, timing of abscission and the amount of shade a plant received (Killingbeck 2004). The capacity of resorption will be firstly determined by the nutrient biochemical limitation, which is the level of nutrient immobilization in the leaf compounds (compounds of which each nutrient is a constituent: enzymes, proteins, DNA, RNA, etc), that will prevent mobilization from senescing leaves. Nutrients in structural and recalcitrant compounds may require high energetic costs to be reabsorbed, and those used for enzymatic machinery for foliar senescence may be unavailable for export from leaves (Killingbeck 2004).

Highly efficient nutrient cycling in nutrient-poor ecosystems has been postulated as a response of species to nutrient-limiting conditions, but the relationship between soil nutrient concentration and nutrient resorption in leaves is not clear (Killingbeck 1996; Aerts and Chapin 2000; Wright and Westoby 2003; Yuan and Chen 2009; Brant and Chen 2015). An analysis of global data conducted by Yuan and Chen (2009) reported an increase in the P resorption efficiencies with temperature and precipitation, suggesting P resorption efficiency as an indicator of P limitation in the tropics. Water availability is clearly a major driver of resorption, the reduction in phloem flux transport and water scarcity lead to lower nutrient resorption due the advance of foliar abscission (Killingbeck 2004; Estiarte and Peñuelas 2015). Phenology substantially affect resorption because the flower or fruit production will act as a sink strength for the nutrient in senescent leaves (Killingbeck 2004; Estiarte and Peñuelas 2015). Phylogenetic relatedness is expected to also play a role in understanding the efficiency of nutrient resorption among plant species, with closely related taxa often having similar resorption efficiencies (Aerts and Van der Piejl 1993; Killingbeck 2004; Sua et al. 2005).

Nitrogen (N), phosphorus (P) and potassium (K) are the most important nutrients for plant development (Aerts and Chapin 2000). Nitrogen is the principal constituent of proteins and nucleic acids in plant cells and is acquired through the plant root uptake as nitrate or ammonia present in the soil and return to the soil through litter inputs, which is why young natural ecosystems are generally poor in N (Walker and Syers 1976; Turner and Condron 2013). Also, nitrogen fixing species are able to fix atmospheric molecular N (N_2) that can be used in the ecosystem. Nitrogen fixing species are common in all terrestrial ecosystems and provide large inputs of N to support plant growth (Chapin 1980), which is important for tropical forests, especially during rapid biomass accumulation (Hedin and Brookshire 2009; Batterman et al. 2013a). Phosphorus is present in plant tissue mainly in energetic forms (ATP and NADPH) and in nucleic acids and

is provided almost completely by apatite-bearing rocks in the parental material (Chapin 1980). Old-growth tropical forests located on geologically ancient soils are generally described as P-limited ecosystems (Bruijnzeel 1991; Vitousek et al. 2010), because of their limited P availability and total P, low cation exchange capacity and typically high concentrations of ferric sesquioxides. These occlude the little P that is left in the soil system, making it inaccessible to most organisms, a process that is known as 'terminal steady state' (Walker and Syers 1976). The deposition of atmospheric dust, however, may represent an important P input in some tropical forests (Gross et al. 2016). Typically, N is not considered to be limiting in these tropical forests (Walker and Syers 1976; Vitousek and Sanford 1986; Tanner et al. 1998), thus we expect that P resorption will be more efficient than N in old-growth tropical forests.

Potassium represents the most abundant cation (K^+) in plant cells and plays a central role in water economy, photosynthetic capacity and nutrient transport in plants, and is thus essential for plant development (Aerts and Chapin 2000; Sardans and Peñuelas 2015a). Despite their importance in plant performance, K resorption efficiencies have been poorly studied (but see Ławniczak 2011; Vergutz et al. 2012). Potassium is highly mobile in plant tissues and is almost completely provided by the weathering of soil parental material. Old tropical soils, especially those of the Precambrian shield, contain little available K, as described for P, due to the weathering and leaching of soil over a very long time (Rosolem et al. 2010; Sayer and Banin 2016). Potassium has been described as a co-limiting nutrient for tree growth in wet lowland tropical forests in Costa Rica (Baribault et al. 2012) and the addition of both K and N increased sapling growth in a long-term fertilization experiment in Panama (Wright et al. 2011). We may thus expect that K will be highly reabsorbed in tropical forests, as expected for P, because their availabilities are similarly low in old tropical soils (Rosolem et al. 2010; Wright et al. 2011). Resorption efficiency in trees is generally higher for P than N (Yuan and Chen 2009; Vergutz et al. 2012), but very few studies have reported K resorption efficiencies (but see Ławniczak 2011; Vergutz et al. 2012).

In this study, we explored the stocks (C, N, P and K) and the resorption efficiencies (N, P and K) of essential elements in aboveground (leaves and leaf-litter) and soil compartments in two old-growth tropical forests growing on old, nutrient-poor soils. We first calculated the stocks (kg ha^{-1}) of carbon (C) and nutrients (N, P and K) of tree leaves, leaf-litter at the soil surface, and soil at two depths (0-15 and 15-30 cm). We then calculated the efficiencies of nutrient (N, P and K) resorption for 39 tropical tree species in the dry season and for a subset of 18 species in the wet and dry seasons to determine if soil nutrient concentration and seasonality affected nutrient resorption. We investigated the possible relationships between the nutrient resorption and some functional characteristics: growth rate, wood density, diameter at breast height (DBH) and specific leaf area (SLA). We also investigated the possible variability in the nutrient resorption efficiencies due to the phylogeny. We calculated the phylogenetic signals of the species (Pagel's λ and Blomberg's K) to determine whether closely related species tended to have more similar resorption efficiencies than distantly related species.

We hypothesized that: 1) nutrients that has been lost greatly by soil leaching would be stored in larger quantities (kg ha^{-1}) in the aboveground biomass (leaves

and leaf-litter) than in the soil, whereas soil would store larger quantities of C and N than the aboveground biomass; 2) K and P would have higher resorption efficiencies than N, because they are less available in the soil and more mobile in the plant; 3) nutrient resorption efficiencies would be higher in the wet than the dry season, because water conductance and the capacity to transport nutrients would be higher in the wet season; 4) species with slow growth rates and high wood densities (a more conservative strategy) would reabsorb more nutrients than species with high growth rates and low wood densities (a more acquisition strategy), which would probably depend more on the nutrients in the litter and soil; and 5) resorption efficiencies would be more similar among closely than distantly related species.

4.2 Material & Methods

Study area

The study was conducted in French Guiana, a tropical region in the north-eastern part of South America. French Guiana is almost completely covered by tropical forest and has an old, nutrient-poor Precambrian substrate, characteristic of the Guiana Shield formation (Bongers et al. 2001; Epron et al. 2006; Courtois et al. 2018). Two study sites were selected: Paracou (5°18N, 52°53W) and Nouragues (4°05'N, 52°41'W) (Fig. A.4.1a). Paracou is located in Sinnamary, 15 km inland from the coast, in an undisturbed forested area characterized by smooth mosaic hills (Janssens et al. 1998; Epron et al. 2006). The Nouragues Research Station is situated in the center of the country, which is covered by extensive primary forest with granitic hills (Bongers et al. 2001). The climate at both sites is typical of tropical rainforests, with two marked seasons: a rainy season from December to July and a dry season from August to November. Mean annual rainfall is similar in both study sites (2990 and 3041 mm y^{-1} at Nouragues and Paracou, respectively) (Bongers et al. 2001; Aguilos et al. 2018). The soils at both sites are classified as Oxisols in the USDA soil classification, typically strongly weathered nutrient-poor red or yellow soils from old, stable geomorphic surfaces rich in sesquioxides (IUSS Working Group 2014).

Experimental design

Two field campaigns were carried out in 2015, from May to the end of June in the rainy season and from the beginning of October until late November in the dry season. Twelve experimental plots were established at each site to represent the spatial variability, including the top of the hills (top plots), the slope of the hills (slope plots) and the bottom of the hills next to rivulets (bottom plots) (Fig. A.4.1b). We established four plots of 50 × 50 m (20-100 m the between plots) at each topographic position and an inner 20 × 20 m sampling area in each plot. We marked, tagged and identified all trees in each plot (50 × 50 m) and measured the DBH of all trees with DBHs >10 cm.

We established five sampling points within each plot, one in the center and one in each corner of the square, to collect leaf-litter at the soil surface and the soil samples (Fig. A.4.1c). We collected all mixed leaf-litter at the soil surface (leaf-litter hereafter) in an area of 20 × 20 cm at these 5 sampling points within each plot. These samples where oven-dried at 70 °C to constant weights. Soil samples were collected in each sampling points with a soil auger 4 cm in diameter from two layers (0-15 and 15-30 cm) and sieved through a 2 mm sieve. Soil weight was then determined after drying at 105°C for 24 h and divided by the corer volume to obtain the soil bulk density.

In each plot, we collected leaves from three canopy emergent trees and two subcanopy trees in the sampling areas based on their DBH, trying to maximize the number of species sampled (if two trees belonged to the same species, we chose the next tree of a different species but with similar size). Green leaves were collected (between 5 or 10 leaves by tree depending of the leaf size) by tree climbers from the upper, mostly sunlit leaves and lower, mainly shaded leaves of the canopy to obtain two contrasting canopy conditions. The leaves were immediately frozen in liquid N. If the climbers could not reach trees in the sampling areas, then we chose trees in the buffer zones (between the inner 20 × 20 m square and the outer 50 × 50 m areas) following the same criteria.

Tree climbers also collected senescent leaves when still attached to the tree and could be easily collected by shaking the branch. Taking both study sites together, we were able to collect senescent leaves from a total of 39 species during the dry season (Appendix 4, Table A.4.1). A subset of 18 species from the 39 collected in dry season were collected also in the wet season (Table A.4.2).

Chemical analysis

Samples were freeze-dried (Christ Freeze Dryer ALPHA 1-2 LDplus. Osterode am Harz, Germany), ground with a ball mill (Retsch, model MM400. Restch GmbH, Haan Germany), and weighed with an AB204 Mettler Toledo (Mettler Toledo, Barcelona, Spain) balance. Leaf C and N (dry weight (dw) of nutrient/dw of sample × 100) were determined by gas chromatography. The amount of sample used was based on % C and % N in each type of sample. For the leaves, senescent leaves and the leaf-litter, 4.5 mg of pulverized dry sample were weighed and sealed in tin capsules. For the soil samples, 9 mg for the 0-15 cm layer (samples with high organic-matter content) and 11 mg for the 15-30 cm layer (samples with moderate-low organic-matter content) were used. Leaves, senescent leaves and leaf-litter samples were then analyzed with a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd. Cheshire, UK), and soils samples with an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH. Hanau, Germany). The samples were combusted at 1000°C (plant material) in a reactor packed with chromium oxide and silvered copper oxide and at 1080 °C (soils) in a reactor packed with copper oxide and tungsten (VI) oxide. Oxides were then removed in a reduction reactor (reduced copper at 650 °C). The samples were interspersed with several replicates of at least two laboratory standards during the analysis. These laboratory standards, selected to be compositionally similar to the samples being

analyzed, had been previously calibrated against National Institute of Standards and Technology (NIST) Standard Reference Materials (IAEA-N1, IAEA-N2, IAEA-N3, USGS-40 and USGS-41).

Pulverized samples were oven-dried at 70 °C for 48 h for determining the concentrations of P and K. For the senescent leaves and leaf litter, 0.25 g of dry material was used, and 0.1 g was used for soil. Samples were diluted in 5 mL of concentrated HNO_3 and digested in a Milestone Ultrawave digestor (Sorisole (BG), Italy). The solution generated was analyzed by inductively coupled plasma (ICP) mass spectrometry to determine the elemental concentrations. The accuracies of the biomass digestions and analytical procedures were assessed using the certified NIST 1573a standard (tomato leaf; NIST, Gaithersburg, USA) for biomass and NIST 2711a (Montana Soil) and CRM005 (Sewage Amended Soil) for soil. Blank solutions (5 mL of HNO_3 and 2 mL of H_2O_2 with no sample) were also regularly analyzed.

Available P was determined by two methods, Bray-P acid fluoride extraction (Bray and Kurtz 1945) and Olsen-P bicarbonate extraction (Olsen et al. 1954) on soils that had been previously sieved (2 mm) and dried at 60 °C. P concentrations in both extracts were measured using an iCAP 6300 Duo ICP optical emission spectrometer (Thermo Fisher Scientific, Germany).

For calculating nutrient stocks per hectare, foliar elemental composition (% C, N, P and K) for trees that were not sampled but were present in our sampling area (20 × 20 m), we used the BRIDGE database (Baraloto et al. 2010), which compiled data on foliar chemistry for many tree species in French Guiana. Mean wood density and SLA were also obtained from the same database, whereas mean growth rate (mm of DBH increase y^{-1}) was obtained from the Guyafor database (Grau et al. 2017), which compiles data on growth rates for many tree species and for several sites across French Guiana, including Paracou and Nouragues.

Leaf weight for each species was calculated as:

$$Leaf\,weight(kg) = 0.02634434 \times basal\,area \tag{4.1}$$

where the $basal\ area\ = \pi \times (\frac{DBH}{2})^2$

The allometric coefficient (0.02634434) was obtained by the power-law fit with the DBH (cm) as a predictor of leaf weight (kg), using the data gathered by Chave et al. 2014. This dataset contained data for 2013 trees (180 in Africa, 942 in the Neotropics and 891 in tropical Asia) at 31 tropical rainforest sites. Dry forests and woodlands were excluded to minimize biases due to the occurrence of deciduous trees (see the Appendix 4 for the coefficient deduction). We then calculated foliar C and nutrient stocks per tree as the product of leaf weight and the foliar C and nutrient concentration. Finally, foliar C and nutrient stocks per unit area were calculated as the sum of all the stocks contained on leaves in trees inside the 20 x 20 m sampling area:

$$Y(kgha^{-1}) = \sum_{n=1}^{n} X \tag{4.2}$$

where Y represents the stocks of a given element (C, N, P or K; stocks for all leaves present in a plot) and n represents the number of trees in the 20 × 20 m sampling area, and X represent the stocks of a given element in the leaves of each tree.

Leaf-litter nutrient stocks were calculated as the product of the sample dw per unit area and the C and nutrient concentrations in the sample.

Soil weight per area was calculated as the product of bulk density (g cm^{-3}) and core depth (15 cm), and nutrient stocks in the soil were calculated as the product of soil weight and the C and nutrient concentrations in the sample.

Foliar C and nutrient concentrations did not differ significantly between the upper and lower parts of the canopy (Fig. A.4.2), so we used the means of both canopy levels.

We calculated nutrient-resorption efficiencies as described by Killingbeck 1996:

$$\frac{(X_{Gl} - X_{Sl})}{X_{Gl}} \times 100 \tag{4.3}$$

where X_{Gl} and X_{Sl} represent the nutrient concentrations of green and senescent leaves, respectively. We unfortunately did not measure weight loss during senescence, so we were not able to apply the mass-area loss correction to the resorption efficiency (van Heerwaarden et al. 2002), which could lead to underestimates of the resorption efficiencies in our study. Negative efficiencies were possibly due to this lack of correction.

Statistical analyses

We performed a principal component analysis (PCA) using the 'FactoMineR' package (Le et al. 2008) in R to investigate the relationship between the N, P and K resorption efficiencies, foliar and soil chemical compositions (C, N, P and K) and functional characteristics (mean growth rate, wood density, DBH and SLA).

We constructed linear models using the 'nlme' package (Pinheiro et al. 2017) in R to identify possible differences in C, N, P or K stocks between sites for each compartment (leaf, leaf-litter, upper soil layer and lower soil layer), with the combination of element and compartment (e.g. foliar N stocks) as the response variable and site as the predictor. We also constructed linear mixed models using the 'nlme' package (Pinheiro et al. 2017) to identify possible differences in the nutrient resorption efficiencies between different seasons. In order to identify differences in the percentage of nutrient reabsorbed, we first constructed a mixed model for the species sampled in both study sites only in the dry season (N = 39), with resorption efficiency (% of resorption) as the response variable, the nutrient reabsorbed (N, P or K as categorical factors) as predictor and species identity added as a random effect. Differences between N, P and K were tested using Tukey post hoc analysis. We tested the effect of season by constructing three mixed models for the species sampled in both seasons (N = 18), one for each nutrient, with resorption efficiency (N, P and K resorption efficiencies) as the response variable, season as the predictor and species identity added as a random effect. We also

used mixed models to test the relationships between nutrient resorption (N, P, and K resorption efficiencies as response variable) and the nutrient concentrations in leaves and soil and functional traits (predictors), with the species identity added as a random effect. All analyses were performed in R 3.5.2 (RCoreTeam 2017).

Phylogenetic analysis

We tested for possible phylogenetic effects on the nutrient resorption efficiencies by calculating phylogenetic signals using Pagel'sλ and Blomberg's K (Münkemüller et al. 2012), two common indices used to quantify the tendency of related species to resemble each other more than species drawn at random from the same phylogenetic tree. Values near 1 indicate a significant phylogenetic effect. We based our phylogenetic tree on only 31 of the 39 species due to the lack of precise phylogenetic information for eight of the species: *Aniba rosaeodora* Ducke., *Chrysophyllum poniferum* (Eyma), *Eugenia culcullata* Amshoff, *Inga jenmanii* Sandwith, *Licania densiflora* Kleinh, *Myrcia splendens* (Sw.)DC., *Paloue guianensis* Aubl. and *Vochysia sabatieri* Marc.-Berti. This tree was obtained from a phylogenetic tree constructed by Chave et al. (unpublished data) for many species present in French Guiana. All analyses were conducted using the 'picante' (Kembel et al. 2010) and 'phytools' (Revell 2012) packages in R 3.5.2 (RCoreTeam 2017).

4.3 Results

P was the scarcest nutrient stored in the leaves, leaf-litter and soil in both study sites (Table 4.1, Fig. 4.1). C, N and K stocks in both soil layers were higher at Nouragues than Paracou, but soil P stocks did not differ significantly between sites. The leaf-litter mass per area at the soil surface was higher in Nouragues (9544.03 ± 1410.83 kg ha^{-1}) than Paracou (8641.36 ± 1602.93 kg ha^{-1}); the leaf-litter stocks of N, P and K were higher at Nouragues than Paracou, but foliar C and nutrient stocks did not differ significantly between sites. Soil N:P and K:P ratios (on a mass basis) were high in both soil layers and sites but were higher at Nouragues than Paracou (Fig.A.4.3). At Nouragues, soil N:P (mean ± sd) was 23.20 ± 11.29 in the 0-15 cm layer and 14.62 ± 7.84 in 15-30 cm layer and soil K:P was 16.67 ± 18 in the 0-15 cm layer and 22.22 ± 24.37 in the 15-30 cm layer. At Paracou, soil N:P (mean ± sd) was 17.75 ± 6.3 in the 0-15 cm layer and 10.03 ± 4.03 in the 15-30 cm layer, while soil K:P was 7.19 ± 3.74 in the 0-15 cm layer and 10.49 ± 8.68 in the 15-30 cm layer.

Table 4.1: Mean values for C, N, P and K stocks (kg ha^{-1}) SD in parentheses, in the plant compartments (leaves and leaf-litter at the soil surface) and in the 0-15 and 15-30 cm soil layers at the two sites. Asterisks indicate significant and dots marginally differences between sites.

Compartment	Site	C (kg ha^{-1})	N (kg ha^{-1})	P (kg ha^{-1})	K (kg $ha^{-}1$)
Leaf	Nouragues	4111.82 (2143.56)	158.11 (80.25)	4.65 (2.50)	41.42 (24.20)
Leaf-litter	Nouragues	4564.67 (719.91)	138.86 * (19.20)	2.21 · (0.47)	16.37 * (5.54)
Soil (0-15 cm)	Nouragues	50103.94 * (4435.59)	3582.78 * (289.74)	204.82 (136.93)	2032.82 · (83.64)
Soil (15-30 cm)	Nouragues	28209.03 * (4409.40)	2222.35 * (278.64)	205.38 (140.11)	2551.29 · (80.44)
Leaf	Paracou	5688.56 (5387.90)	207.34 (216.85)	6.52 (6.41)	54.63 (62.60)
Leaf-litter	Paracou	4156.70 (819.51)	113.26 * (21.55)	1.84 · (0.53)	9.68 * (6.22)
Soil (0-15 cm)	Paracou	36320.06 * (8391.08)	2565.75 * (629.95)	158.47 (61.22)	1008.78 · (181.85)
Soil (15-30 cm)	Paracou	17171.39 * (4551.76)	1361.19 * (375.06)	141.64 (49.13)	1264.61 · (108.27)

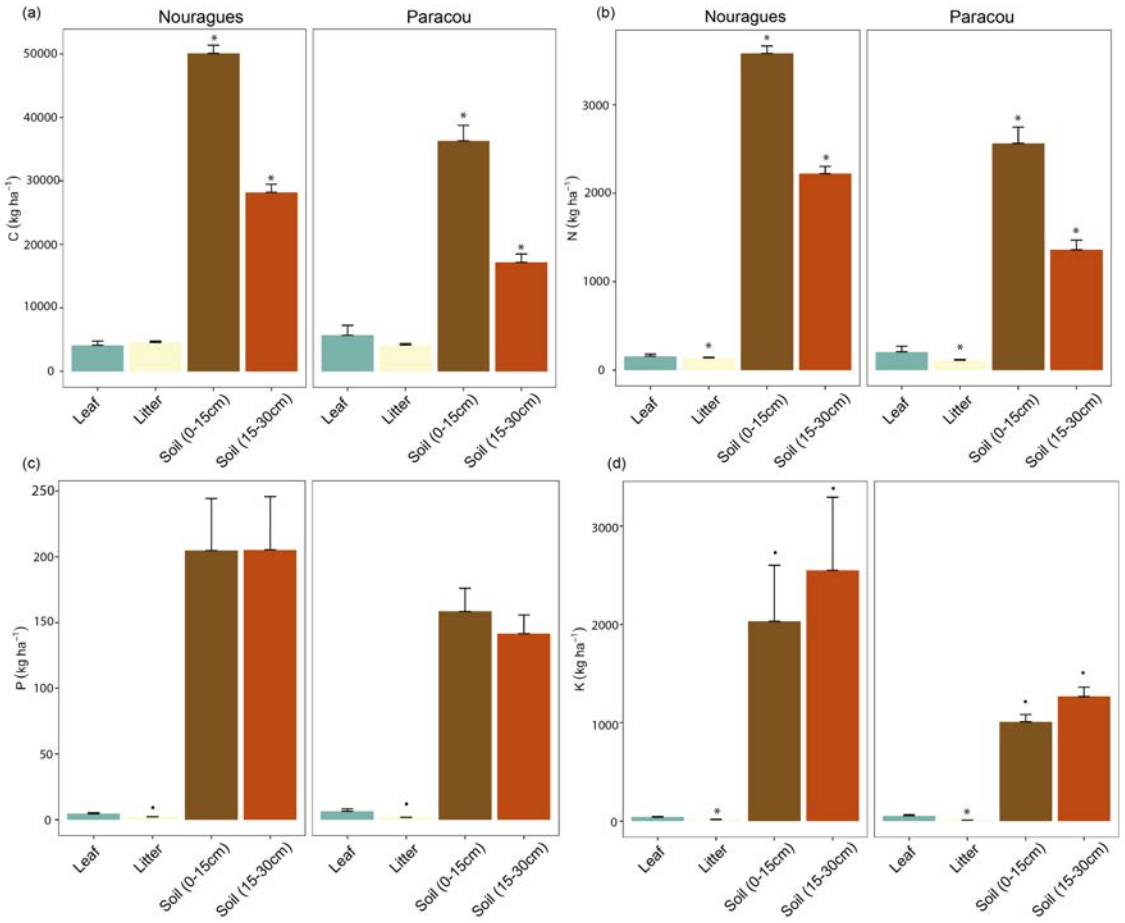


Figure 4.1: Nutrient stocks in aboveground plant organs and soil (kg ha^{-1}) for Nouragues (left panels) and Paracou (right panels).(a) Carbon, (b) nitrogen, (c) phosphorus and (d) potassium. Error bars indicate standard deviation, asterisks indicate significant and dots marginally differences between sites.

