GLOBAL CHANGE AND FOREST NUTRIENT STOICHIOMETRY. THE FOLIAR ELEMENTAL COMPOSITION OF WOODY PLANTS AND ITS DRIVERS

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Doctoral Thesis

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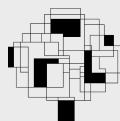
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*A la meva mare, la meva germana, la meva
esposa i a totes les dones d'esperit crític.
Que aquest camí també és nostre.*

Content

1

Introduction

1.1 The Earth system and the biogeochemical cycles

Following the bases of physics and chemistry the Earth is considered a closed system, where energy enters and escapes freely but matter conforming it does not, excepting for the eventual income of rare meteorites. At the same way as energy, matter is not created nor destroyed but it is transformed (Lavoisier, 1785) so all the matter conforming the Earth can flow around the system without disappearing. In other words, there is a limited and static number of atoms of each element conforming our planet. Thus, all this matter can move around the abiotic (atmosphere, lithosphere, hydrosphere) and biotic (biosphere) compartments of the Earth changing in estate (solid, liquid and gas) and conforming what is known as biogeochemical cycles. Each chemical, depending on its atomic properties and how the evolution used them conform its own cycle, subjected to different processes and paths to cross the compartment boarders. To understand and describe each cycle, science characterized them through their reservoirs, where the matter is stored, and fluxes, which are the different ways that each element can "escape" from each reservoir to flow into another. The carbon (C), nitrogen (N), phosphorus (P) and potassium (K) cycles have enormous impacts in biosphere and its development and due to that are the most studied matter cycles on Earth.

C-cycle

Carbon is the 15th most abundant element in Earth's crust, relatively light, solid in ambient temperature and is capable to establish stable and unstable bounds with a vast number of compounds. We can consider carbon as the base of live up the point that chemistry based on C is called organic chemistry. On the Earth, the biggest C reservoir is the ocean, containing more than 37000 Gt of C. Then follows the soil, with 2300 Gt of C, the atmosphere with almost 800 Gt and finally plants, with about 550 Gt of C. All these reservoirs are connected through biotic and/or abiotic reactions, exchanging C between them. Roughly speaking, oceans and atmosphere are connected both-sides through different processes: gas exchange, which produces carbonic acid when CO_2 dissolves into the ocean; through living beings' respiration, emitting CO_2 from the

ocean to the atmosphere; and finally with autotrophs photosynthesis, absorbing CO_2 to the ocean and incorporating it to oceanic life matter. Atmosphere interacts with plants, animals and microbes through respiration, where they emit CO_2 to atmosphere, and through photosynthesis, where plants incorporate CO_2 from atmosphere becoming organic matter. Finally, soil interacts with atmosphere, through soil organisms respiration and through the trophic webs with animals, fungi and microbes that emit great part of the CO_2 previous fixed by plants as organic matter. Soil also interact with plants, incorporating C from organic matter decomposition (Figure 1.1). Despite the Earth system is more complex than that, the aforementioned relations are the most important in terms of quantity moving almost all C in our planet.
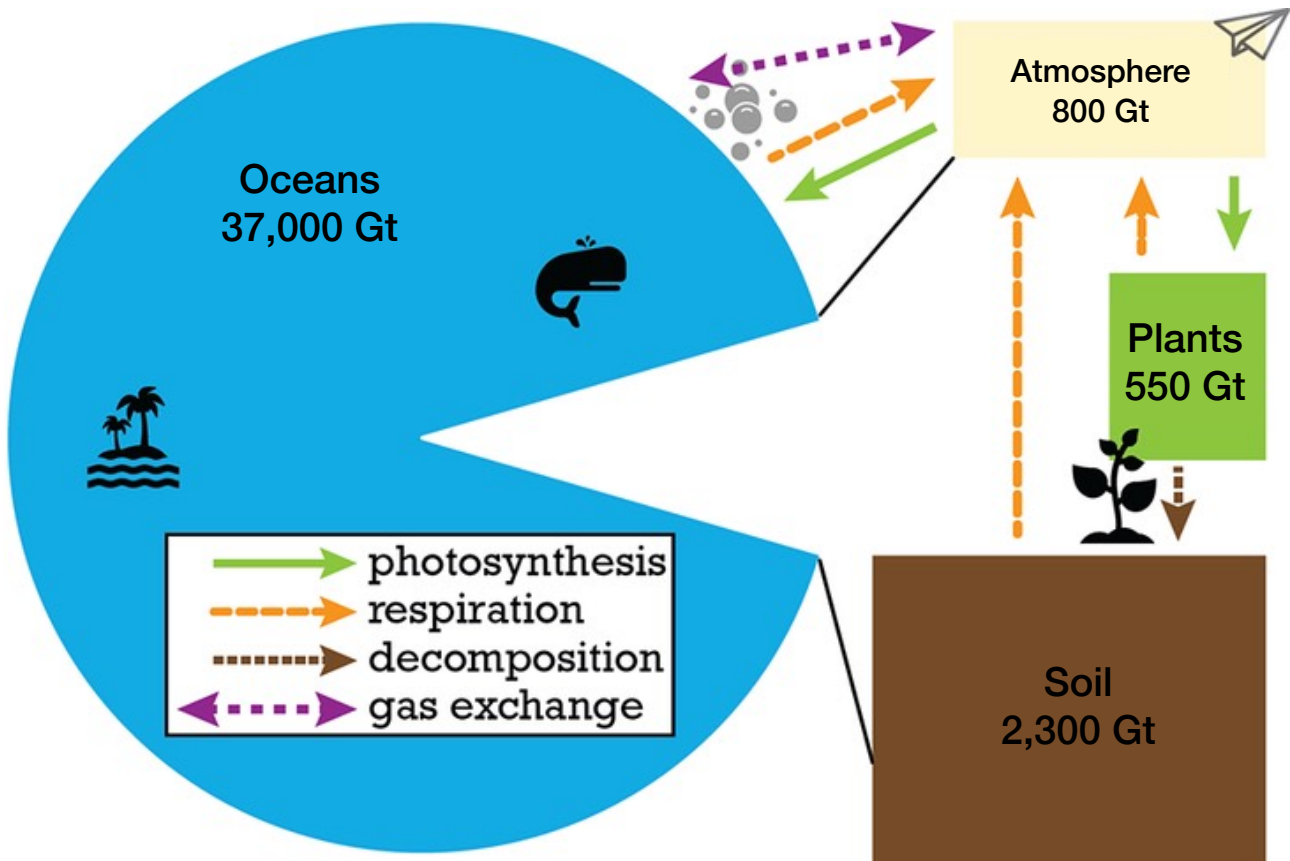
Figure 1.1. Schematic C cycle with compartments estimated Gt.
Ref: futurelearn.com (23/07/21) Tim Doheny-adams

N-cycle

Nitrogen is the 7th most abundant element in Via Lactea but due to the stability of its di-atomic covalent bond that yields very stable gas forms is difficult to be found in solid from in Earth surface conditions of pressure and temperature. On the Earth, 78% of the atmosphere is N, where is found as N_2 gas bonded with a triple covalent bond, one of the strongest union types which make it difficult to move between reservoirs. Paradoxically, N is also crucial to life on Earth, being part of structures as important as nucleic acids, proteins and amino acids. All living being on Earth needs from this difficult to achieve element. Consequently, the strategy that life has had to develop in order to incorporate N into the trophic chain has been challenging. To better
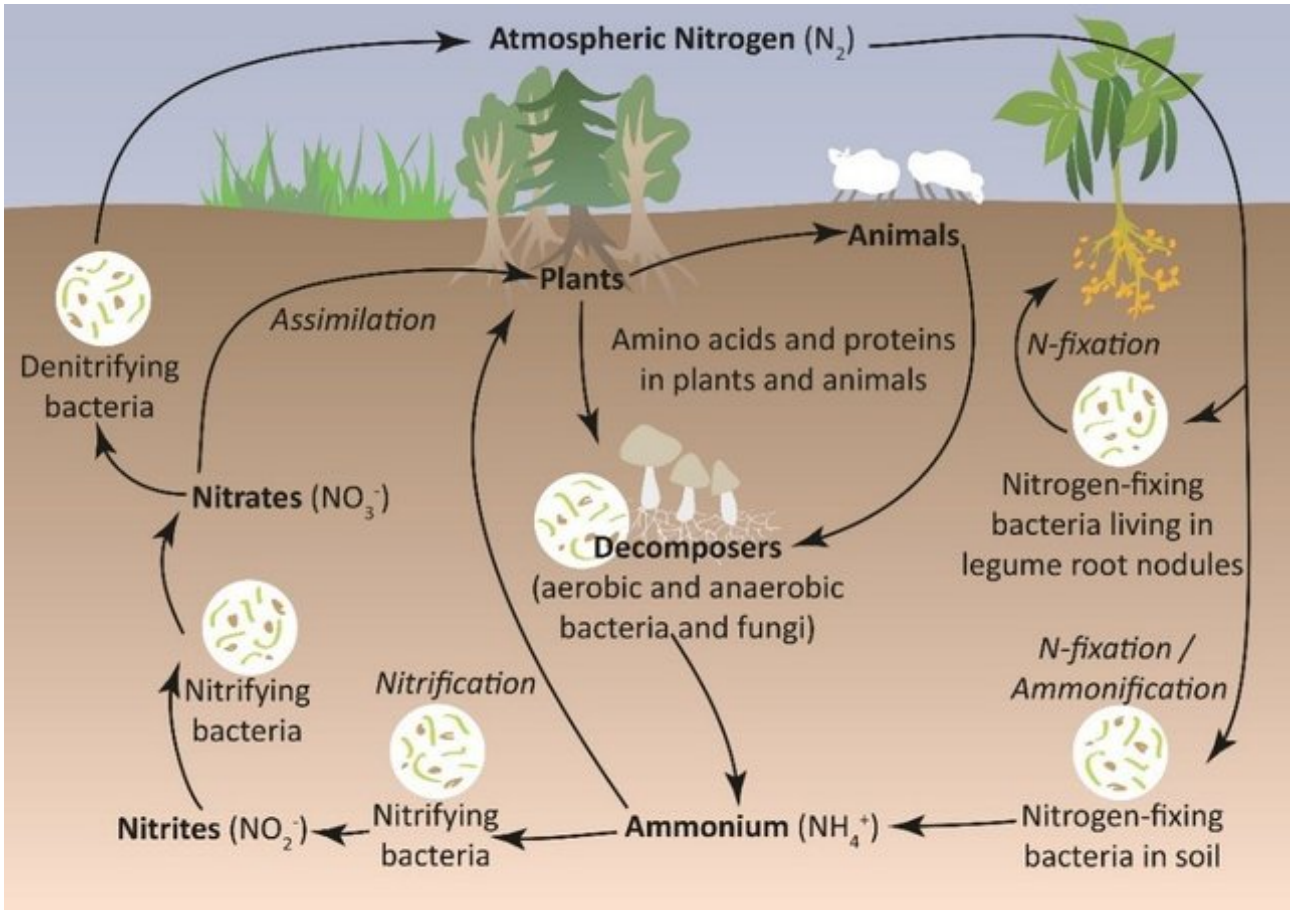
Figure 1.2. Schematic Nitrogen cycle. Ref: Lappalainen et al., 2016.

understand N cycle, it is useful to separate their reservoirs between atmosphere, plants (including both marine and terrestrial) and soil with high importance of microorganisms. The main mechanism life has to transform this inorganic N_2 into easier to absorb nitrogen compounds, such as nitrate, nitrite or ammonia is called nitrogen fixation and is conducted in more than 90% by marine or terrestrial microorganisms. This transformed N can now be assimilated by plants and animals which will return to the ground through decomposition. The return of N to the atmosphere can occur through denitrification process, also done by bacteria, or through organic matter combustion (Figure 1.2).

P-cycle

Phosphorus is the eleventh most abundant element in Earth crust and it is also a vital element for life. All living beings need P on their structures as far as it is part of nucleic acids, such as DNA and RNA, ATP, which is responsible for biological information storing, organisms energy storage and release and part of membrane cells as phospholipids. Inorganic phosphorus lays on the soil crust generally in orthophosphate form (PO_4^{3-}) and it is simply absorbed by plant roots. Different from C and N, phosphorus organic form is not transformed chemically by living beings and is always ortophosphate form, surrounded by 4 oxygens. The rest of successional trophic levels incorporate P by ingesting this biomass which already incorporated P. On one side this P come back to the soil crust through decomposition but on the other side soils are also renovated at

geological scale through soil creation process of weathering, deposition and geological uplift. The concern regarding P is that through years of life succession the soils can be P depleted due to biomass removal or leaching and become P limited, constraining life development. Consequently, young soils have more available P because their reservoirs have not been spent yet (Walker and Syers, 1976) (Figure 1.3).
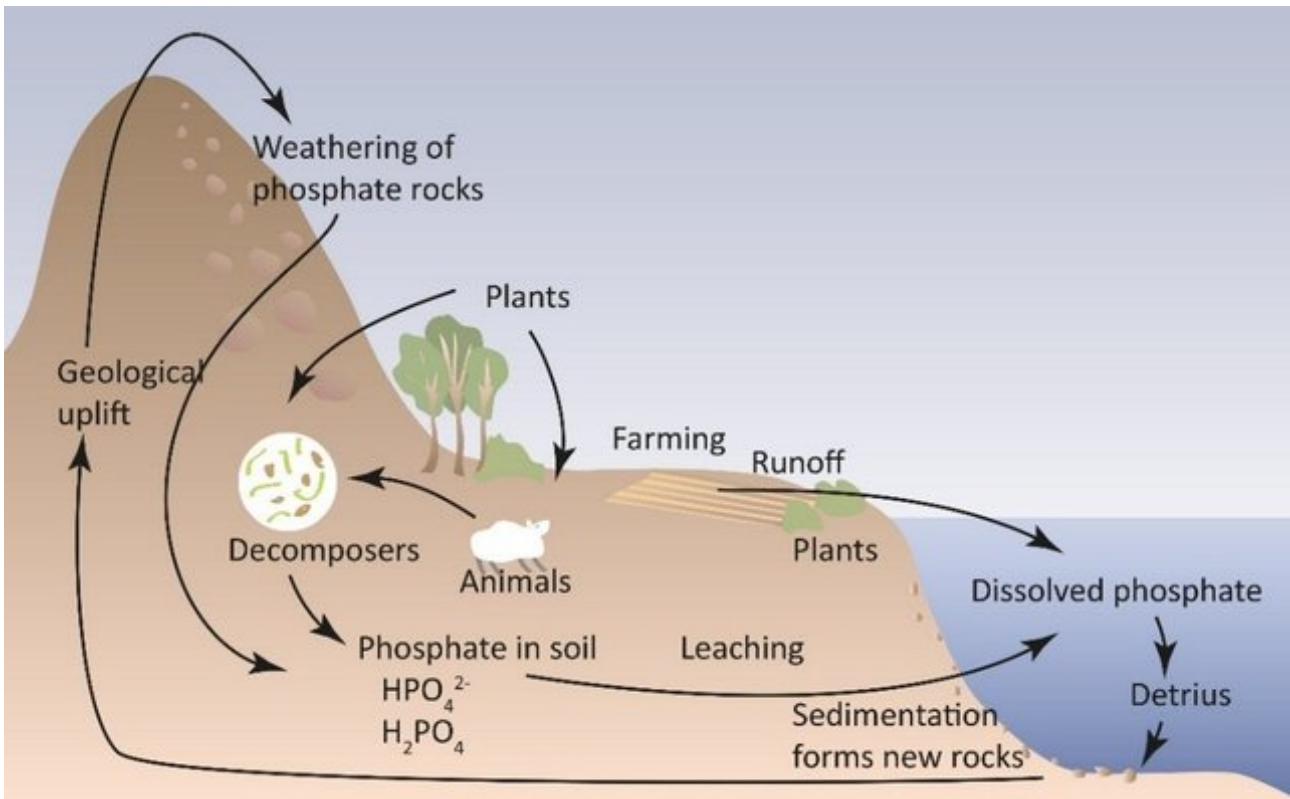
Figure 1.3. Schematic phosphorus cycle. Ref: Lappalainen et al., 2016.