In general, K and P resorption efficiencies were higher than N for all species studied (Fig. 4.2). Resorption efficiencies (mean across all species at both study sites in dry season, N=39) were higher for K and P (total mean of 44.6% and 35.9%, respectively) than N (total mean of 10.3%) (Tables A.4.3 and A.4.4). Foliar N:P ratio (mean across all species at both study sites in dry season, N=39) was 29.4 ± 7.01. The range of variation in the resorption efficiencies was smaller for N (between -31 and 69.5%) than for P (between - 91.6 and 86.7%) and K (between -91.6 and 90.6 %). Resorption efficiency presented more negative values for N than the other elements (Fig.4.2 and see Table A.4.1 for resorption efficiencies by species). Nitrogen and P resorption efficiencies for all species studied were positively correlated with foliar N and P concentrations (R^2=0.27, p<0.0001 and R^2=0.15, p=0.013, respectively), while K resorption efficiency was only marginally significantly correlated with foliar K concentration (R^2=0.08, p=0.08) (Fig. A.4.4). Phosphorus resorption efficiency was negatively correlated with total soil P (R^2=0.14, p=0.02) but not with available P (Olsen or Bray method) (R^2=0.007, p=0.6 and R^2=0.073, p=0.09 respectively) (Fig. A.4.5). Nitrogen and K resorption efficiencies were not correlated with N and K soil concentrations (R^2=0.0039, p=0.7 and R^2=0.0045, p=0.6 respectively) (Fig. A.4.6).

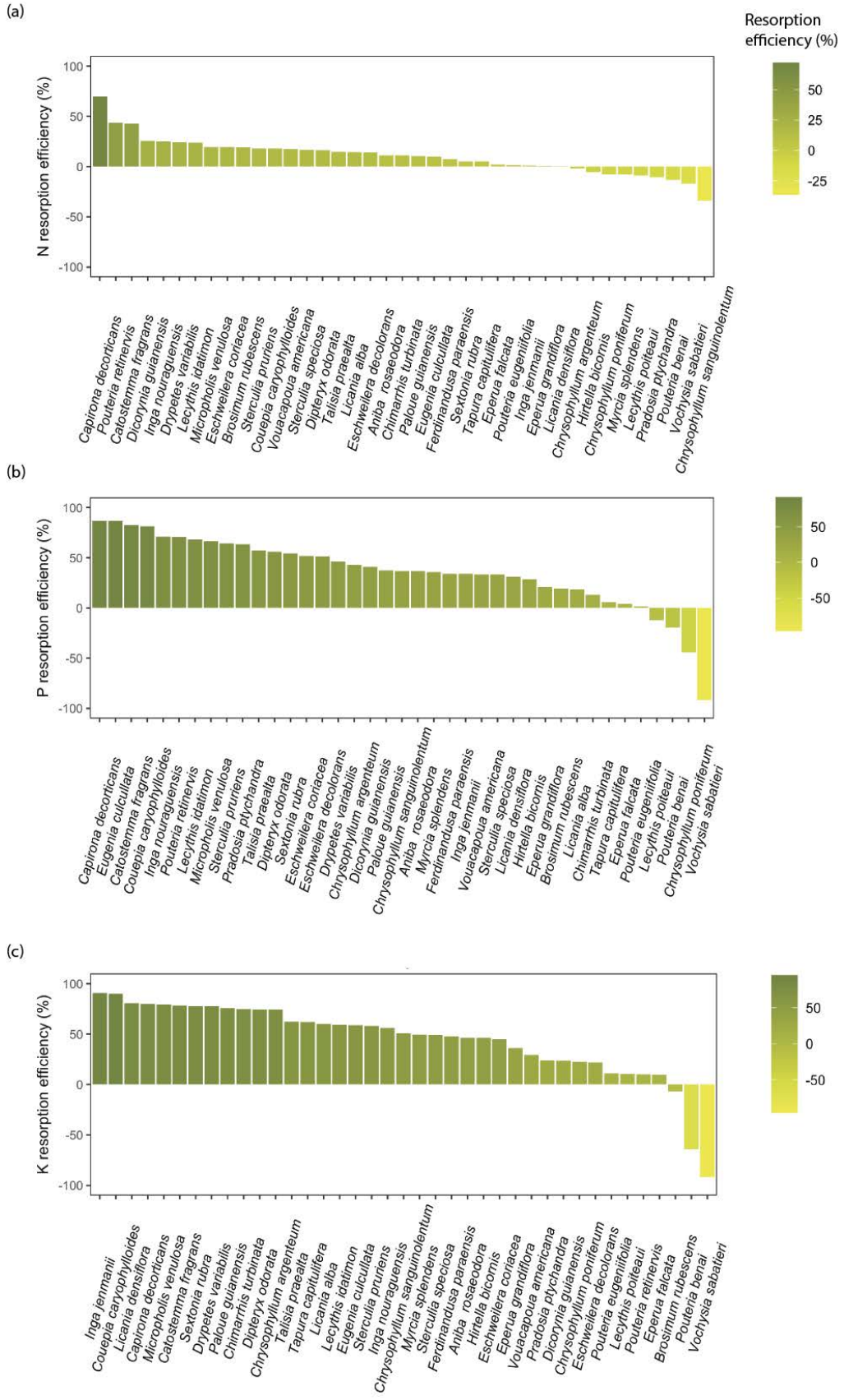


Figure 4.2: (a) Nitrogen, (b) phosphorus and (c) potassium resorption efficiencies for the 39 tropical tree species sampled in the dry season at both study sites.

Potassium, P and N resorptions tended to be higher in the wet than in the dry season for all the species (Fig. 4.3). K resorption was significantly higher in the wet (70.19 % ± 18.7) than the dry season (41.7 % ± 25.26) when all the species were averaged (total mean across all the species for each season, N=18), whereas N and P resorption efficiencies did not differ significantly between seasons (Table A.4.5, see Table A.4.2 for resorption efficiencies by species in each season).

The PCA indicated that foliar C, N and P concentrations were uncoupled from soil C, N and P concentrations. Foliar K was coupled positively with soil K and mean growth rate but negatively with soil C, N and P (Fig. 4.4). N and P resorption efficiencies were positively coupled with their foliar concentrations, but K resorption was not. The relationships between nutrient resorption efficiencies and functional characteristics of the species were usually weak but statistically significant (Fig. A.4.7). Phosphorus and K resorption efficiencies were slightly negatively correlated with wood density (R^2=0.16, p=0.026 and R^2=0.12, p=0.052, respectively), N resorption efficiency was negatively correlated with mean DBH (R^2=0.14, p=0.037) and P resorption efficiency was marginally positively correlated with SLA (R^2=0.13, p=0.096) (Fig. A.4.7). Mean growth rate was not significantly correlated with nutrient resorption. Mean and sd values for nutrient concentrations in leaves and senescent leaves for the all the species studied are summarized in the Table A.4.6 and mean and sd values for soil element concentration in the Table A.4.7.

Pagel's λ and Blomberg's K indices showed no significant differences in the resorption efficiencies in relation to the phylogenetic distances between species. Thus, resorption efficiencies were not more similar between closely than distantly related species (Table A.4.8 and see Fig A.4.8 for the phylogenetic tree).

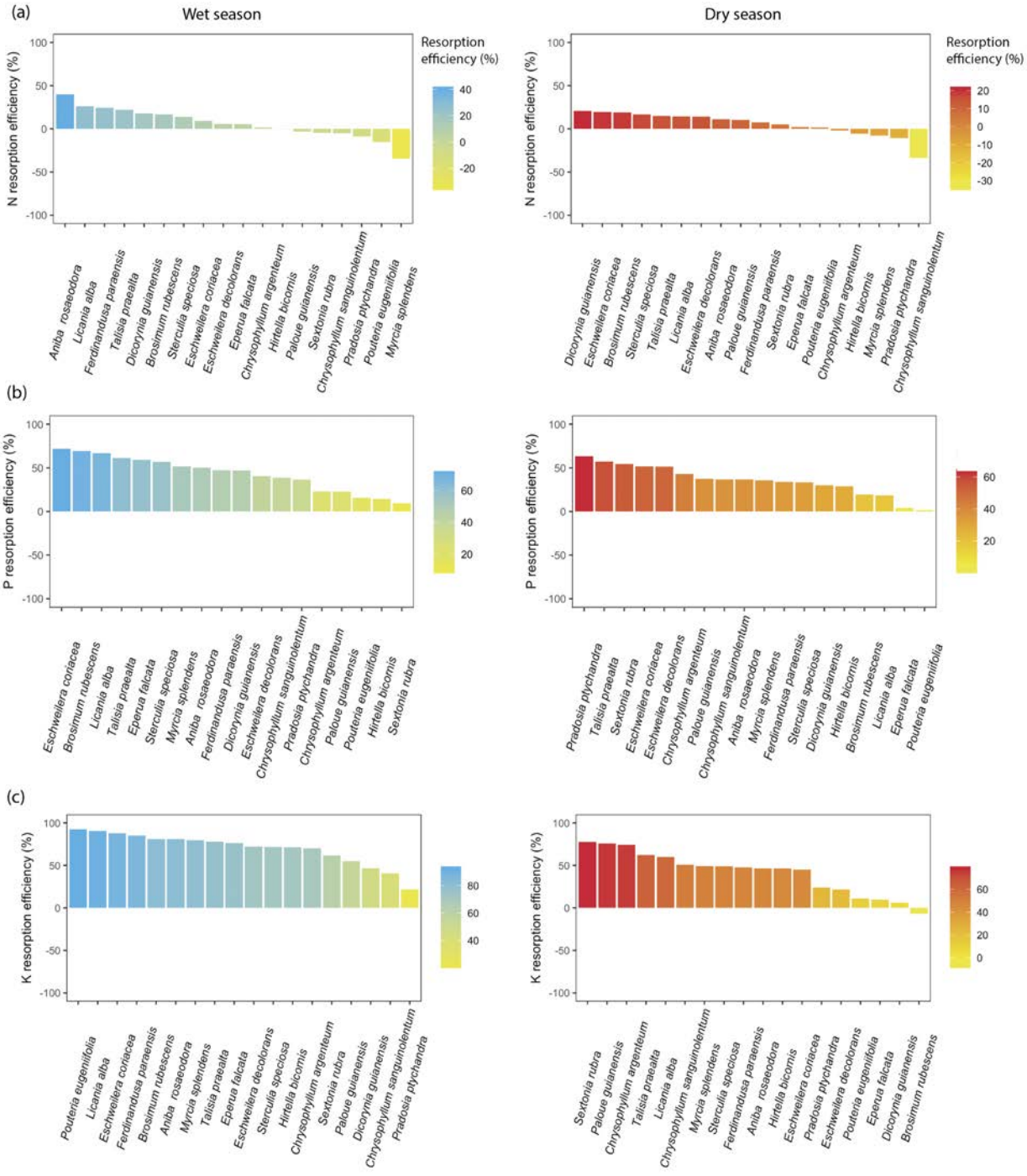


Figure 4.3: (a) Nitrogen, (b) phosphorus and (c) potassium resorption efficiencies for the 18 tropical tree species sampled in the wet (left panels) and dry (right panels) seasons at both study sites.

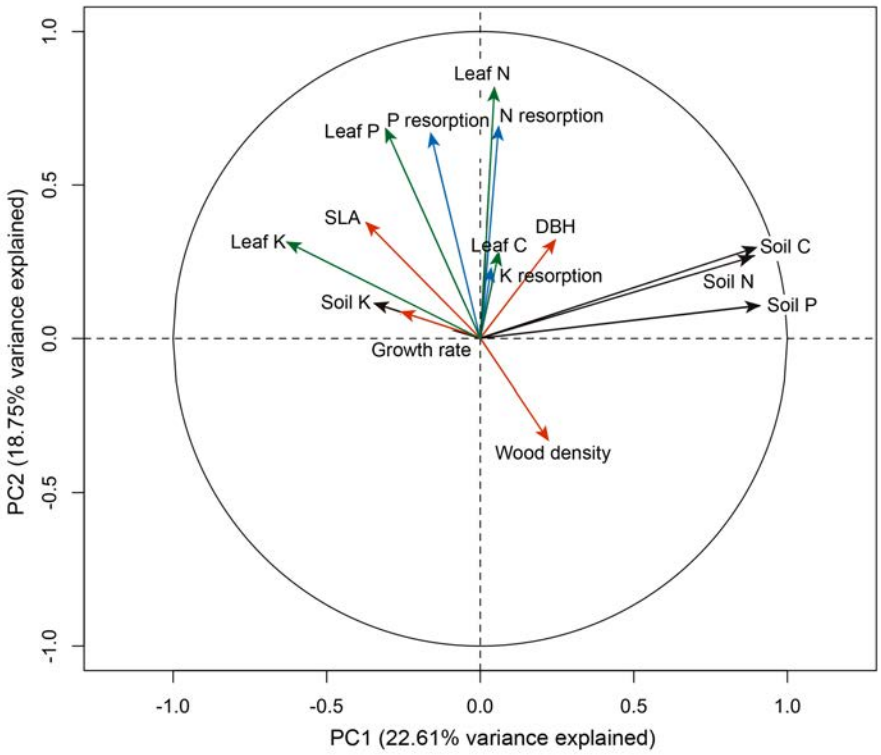


Figure 4.4: Principal component analysis (PCA) of foliar C, N, P and K concentrations (green arrows), soil C, N, P and K concentrations (black arrows), N, P and K resorption efficiencies (blue arrows) and growth rate, DBH, wood density and SLA functional traits (red arrows).

4.4 Discussion

Nutrient stocks

Our results showed that soil C, N, P and K stocks were significantly higher than in aboveground biomass, and that P was the scarcest nutrient stored in leaves, leaf-litter and both soil layers (Fig. 4.1, Table 4.1). Thus, our first hypothesis, that nutrients that have been greatly leached (P and K) would be stored mostly in leaves and leaf-litter, while C and N would be stored mostly in soil, was accomplished only for C and N. Soil C, N and K stocks and leaf-litter N, P and K stocks were higher at Nouragues than Paracou, indicating more nutrient inputs from leaf-litter to the soil at Nouragues. Soil P stocks did not differ significantly between sites, suggesting an important scarcity of this nutrient at both sites (Table 4.1). Furthermore, high soil N:P and K:P ratios at both sites indicated soil P limitation in these tropical forests, as expected in this tropical region (Vitousek and Farrington 1997; Oliveira et al. 2015; Sayer and Banin 2016; Grau et al. 2017). Our ratios values are similar to those reported in other tropical forest growing on old, weathered soils at Brazil and Costa Rica, with soil N:P ratios around 23.5 for Oxisols and 16.7 for Ultisols (Townsend et al. 2007).

The stocks allocation patterns of C, N, P and K were consistent with what has been described for other tropical forests in South America and Africa (Greenland and Kowal 1960; Bruijnzeel 1991; Yavitt et al. 2009; Sayer and Banin 2016). Our study, however, did not account for the stocks in woody tissue, which represent a large part of the C and nutrients stored in aboveground biomass in tropical forests (Tanner 1985; Heineman et al. 2016). The higher N stocks in soil than leaves and leaf-litter was expected, because soil N pools increase as ecosystems mature, so old tropical soils are comparatively more limited by P and K than by N (Vitousek et al. 2010; Turner and Condron 2013; Sayer and Banin 2016). We found similar quantities for total K and N in the soil. Soil K is present in available (in solution or exchangeable) or unavailable (fixed or structural) forms, which vary with soil type (Rosolem et al. 2010). Structural K contained in feldspar and silicate mineral rocks is the predominant form of K in soil, but K pools in clay material and soil colloids and solutions available to plants are relatively low (Rosolem et al. 2010; Baribault et al. 2012). French Guianese soils are classified as Oxisols rich in metal oxides, silicates and feldspar, so the soil is relatively rich in total K. The high content of K in mineral soil (mainly in silicates), however, can make tropical soils relatively rich in total K but poor in plant-available K.

Nutrient resorption and soil nutrient concentration

Foliar N:P ratio in our study varies between 14.6 and 44.9, suggesting P limitation (Mo et al. 2018) for the plant development in these tropical forests. Plant adaptations to scarcity of soil nutrients, such as mycorrhizal associations and phosphatase production, are common in tropical forests (Orwin et al. 2011; Bucking et al. 2012; Hofmann et al. 2016; Sheldrake et al. 2017; Batterman et al. 2018). High nutrient resorption from senescent leaves, however, allows plants to be more independent from external resources and saves the metabolic cost

of symbiotic associations or the production of enzymes (Brant and Chen 2015). Nitrogen is incorporated into plants by root uptake and symbiotic fixation and is returned to the system mostly via litter inputs to the soil, which implies important flows between the atmosphere, plants and soil (open cycle). Phosphorus and K, however, can only be acquired from the parent material and must be taken up by plant roots before being leached from the soil, which implies a lower flow between plants and soil (closed cycle) than for N. Resorption efficiencies for all the species in our study were generally higher for K and P than N, suggesting that the higher resorption of K and P is probably a species response to the large limitation of these nutrients in the soil (Fig. 4.2 and Table A.4.1). In contrast, N is not expected to be limiting in these old tropical forests (Lambers et al. 2008), so the lower resorption efficiency of N than P or K was expected.

In a global meta-analysis Yuan and Chen (2009) reported that P resorption efficiency increase from high to low latitudes, and they interpret the high P resorption values as a proxy of soil P limitation in the tropics. Similarly, Reed et al. 2012 showed that N:P resorption efficiency increase with the latitude and decrease with mean annual temperature and precipitation, reflecting variation in soil type and nutrient status. We found that P resorption efficiencies, however, were only weakly negatively correlated with total soil P but not with available P, despite the low total soil and available P contents (overall mean total: 119.8 ppm and available: 1.4 and 1.06 ppm, Olsen and Bray extractions, respectively). K and N resorption efficiencies were similarly not correlated with soil K and N concentrations. Several studies have reported this lack of correlation between nutrient resorption and soil nutrient concentration along fertility gradients and in fertilization experiments (Chapin 1980; Aerts 1996; Killingbeck 2004; Brant and Chen 2015), while other studies reported a decrease in the N and P resorption efficiencies with N, P fertilizations respectively(Yuan et al. 2015).

The range of total soil P at our sites varied between 20 and 470 ppm, and available P overall mean is 1.12 ppm (Olsen and Bray method together). These values are lower than in other tropical forest that had been described as forests growing on weathered, poor soils. For example, values for total soil P in Panama, in 3 different parent material, varies between 610 and 720 ppm, and available soil P between 3.1 and 4.3 ppm (using Olsen P method) (Yavitt 2006) and 2.2 ppm (using Bray method) (Yavitt et al. 2009). A study conducted by John et al. 2007 including more fertile soils reported values for available P in Colombian, Ecuadorian and Panama tropical forests of 20, 6.34 and 2.9 ppm, respectively (using Mehlich-III method). Then, our resorption efficiencies values correspond to very low soil P concentrations in tropical forests. This small range of soil P may account for the absence of correlation between P resorption and soil P concentration. However, studies including data from different tropical forests suggest that resorption efficiency is higher for P than N in species growing in old weathered soils (Reed et al. 2012). Thus, we interpret that high P resorption efficiencies in these very P-poor ecosystems is likely an adaptive strategy that plant species use to retain this essential nutrient for plant development, but further studies with larger ranges of soil nutrient concentrations are needed to better understand the resorption of nutrients as a response to nutrient scarcity.

Nutrient resorption and biochemical limitation

The order of average nutrient resorption efficiencies for all species (K ≥ P > N) was opposite to the immobilization of each of these nutrients in plant tissues (N > P > K). The level of immobilization of nutrients in foliar tissue will largely determine the capacity of nutrient recovery from senescent leaves (Killingbeck 2004; Kobe et al. 2005). N is highly immobilized in plants e.g. in proteins, enzymes and nucleic acids (Chapin 1980; Sua et al. 2005), P is immobilized mainly in energetic forms (less than N but more than K) (Walker and Syers 1976; Condron et al. 2013) and K is a free cation in plant cells (Sardans and Peñuelas 2015a). Nutrients more involved in the enzymatic metabolism of foliar senescence, such as N, or energetic forms necessary to export nutrients from leaves, such as P, will be partially unavailable for resorption (Killingbeck 2004). K and P may thus be reabsorbed more easily than N, matching the pattern we found. Resorption efficiency was more variable for P and K than N, and N resorption efficiency present more negative values. Aerts and Chapin (2000) previously reported this difference in the variation of P and N resorption efficiencies for different growth forms of plants, but the explanation of this difference was not addressed. Perhaps, the higher immobilization of N than P in leaves compounds probably accounts for the variability of N resorption efficiency. Additionally, Han et al. (2013) also reported higher variability in the P resorption efficiency than N in a global dataset. They relate this variability with the comparably greater importance of P resorption for plant nutrient conservation than N.

Furthermore, we found that N and P resorption showed a positive correlation with their concentration in leaves, while K was only marginally correlated. This is contrary with what has been reported in global meta-analyses, where nutrient resorption efficiency decrease with the increase in leaf nutrient status (Kobe et al. 2005; Vergutz et al. 2012). Our result indicated that in general, for our sites, trees with higher nutrient concentration in leaves are able to reabsorb more nutrients than trees with low nutrient concentration in their foliage. This is probably a general plant strategy to avoid nutrient losses in this very soil poor tropical forest. However, high variation in the leaves and leaf-litter chemistry in these tropical forests (Hättenschwiler et al. 2008) may imply contrasting resorption efficiencies. More studies with species with contrasting leaf chemistry will clarify the relationship between nutrient resorption efficiency and the concentration of elements in leaves in these tropical forests with nutrient-poor soils.