K-cycle

Potassium is the seventh most abundant element in the soil crust. In living beings potassium is responsible for nucleic acids stabilization and it is used as ion to control osmotic pressure. It is very important in plant physiology contributing to photosynthesis through stomatal control and linked to water use and efficiency. In animals, K is responsible to transmit neurological impulse and for muscle contraction. The biggest K reservoir in biosphere are the oceans where K lays in water solution. Through geomorphic processes this K is incorporated into the lithosphere where generally it conform some minerals such as illite and mica or can be absorbed onto clay. Plants have the ability of pumping the exchangeable K from the lower soil layers to the top layers increasing its availability. This K can be dissolved in water and leached back to the oceans. Also some ocean water spray can be transported by air generating some K deposition which could be important in some coastal ecosystems (Figure 1.4). Differently to C, N and P which conform tissues and remain typically longer times in vegetal tissues K can be absorbed and released faster, as being used normally as ion.
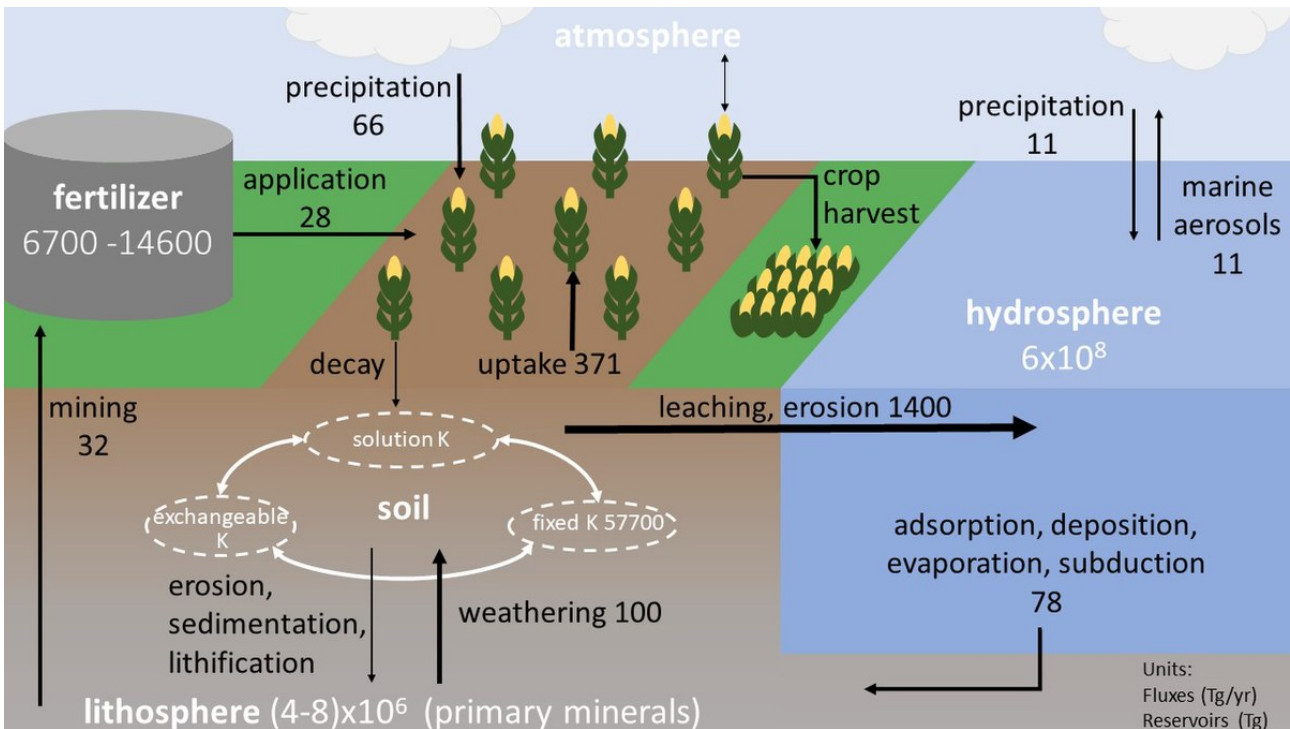
Figure 1.4. Schematic potassium cycle. Ref: wikipedia.

1.2 Climate change and plants

The last centuries and especially since industrial revolution, human being have been postulated as one of the major disruptors of biogeochemical cycles in modern times, endangering their stability (Canadell et al., 2007). Our massive production system based on exponential growth demands huge interactions with this biogeochemical cycles altering its reservoirs and creating new artificial fluxes that rarely occur in natural conditions or modifying existing ones. Is true that the Earth faced climate changes and unstable periods in the past which affected biogeochemical cycles, but not in this short time period and intensity. As a clear example we can have a look in C biogeochemical cycle, which is a recurrent topic in human's agenda. Massive amount of C has been released from the lithosphere to the atmosphere lately, provoking an atmospheric CO_2 increase reaching levels never seen before (Monastersky, 2013; IPCC 2018) (Figure 1.5). These biogeochemical cycles alteration directly affect humans development but also have many direct and indirect implications in other live forms such as plants, which are going to be protagonists in this thesis.

One of the biogeochemical cycles alteration important for plants communities comes from the atmosphere CO_2 rise. Plants absorb CO_2 from the atmosphere to incorporate C into their structures in sugar form through photosynthesis process. This autotroph process is the base of C incorporation to organic chain. Initially, this CO_2 rise is linked to an increase of C absorption by plants called "Greening effect" or "CO_2 fertilization", due to this major C availability for photosynthesis (Keenan et al., 2016) allowing plants to tampon partially the atmospheric CO_2

increase. Sooner, though, this CO_2 absorbing capacity from plants is reduced due to other elements and/or soil moisture limitation (Wang et al., 2020). This phenomenon was already described by Sprengel in 1840 but popularized by Liebig in its low of the minimum. This law postulates that plants productivity or growth is limited by its scarce element, which could be N, P or many others, and hinders plants efficiency to buffer atmosphere C increase in medium/long therm. Furthermore, this increasing unbalance between C and the rest of elements available would also generate plant nutrition deficiencies. By now we are already detecting a dilution of N, P, K, Ca, Mg and S in plant foliar tissues in Europe (Peñuelas et al., 2020a) and we still do not know for sure which additional effects this would cause.
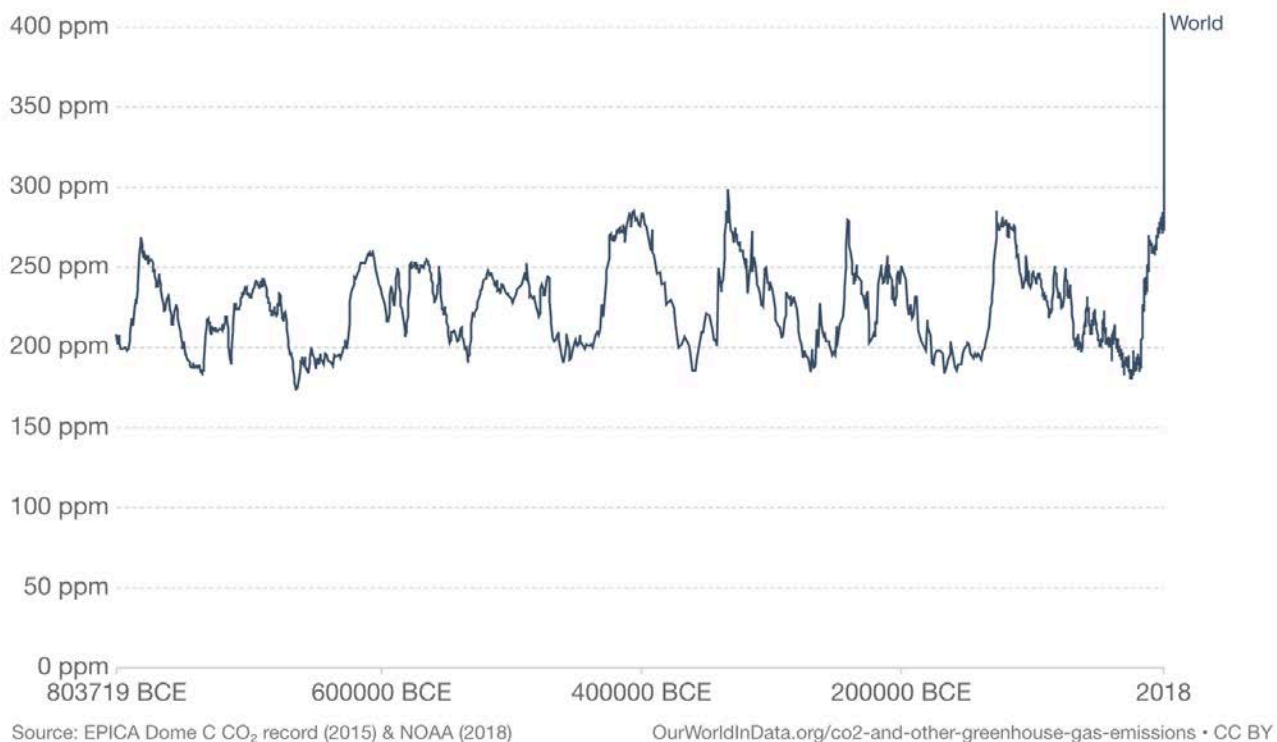
Figure 1.5 Global average long-term atmospheric concentration of carbon dioxide (CO_2), measured in parts per million (ppm). Long-term trends in CO_2 concentrations can be measured at high-resolution using preserved air samples from icecores.

The aforementioned atmospheric CO_2 and other gases increase also participates into the "greenhouse effect" keeping the heat from escaping back to the space. The greenhouse effect is responsible for global warming which have multiple consequences such as poles melting, temperature increase, risk of alteration of water fluxes in the ocean, sea level rise, alteration of pluvial regimes, increase of extreme climatic events between many others which contribute to the climate change (IPCC, 2018). Thus, the climate change is a multifactorial pool of alterations that differently affect plants depending on the species, placement and ecological pressure (Vallicrosa et al., 2021). For instance, an Oak into the Mediterranean will not be equally affected by temperature rise as a pine in Finland or Amazonian tree species will not be equally affected by torrential rains as a cactus in Atacama. Hence, the challenge for the scientists is to assess how

the conditions are going to be modified by climate change and how those conditions are going to affect the different species.

Nitrogen cycle is another biogeochemical cycle highly modified by humans. Some paragraphs before we talked about the difficulties of N_2 fixation and its importance for plant development, so we became able to artificially synthesize N in industrial fertilizers to fertilize crops. This industrialized process which fixes N_2 from the atmosphere through high pressures is called Haber-Bosch process. The volatilization of a portion of this N fertilizers applied into agriculture in conjunction of fossil fuels combustion and the continuous increase of N_2 fixing crops generate an elevated income of atmospheric N which through precipitation reaches natural environments (Galloway et al., 2008; Schmitz et al., 2019; Peñuelas et al., 2021). This extra N fertilization reaching natural environments is associated with the alteration of plant-plant, plant-fungus, plant-soil and plant-herbivore relationships (Gilliam, 2006; Friedrich et al., 2012; Ochoa-Hueso et al., 2011), plant growth and C fixation increase (Bontemps et al., 2011; Schulte-Uebbing & de Vries, 2017), plant N increase (Baron et al., 2000), plant P decrease (Kowalenko, 2006) and increase of N:P ratios (Huang et al., 2016) despite its effects vary depending on the intensity and duration of N deposition. Despite all the information generated by scientific community about N deposition there are still some answers to reach as well as how this N deposition is combined with other climate change factors and how this affects intraspecific competition.

The combustion of fossil fuel, besides the increase of atmospheric CO_2 and N also contributes to the increase of S emissions to atmosphere. This S compounds and some N compounds causes the acid rain. This wet deposition with a very acid pH causes tissue damage by direct contact, soils acidification, availability of toxic compounds such as some trace elements, which negative impact on vegetal growth and negative impact in productivity (Singh and Agrawal, 2008). Consequently, big efforts were done to decrease the S and N emissions which were related to a decrease of S and N deposition in the last decades. Despite of this, N and S deposition are still an ecological concern (Schmitz et al., 2019).

The greening effect caused by atmospheric CO_2 increase generates an increase of vegetal biomass which is higher or lower depending on other elements availability. As seen before, the N deposition is also a factor that increases N availability in some regions which can also contribute to increase this greening effect. In contrast, while the availability of C and N increase, P availability and supply remains stable on soil. This factor can generate regional P limitation as well as high N:P imbalances. Consequently, P can become the limiting factor of vegetal development and restrict the C retention capacity (Peñuelas et al., 2013; Carnicer et al., 2015). Furthermore, the changes in N:P ratio can affect species composition, food webs structure and terrestrial ecosystems diversity and functioning (Sterner and Elser, 2002; Paseka and Grunberg, 2019; Peñuelas et al., 2019;2020b; Sardans et al., 2021). Thus, how to identify and mitigate this N:P imbalances and P limitation all over the world is another topic concerning scientific community these days.

1.3 Biogeochemical niche theory

Between this deep sea of ecological pressures and climate change alterations it would be useful to keep in mind the bases of ecology. One of the main objectives in ecology is to understand diversity. Why this species is in this particular site? How do the species interact? What does it drive its presence? And all these questions, of course, are extremely related to environmental conditions and biotic interactions we discussed earlier. In 1957, Hutchinson proposed the ecological niche theory, which comprehends all conditions necessary for an organism to exist (Hardesty, 1972). To make it easy we should imagine a multi-space with endless variables which settle each specie into a specific range of each variable of temperature, species cohabitation, soil texture, predator pressure and all variables you can imagine. Hence, generalist species have a wider range in the variables than specialist species, which are more adapted into particular conditions. Following this theory, species tend to avoid niche overlap between coexisting species in order to avoid competition and be more ecologically successful.

Unfortunately, even supported for the scientific community, this multi-space of variables is almost impossible to sample, quantify and experiment with so, as an alternative, Peñuelas et al., 2008 proposed the biogeochemical niche theory. This theory is based on the same principles than the classical niche theory but using only variables of elemental composition (the amount of each chemical compound), which are finite and quantifiable through chemical analysis. Following biogeochemical niche theory, species are considered as a product of a long-therm natural selection which generates a particular genetic pool. This genetic pool includes the response of each specie to the different conditions which through the different fundamental biological processes such as growth, metabolism, reproduction or storage it is translated to a particular range of chemical compounds. Hence, the chemical composition of each specie could be considered as a biological fingerprint. This approach suggests the study of elemental composition as an indicator of the ecological processes at specie, community and ecosystem level and it is postulated as a very powerful tool to understand plant performance drivers. For example, it aims to relate chemical composition with photosynthesis and growth, reproduction and at the end ecological success. Also, the study of elemental composition in plants allows to include biosphere into the same language as we study the other compartments of biogeochemical cycles and conceive its function as a whole matter flow of chemical elements, which are the smaller particle we can work with.

1.4 Aim of the thesis

Arising from the exposed ecological knowledge and questions this thesis aim to offer new findings regarding:

• The additional effect of drought and N fertilization into foliar elemental composition of dominant species in a Mediterranean forest (Chapter 2)

- How foliar elemental composition and environmental drivers are related with defoliation and forest health in Europe (Chapter 3)
- How the most abundant nutrients (N, P, K) are distributed into leaves of the woody vegetation all over the world and which are the main drivers (Chapter 4, Chapter 5)
- Empirical support for the biogeochemical niche theory (Chapter 2, Chapter 4, Chapter 5, Chapter 6)

1.5 References

Baron, J., Rueth, H., Wolfe, A. et al. Ecosystem Responses to Nitrogen Deposition in the Colorado Front Range. Ecosystems 3, 352–368 (2000). https://doi.org/10.1007/s100210000032

Bontemps, J.-D., Herve, J.-C., Leban, J.-M., Dhote, J.-F., 2011. Nitrogen footprint in a long-term observation of forest growth over the twentieth century. Trees (Berl.) 25, 237e251. https://doi.org/10.1007/s00468-010-0501-2.