The percentage of K, P and N reabsorbed that we found when the resorption of all species was averaged (44.6 %, 35.9 % and 10.3 %, respectively) are lower than reported values from global meta-analysis studies (around 50-60 % for P and N, with generally higher resorption efficiency for P than N, and 70 % for K resorption efficiency) (Aerts 1996; Vergutz et al. 2012). Van Heerwaarden et al. (2002) demonstrated that leaf mass loss and leaf shrinkage during senescence significantly affected the estimations of resorption efficiency. These two factors could lead an important underestimation of resorption efficiency if the correction is not applied. Actually, Han et al. 2013 reported higher N and P resorption efficiencies with mass loss correction (around 10-12 % higher) than values uncorrected. Thus, in our study, low and negative values for nutrient resorption

efficiencies may be a consequence of the lack of correction of these two factors during the leaf senescence. Also, variation in the nutrient concentration in the leaves due the phenology, seasonality, within tree foliage, and timing of sampling could cause negative values for nutrient resorption efficiencies.

Nutrient resorption and the effect of seasonality

The mean K resorption efficiency, obtained from the average of all species together, was higher in the wet than the dry season, suggesting an important effect of water on internal K recycling. In contrast, P and N did not differ significantly between seasons. The lack of an effect of seasonality on N and P resorption is probably due to the higher immobilization of this nutrient in recalcitrant foliar compounds, which may prevent mobilization even when phloem water fluxes are high. Furthermore, the temporal variability in the nutrient concentration in leaves through seasons (Townsend et al. 2007), may cause distortion in the resorption efficiency. Water availability seems to play a role in K resorption. This partly supports our third hypothesis, as seasonality did not relate with N and P resorption.

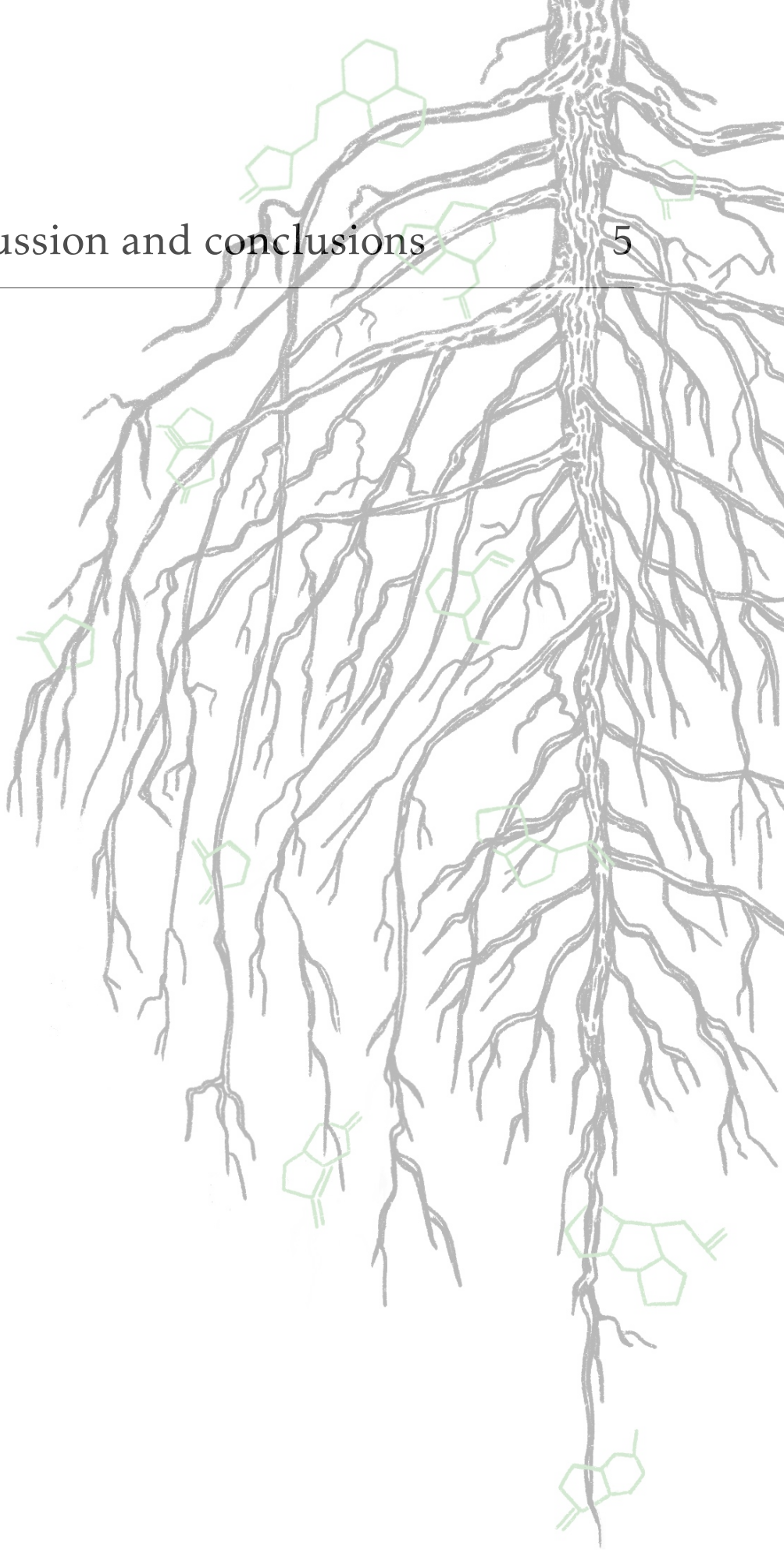
Effects of species functional characteristics and phylogenetic effect on nutrient resorption

Species with high wood densities are associated with slow growth rates and more conservative strategies (Muller-Landau 2004; Nascimento et al. 2005; Chave et al. 2009). We hypothesized that more conservative species, with slow growth rates and high wood density, would have higher resorption efficiencies, as a persistence strategy, than fast-growing species. In contrast, we found that species with high wood densities reabsorbed less K and P and that growth rate was not correlated with N, P or K resorption efficiency. Reabsorbed nutrients may thus be stored in cells (vacuoles) (Sterner and Elser 2002) and various compartments such as wood (Chapin 1980; Heineman et al. 2016) and not used immediately for plant grow. Our data did not provide strong evidence for a relationship between resorption efficiency and other functional characteristics, i.e. DBH or SLA. More studies that explore the relationship between functional traits and nutrient resorption are needed to clarify the role of resorption in plant performance. Furthermore, closely related species did not tend to have more similar resorption efficiencies than distantly related species. The lack of differences in resorption efficiencies between the taxa in these tropical forests probably indicates the selection of an environmental filter at the ecosystem level, which would establish a pool of species with similarly high resorption efficiencies, at least for K and P, due to their scarcity in the soil. In fact, the high P and K resorption efficiencies for all species suggest that resorption is a key process in these tropical forests to conserve nutrient that are less available in soil.

4.5 Conclusion

Our results showed that P was the scarcest nutrient stored in leaves, leaf-litter and soil, both at Paracou and Nouragues. The soil N:K and K:P ratios were high, and total and available P were very low, indicating the significant scarcity of P in these tropical soils. Higher resorption efficiencies for K and P than for N suggest higher plant internal nutrient recycling of K and P, likely due to their scarcity in the soil. The level of immobilization of each nutrient in leave compounds may determine significantly the resorption capacity; seasonality affects the resorption depending on the nutrient mobility inside the plant. The plant functional characteristics (growth rate, wood density, DBH and SLA) were not clearly correlated with nutrient resorption. Our results suggest that nutrient resorption from senescent leaves may be a general adaptive strategy for the conservation of nutrients by plants in these old-growth tropical forests growing on nutrient-poor soils.

General discussion and conclusions 5



5.1 Discussion

One of the main reasons that brought me into the study of chemical ecology for my doctoral thesis is that chemistry is omnipresent. Chemical elements are the fundamental part of all inert and living matter in the world, and how different reactions eventually generate life in millions of different forms is something that stimulated my curiosity to know more. Now, after being immersed during four years in this topic, seeing new leaves in spring is a marvel to me, I can almost see carbon chains in continuous arrangements and nutrient fluxes making life possible. It is truly amazing.

Throughout this Thesis, I explore the effects of the biotic and abiotic factors in the elemental composition of plants and soils in contrasting terrestrial ecosystems. Fluxes of energy and matter completely change depending on the biotic and abiotic characteristics, which determine the living organism communities (e.g. microbe, fungal, plant and animal) adapted to those specific conditions. This is nothing more than natural selection. Thus, different ecosystems represent specific ecological interactions and in consequence contrasting elemental compositions. I will make an overview of each chapter trying to organize the ideas across different levels of biological organization, from ecosystem level (by focusing on the plant-soil systems) to plant organs and cells.

The amount and ratios of elements in different components of ecosystems are determined by processes shaping the ecosystem structure (which determine biomass patterns) as well as by the differences in stoichiometry among species (which determine relative changes in elements) (Sterner and Elser 2002). Differences in stoichiometry among species were studied in the second chapter of this Thesis. Specifically, we used the species foliar elemental composition as a proxy of the species biogeochemical niche. Most of the stoichiometry studies in terrestrial ecology have focused on C, N, and P concentration, the main elements for plant development. However, other elements in plants, like K, Mg, Ca and Fe, play a pivotal role in the physiological functions such as photosynthesis, stomatal control, nutrients transport through the phloem, cellular osmotic regulation, enzymatic catalysis and secondary metabolism (Aerts and Chapin 2000; Sterner and Elser 2002; Dehui and Guangsheng 2005; Sardans and Peñuelas 2015; Peñuelas et al. 2019). Thus, including a broad stoichiometry spectrum in the ecological stoichiometry studies allows us to identify a wider range of functions and processes occurring in an ecosystem.

The species biogeochemical niche is determined by the different requirements and the specialization of each species to different abiotic and biotic conditions (Peñuelas et al. 2008, 2019). In the second Chapter we demonstrated that species growing with the same substrate supply (equal soil conditions) were able to change their biogeochemical niche depending on the neighboring plants with which they grew. This shift was explained by the *biogeochemical niche displacement* hypothesis, which states that the species readjust some physiological functions to cope with changes in the competitive conditions. A key concept in plant stoichiometry is that the acquisition and losses of C and nutrients are not perfectly coupled (they do not exhibit balance growth), they are not constrained to be in strict homeostasis (physiological regulation of an organism's internal environment) and they present

luxury consumption of nutrients, which can be stored in large vacuoles in the cells or in different plant compartments and not immediately invested in growth or reproduction (Sterner and Elser 2002). The shift in the species' biogeochemical niche in response to changes in community composition reflects the stoichiometry flexibility nature of plant stoichiometry. This capacity to adapt the elemental composition to biotic and abiotic changes allows plants to be highly adaptable to different environmental and competitive conditions. We consider the results of this chapter as a piece of evidence of the biogeochemical niche concept, a novel approach in chemical ecology.

Certainly, experiments under controlled conditions allow us to answer specific question about processes that are less known. On the other hand, field studies under natural conditions challenge us to observe, measure and describe as best as possible what is really happening in nature. In the third Chapter we jump to studies under natural conditions in the field. Subalpine grassland ecosystems in the Pyrenees have been used for humans' profit for millennia (Gassiot et al. 2016). Pastoralism and frequent fires related to this traditional activity have had important impacts on the functioning of this ecosystem, favoring the acceleration of the biogeochemical cycle of mountain areas (Catalan et al. 2017). The abandonment of the pastoralism activities in the subalpine grasslands over the last century is the main cause of the current shrub encroachment in the Pyrenees grasslands (Ninot et al. 2010). The shrub expansion into grasslands represents a major change in the elemental composition of the plant aboveground compartments and soil of these ecosystems. Our results showed an increase in the C:nutrient ratios and allocation of C and nutrients in the aboveground biomass with an associated decrease in the soil nutrient concentration along the succession from grassland to shrubland. These changes in chemical composition are related to the change in the chemical composition of the species along the succession, the higher foliar N:P ratios of herbaceous spp. in contrast to higher C:nutrient ratios in shrubs, the increase in lignified litter inputs from shrubs and the reduction of cattle feces inputs to the soil.

Changes in the nutrient stocks (g m^{-2}) in the aboveground biomass along the succession from grassland to shrubland correspond mainly to a shift in the biomass dominance, from the photosynthetic (herbaceous spp.) to structural tissue (shrub's woody biomass). This highlights the important role of shrubs as C and nutrient sink. Also, herbaceous spp. co-habiting with shrubs showed similar plant-soil stoichiometry spectrum, reflecting the specific ecosystem functioning in this stage of the succession. We also found evidence suggesting that shrubs encroachment implies a change in the N uptake strategy by plants, with an increasing dependence of mycorrhizal associations along the succession, which suggest a different N cycle in the shrub communities. Thus, all in all, the current shrub encroachment into subalpine grassland would slow down the biogeochemical cycle at ecosystem level through higher nutrient sequestration in the aboveground biomass, more recalcitrant organic matter associated to lower decomposition rates and the reduction of soil nutrient concentrations. Our results provide valuable information about the biogeochemical changes linked to the advance of shrub encroachment, that is expected to expand in the upcoming years due the land abandonment and the climate warming predicted for the Pyrenees mountain areas

(IPCC 2013; Catalan et al. 2017).

Descriptive studies like those described in chapter 2 and 3 provide relevant information about processes at species and ecosystem level. This is one of the strengths of ecological stoichiometry, the capacity to explore and find chemical patterns in the ecological interactions, thinking of species as chemical complexes product of millions years of evolution and the ecological interactions as chemical reactions and rearrangements. We are aware, though, that this work should be complemented in the future by experimental studies to better understand the biological mechanisms behind the stoichiometric patterns described.

In the fourth chapter, we explored the distribution of C, N, P and K at ecosystem level and the nutrient resorption process in an old-growth tropical forest. Old-growth tropical forests hold huge amounts of aboveground biomass, while soils are extremely poor (Aerts and Chapin 2000; Sayer and Banin 2016; Grau et al. 2017). Plants adaptations to these nutrient-limiting conditions, such as nitrogen fixing species, mycorrhizal associations and production of phosphatases are quite common (Moyersoen et al. 1998; Rillig et al. 2001; Batterman et al. 2013, 2018; Sheldrake et al. 2018). However, the resorption process has been poorly explored in these ecosystems. Nutrient resorption is the active process of nutrient withdrawal from the senescent leaves before the abscission, and represents an important mechanism for the nutrition of plants, that makes them more independent form external conditions (Killingbeck 2004). Nutrient resorption can be calculated as resorption efficiency, defined as the percent reduction of a nutrient between green and senesced leaves (Killingbeck 2004).

Our results show that P is the scarcest nutrient stored in the leaves, leaf-litter and soil in the two tropical forests studied in the French Guiana. Also, the soil N:P and K:P ratios were high in both study sites. This highlights the extreme P scarcity in the soil of these tropical forests. These results are consistent with what has been described widely for the tropical forest ecosystems, characterized to have very old and weathered, P poor soils (Walker and Syers 1976; Vitousek 1984; Vitousek et al. 2010). On the other hand, we found that the more reabsorbed nutrients are those that have been more leached from soil. Resorption efficiencies, when all tree species were averaged, were higher for K and P than N. Additionally, the high resorption efficiencies for P and K support nutrients being recycled more efficiently in tropical forests than in other ecosystems (Chapin 1980; Hedin et al. 2003; Grau et al. 2017). These results provide novel insights on this relatively unknown plant nutrition mechanism in poor soil tropical forests. The narrow range of soil concentrations for some nutrients, such as that of P, made, however, difficult to robustly test the relationship between the resorption efficiencies and the concentration of nutrients in the soil.

Recent studies of a long-term fertilization experiment in the Panama tropical forest demonstrate that P limitation for primary productivity is restricted at tree species, and not at community level (Mo et al. 2018; Turner et al. 2018). This could also apply for the resorption process. We found that, at the community level, K and P were reabsorbed more than N. Unfortunately, our sampling design did not allow testing the differences in the nutrient resorption amongst species with contrasting functional traits growing in the same community, since collecting leaves and senescent leaves from different replicate individuals was not possible for most

species. Despite these limitations we attempted to establish a possible relationship between nutrient resorption and growth, but we did not find any significant correlation, even when we classify our species in categorical or functional groups based on e.g. their relative growth rate. This lack of relationship could though be also explained by the fact that reabsorbed nutrients are stored in cell vacuoles or in different organs (e.g. in wood) and not necessarily used immediately for growth. Long-term field studies measuring systematically both processes and the inclusion of different tree age ranges (saplings and adult trees) is necessary to clarify this relationship between nutrient resorption and functional characteristics.

Furthermore, the order that we found for the resorption efficiencies of the nutrients analyzed ($K \geq P > N$) is inverse to the level of immobilization of each nutrient in the plant tissue ($N > P > K$) (Chapin 1980; Killingbeck 2004). N is highly immobilized in plant cells in form of proteins, enzymes and nucleic acids; P is present in energy compounds (ATP, NADPH) and nucleic acids, while K is the most abundant free cation in the plant cell (K^+) (Sardans and Peñuelas 2015). This shows that the level of immobilization of each nutrient in the leaf's compounds would play an important role in the resorption process, as expected. K resorption efficiency was higher in the wet than in the dry season, but P and N did not show differences between season. We speculate that water availability affects more significantly the K resorption due to the higher mobility of this element in the plant cell.

Phylogenetic relatedness did not explain the observed differences in resorption efficiencies. We interpret this lack of variability amongst different plant species as a generalized strategy to cope with nutrient scarcity. Nutrient resorption probably represents a key mechanism in plant nutrient conservation in these tropical forests, especially for K and P, the scarce nutrients in soil. Moreover, tropical forests present a great variation in soil types and different species may not be limited by the same nutrient (Sayer et al. 2012). Actually, recent studies from a long-term fertilization experiment in tropical forest reported great variation in the species responses to different nutrient addition after 7 years of fertilization (Kaspari et al. 2008; Wright et al. 2011, 2018; Sayer and Banin 2016). This fact makes it difficult to draw general conclusions about common strategies to adapt to nutrient limitation and nutrient cycles in tropical forests.

In view of the above, understanding the cycling of nutrients in tropical forests is a challenging topic, and many questions remain with no clear response. The resorption process is one of them. Nevertheless, the results reported in the fourth chapter confirm previous findings on the C, N, P and K distribution in tropical forests and add new evidence to our knowledge of the resorption process, a mechanism that plays a fundamental role in the cycling of nutrients in old-growth tropical forest growing on weathered soils.

Lastly, I would like to propose some possible future directions and experiments in order to enhance the results reported in this work. The fact that biotic interactions and changes in the competitive conditions shaped the species' biogeochemical niche (Chapter 2) offers many promising ideas in the field of the ecological stoichiometry. For example, to link diversity indices with the species foliar elemental composition and physiological processes under different competitive conditions. Future studies as long-term experiment in controlled conditions

with more plant species, physiological measurements (e.g. photosynthesis and growth), and a final fertilization would strengthen the concept of the species biogeochemical niche. This long-term experiment would allow corroborating the different physiological responses underlying foliar stoichiometry and relating this to the differential resource exploitation of plants in front of different competitive conditions, and indentify differences in the nutrient cycling in contrasting communities.

Regarding the third Chapter, it would be necessary to conduct a similar study while accounting for plant belowground biomass. Roots exudations and turnover represent a significant C input to the soil (Garcia-Pausas et al. 2007). The inclusion of the belowground plant compartment in future stoichiometry studies is fundamental to understand the impact of the shrub encroachment on the complete C and nutrient cycle. Also, sampling at two different soil depths would allow us to properly differentiate the organic and mineral soil layer and reach more robust conclusions about the C and nutrient sequestration in soil. Regarding the fourth chapter, in order to better understand the physiology of the resorption mechanism, future experiments under controlled conditions would be required. From my point of view, there are two main issues to explore: first, to what extent the level of immobilization of each nutrient in the plant compounds (proteins, DNA, RNA, energy forms, free cations) are determining the resorption processes? Answering this question would improve our understanding of the structural-chemical controls of this mechanism. Isotope labeling experiments could be a useful approach to answer this question. A controlled experiment with labelled N and K would allow tracking the mobilization of these nutrients in the senescent leaves at the moment of the leaf death. Second, it would be interesting to explore in depth to what extent the soil nutrient availability condition the resorption process. In order to answer this, a fertilization experiment under controlled conditions with different plant species could help to answer if the increase in the nutrient availability affect the nutrient resorption of the species.

Summarizing, in this Thesis we have demonstrated whether ecological interactions and processes are reflected in the elemental composition of the plant-soil system. We analyzed intraspecific and interspecific plant interactions (Chapter 2), plant mechanisms that are still poorly explored like the nutrient resorption (Chapter 4), as well as the importance of stoichiometry studies for describing changes at ecosystem level and predicting future scenarios (Chapter 3). Certainly, these studies add new knowledge in the field of ecological stoichiometry and highlight the importance of this approach in ecological studies. Furthermore, these results open new questions in each of the topics studied.

I would like to finish with a Walt Whitman quote:

'I like the scientific spirit—the holding off, the being sure but not too sure, the willingness to surrender ideas when the evidence is against them: this is ultimately fine—it always keeps the way beyond open—always gives life, thought, affection, the whole man, a chance to try over again after a mistake—after a wrong guess.'

I hope that all the results reported in this work will be useful and will open

new questions inspiring future ecological studies for people interested in the field of ecological stoichiometry.

5.2 Conclusions

- **Chapter 2: Plant community composition and species' biogeochemical niche**
  - Species are able to modify their biogeochemical niche in response to changes in plant community composition and these changes take place by a displacement of their foliar stoichiometry spectrum, which suggest the specialization of some physiological functions in different competitive conditions.
  - The biogeochemical niche is a proxy of the species-specific need and use of elements in a given abiotic and biotic environment, reflecting the different plant strategies when the patron of coexistence change. The biogeochemical niche concept is a promising new approach for future ecological studies
- **Chapter 3: Shrub encroachment into subalpine grasslands**
  - Shrub expansion into the subalpine grasslands in the Pyrenees represents the transition from an ecosystem characterized by faster turnover of nutrients between the plant-soil system, high N, P and K concentrations in the plant aboveground biomass and soil, but limited biomass accumulation; to shrubland, an ecosystem with slower turnover of nutrients between the plant-soil system, high C:nutrient ratios and high stocks of C and nutrients in the plant aboveground biomass, and low soil nutrient concentrations.
  - Shrub encroachment may affect the functional strategy of N acquisition by plants from the pure grassland to the shrubland, increasing dependence of mycorrhizal associations along the succession
  - Shrubs play an important role in the C and nutrient sequestration in the aboveground biomass along the succession, where the woody tissues play a main role as storage compartment.
  - The changes in plant-soil elemental composition and stocks along the grassland encroachment succession suggest that the shrub encroachment into subalpine grassland will promote a slowdown of biogeochemical cycle at ecosystem level.
- **Chapter 4: Nutrient stocks and resorption in old-growth tropical forests**
  - P is the scarcest nutrient stored in the leaves, leaf-litter and soil in the old-growth tropical forests in French Guiana.
  - At community level, general higher K and P resorption efficiencies than N, suggest a higher limitation of those nutrients in the soil.

- The level of immobilization of each nutrient in leaves and the water availability seem to determine significantly the capacity to reabsorb nutrients.
- Nutrient resorption from senescent leaves appears as a general adaptive strategy for plant nutrient conservation to cope with the scarcity of some nutrients in old-growth tropical forests growing on highly weathered soils.

Appendix 2: Chapter 2 - Plant community composition affects the species biogeochemical niche

Table A.2.1: Elemental concentrations of the soil used in EVENT 1 experiment. No significant differences ($p > 0.05$) were found in the ANOVA conducted with element concentrations as dependent variables and community as categorical factor. The depicted values are the mean and standard error of 28 soil samples.

Element	Concentration (mean (SE))
C (%dw)	4.04 (0.081)
N (%dw)	0.23 (0.004)
P (mg g^{-}1)	0.21 (0.005)
K (mg g^{-}1)	0.40 (0.005)
Ca (mg g^{-}1)	0.50 (0.018)
Mg (mg g^{-}1)	0.28 (0.004)
S (mg g^{-}1)	0.12 (0.001)
Fe (mg g^{-}1)	6.0 (0.1)

Table A.2.2: PERMANOVA results (p-values) for the species elements concentration in different communities.

Species	Community
Calluna vulgaris	=0.05
Vaccinium myrtillus	< 0.001
Holcus lanatus	< 0.0001
Arrhenatherum elatius	n.s

Table A.2.3: PERMANOVA results (p-values) for the species and community effect.

Factor	Shrubs	Grasses
Species	< 0.0001	< 0.001
Community	< 0.0001	< 0.01
Species x Community	< 0.0001	n.s

Table A.2.4: ANOVA one way results for the species element concentration in different communities. Community effect whiting each specie (p-values).

Species	C	N	P	K	Ca	Mg	S	Fe
Calluna vulgaris	n.s	n.s	=0.05	n.s	n.s	n.s	n.s	=0.05
Vaccinium myrtillus	n.s	n.s	n.s	< 0.01	n.s	=0.05	n.s	< 0.001
Holcus lanatus	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Arrhenatherum elatius	n.s	=0.05	< 0.0001	n.s	n.s	=0.05	< 0.001	< 0.01

Table A.2.5: ANOVA one way results for the species elements ratios in different communities. Community effect whiting each specie (p-values) .

Species	C:N	C:P	C:K	N:P	N:K	P:K
Calluna vulgaris	=0.05	=0.05	n.s	n.s	n.s	=0.05
Vaccinium myrtillus	n.s	n.s	< 0.01	n.s	< 0.001	< 0.0001
Holcus lanatus	=0.05	< 0.0001	n.s	< 0.001	n.s	< 0.01
Arrhenatherum elatius	n.s	n.s	n.s	n.s	=0.05	n.s

Table A.2.6: Post-hoc analysis for *Vaccinium myrtillus* growing in different communies

Species	Community	K	Fe	Mg	C:K	N:K	P:K
Vaccinium myrtillus							
	H2	a	a	a	a	a	a
	H4	ab	b	b	a	a	b
	MC	b	b	a	b	b	c

Table A.2.7: Post-hoc analysis for *Calluna vulgaris* growing in different communies

Species	Community	P	Fe	C:N	C:P	P:K
Calluna vulgaris						
	H2	ab	a	a	ab	a
	H4	a	a	a	a	a
	MC	b	a	a	b	a

Appendix 3: Chapter 3 - Encroachment of shrubs into subalpine grasslands in the Pyrenees changes the plant-soil stoichiometry

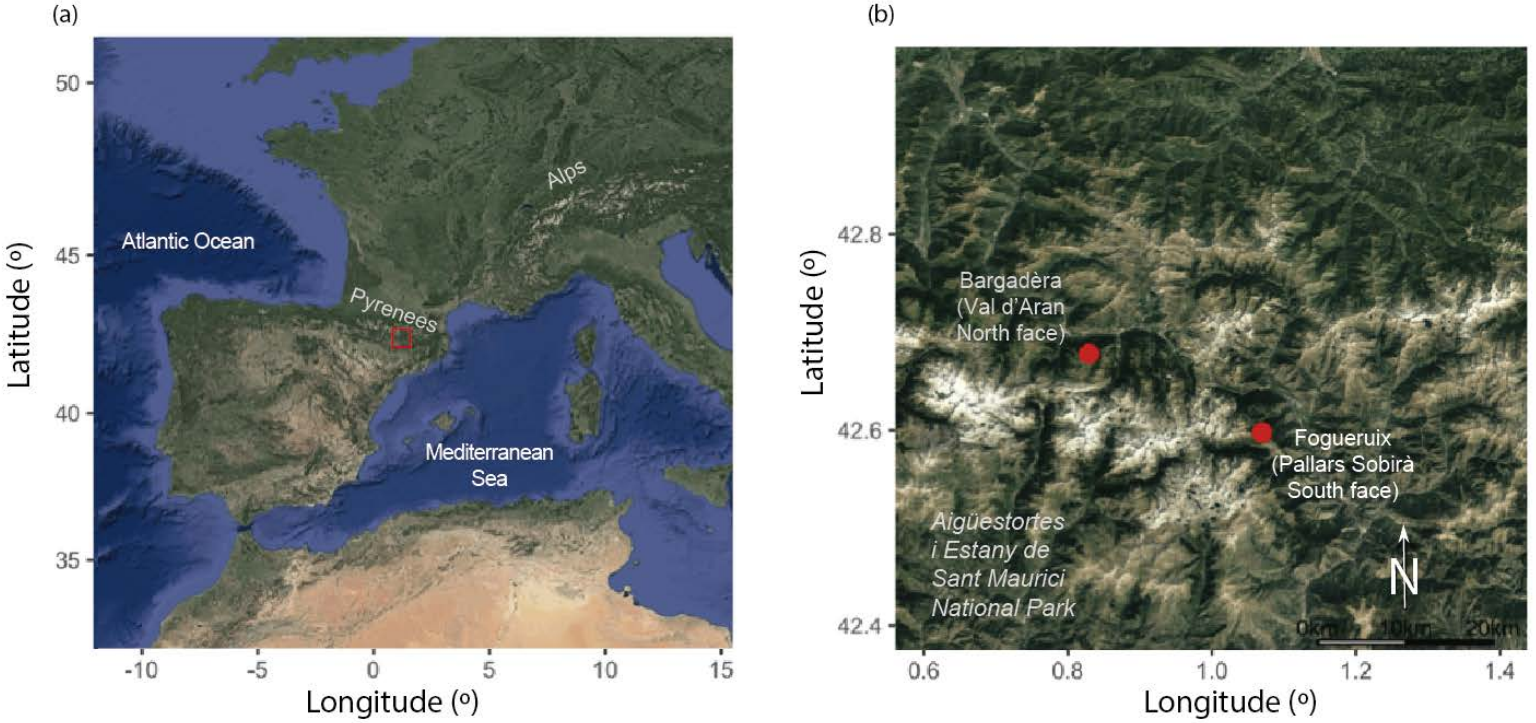


Figure A.3.1: Locations of (a) the study area at the Pyrenees (red square) in southwestern Europe and (b) the two study sites (red dots)

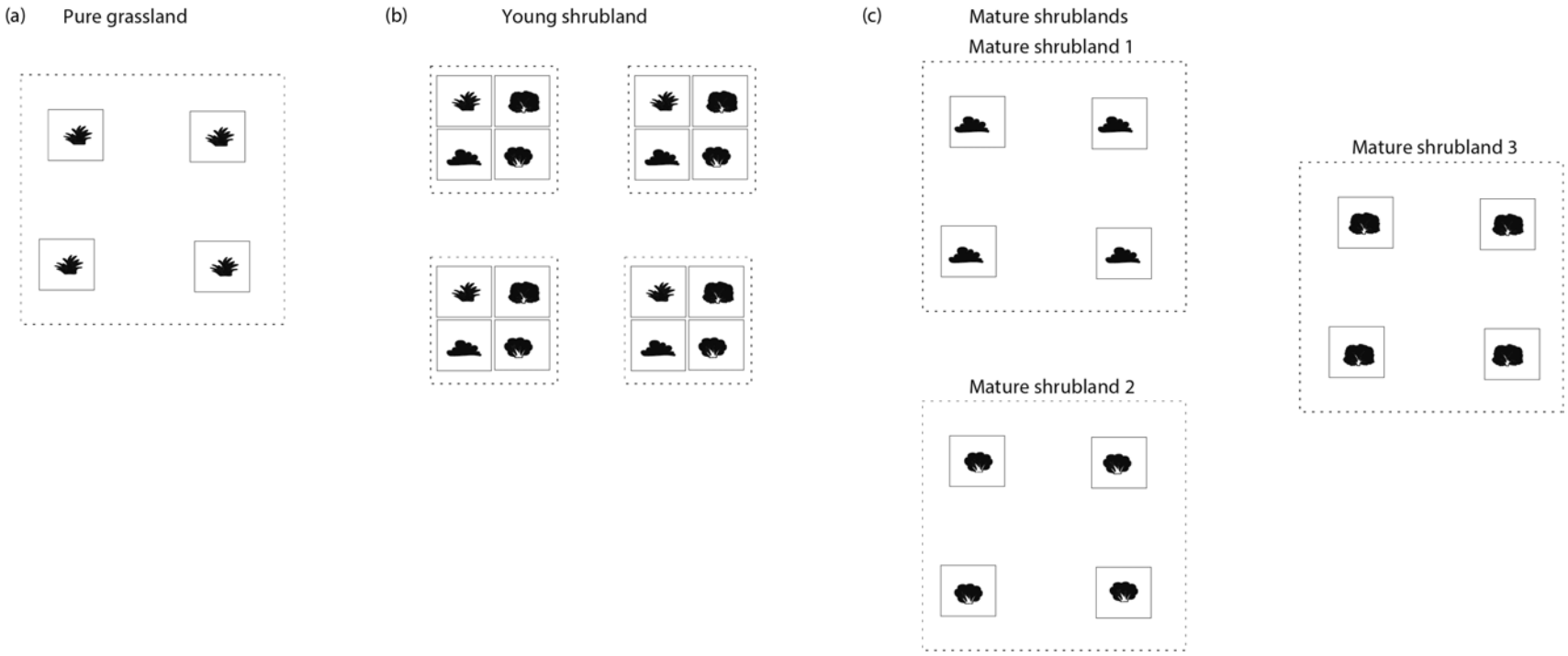


Figure A.3.2: Experimental design. The dotted lines represent the sampling area in each successional stage: (a) pure grassland, (b) young shrubland and (c) mature shrubland. The squares inside each successional stage represent the replicate plots (approximately 2 × 2 m). The distance between the four replicate plots in the pure grassland and the mature shrubland was at least 10 m. In the young shrubland we established four groups of four plots; the herbaceous spp., shrub 1, shrub 2 and shrub 3 were grouped because all vegetation types co-occurred. The distance between the successional stages or among the mature shrublands of each shrub species was > 100 m.

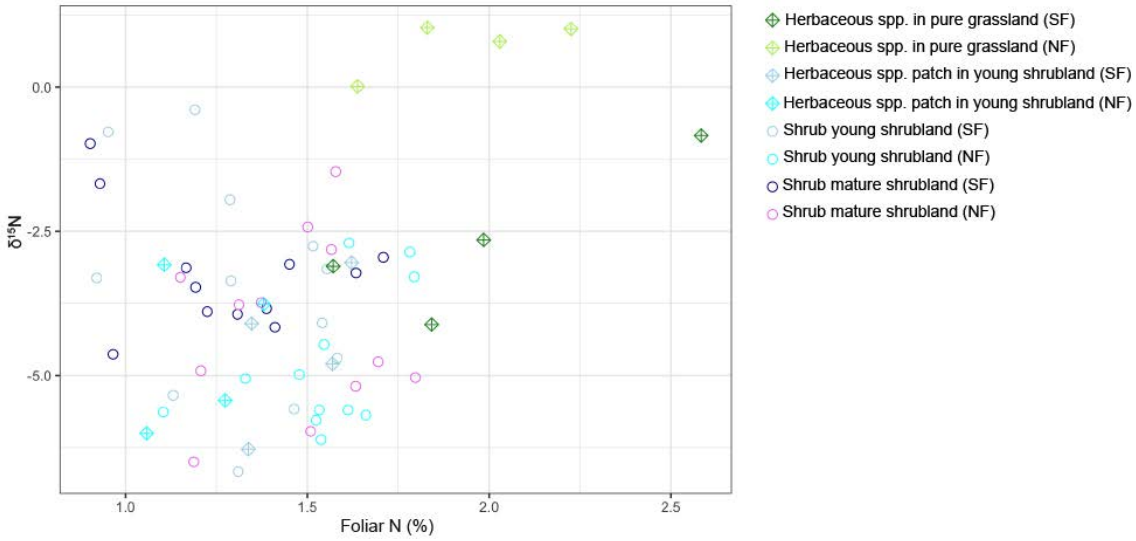


Figure A.3.3: Correlation between foliar N and δ^{15}N for herbaceous spp. and shrubs along the succession from pure grassland to mature shrubland in both study sites. Different shapes show the different vegetation types (herbaceous spp. or shrub) and colors show the successional stage (pure grassland, young shrubland or mature shrubland) in each study site (NF: North face, SF: South face)

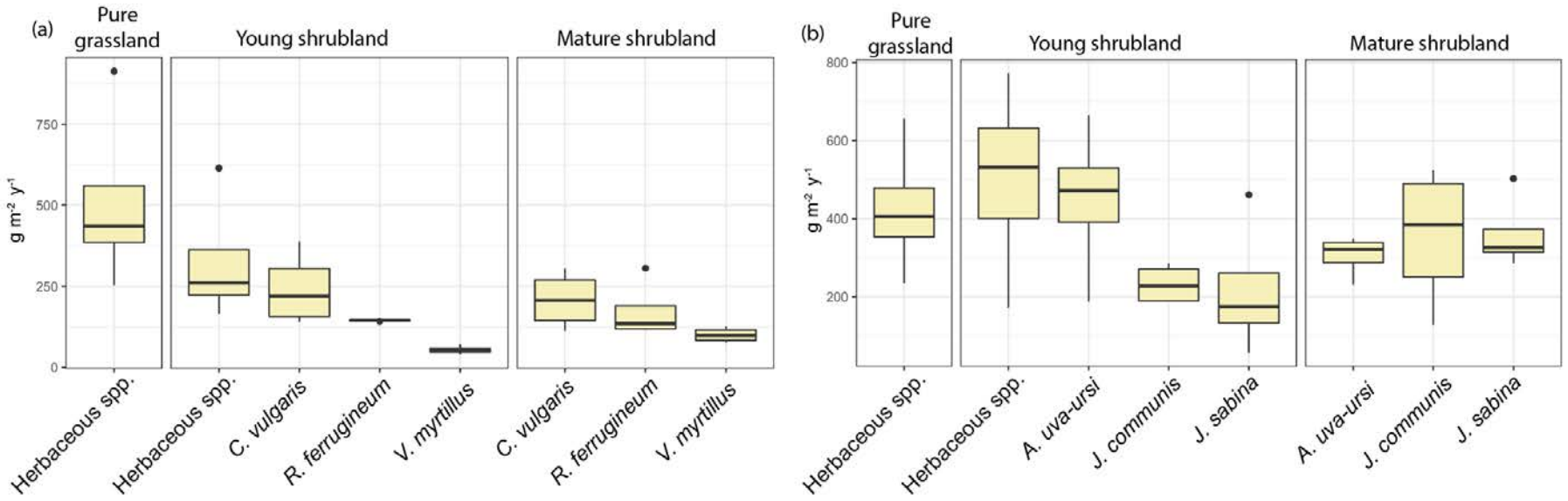


Figure A.3.4: (a) North face and (b) South face in the pure grassland (left panels in each figure), young shrubland (middle panel in each figure) and mature shrubland (right panel in each figure). Leaf-litter production was calculated using the foliar persistence analysis from Ninot et al. (unpublished data). The upper and lower line of the boxes indicates the second and third quartile, respectively; the black line inside de box indicates the median; the bars represent the first and fourth quartiles and dots represent the outliers. Note the different vertical scales for North face and South face.

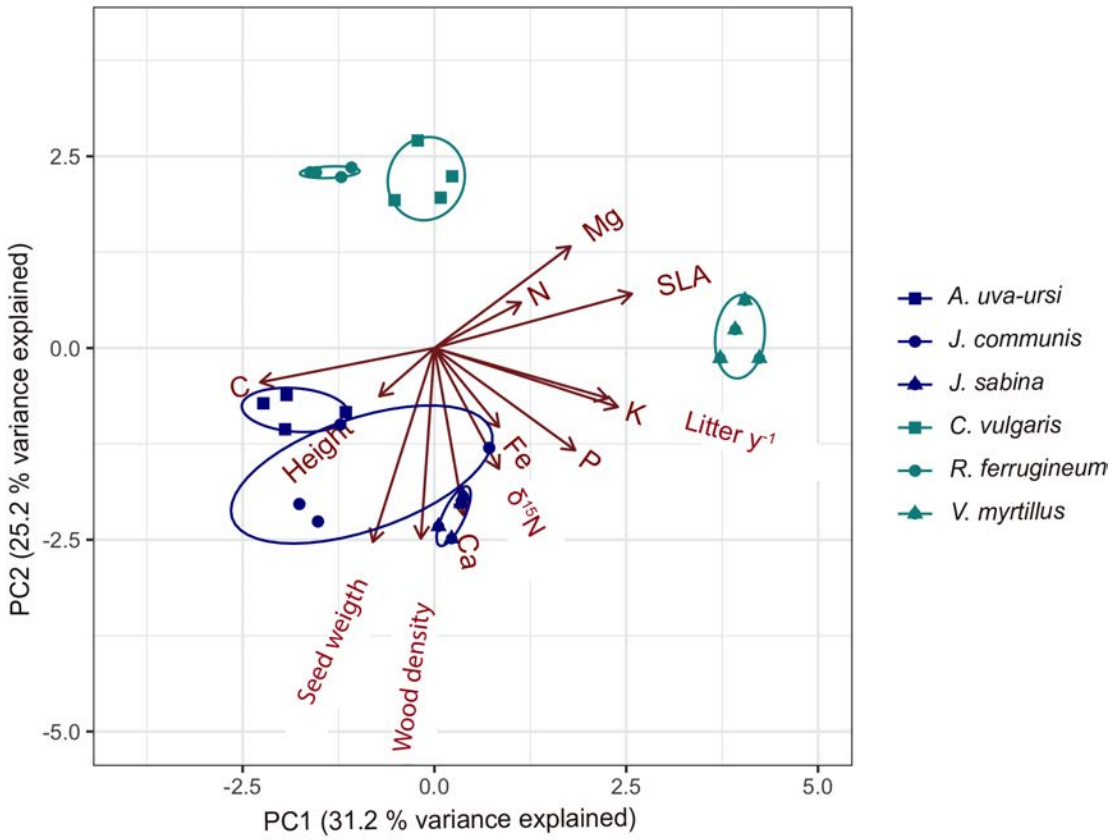


Figure A.3.5: Principal component analysis of the functional traits and foliar elemental composition of the shrub species in the mature shrubland in each study site. The ellipses represent the dispersion around the centroid for each shrub species with a normal probability of 0.85.

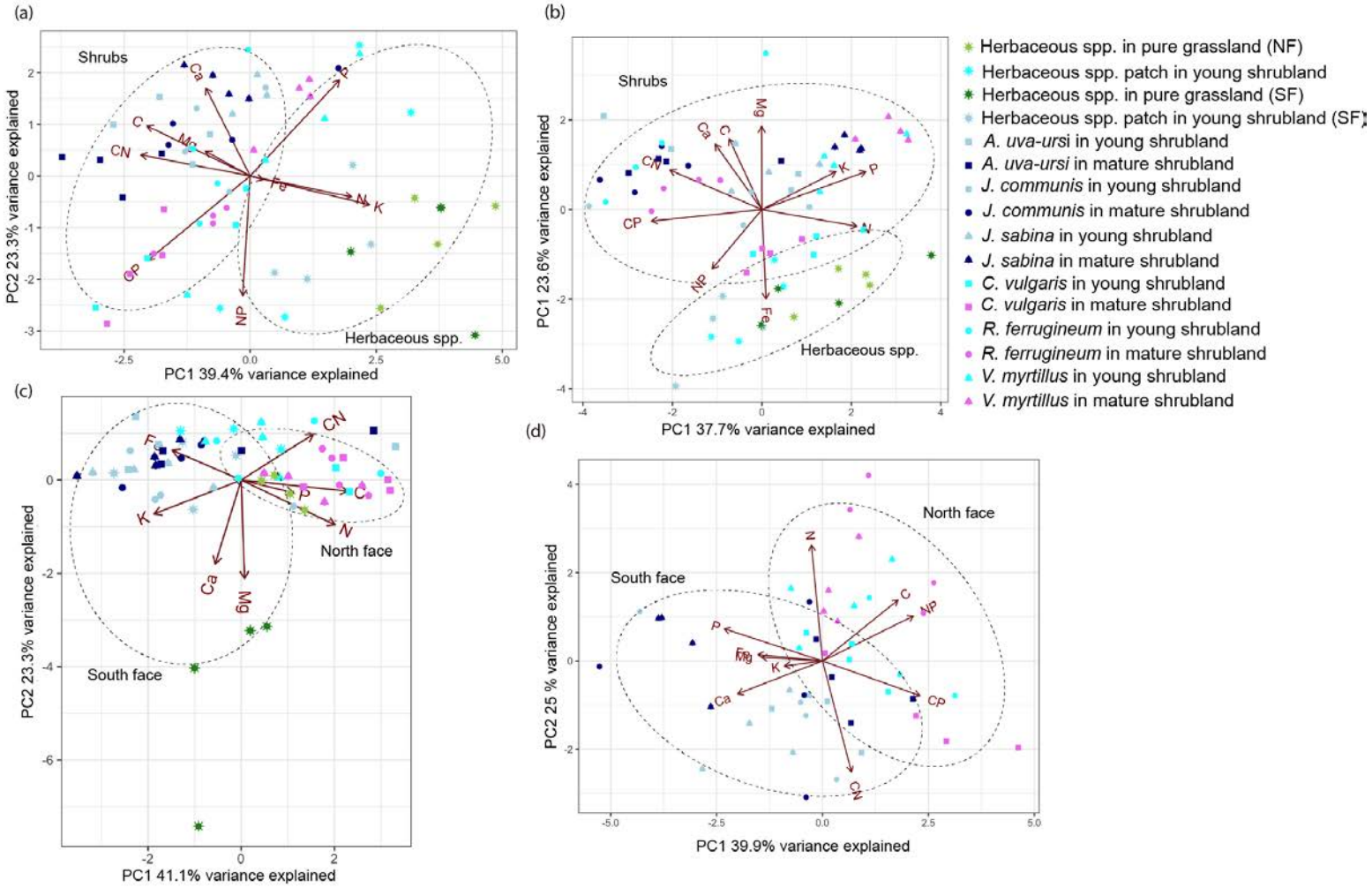


Figure A.3.6: Principal component analysis (PCA) based on the elemental concentrations for (a) leaves, (b) leaf-litter, (c) soil and (d) stems for all vegetation types and successional stages. Only variables that were poorly correlated with each other (Pearson's coefficients <0.6) were included in the analyses. Different colors indicate the successional stages and different shapes indicate the vegetation types. The ellipses in (a) and (b) represent the dispersion around the centroid for herbaceous species and shrubs, and in (c) and (d) for the different sites, with a normal probability of 0.85.