Canadell, J.G., Corinne Le Quéré, Michael R. Raupach, Christopher B. Field, Erik T. Buitenhuis, Philippe Ciais, Thomas J. Conway, Nathan P. Gillett, R. A. Houghton, and Gregg Marland. Contributions to accelerating atmospheric CO2 growth from economic activity, carbon intensity, and efficiency of natural sinks. PNAS November 20, 2007 104 (47) 18866-18870. https://doi.org/10.1073/pnas.0702737104

Carnicer, J., Sardans, J., Stefanescu, C., Ubach, A., Bartrons, M., Asensio, D., & Penuelas, J. (2015). Global biodiversity, stoichiometry and eco- system function responses to human-induced C-N-P imbalances. Journal of Plant Physiology, 172, 82–91. https ://doi.org/10.1016/j. jplph.2014.07.022

Friedrich, U., von Oheimb, G., Kriebitzsch, WU. et al. Nitrogen deposition increases susceptibility to drought - experimental evidence with the perennial grass Molinia caerulea (L.) Moench. Plant Soil 353, 59–71 (2012). https://doi.org/10.1007/s11104-011-1008-3

Galloway, J. N., A. R. Townsend, J. W. Erisman, M. Bekunda, Z. Cai, J. R. Freney, L. A. Martinelli, S. P. Seitzinger, and M. A. Sutton. 2008. Transformation of the nitrogen cycle: recent trends, questions and potential solutions. Science 320: 889–892.

Gilliam, F. S. 2006. Response of the herbaceous layer of forest ecosystems to excess nitrogen deposition. Journal of Ecology 94(6):1176–1191.

Hardesty DL, 1972. The human ecological niche. American Anthropologist, 74: 458- 466.

Huang, Z., Liu, B., Davis, M., Sardans, J., Penuelas, J., & Billings, S. (2016). Long-term nitrogen deposition linked to reduced wáter use effi- ciency in forests with low phosphorus availability. New Phytologist, 210, 431–442. https ://doi.org/10.1111/nph.13785

Hutchinson GE, 1957.Concluding Remarks. Cold Spring Harbor Symposia on Quantitative Biology, 22:415- 422

IPCC, 2018: Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty [V. Masson-Delmotte, P. Zhai, H. O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J. B. R. Matthews, Y. Chen, X. Zhou, M. I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, T. Waterfield (eds.)]. In Press.

Keenan, T. et al. (2016) Recent pause in the growth rate of atmospheric CO2 due to enhanced terrestrial carbon uptake. Nature Communications DOI: 10.1038/ncomms13428

Kowalenko, C.G. The effect of nitrogen and boron fertilizer applications on Willamette red raspberry growth, and on applied and other nutrients in the plant and soil over two growing seasons. Canadian Journal of Plant Science. 86 (1) https://doi.org/10.4141/P04-036

Monastersky, R. Global carbon dioxide levels near worrisome milestone. Nature 497, 13–14 (2013). https://doi.org/10.1038/497013a

Ochoa-Hueso, R., Edith B. Allen, Cristina Branquinho, Cristina Cruz, Teresa Dias, Mark E.

Fenn, Esteban Manrique, M. Esther Pérez-Corona, Lucy J. Sheppard, William D. Stock. Nitrogen deposition effects on Mediterranean-type ecosystems: An ecological assessment, Environmental Pollution,159 (10), 2011, 2265-2279, https://doi.org/10.1016/j.envpol.2010.12.019.

Paseka, R.E. and Grunberg, R.L. (2019), Allometric and trait-based patterns in parasite stoichiometry. Oikos, 128: 102-112. https://doi.org/10.1111/oik.05339

Peñuelas J, Sardans J, Ogaya R, Estiarte M (2008) Nutrient stoichiometric relations and biogeochemical niche in coexisting plant species: effect of simulated climate change. Polish Journal of Ecology 56: 613-622.

Penuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., … Janssens, I. A. (2013). Human-induced nitrogen-phosphorus imbal- ances alter natural and managed ecosystems across the globe. Nature Communications, 4, 2934. https ://doi.org/10.1038/ncomm s3934

Peñuelas, J., Fernández-Martínez, M., Ciais, P., Jou, D., Piao, S., Obersteiner, M., Vicca, S., Janssens, I. A., and Sardans, J.. 2019. The bioelements, the elementome, and the biogeochemical niche. *Ecology* 100 (5):e02652. 10.1002/ecy.2652

Penuelas, J., Fernández-Martínez, M., Vallicrosa, H. et al. Increasing atmospheric CO2 concentrations correlate with declining nutritional status of European forests. Commun Biol 3, 125 (2020a). https://doi.org/10.1038/s42003-020-0839-y

Peñuelas, J., Janssens, I., Ciais, P., Obersteiner, M., Sardans, J. 2020b. Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity, food security, and human health. Global Change Biology 2020;26:1962–1985. doi: 10.1111/GCB.14981.

Sardans, J., Janssens, I.A., Ciais, P., Obersteiner, M., Peñuelas, J. (2021). Recent advances and future research in ecological stoichiometry. Perspectives in Plant ecology, evolution and systematics. 50, 12561.

Singht, A., Agrawal, M. 2008. Acid rain and its ecological consequences. Journal of Environmental Biology. 29 (1), 15-24.

Schmitz, a., Sanders, T.G.M., Bolte, a., Bussotti, F., Dirnböck, T., Johnson, J., Peñuelas, J., Pollastrini, M., Prescher, A-K., Sardans, J., Verstraeten, A., de VriesS, W. 2018. Responses of forest ecosystems in Europe to decreasing nitrogen deposition. Environmental Pollution 244 (2019) 980e994

Schulte-Uebbing, L, de Vries, W. Global-scale impacts of nitrogen deposition on tree carbon sequestration in tropical, temperate, and boreal forests: A meta-analysis. Glob Change Biol. 2018; 24: e416– e431. https://doi.org/10.1111/gcb.13862

Sterner, R., Elser J.J. 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to Biosphere. Princenton University Press. Princenton, New Jersey. pp. 439.

Wang, S., Zhang, Y., Ju, W. et al. Recent global decline of CO2 on vegetation photosynthesis. Science. 370, 6522, 1295-1300. (2020)

2

Short-therm N fertilization differently affects the leaf and leaf litter chemistry of the dominant species in a Mediterranean forest under drought conditions

Helena Vallicrosa, Jordi Sardans, Romà Ogaya, Pere Roc Fernández and Josep Peñuelas

Abstract

Nitrogen (N) deposition is a key driver of global change with significant effects on carbon (C) cycling, species fitness, and diversity; however, its effects on Mediterranean ecosystems are unclear. Here, we simulated N deposition in an N-fertilization experiment with 15N-labeled fertilizer in a montane evergreen Mediterranean holm oak forest, in central Catalonia, to quantify short term impacts on leaf, leaf litter elemental composition, and resorption efficiency in three dominant species (Quercus ilex, Phillyrea latifolia, and Arbutus unedo). We found that even under drought conditions, 15N isotope analysis of leaf and leaf litter showed a rapid uptake of the added N, suggesting an N deficient ecosystem. Species responses to N fertilization varied, where A. unedo was unaffected and the responses in P. latifolia and Q. ilex were similar, albeit with contrasting magnitude. P. latifolia benefited the most from N fertilization under drought conditions of the experimental year. These differences in species response could indicate impacts on species fitness, competition, and abundance under increased N loads in Mediterranean forest ecosystems. Further research is needed to disentangle interactions between long-term N deposition and the drought predicted under future climate scenarios in Mediterranean ecosystems.

Keywords: elemental composition; isotopes; reabsorption; climate change; Arbutus unedo; Phillyrea latifolia; Quercus ilex

2.1 Introduction

Nitrogen (N), which is an essential element for plant nutrition, is involved in complex cycling in terrestrial ecosystems that involves biotic and abiotic processes, such as N2-fixation, mineralization, nitrification, ammonification, and denitrification [1,2,3]. Anthropogenic activities

since the industrial and agricultural revolutions, particularly those associated with fossil fuel combustion, cultivation of N2-fixing crops, and production of N fertilizer using the Haber-Bosch process, increasingly influence the global N-cycle as they are often key sources of N in terrestrial ecosystems, driving fluxes of similar magnitudes to natural fluxes [4,5,6]. Inorganic N deposition in European forests increased on average from about 2.8 kg ha−1a−1 in 1900 to a peak of 10.3 Kg N ha−1a−1 in 1990, followed by a decline to 6.6 Kg N ha−1a−1 by 2018 [7,8]. However, 62% of Europe continues to remain at risk of eutrophication [9] through rises in organic N deposition due to increasing levels of N-fertilization of cropland [8].

In general, N addition to ecosystems alters the first N cycle, increasing N mineralization, nitrification, and nitrate leaching rates, and thereafter other ecosystem variables such as P-cycle [5], and finally is associated with a long-term plant community diversity decrease [10]. Also, in some Mediterranean regions in California with elevated N deposition, native plant species have been replaced by invasive species with higher N performance [11,12,13]. Vegetation responses to increases in N supply are complex and include increased inter-plant competition, modified plant-soil feedbacks, and changes to plant-herbivore and plant-fungus relationships [14,15,16]. Typically, increases in N availability under N deposition are associated with greater plant growth [17,18], C-fixation [19], increased plant N concentrations [20,21], and reduced plant P concentrations [22,23] that contribute to increased plant N:P ratios [24,25,26,27]. While critical N loads have been described for a range of ecosystems and species, including 3–33 kg N ha−1 yr−1 for herbaceous species and shrublands and 4–39 kg N ha−1 yr−1 in forests in the US [10], there is a knowledge gap for Mediterranean Basin ecosystems.

Along with water availability, nutrient supply is a frequent limiting factor for Mediterranean ecosystems [28,29] and is an important factor in the growth, structure, and distribution of plant communities [30,31,32]. Consequently, Mediterranean forest communities tend to be dominated by woody plant species characterized by conservative traits related to tolerance of low water and nutrient conditions, such as slow growth and high levels of sclerophylly, high levels of investment in root biomass, and greater metabolic flexibility [28]. Responses of plant species to long-term N-fertilization under high levels of N deposition vary with life history strategy, where ruderal species are likely to be favored over stress-tolerators [33]. The resulting changes in competitive relationships within plant communities lead to long-term changes in species community composition [10,12,34], which could culminate in the extinction of endemic species and reduce overall levels of species diversity [16].

High levels of N deposition are known to constitute a threat for global plant diversity; as long as Mediterranean ecosystem is considered a diversity hotspot, an increase of N deposition could endanger its species diversity [35,36,37]. Deposition of N has been related to reduced levels

of resilience in Mediterranean plant communities to disturbing climatic conditions [**28**]. However, few field studies have assessed the relationships between increases in N-availability and responses of dominant woody species and shifts in nutrient cycling in Mediterranean plant communities. In particular, even less field studies assessed the impacts of a sudden rise in N deposition on a semi-pristine Mediterranean ecosystem, in which current loads of N deposition are low and especially interacting with drought. Therefore, we aimed to study the short-term effects of N-fertilization to simulate rapid N deposition and availability on: (i) foliar and litter nutrient composition; (ii) foliar nutrient reabsorption; and (iii) foliar and litter carbon (C) and N isotope ratios in three dominant tree species of a semi-pristine, low-nutrient Mediterranean holm-oak forest to increase the understanding of species community composition and dynamics under increased N deposition in the increasingly dry Mediterranean environment.

2.2 Materials and Methods

2.2.1 Study Site

The study was carried out on a south-facing slope (25% incline) of a semi-pristine (*Quercus ilex* L.) forest, where N deposition was 3.5 kg N ha−1 yr−1 between 1981 and 1994 [**38**], in the Prades Mountains, Catalonia, NE Spain (41°21′ N, 1°02′ E). The forest has not been disturbed for the last 70 years and the maximum height of the dominant species is about 6–10 m. Mean annual temperature and precipitation are 12 °C and 658 mm, respectively, with the warmest months occurring between June and August and the rainiest period between September and November (**Figure 1**). These conditions correspond to a mesic-Mediterranean climate, with a pronounced summer drought period that usually lasts for 3 months. During the study period, mean annual temperature and annual precipitation in 2014 were 12.65 °C and 661.38 mm respectively, and 13.25 °C and 355.44 mm respectively in 2015 (Figure 2.1). Air temperature and rainfall were recorded by an automatic meteorological station in the studied area. The soil is a stony Dystric

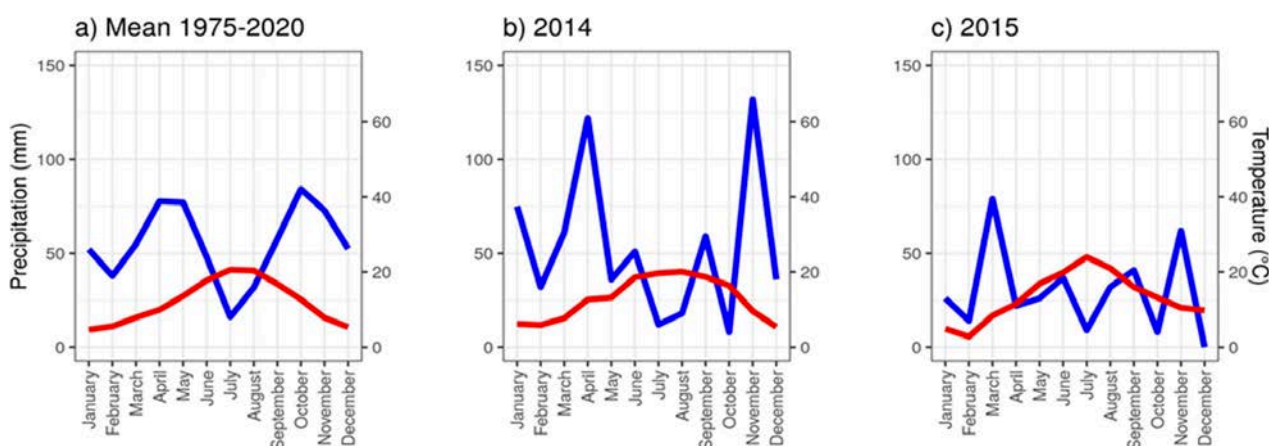**Figure 2.1** Bagnouls-Gaussen ombrotermic diagram of mean climatic temperature (in red) and precipitation (in blue) by month in the study site. (a) Mean monthly data from 1975 to 2020. (b) Mean monthly data for 2014. (c) Mean monthly data for 2015.

Cambisol, on a bedrock of metamorphic sandstone, and ranges between 35 and 100 cm in depth. The dense forest vegetation is dominated by *Q. Ilex*, with abundant *Phillyrea latifolia* L., *Arbutus unedo* L. among other evergreen species that are well-adapted to drought conditions (*Erica arborea* L., *Juniperus oxycedrus* L., and *Cistus albidus* L.) and the occasional occurrence of deciduous species (*Sorbus torminalis* L. Crantz and *Acer monspessulanum* L.). The three tree species (*Q. ilex*, *P. latifolia*, and *A. unedo*) represent 97% of the total aboveground biomass of the forest [39] and they frequently co-occur in Mediterranean *maquis* shrubland and evergreen *Q. ilex* forests. *Q. ilex* is widely distributed across the subhumid areas of the Mediterranean Basin, while *P. latifolia* occurs in warmer and drier Mediterranean areas [40,41] and *A. unedo* exploits forest gaps in which conditions are limiting for *Q. ilex* [42].

2.2.2 Experimental Design

We established eight 15 m × 10 m plots at the same altitude (950 m) [43]; four plots received N-fertilizer and the remainder were untreated controls. In 2015, a total fertilization of 60 kg of N ha−1 a−1 (at a rate of 15 kg N ha−1 in each annual season) was applied as a solution of ammonium nitrate (NH4NO3) with a sprayer. This fertilization was sprayed in 3 different days per season (5 kg N ha−1 each application), with at least 1 week between different applications. This solution was sprayed over the canopy level after climbing to the top of the uppermost tree in each plot. This solution was enriched with 15N as follows: 90% of "normal" ammonium nitrate, and 10% of ammonium nitrate enriched with 15N (the 10% of this enriched ammonium nitrate was 15N) (Sigma-Aldrich, Co., St. Louis, MO, USA).

2.2.3 Sampling

We sampled leaves of *Q. ilex*, *P. latifolia*, and *A. unedo* and foliar litter in mid-May (spring), end of July (summer), and mid-November (autumn) 2014, prior to treatment, and in 2015 during fertilization, sampling the same tree before and after treatment. On each sampling occasion, we randomly selected five trees of each species per plot and collected mature and healthy leaves at a height of 2–3 m from plants with a diameter of 2–12 cm at 5 cm above ground level; these plants represent about the 70% of the community biomass [44,45]. To reduce effects of tissue age and orientation to sunlight, we sampled leaves that were sun-lit and oriented southwards. Mean leaf life span is approximately 18 months [45], so most leaves sampled during 2015 under fertilization were also present during 2014 prior to treatment. Litterfall at the soil surface was collected in 20 circular baskets (27 cm in diameter, with 1.5-mm diameter mesh) that were randomly distributed in each plot. Even though the baskets were permanently placed over the ground, the dense canopy cover almost totally avoided N fertilization reaching the soil or the baskets. The collected litter was not in direct contact with the soil so its conservation state was good and the decomposition process had hardly begun. Once collected, the leaf and litter samples were taken to the laboratory and stored at 4 °C prior to analysis.

Foliar nutrient resorption was calculated as the difference in elemental compound concentration of leaves collected in 2014 and litter collected in 2015, using the equation $100 - (Xl \times 100)/Xf$, where Xl is litter element concentration in 2015 and Xf foliar element concentration in 2014. The result is an inference of proportional (%) element resorption efficiency prior to leaf fall.

2.2.4 Chemical Analyses

The leaf and foliar litter samples were washed with distilled water, as described by Porter (1986) [46], dried in an oven at 60 °C to a constant weight, and then ground using a mill (CYCLOTEC 1093, Foss Tecator, Höganäs, Sweden) for measurement of biomass. Leaf and litter concentrations of carbon (C) and N were quantified by combustion coupled to gas chromatography, by placing 1.4 mg of the milled samples in a tin microcapsule in an elemental analyzer (CHNS-O EuroVector, Milan, Italy), while concentrations of phosphorus (P), potassium (K), and micro-elements were determined by digesting samples of leaf and litter in acid in a microwave (MARSXpress, CEM, Matthews, NC, USA) at high pressure and temperature. Then 250 mg of each ground sample, 5 mL of nitric acid, and 2 mL of H_2O_2 were placed into Teflon tubes; digestate was placed in 50-mL flasks and diluted with Milli-Q water to a volume of 50 mL. Concentrations of P, K, and micro-elements were determined using optic emission spectrometry with inductively coupled plasma and the accuracy of the digestions and analytical biomass procedures was assessed using a certified standard biomass (NIST 1573a, tomato leaf; NIST, Gaitherburg, MD, USA) as a reference. Isotopic analyses of $\delta13C$ (13C/12C) and $\delta15N$ (15N/14N) of the leaf and litter samples were conducted at the Stable Isotope Facility at the University of California, Davis using a PDZ Europa ANCA-GSL elemental analyzer connected to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

2.2.5 Statistical Analyses

Between year treatment differences in plot, species, and season values were calculated based on proportional change in values between 2014 and 2015, and then linear mixed models were used to test for treatment differences in leaf and litter macro- (C, N, P, K) and micro-element concentrations (calcium, Ca; magnesium, Mg; chrome, Cr; iron, Fe; manganese, Mn; copper, Cu; zinc, Zn, strontium, Sr; nickel, Ni; and, lead, Pb) and reabsorption, and in foliar N and C isotopes by species, with plot and season as random factors. Models were defined using lme4 [47] and lmerTest [48] and r2 was calculated in MuMIn [49] R packages. Treatment differences in leaf and litter isotope content by species was tested using Student's *t*-test.

Overall treatment differences in leaf and litter macro- (N, P, K) and micro-element (Ca, Mg, Fe, Mn, Cu, Zn) composition and isotope content ($\delta13C$ and $\delta15N$) by species were tested using general discriminant analysis (GDA) in Statistica 8.0 (StatSoft, Inc., Tulsa, OK, USA) that identifies

variables most responsible for group differences, while controlling variance due to other categorical variables (here, season).

In order to detect the effect of 2015's exceptional arid conditions, we calculated a *t*-test between 2015 and 2014 for leaves and litter from only control plots (which have no treatment applied in 2015).

2.3 Results

2.3.1 Fertilization Effects on Foliar Element Concentrations

There were between-year differences in C:P ratios in *P. latifolia*, where there were increases of 4.83 ± 3.36% and 3.05 ± 0.86% in untreated control and fertilized plots, respectively (difference between treatments: $p < 0.05$; ±standard error (SE) showed; Figure 2.2a, **Table S1**), and ratios of N:P differed for *A. unedo*, where there were 1.64 ± 1.65% decreases and 1.12 ± 1.38% increases in the untreated and treated plots from 2014 (pretreatment) to 2015 (post-treatment), respectively, (difference between treatments: $p < 0.05$; ±standard error (SE) showed; Figure 2.2b, **Table S1**). There were no treatment differences in leaf concentration of N, P, or K or in C:N ratios of the three species.
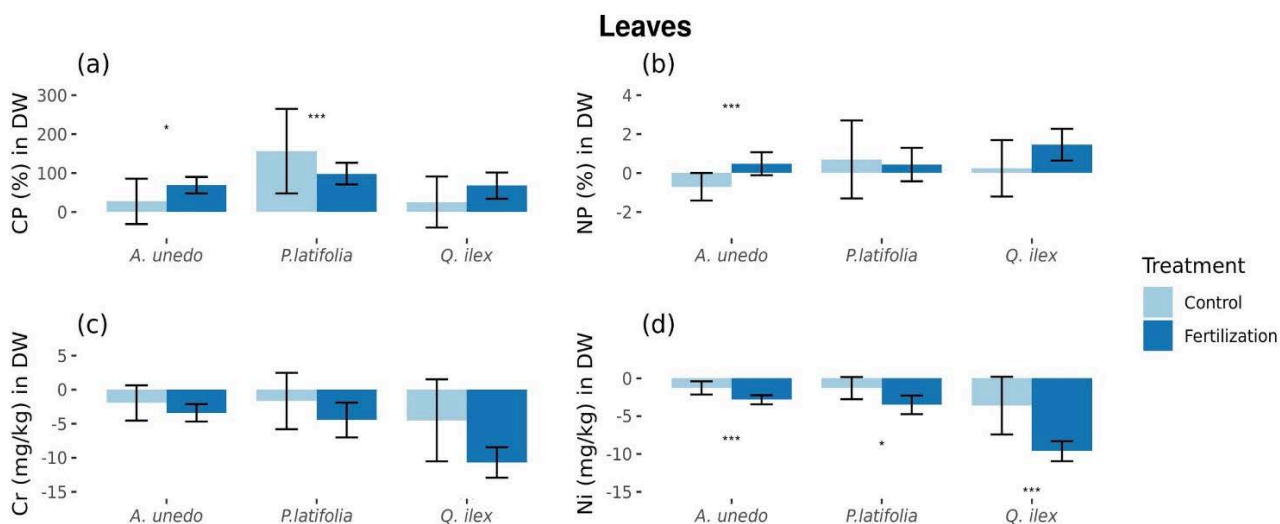
Figure 2.2 Foliar elemental ratios and microelements statistically different between control and fertilized plots. (a) C:P % in dry weight in leaves. (b) N:P % in dry weight in leaves. (c) Cr mg/kg in dry weight in leaves. (d) Ni mg/kg in dry weight in leaves. Values are relatives to original values in 2014 expressed in % of change. * = Marginal significance (p = [0.05, 0.1]). *** = Significant differences (p < 0.05). Figures with standard errors.

Among the micro-elements, there were differences in leaf concentrations of chromium (Cr) in *Q. ilex*, where there were decreases of 1.68 ± 1.83% and 3.56 ± 0.68% in the untreated and treated plots, respectively (difference between treatments: $p < 0.05$; ±standard error (SE)

showed; Figure 2.2c), while leaf concentration of nickel (Ni) in *A. unedo* decreased by 0.76 ± 0.41% and 1.49 ± 0.28%, respectively (difference between treatments: $p < 0.05$; ±standard error (SE) showed), and decreased in *Q. ilex* by 1.86 ± 1.79% and 4.67 ± 0.62% (difference between treatments: $p < 0.05$; ±standard error (SE) showed; Figure 2.2d) (**Table S1**). There were no treatment differences in leaf concentrations of Ca, Mn, Fe, Cu, Zn, Sr, or Pb in the three species.

2.3.2 Fertilization Effects on Leaf Litter Element Concentrations

Treatment affected leaf litter concentration of macro-elements only in *P. latifolia* ($p < 0.05$; ±standard error (SE) showed), where P concentration increased by 0.49 ± 0.59% in untreated plots and decreased by 1.80 ± 0.72% in fertilized plots (Figure 2.3a), while C:P ratios were
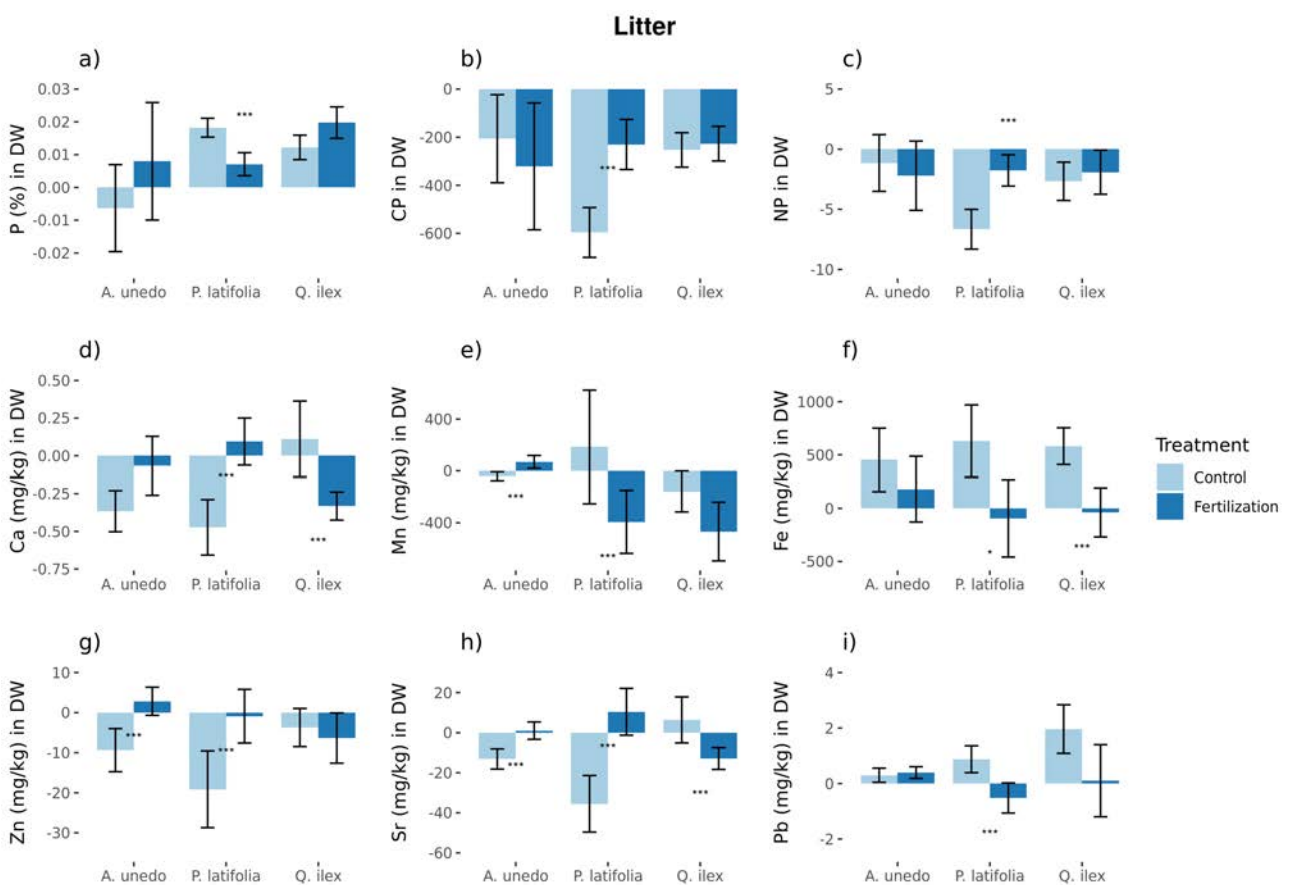
Figure 2.3. Litter elemental composition, ratios, and microelements statistically different between control and fertilized plots. The showed elements are: (a) P % in litter. (b) C:P in litter. (c) N:P in litter (d) Ca mg/kg in litter. (e) Mn mg/kg in litter. (f) Fe mg/kg in litter. (g) Zn mg/kg in litter. (h) Sr mg/kg in litter. (i) Pb mg/kg in litter. Values are relatives to original values in 2014 and are expressed in % of change. * = Marginal significance (p = [0.05, 0.1]). *** = Significant differences (p < 0.05). Figures with standard error.

decreased by 18.44 ± 3.21% and 7.12 ± 3.22% in untreated and fertilized plots, respectively (Figure 2.3b), and N:P ratios were 15.52 ± 3.85% and 4.11 ± 3.04% lower, respectively (Figure 2.3c) (**Table S2**). There were no differences in leaf litter concentration of the remaining macro-elements in the three species.

There were differences in litter concentration of micro-elements among the species (Figure 2.3d–i, **Table S2**). *A. unedo* leaf litter of Mn, Zn, and Sr increased from −1.29 ± 0.82%, −12.25 ± 3.57%, and −9.44 ± 2.7% in the untreated plots to 1.44 ± 1.22%, −4.16 ± 2.33%, and −1.86 ± 2.33%, respectively, in the N fertilized plots (difference between treatments: $p < 0.05$; ±standard error (SE) showed), while *P. latifolia* leaf litter concentration of Ca, Zn, and Sr increased from −19.88 ± 5.35%, −18.73 ± 6.35%, and −21.44 ± 7.58% in untreated plots to −3.39 ± 4.53%, −6.64 ± 4.44%, and 3.19 ± 6.27%, respectively, in N fertilized plots and concentration of Mn and Pb decreased from 4.33 ± 10.88% and 4.04 ± 2.52% to −10.01 ± 6.04% and −3.24 ± 2.83%, in the untreated and treated plots, respectively (differences between treatments: $p < 0.05$; ±standard error (SE) showed). *Q. ilex* leaf litter concentration of Ca, Fe, and Sr decreased from −2.89 ± 7.32%, 16.58 ± 5.21%, and 1.02 ± 6.15% in control plots, respectively, to −15.77 ± 2.70%, −2.17 ± 6.95%, and −9.32 ± 2.91% in N fertilized plots, respectively (differences between treatments: $p < 0.05$; ±standard error (SE) showed).