Table A.3.1: Ecological characterization shrub species at Bargadèra (North face) and Fogueruix (South face).

Name of site	Site	Species	Altitudinal distribution in the Pyreenes	Abundance at regional scale	Substrate preference	Ecological ubiquity	Dominance	Colonization capacity
Bargadèra	North face	*Rhododendron ferrugineum*	100-2800	very high	acidic	medium	high	medium
Bargadèra	North face	*Vacciniun myrtillus*	400-2800	very high	acidic	high	high	hig
Bargadèra	North face	*Calluna vulgaris*	0-2800	very high	acidic	medium	medium	high
Fogueruix	South face	*Arctostaphylos uva-ursi*	400-2800	very high	no preference	medium	high	medium
Fogueruix	South face	*Juniperus communis*	1200-1800	high	no preference	high	medium	high
Fogueruix	South face	*Juniperus sabina*	800-2400	rare	no preference	low	low	low

Table A.3.2: (a) Functional traits of shrub species at Bargadèra (North face) and Fogueruix (South face). Standard deviation in parentheses.

Name of site	Exposure	Species	Shrub type	Canopy heigh (cm)	Wood density (mg cm^{-3})	Leaf area (mm^2)
Bargadèra	North face	*Rhododendron ferrugineum*	evergreen	44.8 (4.10)	457.18 (19)	162.45 (19.38)
Bargadèra	North face	*Vacciniun myrtillus*	deciduos	21.25 (3.06)	571.89 (32.63)	85.88 (32.25)
Bargadèra	North face	*Calluna vulgaris*	evergreen	19.15 (3.25)	525.59 (24.42)	1.42 (0.24)
Fogueruix	South face	*Arctostaphylos uva-ursi*	evergreen	14.45 (3.03)	539.38 (39.45)	126.55 (41.05)
Fogueruix	South face	*Juniperus communis*	evergreen	47.5 (8.35)	629.01 (54.27)	10.95 (1.76)
Fogueruix	South face	*Juniperus sabina*	evergreen	40.73 (16.33)	667.28 (44.91)	43.06 (12.91)

Table A.3.3: (b) Functional traits of shrub species at Bargadèra (North face) and Fogueruix (South face). Standard deviation in parentheses.

Name of site	Exposure	Species	Foliar N (%)	Seed weight (mg)	Foliar dry mass (mg g^{-1})	Specific leaf area (mm^2 mg^{-1})
Bargadèra	North face	*Rhododendron ferrugineum*	1.62 (0.09)	0.026 (0.003)	493.21 (16.29)	6.10 (0.44)
Bargadèra	North face	*Vacciniun myrtillus*	1.57 (0.14)	0.26 (0.02)	326.33 (30.16)	22.17 (4.02)
Bargadèra	North face	*Calluna vulgaris*	1.30 (0.18)	0.035 (0.003)	430.02 (157.69)	15.33 (1.52)
Fogueruix	South face	*Arctostaphylos uva-ursi*	1.04 (0.15)	9.55 (2.05)	417.44 (55.55)	5.35 (1.66)
Fogueruix	South face	*Juniperus communis*	1.36 (0.20)	9.75 (1.47)	464.14 (62.59)	6.14 (0.96)
Fogueruix	South face	*Juniperus sabina*	1.46 (0.12)	16.88 (4.88)	480.38 (29.11)	9.39(0.98)

Table A.3.4: Means for element concentrations (dw/dw), ratios and stokcs (g m^{-2}) in each plant compartments and soil for the different vegetation types in each successional stage. Standard deviation in parentheses. Leaf-litter values correspond to productivity per year (g m^{-2} $year^{-1}$)

Site	Vegetation type	Successional stage	Compartment	mass (g m^{-2})	C (%)	N (%)
North face	Herbaceous spp.	Pure grassland	leaf	509.47(282.47)	45.19 (0.45)	1.93(0.25)
North face	Herbaceous spp.	Pure grassland	litter	509.47(282.47)	45.08 (0.81)	1.09(0.12)
North face	Herbaceous spp.	Pure grassland	soil	34904.46(14647.67)	10.73(1.49)	0.86(0.12)
North face	Herbaceous spp.	young shrubland	leaf	324.96 (198.69)	44.70 (0.24)	1.21(0.15)
North face	Herbaceous spp.	young shrubland	litter	324.96 (198.69)	45.86 (3.96)	0.78(0.06)
North face	Herbaceous spp.	young shrubland	soil	34412.60(2166.91)	7.83(3.66)	0.44(0.23
North face	*Calluna vulgaris*	young shrubland	leaf	281.44(133.73)	50.04 (0.72)	1.40(0.3)
North face	*Calluna vulgaris*	young shrubland	litter	92.88(4.01)	50.58 (0.81)	1.34(0.18)
North face	*Calluna vulgaris*	young shrubland	soil	19705.41(2420.83)	16.13(0.68)	1.00(0.12)
North face	*Calluna vulgaris*	young shrubland	stems	630.44(151.42)	48.84(0.32)	0.48(0.04)
North face	*Calluna vulgaris*	mature shrubland	leaf	241.19(103.52)	49.91(0.29)	1.22(0.07)
North face	*Calluna vulgaris*	mature shrubland	litter	79.59(3.11)	50.82(0.36)	1.06(0.13)
North face	*Calluna vulgaris*	mature shrubland	soil	58136.94(18017.81)	16.20(4.18)	0.91(0.20)

Site	Vegetation type	Successional stage	Compartment	mass (g m^{-2})	C (%)	N (%)
North face	*Calluna vulgaris*	mature shrubland	stems	941.71(234.51)	48.52(0.39)	0.41(0.05)
North face	*Rhododendrum ferrugineum*	young shrubland	leaf	320.16(3.22)	51.96(0.10)	1.58(0.06)
North face	*Rhododendrum ferrugineum*	young shrubland	litter	144.07(1.45)	52.36(0.22)	0.62(0.10)
North face	*Rhododendrum ferrugineum*	young shrubland	soil	28598.73(18288.66)	12.17(4.61)	0.69(0.26)
North face	*Rhododendrum ferrugineum*	young shrubland	stems	2229.20(195.90)	50.79(0.19)	0.47(0.06)
North face	*Rhododendrum ferrugineum*	mature shrubland	leaf	383.63(199.53)	51.99(0.25)	1.66(0.12)
North face	*Rhododendrum ferrugineum*	mature shrubland	litter	172.63(89.79)	52.34(0.41)	0.88(0.07)
North face	*Rhododendrum ferrugineum*	mature shrubland	soil	46058.92(12133.86)	16.69(3.53)	0.95(0.19)
North face	*Rhododendrum ferrugineum*	mature shrubland	stems	3410.09(587.80)	51(0.82)	0.64(0.11)
North face	*Vaccinium myrtillus*	young shrubland	leaf	53.80(13.35)	48.60(0.09)	1.65(0.16)
North face	*Vaccinium myrtillus*	young shrubland	litter	53.80(13.35)	49.75(0.46)	1.08(0.08)
North face	*Vaccinium myrtillus*	young shrubland	soil	27251.19(6717.61)	9.62(2.39)	0.54(0.17)
North face	*Vaccinium myrtillus*	young shrubland	stems	251.72(54.02)	48.45(0.67)	0.57(0.06)
North face	*Vaccinium myrtillus*	mature shrubland	leaf	99.85(23.34)	48.42(0.12)	1.51(0.09)

Site	Vegetation type	Successional stage	Compartment	mass (g m^{-2})	C (%)	N (%)
North face	*Vaccinium myrtillus*	mature shrubland	litter	99.85(23.34)	49.62(0.23)	1.05(0.12)
North face	*Vaccinium myrtillus*	mature shrubland	soil	37416.40(24137.04)	13.11(3.67)	0.78(0.24)
North face	*Vaccinium myrtillus*	mature shrubland	stems	543.59(119.43)	48.20(0.38)	0.59(0.06)
South face	Herbaceous spp.	Pure grassland	leaf	425.55(173.57)	44.93(0.19)	2(0.43)
South face	Herbaceous spp.	Pure grassland	litter	425.55(173.57)	44.40(0.14)	1.16(0.41)
South face	Herbaceous spp.	Pure grassland	soil	76421.58(26473.47)	10.73(1.50)	0.93(0.12)
South face	Herbaceous spp.	young shrubland	leaf	501.88(251.04)	45.03(0.51)	1.47(0.15)
South face	Herbaceous spp.	young shrubland	litter	501.88(251.04)	48.27(7.86)	0.77(0.16)
South face	Herbaceous spp.	young shrubland	soil	94320.60(26877.40)	7.39(2.44)	0.57(0.15)
South face	*Arctostaphylos uva-ursi*	young shrubland	leaf	616.68(294.47)	51.45(0.58)	1.09(0.18)
South face	*Arctostaphylos uva-ursi*	young shrubland	litter	448.69(108.96)	51.32(0.67)	0.58(0.24)
South face	*Arctostaphylos uva-ursi*	young shrubland	soil	109766.46(15402.34)	8.52(8.80)	0.47(0.31)
South face	*Arctostaphylos uva-ursi*	young shrubland	stems	590.12(482.74)	47.45(0.43)	0.42(0.03)
South face	*Arctostaphylos uva-ursi*	mature shrubland	leaf	825.76(142.34)	52.12(0.50)	1.01(0.15)

Site	Vegetation type	Successional stage	Compartment	mass (g m^{-2})	C (%)	N (%)
South face	*Arctostaphylos uva-ursi*	mature shrubland	litter	305.53(52.67)	51.98(0.66)	0.59(0.15)
South face	*Arctostaphylos uva-ursi*	mature shrubland	soil	65924.36(24261.53)	10.27(6.48)	0.57(0.21)
South face	*Arctostaphylos uva-ursi*	mature shrubland	stems	952.19(159.33)	47.89(0.89)	0.45(0.04)
South face	*Juniperus communis*	young shrubland	leaf	1353.00(310.77)	50.12(0.55)	1.37(0.20)
South face	*Juniperus communis*	young shrubland	litter	232.64(77.69)	50.68(0.40)	0.86(0.26)
South face	*Juniperus communis*	young shrubland	soil	98992.57 (18745.13)	7.79(4.85)	0.49(0.16)
South face	*Juniperus communis*	young shrubland	stems	1041.68(311.29)	47.05(0.45)	0.45(0.09)
South face	*Juniperus communis*	mature shrubland	leaf	1420.92(728.07)	50.36(0.37)	1.36(0.25)
South face	*Juniperus communis*	mature shrubland	litter	355.23(182.02)	50.96(0.53)	0.57(0.06)
South face	*Juniperus communis*	mature shrubland	soil	77357.88(28341.60)	6.24(1.26)	0.40(0.05)
South face	*Juniperus communis*	mature shrubland	stems	2975.57(2320.06)	47.29(1.13)	0.46(0.10)
South face	*Juniperus sabina*	young shrubland	leaf	1013.40(314.69)	50.66(0.36)	1.48(0.12)
South face	*Juniperus sabina*	young shrubland	litter	566.22(22.03)	50.75(0.34)	0.91(0.16)
South face	*Juniperus sabina*	young shrubland	soil	94466.56(24995.46)	5.60(2.07)	0.43(0.12)

Site	Vegetation type	Successional stage	Compartment	mass (g m^{-2})	C (%)	N (%)
South face	*Juniperus sabina*	young shrubland	stems	1004.40(610.00)	46.57(0.27)	0.41(0.05)
South face	*Juniperus sabina*	mature shrubland	leaf	1091.85(293.28)	51.85(0.34)	1.45(0.14)
South face	*Juniperus sabina*	mature shrubland	litter	938.99(20.53)	52.03(0.43)	1.31(0.18)
South face	*Juniperus sabina*	mature shrubland	soil	88398.09(17150.67)	5.81(2.04)	0.45(0.09)
South face	*Juniperus sabina*	mature shrubland	stems	3254.98(1555.61)	46.28(0.47)	0.54(0.06)

Site	Vegetation type	Succession stage	Compartment	N:P	C:P	C:N	P (%)	K (%)
North face	Herbaceous spp.	Pure grassland	leaf	14.46 (3.14)	339.64 (65.36)	23.72 (3.20)	0.14 (0.02)	1.59 (0.30)
North face	Herbaceous spp.	Pure grassland	litter	13.89 (0.90)	583.83 (110.08)	41.79 (5.18)	0.08 (0.01)	0.39 (0.18)
North face	Herbaceous spp.	Pure grassland	soil	6.37 (0.98)	79.04 (12.18)	12.42 (0.20)	0.14 (0.01)	1.13 (0.03)
North face	Herbaceous spp.	young shrubland	leaf	11.07 (5.28)	416.88 (211.35)	37.53 (4.65)	0.13 (0.07)	1.19 (0.21)
North face	Herbaceous spp.	young shrubland	litter	15.25 (5.05)	881.62 (253.46)	58.59 (4.01)	0.06 (0.02)	0.12 (0.01)
North face	Herbaceous spp.	young shrubland	soil	4.70 (3.03)	83.30 (48.49)	18.16 (1.32)	0.10 (0.02)	1.29 (0.16)

Site	Vegetation type	Succession stage	Compartment	N:P	C:P	C:N	P (%)	K (%)
North face	*Calluna vulgaris*	young shrubland	leaf	16.17 (2.06)	667.11 (105.00)	41.18 (2.12)	0.09 (0.02)	0.50 (0.09)
North face	*Calluna vulgaris*	young shrubland	litter	18.41 (0.55)	890.37 (120.87)	48.26 (5.33)	0.08 (0.02)	0.22 (0.05)
North face	*Calluna vulgaris*	young shrubland	soil	8.18 (1.56)	145.88 (34.84)	17.71 (1.24)	0.10 (0.01)	1.02 (0.04)
North face	*Calluna vulgaris*	young shrubland	stems	15.00 (3.66)	1819.55(596.16)	119.19 (13.00)	0.04 (0.01)	0.29 (0.04)
North face	*Calluna vulgaris*	mature shrubland	leaf	16.00 (1.50)	594.07 (163.99)	36.68 (6.73)	0.08 (0.01)	0.41 (0.06)
North face	*Calluna vulgaris*	mature shrubland	litter	18.29 (2.21)	707.70 (189.49)	38.21 (5.47)	0.06 (0.01)	0.28 (0.04)
North face	*Calluna vulgaris*	mature shrubland	soil	10.52 (0.30)	170.89 (18.12)	16.23 (1.26)	0.11 (0.01)	0.92 (0.06)
North face	*Calluna vulgaris*	mature shrubland	stems	13.00 (1.43)	1331.12 (227.66)	101.93 (8.93)	0.03 (0.01)	0.19 (0.04)
North face	*Rhododendrum ferrugineum*	young shrubland	leaf	16.99 (1.15)	536.05 (71.27)	31.47 (2.19)	0.12 (0.03)	0.36 (0.02)
North face	*Rhododendrum ferrugineum*	young shrubland	litter	21.66 (2.39)	1298.73 (221.47)	59.69 (4.16)	0.06 (0.04)	0.11 (0.02)
North face	*Rhododendrum ferrugineum*	young shrubland	soil	9.52 (0.45)	166.18 (6.53)	17.46 (0.39)	0.10 (0.03)	1.23 (0.19)
North face	*Rhododendrum ferrugineum*	young shrubland	stems	15.24 (1.04)	1239.20 (225.82)	81.27 (13.42)	0.04 (0.01)	0.14 (0.02)
North face	*Rhododendrum ferrugineum*	mature shrubland	leaf	13.56 (3.16)	442.63 (88.72)	32.85 (1.33)	0.10 (0.01)	0.37 (0.02)
North face	*Rhododendrum ferrugineum*	mature shrubland	litter	16.10 (9.16)	1292.63 (636.49)	86.02 (14.13)	0.04 (0.01)	0.15 (0.03)

Site	Vegetation type	Succession stage	Compartment	N:P	C:P	C:N	P (%)	K (%)
North face	*Rhododendrum ferrugineum*	mature shrubland	soil	7.29 (2.00)	125.39 (26.55)	17.66 (3.08)	0.10 (0.03)	1.10 (0.18)
North face	*Rhododendrum ferrugineum*	mature shrubland	stems	12.10 (1.64)	1323.87(268.82)	109.20 (13.26)	0.04 (0.01)	0.16 (0.03)
North face	*Vaccinium myrtillus*	young shrubland	leaf	10.52 (1.00)	341.09 (54.67)	32.28 (2.04)	0.13 (0.05)	0.93 (0.16)
North face	*Vaccinium myrtillus*	young shrubland	litter	9.09 (1.75)	433.85 (108.69)	47.59 (5.03)	0.08 (0.03)	0.74 (0.08)
North face	*Vaccinium myrtillus*	young shrubland	soil	5.94 (1.72)	99.66 (27.63)	16.83 (0.70)	0.11 (0.03)	1.23 (0.08)
North face	*Vaccinium myrtillus*	young shrubland	stems	13.06 (2.18)	1064.30 (74.67)	82.42 (7.65)	0.05 (0.01)	0.17 (0.05)
North face	*Vaccinium myrtillus*	mature shrubland	leaf	13.92 (4.47)	421.15 (173.81)	29.67 (2.89)	0.14 (0.02)	0.89 (0.11)
North face	*Vaccinium myrtillus*	mature shrubland	litter	14.05 (4.44)	644.10 (172.42)	46.40 (3.05)	0.12 (0.02)	0.69 (0.15)
North face	*Vaccinium myrtillus*	mature shrubland	soil	5.22 (2.05)	92.51 (26.20)	18.16 (1.61)	0.13 (0.02)	1.08 (0.03)
North face	*Vaccinium myrtillus*	mature shrubland	stems	12.50 (2.86)	1060.30 (182.42)	85.73 (8.04)	0.05 (0.001)	0.14 (0.01)
South face	Herbaceous spp.	Pure grassland	leaf	15.67 (3.96)	353.64 (48.83)	23.25 (4.61)	0.13 (0.02)	1.59 (0.24)
South face	Herbaceous spp.	Pure grassland	litter	14.34 (2.31)	601.17 (224.25)	41.44 (12.61)	0.08 (0.04)	0.18 (0.06)
South face	Herbaceous spp.	Pure grassland	soil	8.80 (1.08)	101.17 (13.01)	11.51 (0.66)	0.11 (0.001)	1.50 (0.25)
South face	Herbaceous spp.	young shrubland	leaf	13.82 (2.73)	424.29 (80.92)	30.89 (3.15)	0.11 (0.02)	1.05 (0.27)

Site	Vegetation type	Succession stage	Compartment	N:P	C:P	C:N	P (%)	K (%)
South face	Herbaceous spp.	young shrubland	litter	17.68 (2.13)	1135.39(236.08)	63.98 (8.35)	0.04 (0.01)	0.19 (0.05)
South face	Herbaceous spp.	young shrubland	soil	6.49 (1.78)	83.61 (22.94)	12.89 (0.98)	0.09 (0.03)	1.37 (0.39)
South face	*Arctostaphylos uva-ursi*	young shrubland	leaf	11.10 (0.80)	580.34 (55.11)	52.56 (6.86)	0.11 (0.01)	0.45 (0.12)
South face	*Arctostaphylos uva-ursi*	young shrubland	litter	12.29 (1.73)	1151.67 (342.95)	92.34 (19.78)	0.06 (0.02)	0.29 (0.09)
South face	*Arctostaphylos uva-ursi*	young shrubland	soil	9.04 (2.13)	158.31 (79.41)	16.76 (4.25)	0.08 (0.02)	1.21 (0.33)
South face	*Arctostaphylos uva-ursi*	young shrubland	stems	12.32 (2.45)	1316.90(259.29)	107.23 (9.28)	0.04 (0.00)	0.24 (0.07)
South face	*Arctostaphylos uva-ursi*	mature shrubland	leaf	9.69 (1.52)	459.38 (44.09)	48.19 (7.50)	0.09 (0.01)	0.44 (0.13)
South face	*Arctostaphylos uva-ursi*	mature shrubland	litter	9.08 (1.74)	909.90 (373.08)	102.57 (43.18)	0.05 (0.02)	0.19 (0.05)
South face	*Arctostaphylos uva-ursi*	mature shrubland	soil	6.25 (3.89)	111.24 (103.51)	15.68 (5.35)	0.06 (0.01)	1.19 (0.24)
South face	*Arctostaphylos uva-ursi*	mature shrubland	stems	10.19 (0.57)	1169.19 (89.90)	114.82 (7.42)	0.04 (0.01)	0.16 (0.03)
South face	*Juniperus communis*	young shrubland	leaf	11.20 (1.14)	427.91 (106.34)	37.82 (6.53)	0.12 (0.02)	0.43 (0.06)
South face	*Juniperus communis*	young shrubland	litter	14.51 (2.54)	1319.66 (272.81)	91.01 (9.14)	0.06 (0.02)	0.18 (0.09)
South face	*Juniperus communis*	young shrubland	soil	4.52 (0.26)	69.49 (9.96)	15.33 (1.63)	0.09 (0.05)	1.43 (0.29)
South face	*Juniperus communis*	young shrubland	stems	9.72 (3.52)	1002.13 (313.68)	105.49 (22.70)	0.04 (0.01)	0.17 (0.06)