2.3.3 Foliar Nutrient Resorption

There were no effects of treatment on resorption of N, P, or K. While there were no differences in micro-element resorption in *A. unedo*, resorption of Mg and Ca in *P. latifolia* decreased from −2.91 ± 5.28%, and −90.2 ± 50.17% in untreated plots, respectively, to −19.71 ± 8.12% and −171.1 ± 20.99%, respectively, in fertilized plots (differences between treatments: $p < 0.05$; ±standard error (SE) showed; Figure 2.4, **Table S3**).
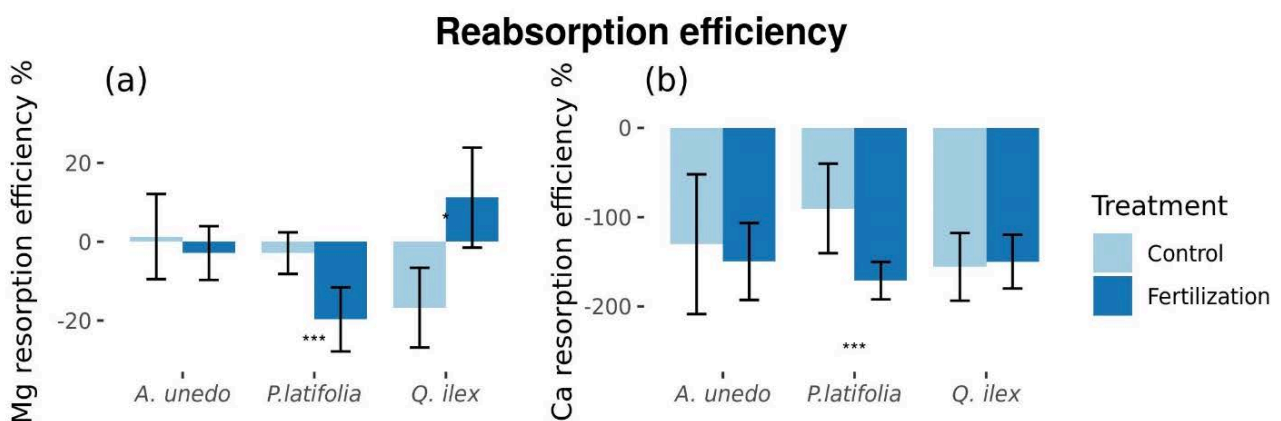
Figure 2.4. Microelements resorption efficiency % statistically different between control and fertilized plots. (a) Mg resorption efficiency. (b) Ca resorption efficiency. * = Marginal significance (p = [0.05, 0.1]). *** = Significant differences (p <0.05). Figures with standard error.

2.3.4 Fertilizer N-Uptake

While there were no differences between year treatment differences in leaf and litter δ13C in the three species, we found that leaf δ15N varied between the untreated and treated plots in *P.*

latifolia (0.25 ± 8.28‰ and 34.2 ± 8.52‰, respectively) and *Q. ilex* (1.42 ± 7.78‰ and 37.49 ± 6.11‰, respectively) (differences between treatments: *p* < 0.05; ±standard error (SE) showed; Figure 2.5a). No leaf differences in foliar δ15N in A. unedo. Fertilization with 15N-enriched fertilizer increased leaf litter δ15N in *A. unedo* (from 0.072 ± 14.57‰ to 57.0 ± 10.42‰), *P. latifolia* (from 9.01 ± 18.30‰ to 58.5 ± 13.68‰), and *Q. ilex* (from −0.34 ± 20.68‰ to 81.1 ± 17.47‰) (differences between treatments: *p* < 0.05; ±standard error (SE) showed; Figure 2.5b) (**Table S4**). Content of δ15N in *A. unedo* and *Q. ilex* was greater in leaf litter than in leaf material.
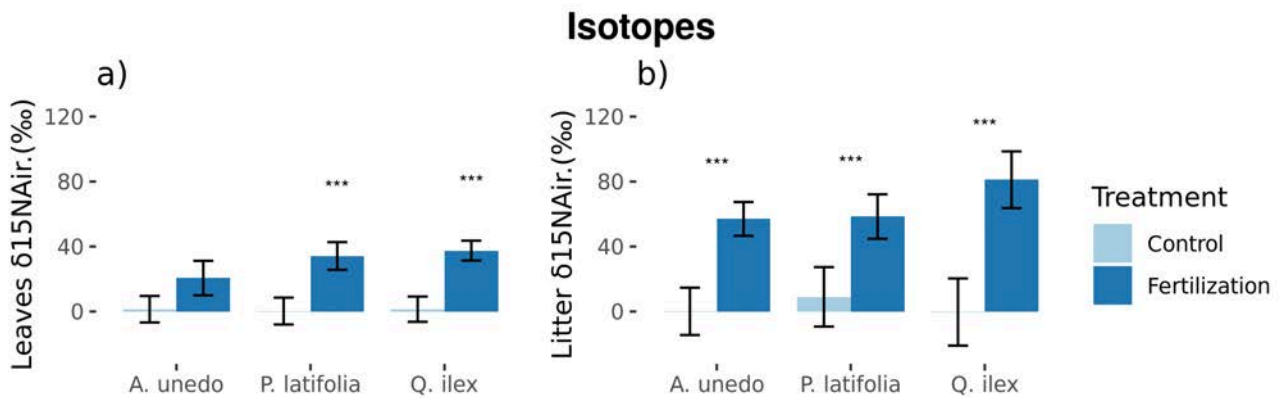
Figure 2.5 Isotopes statistically different between control and fertilized plots. a) Foliar tissue b) Foliar-litter tissue. *** = Significant differences (*P* < 0.05). Figures with standard error. A. unedo and Q. ilex foliar δ15N is significantly different from litter δ15N.

2.3.5. Species Differences in Leaf and Leaf Litter Element Concentrations
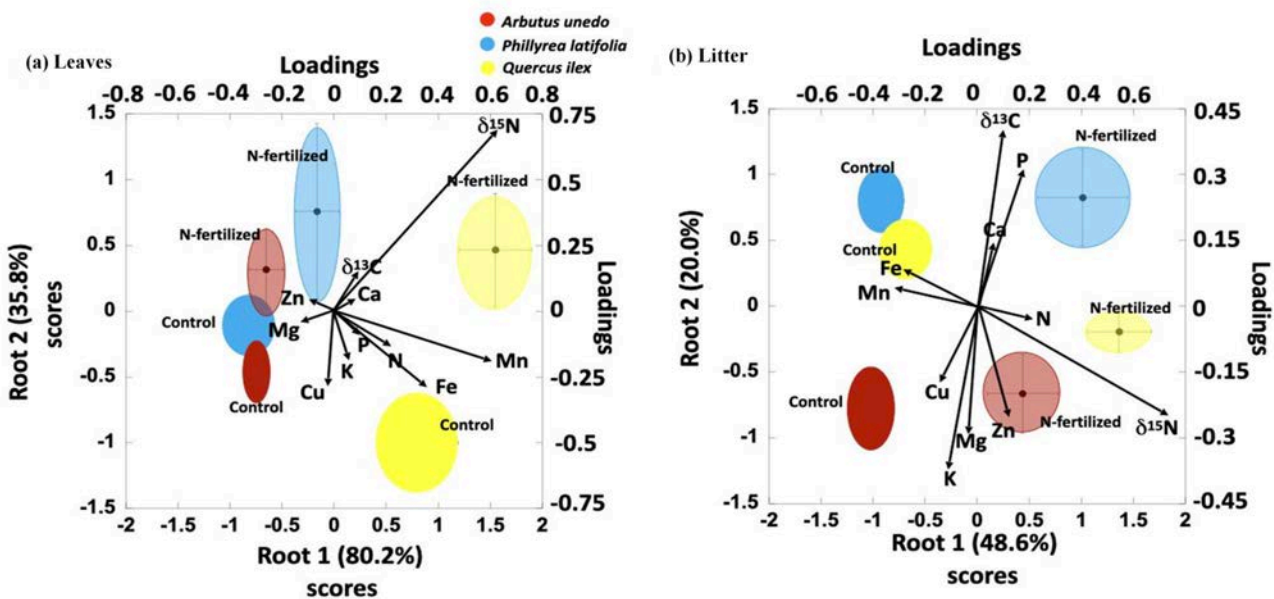
Figure 2.6 Generalized discriminant analysis (GDA) results representation of root 1 and root 2. Vectors representing variables, and ellipses representing control and N-fertilized plots measurements. (a) Leaves data. (b) Litter data.

The GDA driven by variation in δ13C, δ15N, K, Ca, Mn, Fe, Cu, and Zn concentrations (Table 2.1a) clearly separated the foliar elemental concentrations by species within and between treatments (Table 2.1b, Figure 2.6a). Similarly, there was clear separation of species in foliar litter within and between treatments (**Table 2**b, **Figure 6**b), driven by variation in δ13C, δ15N, N, P, K, Ca, Mn, and Fe concentrations (**Table 2**a). The variable loading most strongly in fertilized plots, separating them from control plots in leaves, is clearly δ15N and with lower intensity δ13C, while Cu, Mg,

Table 2.1 (**a**) Foliar variables effect in GDA analysis in leaves. (**b**) Foliar variables effect in GDA analysis in litter. Significant effects ($p < 0.05$) are highlighted in bold type.

(a) Leaves			
Test	**Wilks Lambda**	**F**	**_p_-Value**
^{13}C	**0.953**	**2.67**	**0.023**
^{15}N	**0.748**	**18.1**	**<0.0001**
N	0.989	0.615	0.69
P	0.962	2.13	0.062
K	**0.959**	**2.24**	**0.049**
Mg	0.962	2.14	0.061
Ca	**0.938**	**3.57**	**0.0038**
Mn	**0.742**	**18.7**	**<0.0001**
Fe	**0.906**	**5.6**	**<0.0001**
Cu	**0.946**	**3.1**	**0.0098**
Zn	**0.941**	**3.36**	**0.0058**
season	0.941	1.66	0.087
(b) Litter			
Variables	**Wilks Lambda**	**F**	**_p_-Value**
^{13}C	**0.917**	**4.36**	**0.00081**
^{15}N	**0.655**	**25.3**	**<0.00001**
N	**0.948**	**2.65**	**0.023**
P	**0.827**	**10.1**	**<0.00001**
K	**0.869**	**7.26**	**<0.00001**
Mg	0.959	2.07	0.07
Ca	**0.85**	**8.51**	**<0.00001**
Mn	**0.842**	**9.04**	**<0.00001**
Fe	**0.947**	**2.68**	**0.022**
Cu	**0.955**	**2.28**	**0.048**
Zn	**0.932**	**3.52**	**0.0043**
season	0.9	2.6	0.0045

Table 2.2. (a) Squared Mahalanobis distances between species-treatment in leaves. (b) Squared Mahalanobis distances between species-treatment in litter. AC = *A. unedo* control plot. PC = *P. latifolia* control plot. QC = *Q. ilex* control plot. AN = *A. unedo* N-fertilized plot. PN = *P. latifolia* N-fertilized plot. QN = *Q. ilex* N-fertilized plot.

(a) Leaves

	PC	AC	AN	QN	PN
QC	M = 3.83 F = 6.77 $p < 0.0001$	M = 3.08 F = 5.44 $p < 0.0001$	M = 4.40 F = 7.69 $p < 0.0001$	M = 2.96 F = 5.24 $p < 0.0001$	M = 4.21 F = 7.44 $p < 0.0001$
PC		M = 1.03 F = 1.82 $p = 0.041$	M = 1.37 F = 2.39 $p = 0.0046$	M = 6.27 F = 11.09 $p < 0.0001$	M = 1.76 F = 3.10 $p = 0.0002$
AC			M = 1.29 F = 2.26 $p = 0.0077$	M = 6.28 F = 11.1 $p < 0.0001$	M = 2.31 F = 4.08 $p < 0.0001$
AN				M = 5.28 F = 0.23 $p < 0.0001$	M = 1.75 F = 3.07 $p = 0.0003$
QN					M = 3.64 F = 6.43 $p < 0.0001$

(b) Litter

	AC	PC	AN	QN	PN
QC	M = 2.62 F = 4.61 $p < 0.000001$	M = 3.41 F = 5.21 $p < 0.000001$	M = 4.09 F = 7.12 $p < 0.000001$	M = 5.9 F = 10.2 $p < 0.000001$	M = 4.03 F = 5.77 $p < 0.000001$
AC		M = 3.63 F = 5.56 $p < 0.000001$	M = 3.57 F = 6.21 $p < 0.000001$	M = 6.50 F = 11.2 $p < 0.000001$	M = 7.25 F = 10.38 $p < 0.000001$
PC			M = 5.23 F = 7.93 $p < 0.000001$	M = 7.29 F = 10.9 $p < 0.000001$	M = 5.39 F = 6.89 $p < 0.000001$
AN				M = 3.19 F = 5.44104 $p < 0.000001$	M = 3.24 F = 4.60059 $p < 0.000001$
QN					M = 2.551809 F = 3.60 $p < 0.000001$

and Ca load to control plots. In litter, δ15N is the strongest variable loading in fertilized plots with N in lower intensity. Contrarily, Fe and Mn load to control plots.

2.3.6 Drought Effects

All species have differences in control plots elemental composition between 2014 and 2015 in foliar tissue and respond similarly to drought. In foliar tissue, all macro-nutrients decrease

significantly in 2015 in all three studied species except C in *P. latifolia*, which have no significant differences. In foliar micro-nutrients, Mg and Cu decrease significantly in 2015 in all three species, Ca increases significantly in *P. latifolia* and *Q. ilex*; Cr and Ni decreases in *A. unedo* and *Q. ilex*; Mn and Sr increase significantly in all three species; Fe decreases in *A. unedo*, increases in *Q. ilex* and has no differences in *P. latifolia*; Zn decreases in *Q. ilex*; and Pb decreases in *A. unedo* and *P. latifolia* and has no differences in *Q. ilex* (Figure 2.7).
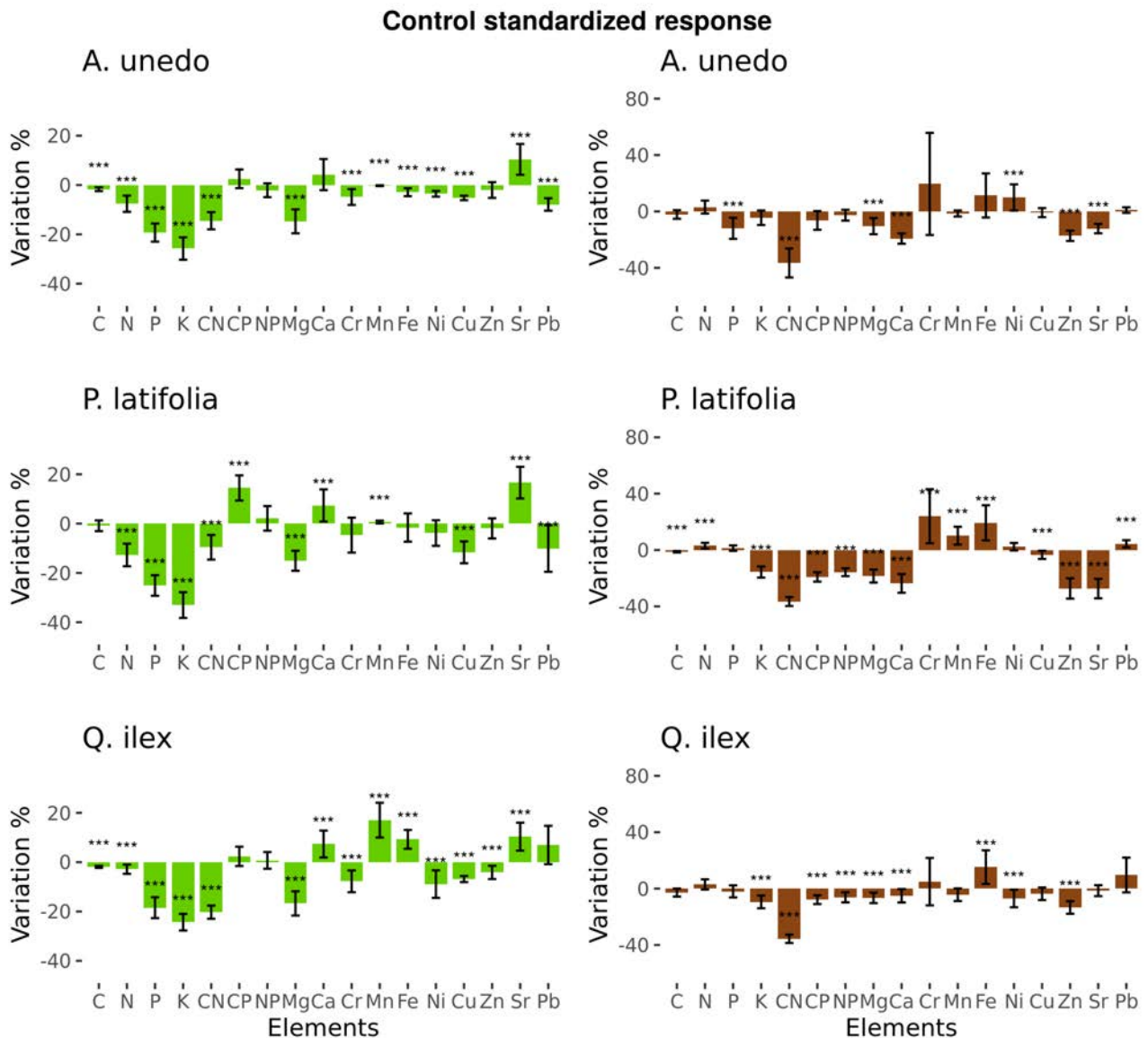
Figure 2.7 Foliar elemental composition; ratios and microelements (green); and litter elemental composition, ratios, and microelements (brown) by specie control plots data. Bars show the effect of drought through the values difference between 2015 and 2014. Values are relative to original values in 2014 so are expressed in % of change. *** = Significant differences from 0 according to t-test (*p* < 0.05). Figures with standard deviation.