Site	Vegetation type	Succession stage	Compartment	N:P	C:P	C:N	P (%)	K (%)
South face	*Juniperus communis*	mature shrubland	leaf	11.86 (1.72)	438.77 (68.33)	37.18 (5.19)	0.12 (0.04)	0.59 (0.19)
South face	*Juniperus communis*	mature shrubland	litter	15.41 (2.02)	1025.89(537.39)	64.44 (25.32)	0.04 (0.01)	0.20 (0.05)
South face	*Juniperus communis*	mature shrubland	soil	6.25 (2.97)	96.92 (67.25)	15.09 (4.60)	0.09 (0.01)	1.34 (0.15)
South face	*Juniperus communis*	mature shrubland	stems	10.11 (0.82)	1093.78(192.40)	108.82 (22.35)	0.05 (0.02)	0.17 (0.06)
South face	*Juniperus sabina*	young shrubland	leaf	11.06 (0.59)	396.26 (17.15)	35.97 (3.27)	0.13 (0.02)	0.59 (0.05)
South face	*Juniperus sabina*	young shrubland	litter	11.84 (1.23)	471.03 (23.38)	40.17 (5.25)	0.07 (0.02)	0.32 (0.05)
South face	*Juniperus sabina*	young shrubland	soil	7.51 (2.62)	93.65 (32.46)	12.75 (3.51)	0.09 (0.02)	1.47 (0.35)
South face	*Juniperus sabina*	young shrubland	stems	9.28 (0.29)	803.55 (72.17)	86.79 (10.22)	0.05 (0.01)	0.20 (0.03)
South face	*Juniperus sabina*	mature shrubland	leaf	11.72 (1.30)	404.64 (64.34)	34.46 (2.88)	0.13 (0.01)	0.56 (0.03)
South face	*Juniperus sabina*	mature shrubland	litter	13.03 (1.10)	745.04 (169.52)	56.72 (9.24)	0.11 (0.01)	0.30 (0.07)
South face	*Juniperus sabina*	mature shrubland	soil	5.04 (0.98)	65.49 (16.10)	12.96 (1.46)	0.06 (0.02)	1.54 (0.18)
South face	*Juniperus sabina*	mature shrubland	stems	9.31 (2.85)	1046.16(250.82)	115.03 (12.68)	0.06 (0.01)	0.21 (0.05)

Site	Vegetation type	Succession stage	Compartment	Mg (%)	Ca (%)	Fe (%)	C (g m^{-2})	N (g m^{-2})
North face	Herbaceous spp.	Pure grassland	leaf	0.10 (0.03)	0.22 (0.04)	0.01 (0.01)	230.08 (127.94)	9.44(4.11)
North face	Herbaceous spp.	Pure grassland	litter	0.10 (0.03)	0.34 (0.11)	0.05 (0.02)	230.44 (128.82)	5.47(2.98)
North face	Herbaceous spp.	Pure grassland	soil	0.03 (0.001)	0.09 (0.03)	4.07 (0.17)	3645.71(1502.85)	294.79(125.00)
North face	Herbaceous spp.	young shrubland	leaf	0.10 (0.03)	0.24 (0.04)	0.01 (0.001)	145.28(89.01)	3.75(1.91)
North face	Herbaceous spp.	young shrubland	litter	0.05 (0.01)	0.35 (0.09)	0.05 (0.01)	146.89(83.96)	2.53(1.46)
North face	Herbaceous spp.	young shrubland	soil	0.01 (0.001)	0.16 (0.02)	3.88 (0.89)	2672.34(1156.42)	148.60(70.15)
North face	*Calluna vulgaris*	young shrubland	leaf	0.25 (0.03)	0.49 (0.06)	0.01 (0.001)	140.37(65.64)	4.14(2.51)
North face	*Calluna vulgaris*	young shrubland	litter	0.20 (0.03)	0.66 (0.07)	0.05 (0.01)	46.88(2.01)	1.29(0.07)
North face	*Calluna vulgaris*	young shrubland	soil	0.01 (0.001)	0.16 (0.03)	2.76 (0.42)	3170.27(256.71)	195.34(0.69)
North face	*Calluna vulgaris*	young shrubland	stems	0.06 (0.01)	0.14 (0.03)	0.01 (0.001)	307.93(73.89)	3.05(0.84)
North face	*Calluna vulgaris*	mature shrubland	leaf	0.22 (0.03)	0.46 (0.03)	0.01 (0.001)	120.44(51.89)	2.95(1.38)
North face	*Calluna vulgaris*	mature shrubland	litter	0.15 (0.02)	0.55 (0.09)	0.05 (0.02)	40.49(1.59)	0.86(0.04)
North face	*Calluna vulgaris*	mature shrubland	soil	0.03 (0.001)	0.15 (0.04)	2.93 (0.82)	8953.52(1382.65)	507.53(83.77)
North face	*Calluna vulgaris*	mature shrubland	stems	0.05 (0.01)	0.14 (0.01)	0.01 (0.001)	457.22(115.31)	3.94 (1.40)

Site	Vegetation type	Succession stage	Compartment	Mg (%)	Ca (%)	Fe (%)	C (g m^{-2})	N (g m^{-2})
North face	*Rhododendrum ferrugineum*	young shrubland	leaf	0.25 (0.04)	0.72 (0.13)	0.01 (0.001)	166.36 (1.62)	5.07 (0.17)
North face	*Rhododendrum ferrugineum*	young shrubland	litter	0.27 (0.02)	1.18 (0.11)	0.01 (0.001)	75.43 (0.63)	0.89 (0.13)
North face	*Rhododendrum ferrugineum*	young shrubland	soil	0.01 (0.001)	0.13 (0.04)	2.98 (1.02)	3123.01 (1362.05)	185.91 (94.50)
North face	*Rhododendrum ferrugineum*	young shrubland	stems	0.05 (0.01)	0.17 (0.01)	0.01 (0.001)	1132.12 (99.95)	10.48 (1.49)
North face	*Rhododendrum ferrugineum*	mature shrubland	leaf	0.20 (0.02)	0.69 (0.17)	0.01 (0.001)	199.32(103.30)	6.40 (3.46)
North face	*Rhododendrum ferrugineum*	mature shrubland	litter	0.23 (0.02)	1.18 (0.10)	0.01 (0.001)	90.09 (46.00)	1.51 (0.74)
North face	*Rhododendrum ferrugineum*	mature shrubland	soil	0.02 (0.01)	0.12 (0.02)	3.27 (1.23)	7388.76(518.27)	423.67(35.42)
North face	*Rhododendrum ferrugineum*	mature shrubland	stems	0.04 (0.01)	0.20 (0.04)	0.01(0.001)	1741.60 (321.94)	21.73(4.12)
North face	*Vaccinium myrtillus*	young shrubland	leaf	0.25 (0.04)	1.02 (0.08)	0.01 (0.001)	26.15(6.50)	0.89(0.22)
North face	*Vaccinium myrtillus*	young shrubland	litter	0.20 (0.03)	0.69 (0.10)	0.01 (0.001)	26.74(6.46)	0.57(0.10)
North face	*Vaccinium myrtillus*	young shrubland	soil	0.01 (0.01)	0.16 (0.02)	4.32 (1.04)	2571.35(767.21)	143.39(48.78)
North face	*Vaccinium myrtillus*	young shrubland	stems	0.04 (0.001)	0.28 (0.05)	0.01 (0.001)	121.90(25.79)	1.45(0.40)
North face	*Vaccinium myrtillus*	mature shrubland	leaf	0.24 (0.03)	0.92 (0.07)	0.01 (0.001)	48.35(11.33)	1.51(0.39)
North face	*Vaccinium myrtillus*	mature shrubland	litter	0.20 (0.02)	0.72 (0.11)	0.01 (0.001)	49.54(11.53)	1.06(0.33)

Site	Vegetation type	Succession stage	Compartment	Mg (%)	Ca (%)	Fe (%)	C (g m^{-2})	N (g m^{-2})
North face	*Vaccinium myrtillus*	mature shrubland	soil	0.03 (0.001)	0.18 (0.06)	3.98 (0.34)	5311.28 (4772.65)	317.73 (287.40)
North face	*Vaccinium myrtillus*	mature shrubland	stems	0.04 (0.001)	0.27 (0.08)	0.02 (0.01)	261.81(56.04)	3.18 (0.62)
South face	Herbaceous spp.	Pure grassland	leaf	0.10 (0.01)	0.40 (0.09)	0.01 (0.01)	191.07 (77.78)	8.24 (3.27)
South face	Herbaceous spp.	Pure grassland	litter	0.08 (0.03)	0.72 (0.17)	0.05 (0.01)	189.02 (77.45)	5.44 (4.09)
South face	Herbaceous spp.	Pure grassland	soil	0.11 (0.10)	0.56 (0.04)	3.41 (0.24)	8047.70 (2203.44)	695.63 (171.84)
South face	Herbaceous spp.	young shrubland	leaf	0.10 (0.02)	0.35 (0.05)	0.01 (0.001)	225.48 (111.77)	7.42 (3.62)
South face	Herbaceous spp.	young shrubland	litter	0.07 (0.01)	0.44 (0.02)	0.10 (0.06)	253.34(160.66)	3.89 (2.51)
South face	Herbaceous spp.	young shrubland	soil	0.01 (0.001)	0.20 (0.03)	5.48 (1.26)	6607.99(1627.22)	513.22(127.03)
South face	*Arctostaphylos uva-ursi*	young shrubland	leaf	0.13 (0.02)	0.61 (0.18)	0.01 (0.001)	317.71(151.69)	6.82 (3.49)
South face	*Arctostaphylos uva-ursi*	young shrubland	litter	0.13 (0.04)	0.81 (0.42)	0.01 (0.001)	158.79(56.94)	1.83 (1.01)
South face	*Arctostaphylos uva-ursi*	young shrubland	soil	0.01 (0.001)	0.19 (0.06)	5.20 (1.91)	9559.75(10343.17)	519.46 (369.38)
South face	*Arctostaphylos uva-ursi*	young shrubland	stems	0.05 (0.02)	0.38 (0.10)	0.02 (0.01)	278.78(226.66)	2.39 (1.89)
South face	*Arctostaphylos uva-ursi*	mature shrubland	leaf	0.16 (0.05)	0.66 (0.16)	0.01 (0.001)	430.39(74.93)	8.38 (2.26)
South face	*Arctostaphylos uva-ursi*	mature shrubland	litter	0.17 (0.03)	0.93 (0.28)	0.01 (0.001)	230.29(27.46)	2.84 (0.71)

Site	Vegetation type	Succession stage	Compartment	Mg (%)	Ca (%)	Fe (%)	C (g m^{-2})	N (g m^{-2})
South face	*Arctostaphylos uva-ursi*	mature shrubland	soil	0.01 (0.001)	0.18 (0.03)	4.21 (0.52)	5878.27(1893.36)	348.19 (70.13)
South face	*Arctostaphylos uva-ursi*	mature shrubland	stems	0.06 (0.02)	0.34 (0.06)	0.01 (0.001)	454.94 (68.30)	4.25 (0.54)
South face	*Juniperus communis*	young shrubland	leaf	0.13 (0.02)	1.08 (0.18)	0.02 (0.02)	678.25 (157.20)	18.61 (5.76)
South face	*Juniperus communis*	young shrubland	litter	0.12 (0.01)	1.36 (0.41)	0.02 (0.001)	180.85 (39.23)	2.06 (1.02)
South face	*Juniperus communis*	young shrubland	soil	0.01 (0.001)	0.31 (0.13)	5.12 (2.20)	7260.72 (3401.21)	470.06 (86.73)
South face	*Juniperus communis*	young shrubland	stems	0.05 (0.02)	1.48 (0.28)	0.04 (0.001)	490.65 (148.94)	4.60 (1.38)
South face	*Juniperus communis*	mature shrubland	leaf	0.12 (0.04)	1.13 (0.32)	0.01 (0.001)	716.91(368.39)	18.46(8.67)
South face	*Juniperus communis*	mature shrubland	litter	0.11 (0.03)	2.07 (0.39)	0.02(0.001)	118.00(92.81)	2.06 (1.17)
South face	*Juniperus communis*	mature shrubland	soil	0.01 (0.001)	0.24 (0.06)	5.31 (0.37)	4968.57(2257.34)	315.76(124.08)
South face	*Juniperus communis*	mature shrubland	stems	0.05 (0.02)	2.08 (0.23)	0.03 (0.01)	1403.08(1076.36)	14.56(11.97)
South face	*Juniperus sabina*	young shrubland	leaf	0.18 (0.04)	1.24 (0.11)	0.01 (0.001)	513.44(159.33)	15.22(5.76)
South face	*Juniperus sabina*	young shrubland	litter	0.17 (0.06)	1.89 (0.29)	0.04 (0.02)	488.04 (10.99)	12.16 (0.32)
South face	*Juniperus sabina*	young shrubland	soil	0.01 (0.001)	0.23 (0.09)	5.32 (1.03)	5500.47(3403.07)	414.91(219.39)
South face	*Juniperus sabina*	young shrubland	stems	0.06 (0.01)	1.99 (0.20)	0.02 (0.001)	467.54(283.06)	3.97(2.14)

Site	Vegetation type	Succession stage	Compartment	Mg (%)	Ca (%)	Fe (%)	C (g m^{-2})	N (g m^{-2})
South face	*Juniperus sabina*	mature shrubland	leaf	0.20 (0.02)	1.33 (0.29)	0.01 (0.001)	566.36 (153.61)	15.66(3.24)
South face	*Juniperus sabina*	mature shrubland	litter	0.20 (0.01)	1.44 (0.13)	0.02 (0.001)	286.54 (10.41)	5.64 (0.21)
South face	*Juniperus sabina*	mature shrubland	soil	0.01 (0.001)	0.17 (0.02)	5.35 (0.76)	5198.33(2264.67)	402.16 (142.23)
South face	*Juniperus sabina*	mature shrubland	stems	0.08 (0.02)	2.46 (0.50)	0.02(0.001)	1505.28(716.55)	17.83(9.94)

Site	Vegetation type	Succession stage	Compartment	P (g m^{-2})	K (g m^{-2})	Mg (g m^{-2})	Ca (g m^{-2})	Fe (g m^{-2})
North face	Herbaceous spp.	Pure grassland	leaf	0.71(0.42)	8.56(5.67)	0.55(0.42)	1.13(0.71)	0.06 (0.02)
North face	Herbaceous spp.	Pure grassland	litter	0.40(0.22)	1.98 (1.28)	0.48 (0.32)	1.67 (0.98)	0.25 (0.08)
North face	Herbaceous spp.	Pure grassland	soil	47.56(20.20)	394.99(168.27)	10.28 (4.48)	28.41 (9.36)	1428.56(605.05)
North face	Herbaceous spp.	young shrubland	leaf	0.36(0.12)	3.65 (1.80)	0.33 (0.20)	0.75 (0.40)	0.02 (0.01)
North face	Herbaceous spp.	young shrubland	litter	0.16(0.05)	0.38 (0.22)	0.17 (0.11)	1.25 (1.08)	0.15 (0.08)
North face	Herbaceous spp.	young shrubland	soil	33.99(9.92)	443.63(68.46)	2.32 (0.75)	54.64 (8.24)	1349.04(392.59)
North face	*Calluna vulgaris*	young shrubland	leaf	0.27(0.19)	1.47 (0.91)	0.71 (0.40)	1.40 (0.82)	0.03 (0.01)
North face	*Calluna vulgaris*	young shrubland	litter	0.07(0.01)	0.22 (0.01)	0.18(0.01)	0.60 (0.02)	0.05(0.0001)
North face	*Calluna vulgaris*	young shrubland	soil	18.58(0.47)	201.27(32.42)	1.87(0.04)	30.31 (1.47)	538.49 (16.02)
North face	*Calluna vulgaris*	young shrubland	stems	0.24 (0.09)	1.83 (0.51)	0.40 (0.10)	0.86 (0.15)	0.07 (0.01)

Site	Vegetation type	Succession stage	Compartment	P (g m^{-2})	K (g m^{-2})	Mg (g m^{-2})	Ca (g m^{-2})	Fe (g m^{-2})
North face	*Calluna vulgaris*	mature shrubland	leaf	0.18 (0.08)	0.96 (0.36)	0.52 (0.20)	1.08 (0.44)	0.03 (0.01)
North face	*Calluna vulgaris*	mature shrubland	litter	0.05 (0.0001)	0.22 (0.01)	0.12 (0.01)	0.46 (0.02)	0.04 (0.0001)
North face	*Calluna vulgaris*	mature shrubland	soil	63.80(17.35)	540.53(191.72)	14.97(3.16)	91.72(56.60)	1594.04(97.57)
North face	*Calluna vulgaris*	mature shrubland	stems	0.29 (0.18)	1.78 (0.50)	0.51 (0.16)	1.33 (0.36)	0.09 (0.03)
North face	*Rhododendrum ferrugineum*	young shrubland	leaf	0.39 (0.09)	1.16 (0.07)	0.81 (0.13)	2.30 (0.43)	0.02(0.002)
North face	*Rhododendrum ferrugineum*	young shrubland	litter	0.08 (0.06)	0.16 (0.03)	0.38 (0.03)	1.70 (0.17)	0.01(0.002)
North face	*Rhododendrum ferrugineum*	young shrubland	soil	25.16(11.43)	361.18(249.26)	1.81(0.74)	35.62(21.50)	877.19 (742.12)
North face	*Rhododendrum ferrugineum*	young shrubland	stems	0.88 (0.19)	3.13 (0.54)	1.10 (0.20)	3.67(0.19)	0.13(0.04)
North face	*Rhododendrum ferrugineum*	mature shrubland	leaf	0.38 (0.22)	1.43 (0.77)	0.77 (0.41)	2.62(1.40)	0.02(0.01)
North face	*Rhododendrum ferrugineum*	mature shrubland	litter	0.07 (0.03)	0.26 (0.14)	0.40 (0.24)	2.11(1.32)	0.02(0.01)
North face	*Rhododendrum ferrugineum*	mature shrubland	soil	44.53(3.80)	489.28(57.90)	7.52(2.47)	55.70(9.82)	1426.29(348.09)
North face	*Rhododendrum ferrugineum*	mature shrubland	stems	1.43 (0.26)	5.28 (0.62)	1.49(0.42)	6.78(1.98)	0.29(0.11)
North face	*Vaccinium myrtillus*	young shrubland	leaf	0.07 (0.03)	0.50 (0.12)	0.13 (0.02)	0.55 (0.16)	0.01(0.002)
North face	*Vaccinium myrtillus*	young shrubland	litter	0.05 (0.02)	0.40 (0.09)	0.11(0.04)	0.38 (0.15)	0.01(0.002)

Site	Vegetation type	Succession stage	Compartment	P (g m^{-2})	K (g m^{-2})	Mg (g m^{-2})	Ca (g m^{-2})	Fe (g m^{-2})
South face	*Juniperus sabina*	young shrubland	leaf	1.30 (0.49)	6.04 (2.13)	1.77 (0.56)	12.83(4.93)	0.11(0.05)
South face	*Juniperus sabina*	young shrubland	litter	1.04 (0.03)	2.93 (0.11)	1.89 (0.05)	11.02 (0.62)	0.19 (0.01)
South face	*Juniperus sabina*	young shrubland	soil	80.90(32.72)	1352.21(324)	10.59(6.65)	233.24(149.64)	5117.25(2102.71)
South face	*Juniperus sabina*	young shrubland	stems	0.51 (0.45)	1.84 (1.01)	0.51 (0.24)	20.41(12.88)	0.20(0.11)
South face	*Juniperus sabina*	mature shrubland	leaf	1.42 (0.33)	6.03 (1.29)	2.21 (0.73)	14.87(6.48)	0.11(0.03)
South face	*Juniperus sabina*	mature shrubland	litter	0.46 (0.02)	1.98 (0.12)	0.83 (0.04)	13.64 (0.36)	0.20 (0.002)
South face	*Juniperus sabina*	mature shrubland	soil	56.24 (8.21)	1364.41(300)	8.82 (2.49)	146.37(31.95)	4732.03(1049.04)
South face	*Juniperus sabina*	mature shrubland	stems	1.92 (1.05)	6.62 (3.02)	2.64 (1.38)	82.66(49.25)	0.75 (0.29)

Appendix 4: Chapter - High foliar K and P resorption efficiencies in old-growth nutrient-poor tropical forests

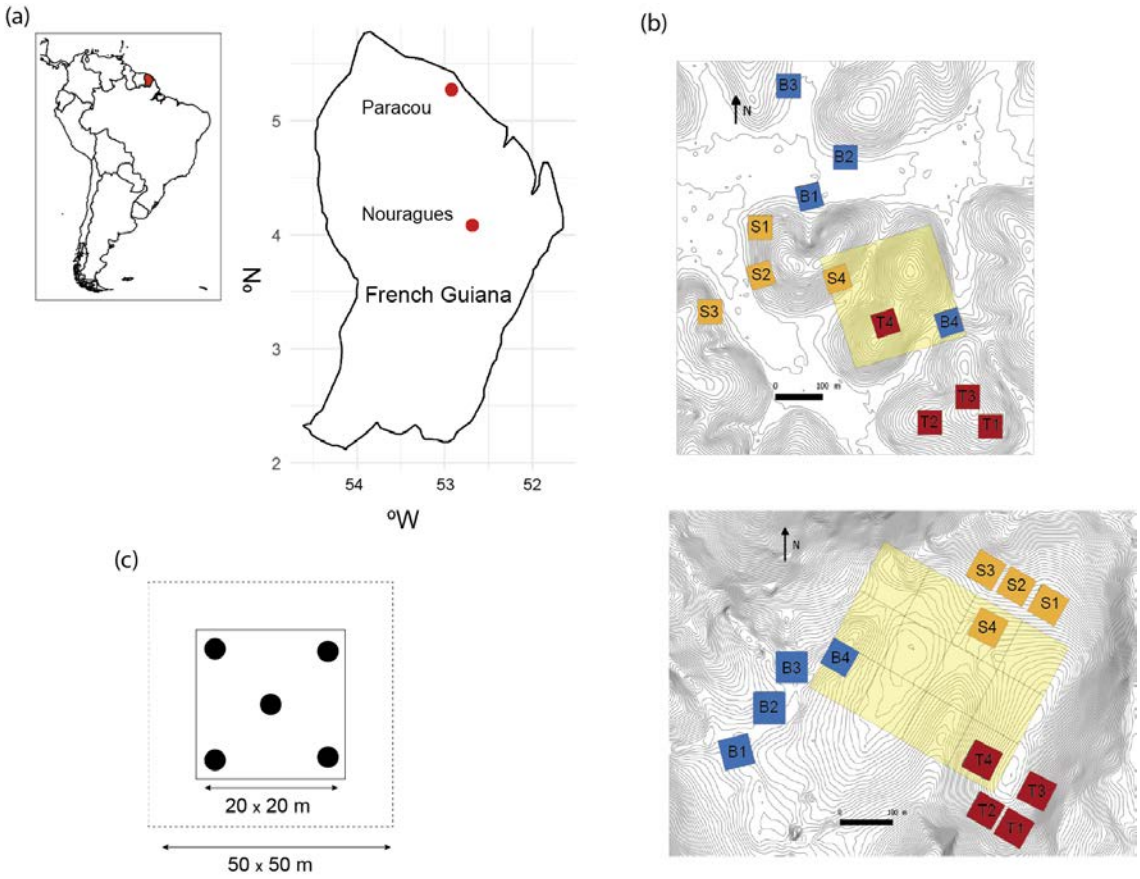


Figure A.4.1: Location of the study sites and experimental design. (a) Location of French Guiana in South America and the two study sites: the Paracou and Nouragues research stations (red dots). (b) Topographic maps of Paracou (top panel) and Nouragues (lower panel). Twelve plots four for each topographic level (in the figure, B = bottom of the hill (blue), S = slope plots in the middle of the hills (yellow) and T = top plots in the top of the hill (red), were established at each site to catch spatial variability. (c) Experimental plots (50 × 50 m), buffer zone and the sampling area (20 × 20 m) (solid line). Circles in the sampling area indicate the sampling points for the soil and leaf litter.