2.4 Discussion

2.4.1 Interannual Differences

The overall macro-elements reduction and micro-elements variation in control plots between 2014 and 2015, as seen in Figure 2.7, are inherent effects of the exceptional drought in 2015. These year to year effects of drought are consistent with the drought experiments carried out in the same studied Mediterranean forest, where reduction of foliar N [50,51], P [52,53,54], and micro nutrients variation [55,56] were described to be associated to the drought treatment. This decrease of nutrient uptake associated to a decrease of soil water content gives evidence of the potential negative effects of drought by decreasing nutrient uptake capacity as well as the reinforced negative feedback of low nutrient concentration in leaves lowering the plant capacity to maintain an adequate water use efficiency [57]. Therefore, to correctly assess the effects of addition of N in this study, the exceptional 2015 arid conditions needs to be considered.

2.4.2 Macro-Elements

In contrast to our results, fertilization with N would be expected to increase total concentration of foliar N; however, we found evidence of an increase in foliar 15N that indicates rapid plant uptake of the applied fertilizer. The contrasting effects of added N on total foliar N concentration and foliar 15N may be explained by two hypotheses. First, added N is highly available, so although plants in the fertilized plots may have taken up similar amounts of total N from soil, there may have been greater uptake of the more easily available rich 15N, and secondly, plants may have taken up greater amounts of N that was rapidly allocated to tissue other than leaf material, such as roots, to stimulate growth. Our results support these hypotheses, because we observed higher foliar concentrations of other elements following fertilization and higher leaf litter 15N concentrations that indicate decreases in leaf N resorption under increased N-availability.

2.4.3 Micro-Elements

In previous studies, N addition was related with a massive incorporation of NO3– into the system, causing cation losses by leaching and soil acidification [58,59,60]. In our study, leaching could have been generated by direct NO3– contact to the leaves, as described for acid rain impacts [61]. The decrease of foliar Cr in *Q. ilex* (Figure 2.2d) and Ni in *A.unedo* and *Q. ilex* (Figure 2.2e) is consistent with foliar leaching theory. In contrast, litter micro-elemental concentrations were not conclusively responding to N addition. The most remarkable response of micro-elements to N addition was the decrease of foliar Cr and Ni, which do not have a known biological benefit to plants and are considered pollutants. In this case, the short-term effects of N addition may be considered beneficial for species fitness, even though the size of effect varied among the three species (greatest in *Q. ilex*, moderate in *A. unedo*, and null in *P. latifolia*).

2.4.4 Nutrient Uptake

The isotope analysis showed rapid incorporation of N to the ecosystem following fertilization, where there were differences in foliar δ15N, but not of δ13C. Foliar uptake of N by *A. unedo* was

unaffected by treatment, whereas fertilization led to greater uptake in *P. latifolia* and *Q. ilex* (Figure 2.6), indicating possible interspecific imbalances in nutrient uptake under drought conditions.

In contrast, leaf litter 15N was consistently greater among the three species under N-fertilization, where the greater concentration in *A. unedo* and *Q. ilex* leaf litter than in leaf material highlights differences in resorption and indicates that, prior to the release of senescent leaves, *A. unedo* and *Q. ilex* reabsorbed smaller or negligible amounts of the applied N enriched with 15N. There were no differences between δ15N of *P. latifolia* leaf material and leaf litter, indicating unchanged levels of N resorption under N addition; this maintenance of greater nutrient use efficiency, even under drought conditions, than in *A. unedo* and *Q. ilex*, is consistent with previous studies that report its greater capacity for the maintenance of optimal function and growth under increasing drought than the other two species [44,62].

2.4.5 N limited Ecosystem

Our analyses showed rapid leaf and leaf litter elemental composition responses to N addition, through the increases in leaf and leaf litter δ15N, allowing us to conclude that this Mediterranean forest ecosystem is N limited, which is consistent with previous studies that showed decreases in foliar N in Mediterranean ecosystems during the 20th century, and associated them to increases in photosynthetic rates and C fixation, as well as growth capacity because of increasing atmospheric CO_2 concentrations [63] and with N limitation reported in the forests we studied [64]. Furthermore, the drought in 2015 also contributed to decreasing foliar N and increased even more the original plant N deficiency phenomenon already described in other studies [65].

These three dominant species of the holm oak forest ecosystem were characterized by smaller decreases in leaf and leaf litter concentrations of several elements under N-fertilization during the extreme high drought conditions of 2015. This rapid response of the mature ecosystem was remarkable and indicates that addition of N to an N-limited ecosystem improves nutrient retention and increases resilience of ecosystem function to drought stress.

Such drought recovery under N fertilization conditions could drive forest managers to consider generalized application of N-fertilization to stimulate C-uptake after droughts. However, N fertilization would be logistically complicated and would cause important N-cycle alterations, nutrient imbalances, decrease of diversity, and also can favor highly competitive invasive species replacing endemic ones. However, these impacts could be improved adding other nutrients such as P and K or even Fe, Mg, and sulfur. In this way, the fertilization probably would not generate important nutrient imbalances, and plants with better nutritional status could improve their water use efficiency. Our results can thus be useful for developing management strategies for Mediterranean forests under increasingly dry conditions projected for coming decades.

Long-term continuous N-fertilization experiments are needed to confirm the wider effects of increased availability of N on growth and stocks and changes in plant-soil cycling of nutrient elements of this Mediterranean ecosystem, including its dominant species. It is likely that sustained N deposition would decrease N limitation and its associated effects, but elements that derive from the bedrock, such as P or K, will increasingly become leached and limiting [5,6].

2.4.6 Biogeochemical Niche Differentiation

The GDA analysis showed a clear differentiation in element concentration among the three studied species and with treatment. This differentiation suggests a particular chemical identity per species as well as a particular species response to the same drivers. This chemical differentiation among species is supported by biogeochemical niche hypothesis [33,66,67]. Biogeochemical niche hypothesis is a derivation of the classic niche theory, which assigns to each species a specific position into a multidimensional space of traits, environmental requirements, and "needs". Biogeochemical niche hypothesis aims to synthesize all these variables into an easily quantifiable multidimensional space of the elemental composition with the "n-dimensional" axes being the concentrations of the different bioelements. This chemical divergence responds to the necessity of avoiding niche overlap that has been driven through evolutive genetic selection and the environmental conditions where the species has been developed.

2.4.7 Species Differences in Response to N Fertilization

A. unedo, *P. latifolia*, and *Q. ilex* coexist at the same stage of ecological succession in the Mediterranean forest ecosystem [42,68], presenting different intraspecific tolerance to different extreme conditions. Despite *P. latifolia* and *Q. ilex* sharing the same strategy to avoid water loss during drought, *P. latifolia* has a higher tolerance, a more efficient recovery to drought [69], and high temperatures than *Q. ilex* [44]. At the same time, *P. latifolia* has lower tolerance to low winter temperatures than *Q. ilex* [69]. Also, previous studies of impacts of long-term drought have shown that *Q. ilex* and *A. unedo* are mostly affected through changes in C and nutrient storage, whereas *P. latifolia* tends to remain unaffected [44,62]. Despite all three species sharing a dimorphic root system, enabling them to access different water level sources [70], these differences seem to be explained through xylem vessels width. *Q. ilex* and *A. unedo* have wider xylem vessels, which allow them to transport greater amounts of sap under well-watered conditions, but makes them more susceptible to embolism under drought conditions [71]. Also, *Q. ilex* has higher capacity to exclude potential toxic ions than *A. unedo* [72].

The intraspecific different responses to N-fertilization observed in this study are consequent with the different species tolerance to drought conditions, where *P. latifolia* is the most drought-resistant species and also the one best at assimilating better N addition. As long as the fertilizer is sprayed to the leaves, its absorption is directly related to stomatal opening. Thus,

better watered species can afford longer stomatal apertures and consequently, higher N absorption [73]. This different N absorption among species may lead to changes in species composition in a drier and N fertilized environment as projected for these Mediterranean ecosystems for the next decades [9,28,74]. Under extreme drought conditions, *P. latifolia* could overtake *Q. ilex* dominance [65].

2.5 Conclusions

We found that added N was rapidly utilized in the three studied species, consistent with previous observations of N limitation in this area; this effect was remarkable, given the exceptional arid conditions during the study. Species responses to sudden increases in N availability varied, where *A. unedo* was unaffected and direction of responses in *P. latifolia* and *Q. ilex* were similar, albeit with contrasting magnitude. The macro-element concentrations were more sensitive in *P. latifolia* than in the other two species. *P. latifolia* was the species that took best advantage of the N fertilization and the species less affected by drought. The chemical composition of the three study species before and after N-fertilization follows the biogeochemical niche hypothesis, where differences in response may trigger changes in species coexistence and community composition.

Supplementary Materials

The following are available online at: https://www.mdpi.com/article/10.3390/f12050605/s1. Table S1: Results of the foliar mixed models. Table S2: Results of the litter mixed models. Table S3: Results of the reabsorption mixed models. Table S4: Results of the isotopes mixed models.

Author Contributions

Funding

Data Availability Statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to the project is not finished yet.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Peoples:, M.B.; Herridge, D.F.; Ladha, J.K. Biological nitrogen fixation: An efficient source of nitrogen for sustainable agricultural production? *Plant Soil* **1995**, *174*, 3–28.

2. Foth, H. Chapter 12: Plant-Soil Macronutrient Relations. In *Fundamentals of Soil Science*, 8th ed.; John Wiley Company: New York, NY, USA, 1990; pp. 186–209.

3. Brady, N.; Weil, R. Nutrient cycles and soil fertility. In *Elements of the Nature and Properties of Soils*, 3rd ed.; Anthony, V.R., Ed.; Pearson Education Inc.: Upper Saddle River, NJ, USA, 2010; pp. 396–420.

4. Galloway, J.N.; ATownsend, A.R.; Erisman, J.W.; Bekunda, M.; Cai, Z.; Freney, J.R.; Martinelli, L.A.; Seitzinger, S.P.; Sutton, M.A. Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. *Science* **2008**, *320*, 889–892.

5. Peñuelas, J.; Poulter, B.; Sardans, J.; Ciais, P.; Van Der Velde, M.; Bopp, L.; Boucher, O.; Godderis, Y.; Hisinger, P.; Llusia, J.; et al. Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. *Nat. Commun.* **2013**, *4*, 2934.

6. Penuelas, J.; Janssens, I.; Ciais, P.; Obersteiner, M.; Sardans, J. Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity, food security, and human health. *Glob. Chang. Biol.* **2020**, *26*, 1962–1985.

7. Engardt, M.; Simpson, D.; Schwikowski, M.; Granat, L. Deposition of sulphur and nitrogen in Europe 1900–2050. Model calculations and comparison to historical observations. *Tellus B Chem. Phys. Meteorol.* **2017**, *69*, 1328945.

8. Schmitz, A.; Sanders, T.G.M.; Bolte, A.; Bussotti, F.; Dirnböck, T.; Johnson, J.; Peñuelas, J.; Pollastrini, M.; Prescher, A.-K.; Sardans, J.; et al. Responses of forest ecosystems in Europe to decreasing nitrogen deposition. *Environ. Pollut.* **2019**, *244*, 980–994.

9. Slootweg, J.; Posch, M.; Hettelingh, J.P. *Modelling and Mapping the Impacts of Atmospheric Deposition of Nitrogen and Sulphur: CCE Status Report 2015*; Coordination Centre for Effects, National Institute for Public Health and the Environment: Bilthoven, The Netherlands, 2015.

10. Pardo, L.H.; Fenn, M.E.; Goodale, C.L.; Geiser, L.H.; Driscoll, C.T.; Allen, E.B.; Baron, J.S.; Bobbink, R.; Bowman, W.D.; Clark, C.M.; et al. Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. *Ecol. Appl.* **2011**, *21*, 3049–3082.

11. Fenn, M.E.; Allen, E.B.; Weiss, S.B.; Jovan, S.; Geiser, L.H.; Tonnesen, G.S.; Johnson, R.F.; Rao, L.E.; Gimeno, B.S.; Yuan, F.; et al. Nitrogen critical loads and management alternatives for N-impacted ecosystems in California. *J. Environ. Manag.* **2010**, *91*, 2404–2423.

12. Rao, L.E.; Allen, E.B. Combined effects of precipitation and nitrogen deposition on native and invasive winter annual production in California deserts. *Oecologia* **2010**, *162*, 1035–1046.

13. Rao, L.E.; Allen, E.B.; Meixner, T. Risk-based determination of critical nitrogen deposition loads for fire spread in southern California deserts. *Ecol. Appl.* **2010**, *20*, 1320–1335.

14. Gilliam, F.S. Response of the herbaceous layer of forest ecosystems to excess nitrogen deposition. *J. Ecol.* **2006**, *94*, 1176–1191.

15. Friedrich, U.; Von Oheimb, G.; Kriebitzsch, W.U.; Schleßelmann, K.; Weber, M.S.; Härdtle, W. Nitrogen deposition increases susceptibility to drought—Experimental evidence with the perennial grass *Molinia caerulea* (L.) Moench. *Plant Soil* **2012**, *353*, 59–71.

16. Ochoa-Hueso, R.; Allen, E.B.; Branquinho, C.; Cruz, C.; Dias, T.; Fenn, M.E.; Manrique, E.; Pérez-Corona, M.E.; Sheppard, L.J.; Stock, W.D. Nitrogen deposition effects on Mediterranean-type ecosystems: An ecological assessment. *Environ. Pollut.* **2011**, *159*, 2265–2279.

17. Eisenlord, S.; Zak, D. Simulated Atmospheric Nitrogen Deposition Alters Actinobacterial Community Composition in Forest Soils. *Soil Biol. Biochem.* **2009**, *74*, 1157–1166.