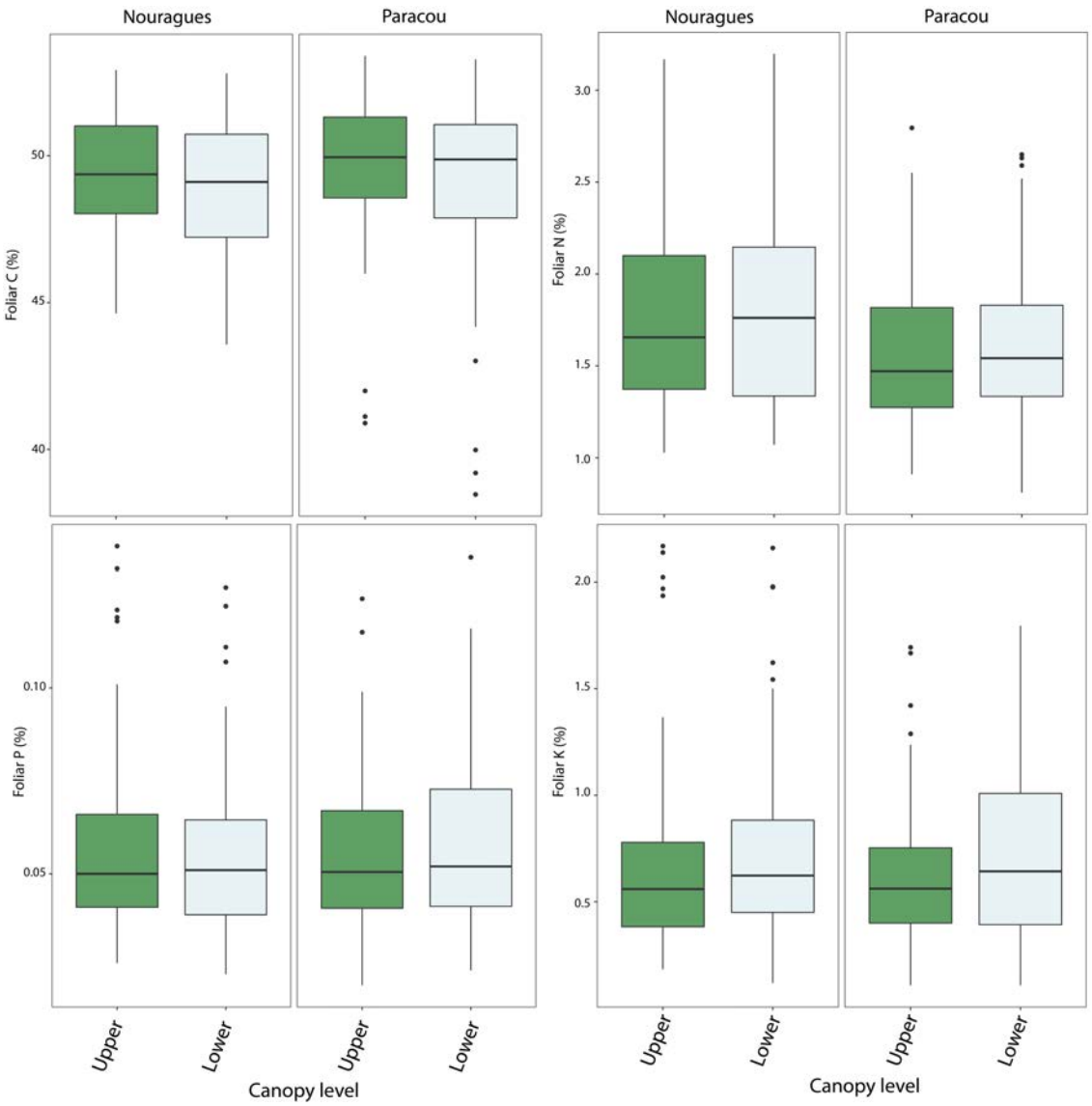


FIGURE A.4.2: C, N, P and K concentrations (dw/dw) in the upper and lower tree canopy for Nouragues (left panels) and Paracou (right panels). Boxes represent the second and third quartiles, the black lines inside the boxes represent medians, bars represent the first (top) and fourth (down) quartiles, and dots represent outliers.

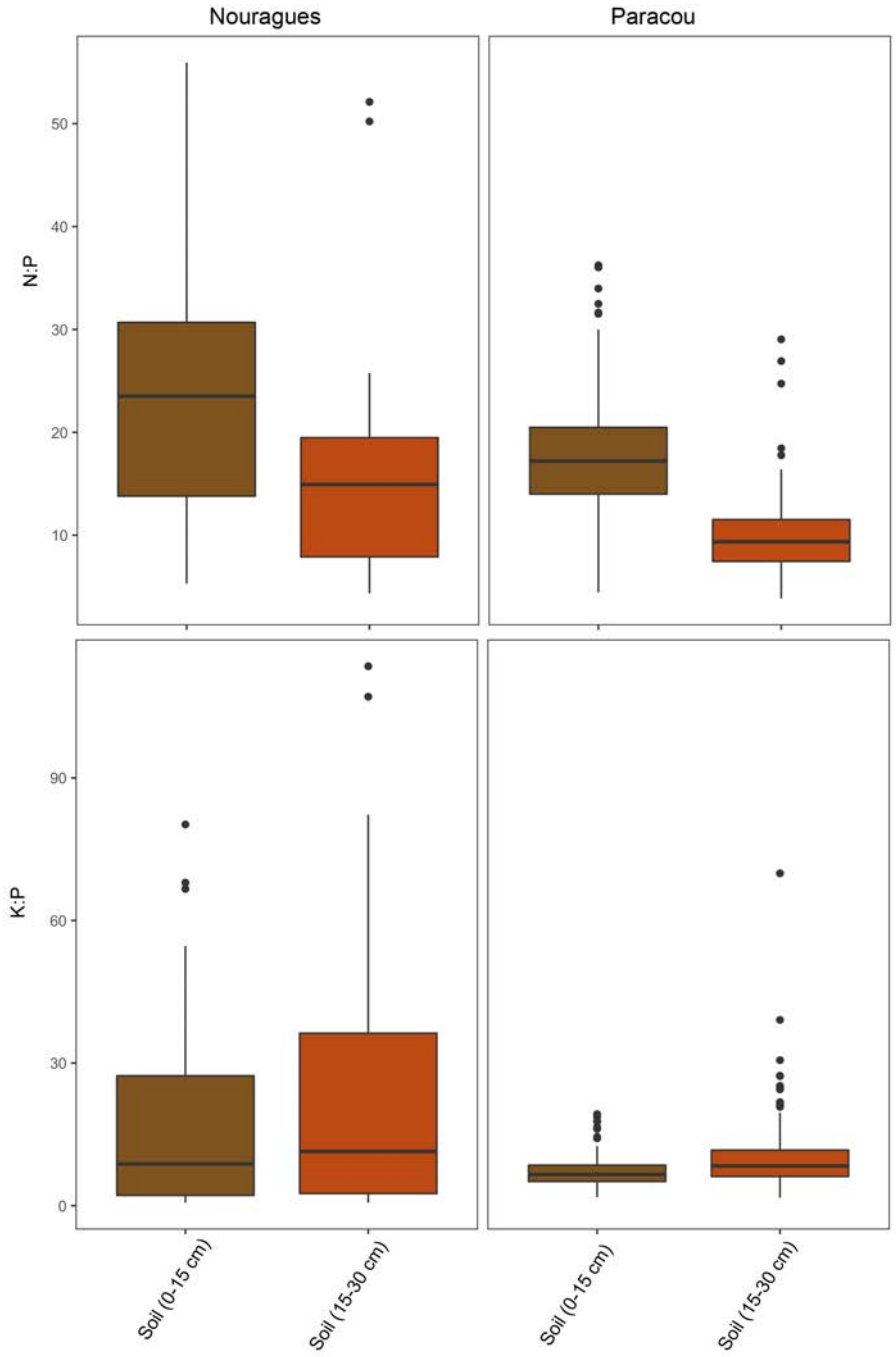


Figure A.4.3: N:P (upper panels) and K:P (lower panels) ratios for the 0-15 and 15-30 cm soil layers at the two sites, Nouragues (left panels) and Paracou (right panels). Boxes represent the second and third quartiles, the black lines inside the boxes represent medians, bars represent the first (top) and fourth (down) quartiles, and dots represent outliers.

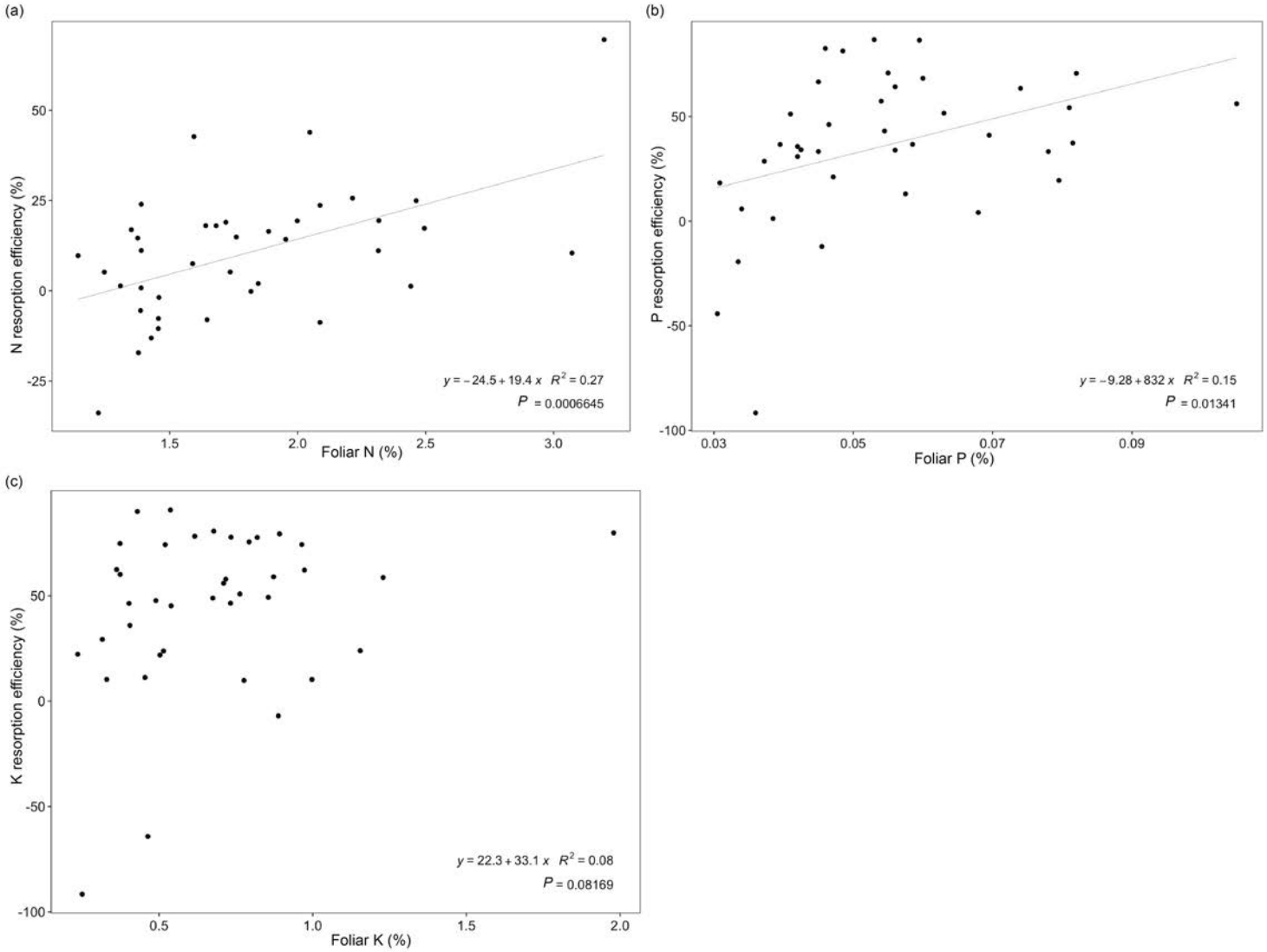


FIGURE A.4.4: Nutrient resorption efficiencies and their correlations with foliar nutrient concentration (dw/dw) based on the 39 tropical tree species sampled at both study sites in the dry season. (a) N resorption efficiency versus N in leaves, (b) P resorption versus P in leaves, and (c) K resorption versus K in leaves. Coefficients for the significant regressions and R^2 are displayed in the lower-right corner of each panel.

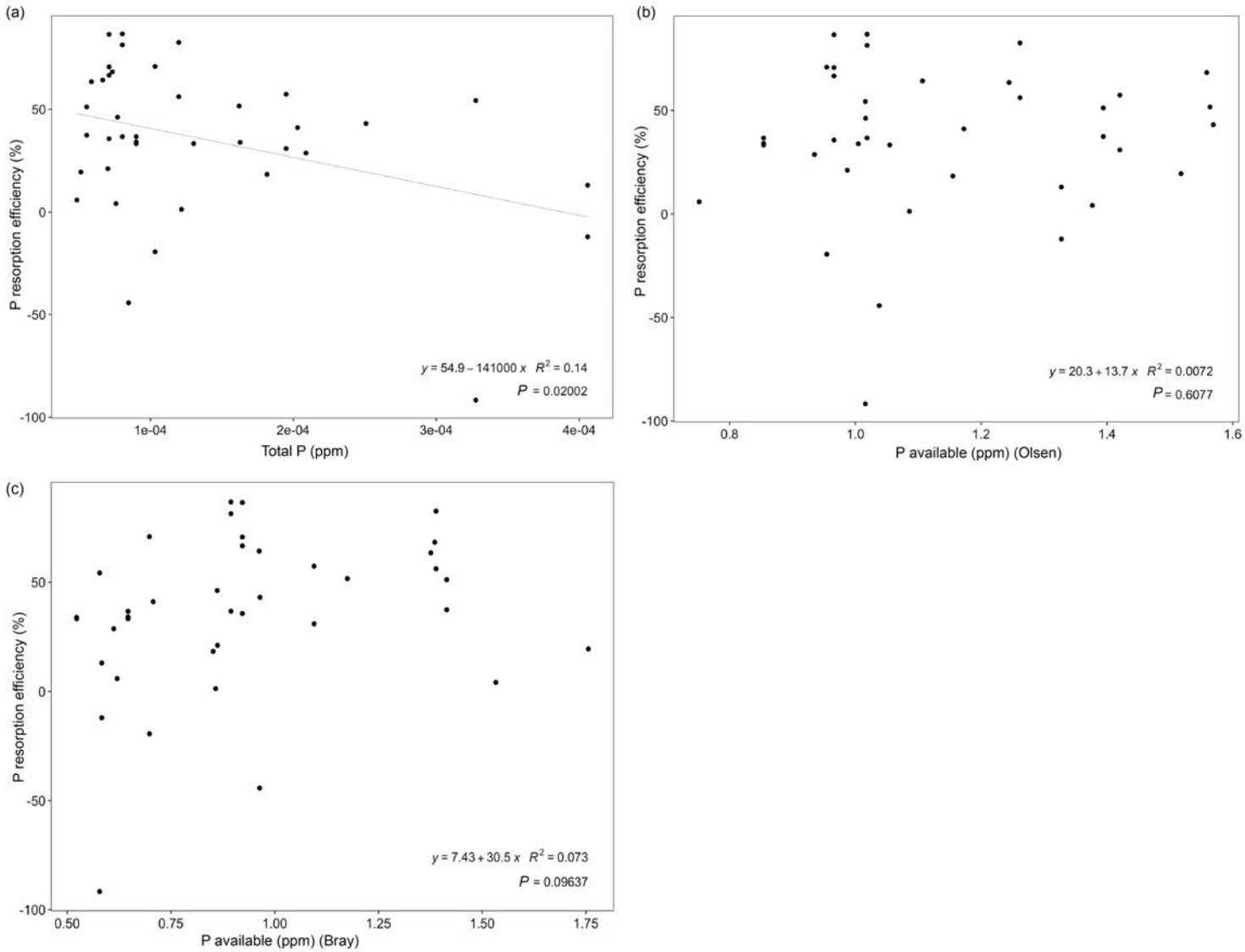


Figure A.4.5: P resorption efficiency and its correlation with soil P (ppm) based on the 39 tropical tree species sampled at both study sites in the dry season. (a) P resorption efficiency versus total soil P, (b) P resorption efficiency versus soil available P (Olsen method) and (c) P resorption efficiency versus soil available P (Bray method). Coefficients for the significant regressions and R^2 are displayed in the lower-right corner of each panel.

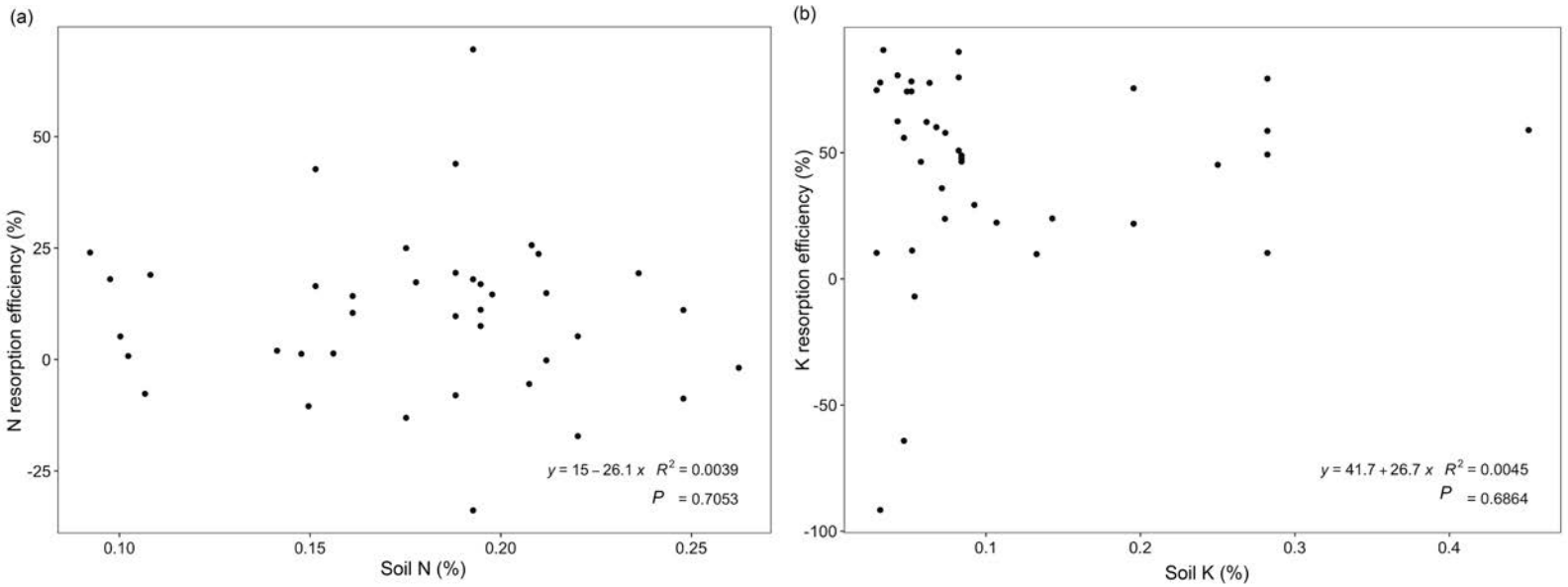


FIGURE A.4.6: N and K resorption efficiencies and their correlations with soil N and K (dw/dw) based on the 39 tropical tree species. (a) N resorption efficiency versus soil N and (b) K resorption efficiency versus soil K. Coefficients for the significant regressions and R^2 are displayed in the lower-right corner of each panel.

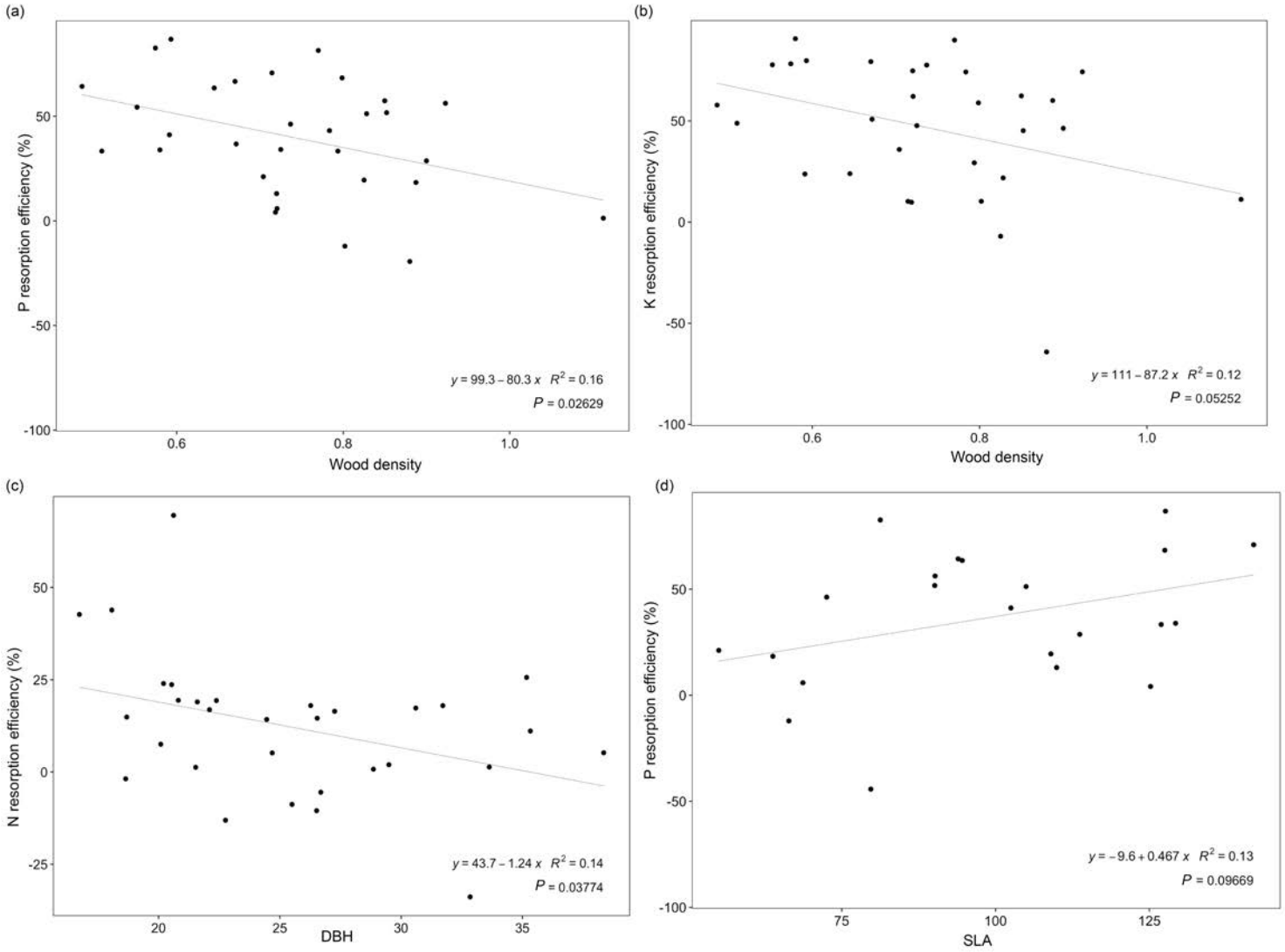


Figure A.4.7: Nutrient resorption efficiency and its relationship with some functional traits based on the 39 tropical tree species. Significant relationships of (a) P resorption versus wood density, (b) K resorption versus wood density, (c) N resorption versus DBH and (d) P resorption versus SLA. Coefficients for the significant regressions and R^2 are displayed in the lower-right corner of each panel.