18. Bontemps, J.D.; Hervé, J.C.; Leban, J.M.; Dhôte, J.F. Nitrogen footprint in a long-term observation of forest growth over the twentieth century. *Trees* **2011**, *25*, 237–251.

19. Schulte-Uebbing, L.; De Vries, W. Global-scale impacts of nitrogen deposition on tree carbon sequestration in tropical, temperate, and boreal forests: A meta-analysis. *Glob. Chang. Biol.* **2018**, *24*, e416–e431.

20. Fenn, M.E.; Poth, M.A.; Aber, J.D.; Baron, J.S.; Bormann, B.T.; Johnson, D.W.; Lemly, A.D.; McNulty, S.G.; Ryan, D.F.; Stottlemyer, R. Nitrogen Excess in North American Ecosystems: Predisposing Factors, Ecosystem Responses, and Management Strategies. *Ecol. Appl.* **1998**, *8*, 706–733.

21. Baron, J.; Rueth, H.; Wolfe, A.; Nydick, K.R.; Allstott, E.J.; Minear, J.T.; Moraska, B. Ecosystem Responses to Nitrogen Deposition in the Colorado Front Range. *Ecosystems* **2000**, *3*, 352–368.

22. Duquesnay, A.; Dupouey, J.L.; Clement, A.; Ulrich, E.; Le Tacon, F. Spatial and temporal variability of foliar mineral concentration in beech (*Fagus sylvatica*) stands in northeastern France. *Tree Physiol.* **2000**, *20*, 13–22.

23. Kowalenko, C.G. The effect of nitrogen and boron fertilizer applications on Willamette red raspberry growth, and on applied and other nutrients in the plant and soil over two growing seasons. *Can. J. Plant Sci.* **2005**, *86*, 213–225.

24. Huang, Z.; Liu, B.; Davis, M.; Sardans, J.; Peñuelas, J.; Billings, S. Long-term nitrogen deposition linked to reduced water use efficiency in forests with low phosphorus availability. *New Phytol.* **2016**, *210*, 431–442.

25. Ferretti, M.; Marchetto, A.; Arisci, S.; Bussotti, F.; Calderisi, M.; Carnicelli, S.; Cecchini, G.; Fabbio, G.; Bertini, G.; Matteucci, G.; et al. On the tracks of Nitrogen deposition effects on temperate forests at their southern European range—An observational study from Italy. *Glob. Chang. Biol.* **2014**, *20*, 3423–3438.

26. Veresoglou, S.D.; Peñuelas, J.; Fischer, R.; Rautio, P.; Sardans, J.; Merilä, P.; Tabakovic-Tosic, M.; Rillig, M.C. Exploring continental-scale stand health e N: P ratio relationships for European forests. *New Phytol.* **2014**, *202*, 422–430.

27. Zhang, X.; Chen, Q.; Han, X. Soil Bacterial Communities Respond to Mowing and Nutrient Addition in a Steppe Ecosystem. *PLoS ONE* **2013**, *8*, e84210.

28. Sardans, J.; Penuelas, J. Plant-soil interactions in Mediterranean forest and shrublands: Impacts of climatic change. *Plant Soil* **2013**, *365*, 1–33.

29. Peñuelas, J.; Sardans, J.; Filella, I.; Estiarte, M.; Llusià, J.; Ogaya, R.; Carnicer, J.; Bartrons, M.; Rivas-Ubach, A.; Grau, O.; et al. Assessment of the impacts of climate change on Mediterranean terrestrial ecosystems based on data from field experiments and long-term monitored field gradients in Catalonia. *Environ. Exp. Bot.* **2018**, *152*, 49–59.

30. Carreira, J.A.; Sánchez-Vázquez, F.; Niell, F.X. Short-termand small-scale patterns of post-fire regeneration in a semi-ariddolomitic basin of Southern Spain. *Acta Oecol.* **1992**, *13*, 241–253.

31. Henkin, Z.; Seligman, N.G.; Kafkafi, U.; Noy-Meir, I. Effective growing days: A simple predictive model of re-sponse of herbaceous plant growth in a Mediterranean ecosystem to variation in rainfall and phosphorus availability. *J. Ecol.* **1998**, *86*, 137–148.

32. Sardans, J.; Peñuelas, J. Tree stoichiometry and growth. *Glob. Ecol. Biogeogr.* **2013**, *22*, 494–507.

33. Peñuelas, J.; Fernández-Martínez, M.; Ciais, P.; Jou, D.; Piao, S.; Obersteiner, M.; Vicca, S.; Janssens, I.A.; Sardans, J. The bioelements, the elementome, and the biogeochemical niche. *Ecology* **2019**, *100*, e02652.

34. Hu, M.; Peñuelas, J.; Sardans, J.; Sun, Z.; Wilson, B.J.; Huang, J.; Zhu, Q.; Tong, C. Stoichiometry patterns of plant organ N and P in coastal herbaceous wetlands along the East China Sea: Implications for biogeochemical niche. *Plant Soil* **2018**, *431*, 273–288.

35. Bobbink, B.; Hicks, K.; Galloway, J.; Spranger, T.; Alkemade, R.; Ashmore, M.; Bustamante, M.; Cinderby, S.; Davidson, E.; Dentener, F.; et al. Global assessment of nitrogen deposition effects on terrestrial plant diversity: A synthesis. *Ecol. Appl.* **2010**, *20*, 30–59.

36. Clark, C.M.; Morefield, P.E.; Gilliam, F.S.; Pardo, L.H. Estimated losses of plant biodiversity in the United States from historical N deposition (1985–2010). *Ecology* **2013**, *94*, 1441–1448.

37. Soons, M.B.; Hefting, M.M.; Dorland, E.; Lamers, L.P.M.; Versteeg, C.; Bobbink, R. Nitrogen effects on plant species richness in herbaceous communities are more widespread and stronger than those of phosphorus. *Biol. Conserv.* **2017**, *212*, 390–397.

38. Escarre, J.; Lepart, J.; Sans, X.; Sentuc, J.; Gorse, V. Effects of herbivory on the growth and reproduction of *Picris hieracioides* in the Mediterranean region. *J. Veg. Sci.* **1999**, *10*, 101–110.

39. Ogaya, R.; Peñuelas, J. Tree growth, mortality, and above-ground biomass accumulation in a holm oak forest under a five-year experimental field drought. *Plant Ecol.* **2007**, *189*, 291–299.

40. Tretiach, M. Photosynthesis and transpiration of evergreen Mediterranean and deciduous trees in an ecotone during a growing season. *Acta Oecol. Int. J. Ecol.* **1993**, *14*, 341–360.

41. Lloret, F.; Siscart, D. Demographic drought effects on holm oak populations. *Cuad. Soc. Esp. Cien. Forest.* **1995**, *2*, 77–81.

42. Panaiotis, C.; Loiser, R.; Paradis, G. Dating natural gaps in the holm oak forest (*Quercus ilex* L.) in Fango MAB Reserve (Corsica) by reading rings of maquis components datation des trouées naturelles en forêt de chênes verts (*Quercus ilex* L.) dans la réserve MAB du Fango (Corse) par la lecture des cernes des essences du maquis. *Ann. Sci. For.* **1995**, *52*, 477–487.

43. Ogaya, R.; Liu, D.; Barbeta, A.; Peñuelas, J. Stem Mortality and Forest Dieback in a 20-Years Experimental Drought in a Mediterranean Holm Oak Forest. *Front. For. Glob. Chang.* **2020**, *2*, 89.

44. Ogaya, R.; Peñuelas, J.; Martínez-Vilalta, J.; Mangirón, M. Effect of drought on diameter increment of *Quercus ilex*, *Phillyrea latifolia*, and *Arbutus unedo* in a holm oak forest of NE Spain. *For. Ecol. Manag.* **2003**, *180*, 175–184.

45. Ogaya, R.; Penuelas, J. Contrasting foliar responses to drought in *Quercus ilex* and *Phillyrea latifolia*. *Biol. Plant.* **2006**, *50*, 373–382.

46. Porter, J. Evaluation of washing procedures for pollution analysis of *Ailanthus altissima* leaves. *Environ. Pollut. Ser. B Chem. Phys.* **1986**, *12*, 195–202.

47. Bates, D.; Maechler, M.; Bolker, B.; Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* **2015**, *67*, 1–48.

48. Kuznetsova, A.; Brockhoff, P.B.; Christensen, R.H.B. lmerTest Package: Tests in Linear Mixed Effects Models. *J. Stat. Softw.* **2017**, *82*, 1–26.

49. Bartoń, K. MuMIn: Multi-Model Inference. R Package Version 1.43.17. 2020. Available online: **https://CRAN.R-project.org/package=MuMIn** (accessed on 6 March 2021).

50. Sardans, J.; Peñuelas, J.; Estiarte, M.; Prieto, P. Warming and drought alter C and N concentration, allocation and accumulation in a Mediterranean shrubland. *Glob. Chang. Biol.* **2008**, *14*, 2304–2316.

51. Sardans, J.; Peñuelas, J.; Ogaya, R. Drought-induced changes in C and N stoichiometry in a Quercus ilex Mediterranean forest. *For. Sci.* **2008**, *54*, 513–522.

52. Sardans, J.; Peñuelas, J. Increasing drought decreases phosphorus availability in an evergreen Mediterranean forest. *Plant Soil* **2004**, *267*, 367–377.

53. Sardans, J.; Peñuelas, J. Drought changes phosphorus and potassium accumulation patterns in an evergreen Mediterranean forest. *Funct. Ecol.* **2007**, *21*, 191–201.

54. Sardans, J.; Peñuelas, J.; Prieto, P.; Estiarte, M. Drought and warming induced changes in P and K concentration and accumulation in plant biomass and soil in a Mediterranean shrubland. *Plant Soil.* **2008**, *306*, 261–271.

55. Sardans, J.; Peñuelas, J.; Ogaya, R. Drough's impact on Ca, Fe, Mg, Mo and S concentration and accumulation patterns in the plants and soil of a Mediterranean evergreen Quercus ilex forest. *Biogeochemistry* **2008**, *87*, 49–69.

56. Sardans, J.; Peñuelas, J.; Prieto, P.; Estiarte, M. Changes in Ca, Fe, Mg, Mo, Na, and S content under warming and drought. *J. Geophys. Res.* **2008**, *113*, G03039.

57. Plett, D.C.; Ranathunge, K.; Melino, V.J.; Kuya, N.; Uga, Y.; Kronzucker, H.J. The intersection of nitrogen nutrition and water use in plants: New paths toward improved crop productivity. *J. Exp. Bot.* **2020**, *71*, 4452–4468.

58. Barak, P.; Jobe, B.O.; Krueger, A.R.; Peterson, L.A.; Laird, D.A. Effects of long-term soil acidification due to nitrogen fertilizer inputs in Wisconsin. *Plant Soil* **1997**, *197*, 61–69.

59. Lu, X.K.; Mao, Q.G.; Gilliam, F.S.; Luo, Y.Q.; Mo, J.M. Nitrogen deposition contributes to soil acidification in tropical ecosystems. *Glob. Chang. Biol.* **2014**, *20*, 3790–3801.

60. Lu, X.K.; Vitousek, P.M.; Mao, Q.G.; Gilliam, F.S.; Luo, Y.Q.; Zhou, G.Y.; Zou, X.; Bai, E.; Scanlon, T.M.; Hou, E.; et al. Plant acclimation to long-term high nitrogen deposition in an N-rich tropical forest. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 5187–5192.

61. Fairfax, J.; Lepp, N. Effect of simulated 'acid rain' on cation loss from leaves. *Nature* **1975**, *255*, 324–325.

62. Sardans, J.; Urbina, I.; Grau, O.; Asensio, L.; Ogaya, R.; Peñuelas, J. Long-term drought decreases ecosystem C and nutrient storage in a Mediterranean holm oak forest. *Environ. Exp. Bot.* **2020**, *177*, 104135.

63. Peñuelas, J.; Matamala, R. Changes in N and S Leaf Content, Stomatal Density and Specific Leaf Area of 14 Plant Species during the Last Three Centuries of CO2 Increase. *J. Exp. Bot.* **1990**, *41*, 1119–1124.

64. Mayor, X.; Rodà, F. Effects of irrigation and fertilization on stem diameter growth in a Mediterranean holm oak forest. *For. Ecol. Manag.* **1994**, *68*, 119–126.

65. Ogaya, R.; Peñuelas, J. Climate Change Effects in a Mediterranean Forest Following 21 Consecutive Years of Experimental Drought. *Forests* **2021**, *12*, 306.

66. Sardans, J.; Janssens, I.A.; Alonso, R.; Veresoglou, S.D.; Rillig, M.C.; Sanders, T.G.; Carnicer, J.; Filella, I.; Farré-Armengol, G.; Peñuelas, J. Biogeochemical niche of European forest trees. *Glob. Ecol. Biogeogr.* **2015**, *24*, 240–255.

67. Sardans, J.; Vallicrosa, H.; Zuccarini, P.; Farré-Armengol, G.; Fernández-Martínez, M.; Peguero, G.; Gargallo-Garriga, A.; Ciais, P.; Janssens, I.A.; Obersteiner, M.; et al. Empirical support for the biogeochemical niche hypothesis in forest trees. *Nat. Ecol. Evol.* **2021**, *5*, 184–194.

68. Capitanio, R.; Carcaillet, C. Post-fire Mediterranean vegetation dynamics and diversity: A discussion of succession models. *For. Ecol. Manag.* **2008**, *255*, 431–439.

69. Gratani, L.; Catoni, R.; Varone, L. Morphological, anatomical and physiological leaf traits of *Q. ilex*, *P. latifolia*, *P. lentiscus*, and *M. communis* and their response to Mediterranean climate stress factors. *Bot. Stud.* **2013**, *54*, 35.

70. Barbeta, A.; Mejía-Chang, M.; Ogaya, R.; Voltas, J.; Dawson, T.E.; Peñuelas, J. The combined effects of a long-term experimental drought and an extreme drought on the use of

plant-water sources in a Mediterranean forest. *Glob. Chang. Biol.* **2015**, *21*, 1213–1225.

71. Martínez-Vilalta, J.; Prat, E.; Oliveras, I.; Piñol, J. Hydraulic properties of roots and stems of nine woody species from a Holm oak forest in NE Spain. *Oecologia* **2002**, *133*, 19–29.

72. Fusaro, L.; Mereu, S.; Brunetti, C.; Ferdinando, M.D.; Ferrini, F.; Manes, F.; Salvatori, E.; Marzuoli, R.; Gerosa, G.; Tattini, M. Photosynthetic performance and biochemical adjustments in two co-occurring *Mediterranean evergreens*, *Quercus ilex* and *Arbutus unedo*, differing in salt-exclusion ability. *Funct. Plant Biol.* **2014**, *41*, 391–400.

73. Hu, Y.; Burucs, Z.; Schmidhalter, U. Effects of foliar fertilization application on the growth and mineral nutrient content of maize seedlings under drought and salinity. *Soil Sci. Plant Nutr.* **2008**, *54*, 133–141.

74. Tramblay, Y.; Koutroulis, A.; Samaniego, L.; Vicente-Serrano, S.M.; Volaire, F.; Boone, A.; Le Page, M.; Llasat, M.C.; Albergel, C.; Burak, S.; et al. Challenges for drought assessment in the Mediterranean region under future climate scenarios. *Earth-Sci. Rev.* **2020**, *210*, 103348.