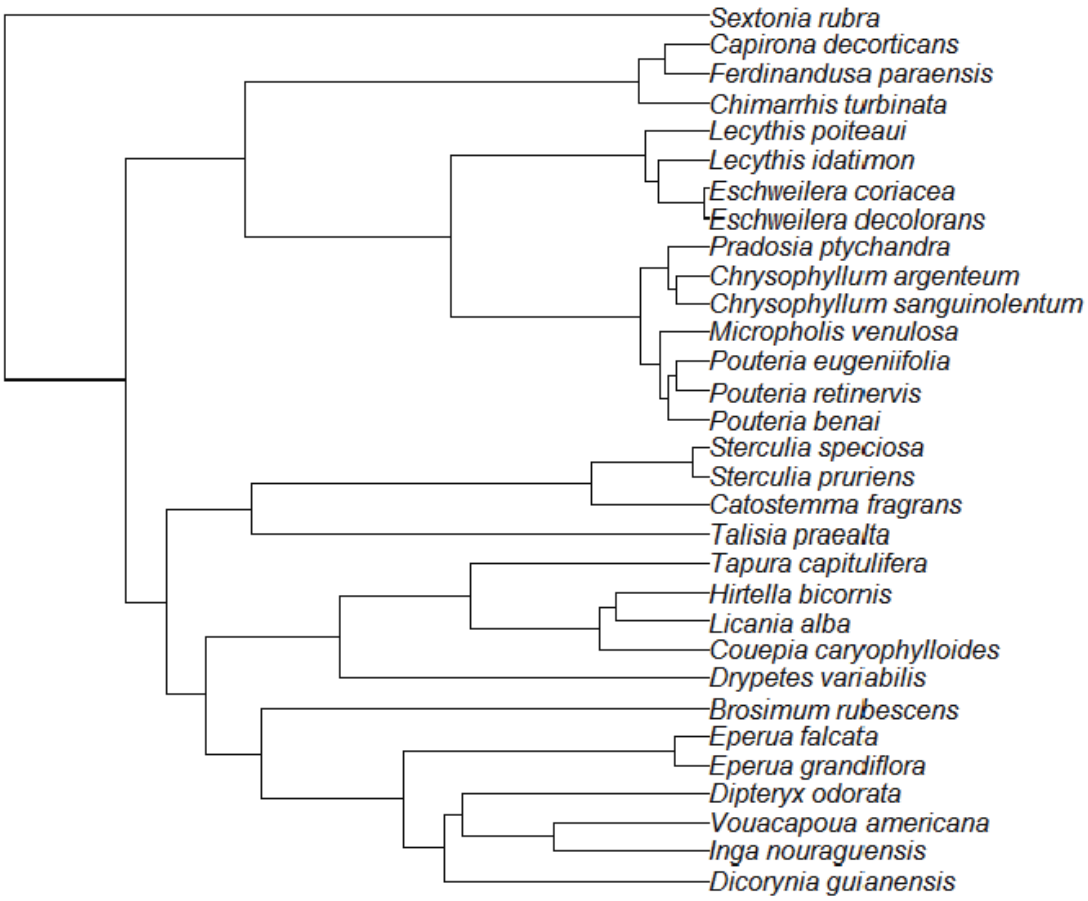


Figure A.4.8: Phylogenetic tree based on the 31 species sampled in this study. *Aniba rosaeodora*, *Chrysophyllum poniferum*, *Eugenia culcullata*, *Inga jenmanii*, *Licania densiflora*, *Myrcia splendens*, *Paloue guianensis*, *Vochysia sabatieri* could not be included in the phylogenetic analysis due to the lack of genetic sequences.

Table A.4.1: N, P and K resorption efficiencies (%) for the 39 species sampled in the dry season at both study sites. N show the number of individual sampled by species. Standard deviation in parentheses and not provided for species with only one individual sampled.

Species	N	Season	N resorption	P resorption	K resorption
Aniba rosaeodora Ducke	1	Dry	11.2	36.7	46.5
Brosimum rubescens Taub.	1	Dry	19.0	19.5	-7.0
Capirona decorticans Spruce	1	Dry	69.5	86.8	79.8
Catostemma fragrans Benth.	1	Dry	42.7	82.6	78.2
Chimarrhis turbinata DC.	1	Dry	11.1	13.0	74.8
Chrysophyllum argenteum Jacq.	1	Dry	-1.9	43.1	74.2
Chrysophyllum pomiferum (Eyma) T.D.Penn.	1	Dry	-7.7	-44.3	22.3
Chrysophyllum sanguinolentum (Pierre) Baehni	1	Dry	-33.8	36.8	50.9
Couepia caryophylloides Benoist	1	Dry	18.0	81.4	90.0
Dicorynia guianensis Amshoff	5	Dry	25.6 (22.2)	41.1 (52.9)	23.8 (57.4)
Dipteryx odorata (Aubl.) Willd.	1	Dry	16.5	56.2	74.3
Drypetes variabilis Uittien	1	Dry	24.0	46.2	77.7
Eperua falcata Aubl.	8	Dry	2.0 (17.0)	4.1	(27.5)
9.8 (34.7)					
Eperua grandiflora (Aubl.) Benth	4	Dry	0.8 (13.6)	21.1 (24.6)	35.9 (28.1)
Eschweilera coriacea (DC.) S.A.Mori	2	Dry	19.4 (3.1)	51.7 (12.5)	45.2 (21.0)
Eschweilera decolorans Sandwith	1	Dry	14.2	51.2	21.9
Eugenia cucullata Amshoff	1	Dry	9.7	86.6	58.7
Ferdinandusa paraensis Ducke	1	Dry	7.5	34.1	47.7
Hirtella bicornis Mart. and Zucc.	2	Dry	-5.5 (6.9)	28.7 (11.0)	46.4 (10.1)
Inga jenmanii Sandwith	1	Dry	1.3	33.9	90.7
Inga nouragensis Poncy	1	Dry	25.0	70.9	55.9
Lecythis idatimon Aubl.	1	Dry	23.7	68.3	59.0
Lecythis poiteaui O.Berg	1	Dry	-8.8	-12.1	10.3
Licania alba (Bernoulli) Cuatrec	7	Dry	14.6 (6.6)	18.3 (32.9)	60.1 (22.1)
Licania densiflora Kleinh	1	Dry	-0.2	31.0	80.7
Micropholis venulosa (Mart. and Eichler ex Miq.) Pierre	1	Dry	19.4	66.7	79.4
Myrcia splendens (Sw.) DC.	1	Dry	-8.0	35.7	49.3
Paloue guianensis Aubl.	1	Dry	10.4	37.4	75.5
Pouteria benai (Aubrév. and Pellegr.)T.D.Penn	1	Dry	-13.1	-19.4	-64.2
Pouteria eugeniifolia (Pierre) Baehni	2	Dry	1.4 (1.4)	1.3 (66.1)	11.2 (100.1)
Pouteria retinervis T.D.Penn.	1	Dry	43.9	70.7	10.3
Pradosia ptychandra (Eyma) T.D.Penn.	1	Dry	-10.5	63.5	23.9
Sextonia rubra (Mez) van der Werff	1	Dry	5.2	54.3	77.8
Sterculia pruriens (Aubl.) K.Schum.	1	Dry	18.0	64.3	57.9
Sterculia speciosa K. Schum.	1	Dry	16.9	33.3	48.9
Talisia praealta Radlk	1	Dry	14.9	57.4	62.4
Tapura capitulifera Baill.	1	Dry	5.2	5.9	62.2
Vochysia sabatieri Marc.-Berti	1	Dry	-17.2	-91.7	-91.6
Vouacapoua americana Aubl.	1	Dry	17.3	33.3	29.3

Table A.4.2: N, P and K resorption efficiencies (%) for the 18 species sampled in both the wet and dry seasons. N show the number of individual sampled by species. Standard deviation in parentheses and not provided for species with only one individual sampled.

Species	N	Season	N resorption	P resorption	K resorption
Aniba rosaeodora Ducke	1	Wet	40.2	50.0	81.0
	1	Dry	11.2	36.7	46.5
Brosimum rubescens Taub.	1	Wet	16.8	69.2	81.0
	1	Dry	19.0	19.5	-7.0
Chrysophyllum argenteum Jacq.	1	Wet	1.4	23.1	69.9
	1	Dry	-1.9	43.1	74.2
Chrysophyllum sanguinolentum (Pierre) Baehni	1	Wet	-5.1	38.7	40.7
	1	Dry	-33.8	36.8	50.9
Dicorynia guianensis Amshoff	3	Wet	18.0 (16.0)	47.0 (20.49)	46.6 (13.9)
	5	Dry	25.6 (22.2)	30.0 (48.7)	6.1 (43.3)
Eperua falcata Aubl.	3	Wet	5.4 (24.4)	59.2 (10.3)	76.4 (12.9)
	8	Dry	2.0 (16.3)	4.1 (26.3)	9.8 (33.2)
Eschweilera coriacea (DC.) S.A.Mori	2	Wet	9.4 (0.2)	72.0 (9.1)	87.7 (6.9)
	2	Dry	19.4 (2.5)	51.7 (10.2)	45.2 (17.2)
Eschweilera decolorans Sandwith	1	Wet	5.8	40.4	72.4
	1	Dry	14.2	51.2	21.9
Ferdinandusa paraensis Ducke	1	Wet	24.3	47.5	85.5
	1	Dry	7.5	34.1	47.7
Hirtella bicornis Mart. and Zucc.	2	Wet	-0.3 (0.9)	14.3 (21.0)	71.4 (18.3)
	2	Dry	-5.5 (5.6)	28.7 (9.0)	46.4 (8.2)
Licania alba (Bernoulli) Cuatrec	5	We t	26.1 (8.9)	67.2 (19.5)	90.7 (3.7)
	7	Dry	14.6 (6.2)	18.3 (30.9)	60.1 (20.8)
Myrcia splendens (Sw.) DC.	1	Wet	-34.6	51.7	79.6
	1	Dry	-8.0	35.7	49.3
Paloue guianensis Aubl.	1	Wet	-3.1	22.8	54.9
	1	Dry	10.4	37.4	75.5
Pouteria eugeniifolia (Pierre) Baehni	1	Wet	-15.2	15.9	92.2
	2	Dry	1.4 (1.4)	1.3 (66.1)	11.2 (100.1)
Pradosia ptychandra (Eyma) T.D.Penn.	1	Wet	-8.8	36.3	21.7
	1	Dry	-10.5	63.5	23.9
Sextonia rubra (Mez) van der Werff	1	We t	-4.5	9.5	61.7
	1	Dry	5.2	54.3	77.8
Sterculia speciosa K. Schum.	1	Wet	13.7	57.1	72.1
	1	Dry	16.9	33.3	48.9
Talisia praealta Radlk	1	Wet	22.3	61.5	77.9
	1	Dry	14.9	57.4	62.4

Table A.4.3: Significant differences at community level in the N, P and K resorption efficiencies. Output of the estimated regression parameters, standard errors , t and *p*-values for the linear mixed model. The estimated standard deviation associated with the random effect, σ species is 23.25.

	Estimate	Standrad Error	df	t-value	*p*-values
Intercept	44.61	5.17	76 8.62	<0.0001	
N	-34.31	5.07	76	-6.75	<0.0001
P	-8.71	5.07	76	1.71	0.09

Table A.4.4: Results of the post hoc test (pairwise comparison) using Tukey's method.

Contrast	Estimate	Standrad Error	df	t-ratio	*p*-values
K-N	34.31	5.08	76	6.75	<0.0001
K-P	8.71	5.08	76	1.71	0.2055
P-N	-25.6	5.08	76	-5.04	<0.0001

Table A.4.5: Significant differences at community level (average of all the species) in the N, P and K resorption efficiencies between different season. Output of the estimated regression parameters, standard errors, t and *p*-values for the linear mixed models for (a) nitrogen, (b) phosphorus and (c) potassium resorption and the effect of seasonality. The estimated standard deviation associated with the random effect for the model σ species, is 11.88 for nitrogen, 0.002 for phosphorus and 0.02 for potassium.

	Estimate	Standrad Error	df	t-value	*p*-values
Nitrogen					
Intercept	6.21	3.71	17	1.67	0.11
dry	-0.78	3.44	17	-0.22	0.82
Phosphorus					
Intercept	43.51	4.35	17	9.98	0.00
dry	-8.11	6.16	17	-1.31	0.20
Potassium					
Intercept	70.19	5.24	17	13.39	0.00
dry	-28.47	7.41	17	-3.84	0.0013

Table A.4.6: Mean and sd values (in parenthese) for N, P and K concentrations in green leaves and senescent leaves (% dw/dw) for the 39 species sampled in the dry season at both study sites. Standard deviation not provided for species with only one individual sampled.

Species	N (%)		P (%)		K (%)	
	leaves	senescent leaves	leaves	senescent leaves	leaves	senescent leafves
Aniba rosaeodora Ducke	1.389	1.234	0.040	0.025	0.733	0.392
Brosimum rubescens Taub.	1.720	1.393	0.080	0.064	0.888	0.950
Capirona decorticans Spruce	3.197	0.974	0.053	0.007	1.979	0.399
Catostemma fragrans Benth.	1.595	0.914	0.046	0.008	0.616	0.134
Chimarrhis turbinata DC.	2.315	2.058	0.058	0.050	0.373	0.094
Chrysophyllum argenteum Jacq.	1.458	1.485	0.055	0.031	0.520	0.134
Chrysophyllum pomiferum (Eyma) T.D.Penn.	1.456	1.568	0.031	0.044	0.236	0.183
Chrysophyllum sanguinolentum (Pierre) Baehni	1.221	1.634	0.059	0.037	0.763	0.375
Couepia caryophylloides Benoist	1.682	1.379	0.049	0.009	0.430	0.043
Dicorynia guianensis Amshoff	2.214 (0.220)	1.614 (0.361)	0.070 (0.013)	0.038 (0.033)	0.514 (0.134)	0.364 (0.236)
Dipteryx odorata (Aubl.) Willd.	1.887	1.576	0.105	0.046	0.965	0.248
Drypetes variabilis Uittien	1.389	1.056	0.047	0.025	0.820	0.183
Eperua falcata Aubl.	1.846 (0.303)	1.782 (0.257)	0.068 (0.011)	0.065 (0.021)	0.776 (0.275)	0.669 (0.279)
Eperua grandiflora (Aubl.) Benth	1.388 (0.036)	1.379 (0.198)	0.047 (0.007)	0.036 (0.006)	0.405 (0.080)	0.255 (0.123)
Eschweilera coriacea (DC.) S.A.Mori	1.998 (0.031)	1.612 (0.086)	0.063 (0.015)	0.030 (0.001)	0.539 (0.139)	0.310 (0.190)
Eschweilera decolorans Sandwith	1.953	1.675	0.041	0.020	0.503	0.393
Eugenia cucullata Amshoff	1.142	1.031	0.060	0.008	1.229	0.508
Ferdinandusa paraensis Ducke	1.590	1.470	0.043	0.028	0.490	0.256
Hirtella bicornis Mart. and Zucc.	1.386 (0.031)	1.461 (0.062)	0.037 (0.008)	0.027 (0.010)	0.402 (0.216)	0.227 (0.156)
Inga jenmanii Sandwith	2.442	2.411	0.056	0.037	0.537	0.050
Inga nouragensis Poncy	2.463	1.848	0.055	0.016	0.710	0.313
Lecythis idatimon Aubl.	2.087	1.593	0.060	0.019	0.873	0.358
Lecythis poiteaui O.Berg	2.087	2.270	0.046	0.051	0.330	0.296

Licania alba (Bernoulli) Cuatrec	1.375 (0.097)	1.175 (0.132)	0.031 (0.005)	0.024 (0.008)	0.373 (0.156)	0.142 (0.085)
Licania densiflora Kleinh	1.818	1.821	0.042	0.029	0.678	0.131
Micropholis venulosa (Mart. and Eichler ex Miq.) Pierre	2.317	1.867	0.045	0.015	0.892	0.184
Myrcia splendens (Sw.) DC.	1.646	1.778	0.042	0.027	0.856	0.434
Paloue guianensis Aubl.	3.072	2.751		0.082		0.051
0.793	0.194					
Pouteria benai (Aubrév. and Pellegr.)T.D.Penn	1.428	1.614	0.034	0.040	0.464	0.761
Pouteria eugeniifolia (Pierre) Baehni	1.307 (0.025)	1.290 (0.043)	0.039 (0.0001)	0.038 (0.025)	0.454 (0.048)	0.379 (0.412)
Pouteria retinervis T.D.Penn.	2.048	1.149	0.082	0.024	0.998	0.895
Pradosia ptychandra (Eyma) T.D.Penn.	1.456	1.608	0.074	0.027	1.155	0.878
Sextonia rubra (Mez) van der Werff	1.737	1.646	0.081	0.037	0.734	0.163
Sterculia pruriens (Aubl.) K.Schum.	1.641	1.345	0.056	0.020	0.717	0.302
Sterculia speciosa K. Schum.	1.350	1.122	0.045	0.030	0.675	0.345
Talisia praealta Radlk	1.760	1.498	0.054	0.023	0.362	0.136
Tapura capitulifera Baill.	1.245	1.180	0.034	0.032	0.973	0.368
Vochysia sabatieri Marc.-Berti	1.378	1.614	0.036	0.069	0.250	0.479
Vouacapoua americana Aubl.	2.495	2.063	0.078	0.052	0.316	0.223

Table A.4.7: Mean and sd values (in parentheses) for C, N, P and K concentraions (% dw/dw) in soil by plot in each study sites

Site	Plot	Depth (cm)	C (%)	N (%)	P (%)	K (%)
Paracou	top	0-15	1.997(0.485)	0.141 (0.029)	0.007 (0.003)	0.059 (0.025)
Paracou	top	15-30	0.789(0.189)	0.062 (0.013)	0.007 (0.003)	0.083 (0.07)
Paracou	slope	0-15	2.480(0.819)	0.171(0.046)	0.013(0.004)	0.074(0.046)
Paracou	slope	15-30	1.33(0.466)	0.107(0.033)	0.011(0.004)	0.091(0.076)
Paracou	bottom	0-15	2.102(0.704)	0.151(0.047)	0.008(0.003)	0.054(0.026)
Paracou	bottom	15-30	0.927(0.492)	0.075(0.029)	0.006(0.004)	0.055(0.013)
Nouragues	top	0-15	4.331(0.919)	0.301(0.048)	0.031(0.009)	0.041(0.01)
Nouragues	top	15-30	2.386(0.584)	0.181(0.038)	0.029(0.009)	0.039(0.008)
Nouragues	slope	0-15	3.083(0.788)	0.212(0.046)	0.008(0.002)	0.123(0.107)
Nouragues	slope	15-30	1.555(0.379)	0.123(0.024)	0.008(0.001)	0.13(0.12)
Nouragues	bottom	0-15	2.8039(1.026)	0.206(0.069)	0.006(0.002)	0.21(0.177)
Nouragues	bottom	15-30	1.269(0.615)	0.104(0.041)	0.005 (0.001)	0.244 (0.202)

Table A.4.8: Results for Pagel's and Blomberg's indices used to calculate the phylogenetic signals effect (value and significance) in the N, P and K resorption (%) based on 31 species. *Aniba rosaeodora*, *Chrysophyllum poniferum*, *Eugenia culcullata*, *Inga jenmanii*, *Licania densiflora*, *Myrcia splendens*, *Paloue guianensis*, *Vochysia sabatieri* could not be included in the phylogenetic analysis due to the lack of genetic sequences.

	N resorption		P resortion		K resorption	
Phylogenetic signal	value	p	value	p	value	p
Pagel's λ	6.64×10^{-5}	1	6.64×10^{-5}	1	6.64×10^{-5}	1
Blomberg's K	0.128	0.393	0.119	0.374	0.119	0.373

Deduction of the **allometric coefficient (C)** for estimating **leaf weight** based on diameter at breast height (DBH) from a global data set containing data for 2013 tropical trees published by Chave et al. (2014).

$$log(leaf\,weight) = \log(basal\,area) + C \tag{5.1}$$

$$basal\ area = \pi \times (\frac{DBH}{2})^2$$

The final model to infer leaf weight from basal area is:

$$Leaf\,weight = \exp(\log(basal\,area) + C') \tag{5.2}$$

where C'= C + $RSE^{2/2}$ (Baskerville correction is needed here, because a Gaussian error in the log-transformed model is no longer Gaussian when exponential; the error becomes log-normal with a mean of $RSE^{2/2}$, hence the correction).

The model fit is: **C = -4.2569** and Residual Standard Error (RSE) =0.8704, such that:

$$C' = \exp(-4.0153 + 0.8704)^{(2/2)} = 0.02634434 \tag{5.3}$$

The resulting allometric equation is:

$$Leaf\,weight = 0.02634434 \times basal\,area \tag{5.4}$$

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