3

Foliar elemental composition and weather as drivers of defoliation rate in European forests

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Abstract

Defoliation is a standard, easily acquirable and widely used variable to assess forest health and vitality. However, its relationship with foliar elemental composition remains uncertain. Using inventory from the ICP Forests programme, we examined the relationships between trends in European defoliation, foliar elemental composition, and environmental factors in Europe as a whole and by latitudinal fringes. We observed a significant trend for increasing defoliation in South and Central Europe, but a slight decreasing tendency in the boreal fringe. Gymnosperms had higher defoliation trends than angiosperms. All studied species showed evidence of increased defoliation over the last decades, excepting *Quercus ilex*, which had decreased defoliation, and *Abies alba*, which was non-significant. Foliar elemental composition was associated with defoliation in European forests, with a clear inverse correlation with foliar N, and additional significant relationships found between defoliation and foliar P, foliar Ca, and foliar S. Climatic legacy, the distribution of precipitation over time, and different environmental pressures also played a role in defoliation, depending on the studied latitudinal fringe.

3.1 Introduction

Defoliation data are broadly used as an indicator of forest health and vitality (De Marco A. et al., 2014; Vacek et al., 2015; Gea-Izquierdo et al., 2019; Gottardini et al. 2020), probably due to its long history of observations (Menzel and Dose, 2005) and effortless data collection (Vliet et al., 2003). Defoliation is a generalist indicator (Ferretti, 1997, 1998) that is particularly related to temperature (Linkosalo, 1999; Peñuelas J. et al., 2004; Lebourgeois et al., 2010; De la Cruz et al., 2014), but is also related to soil water content (Peñuelas et al., 2004; Carnicer et al., 2011), photoperiod (Vitasse and Basler, 2013), rainfall (Peñuelas et al., 2004) and air pollution (Ferretti and Fischer, 2013). Consequently, it has been shown to be efficient for assessing the biological responses of forests to climate change (Menzel et al., 2006; Lebourgeois et al., 2010).

Documentation of the effects of climate change effects in Europe is growing, with consequences that include defoliation and drought-induced mortality in the central and semi-arid areas (Allen et al., 2010; de Vries and Posch, 2011; De Marco et al., 2014), long-term changes in the species compositions of terrestrial ecosystems (Mueller et al., 2005; Andreu et al., 2007; van Mantgem et al., 2009; Allen et al., 2010; Carnicer et al., 2011; Hanewinkel et al., 2013) and increased plant growth associated with warming, especially in the central and northern areas (Bussotti et al., 2014; Kauppi et al., 2014). Also, N deposition in Europe has increased from 2.8 kg ha^{-1}a^{-1} in 1900 to 6.6 Kg N ha^{-1}y^{-1} by 2018 through human activities (Engardt et al., 2017; Schmitz et al., 2019), with 62% of Europe considered to be at risk of eutrophication (Slootweg et al., 2015). This increase is associated with changes in soil condition at the European scale (Wallace et al., 2007; Phoenix et al., 2012), leading to substantial impacts on forest tree growth (Thomas et al., 2010) and nutrient imbalances (Blanes et al., 2013; de Vries et al., 2014). Even though N deposition intensity has decreased in the last 20 to 30 years in some areas of Europe, the long-term accumulation of N in soils remains a concern (Schmitz et al., 2019).

Shifts in soil nutrient availability and its effects on the foliar elemental composition of trees is another important factors related to forest health and vitality. The availability of elements like nitrogen, phosphorus, and potassium in the plant-soil system can substantially modify the impacts of global climate change (Jiang et al., 2017; Sardans & Peñuelas, 2015; Peñuelas et al., 2017, 2020; Sun et al., 2017; Wang et al., 2017; 2018; Terrer et al., 2019) and limit the global capacity for C fixation in the face of rising atmospheric CO_2 concentrations (Vicca et al., 2012; Fernández-Martínez et al., 2014; Zhu et al., 2016; Wang et al 2020). Thus, we expect that soil nutrient concentrations should interact with the effects of warming and drought to affect the defoliation status of forests in some European regions (Eastaugh et al., 2011; Zhang et al., 2014; Castagneri et al., 2015; Zimmermann et al., 2015; Vallicrosa et al., 2021). In this regard, it has been reported that the amounts of foliar elements, including N, P, K, and S, have decreased between 5% and 11% over the last 30 years in European forests, likely because of the effects of increasing atmospheric CO_2 concentrations and climate change (Peñuelas et al., 2020).

Given the above, our goal was to investigate the roles of foliar elemental composition and environmental factors on defoliation and assess tree vitality and drivers of health in (i) forest cover over the whole of Europe; (ii) angiosperms versus gymnosperms; (iii) the European forest cover, subdivided by boreal, temperate, and Mediterranean latitudes; and (iv) the most representative European forest species.

3.2 Methods

3.2.1 Data acquisition

We selected the dataset from International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests) (http://icp-forests.net/) that contained

both plot defoliation and plot foliar elemental composition data. The study dataset included data from 782 plots collected between 1992 and 2015. These data were collected for each plot repeatedly but irregularly over the sampled years, allowing for temporal analyses. Using geographical coordinates, we crossed the defoliation and elemental composition database with climatic gridded data from Climatic Research Unit (CRU) (Harris et al., 2020), the Standardized Precipitation-Evapotranspiration Index (SPEI), which is inverse to the aridity index and is used as a proxy of available water; soil data from the Land-Atmosphere Interaction Research Group at Sun Yat-sen University (Shangguan et al., 2014), and deposition data from CRU (Table S3.1). In addition to using the general database, we split the data on angiosperms and gymnosperms according to species, and by latitude, corresponding to the Mediterranean (latitude < 46°), temperate (from 46 to 58° latitude), and boreal fringes (latitude > 58°). Furthermore, the most commonly sampled species were selected for analysis (defined as > 40 individuals, and present in > 5 plots).

3.2.2 Maps generation and regressions

Using repeated defoliation measurements in the general database that were collected over multiple years, we calculated the defoliation trend by plot and the corresponding significance. We plotted the values of the ICP plots containing defoliation and elemental composition data onto a European map using the *ggspatial* package in R (Dunnington, 2021). We used the same procedure to similarly plot defoliation trends of angiosperms, gymnosperms, and for each focal species. The whole Europe, latitudinal, angiosperm, gymnosperm and by-species defoliation trends were calculated by Theil-Sen regression in the *mblm* package in R (Komsta, 2019) using the corresponding dataset following the Defoliation ~ year form. Comparison of the slopes for angiosperms and gymnosperms was carried out using a Chow test in the R package *gap* (Zhao, 2021).

3.2.3 Data analysis and model creation

To select the most meaningful variables, we built univariate models using the complete database, and for each latitudinal group, the division between gymnosperms and angiosperms, and a subset of individual species. The models were built using the *lme* function in the *nlme* R package (Pinheiro et al., 2020) with the form Defoliation ~ variable, and country, plot, and species included as random factors. We used the temporal autocorrelation corCAR1 in year, country, plot, and species with a velue of 0.2. The significant variables for each subset (P-value < 0.05) were reanalyzed with the *lme* function and the following form: Defoliation ~ significant variable 1 + significant variable 2…, with country, plot, and species included as random factors. A corCAR1 temporal autocorrelation was employed following year, country, plot, and species forms. The models built with significant variables were subjected to the *dredge* function of *MuMIn* R package (Barton, 2020) for model selection using AIC optimization.

3.3 Results

3.3.1 Description

We found a temporal trend toward increasing defoliation in European forests, whith defoliation %
increasing by 0.38% yearly over the study period (Figure 3.1). There was a slight but significant
decrease in defoliation in the Northern latitudes (Figure 3.2a), with defoliation decreasing by
-0.004% each year from 1995 to 2015 (Figure 3.2a). By contrast, increasing defoliation tendencies
were observed in Central and Mediterranean latitudes (0.501% and 0.272%, respectively) with the
central latitudes being most strongly affected by defoliation (Figure 3.2b and Figure 3.2c).
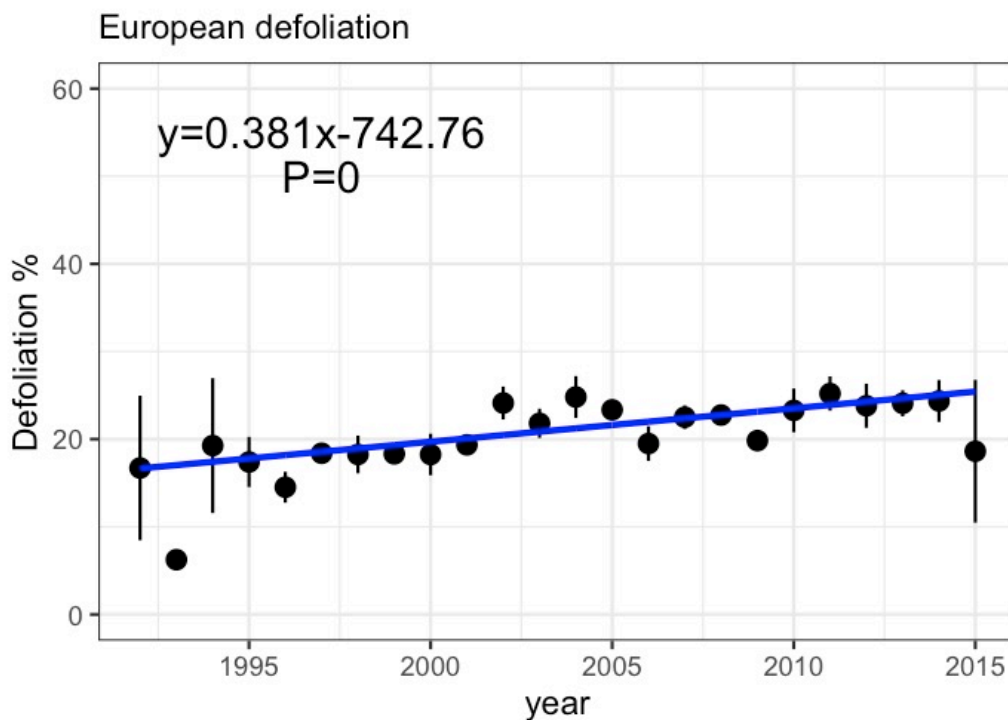
Figure 3.1 Defoliation trend in % defoliation for European plots from 1992 to 2015.
The data summarize the defoliation of 3,869 individual trees. P=0 means < 0.0009.
Bars represent the point range for each year.

Comparing defoliation rates between angiosperms and gymnosperms overall, or by
latitudinal groups, showed that in all cases, gymnosperms had higher rates of defoliation than
angiosperms (Figure 3.3). The results of the Chow test showed that these differences between
angiosperms and gymnosperms were statistically different in all cases (Table S3.2).

Species-specific descriptive analyses revealed significantly increased defoliation ($p < 0.05$)
in *Fagus sylvatica* (0.28% by year), *Picea abies* (0.58% by year), *Pinus pinaster* (0.71% by year),
Pinus sylvestris (0.18% by year), *Quercus cerris* (0.25% by year), *Quercus petraea* (0.47% by
year), and *Quercus robur* (0.35 % by year). A significant decreasing trend in defoliation was found
for *Quercus ilex* (-0.61% a year), and no significant trend was observed for *Abies alba* (Figure 3.3).
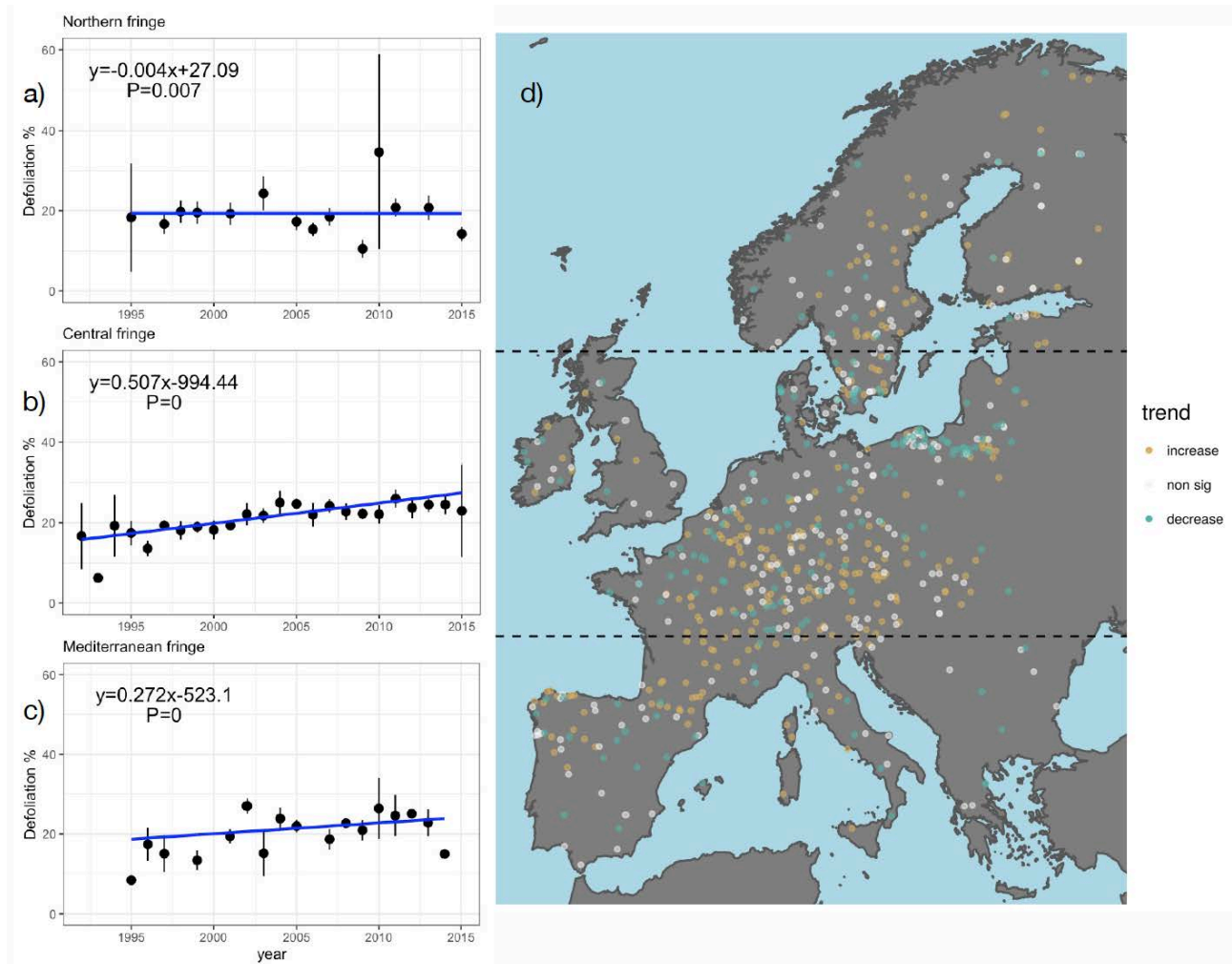
Figure 3.2. Relationship between defoliation % and year in a) the Northern fringe, b) the Central fringe, c) the Mediterranean fringe. d) Map shows each sampled plot coded according to whether defoliation during the study period showed a significantly increasing trend (orange), significantly decreasing trend (green), or non-significant trend (white). The dashed lines indicates the boundaries between the three latitudinal fringes P=0 means P<0.0009.

3.3.2 Global and latitudinal models

According to our model (Table 3.1), European defoliation was directly correlated with spring precipitation, spring temperature and nitrogen oxide deposition; it was also inversely correlated with sulphur oxide deposition and annual SPEI average. Based on our models, in latitudes higher than 58° the main drivers of defoliation were spring precipitation and spring temperature in the previous year, which were directly correlated with defoliation, whereas foliar nitrogen concentration was inversely correlated with defoliation. At the central latitudes (from 46° to 58°), the best model showed that defoliation was directly correlated with spring temperature and summer SPEI, and inversely correlated with precipitation and temperature in the previous year and sulfur oxide deposition. At the Mediterranean latitudes (< 46°), the main driver of defoliation was the mean precipitation in spring, which, in contrast to the northern latitudes, was inversely correlated with defoliation.
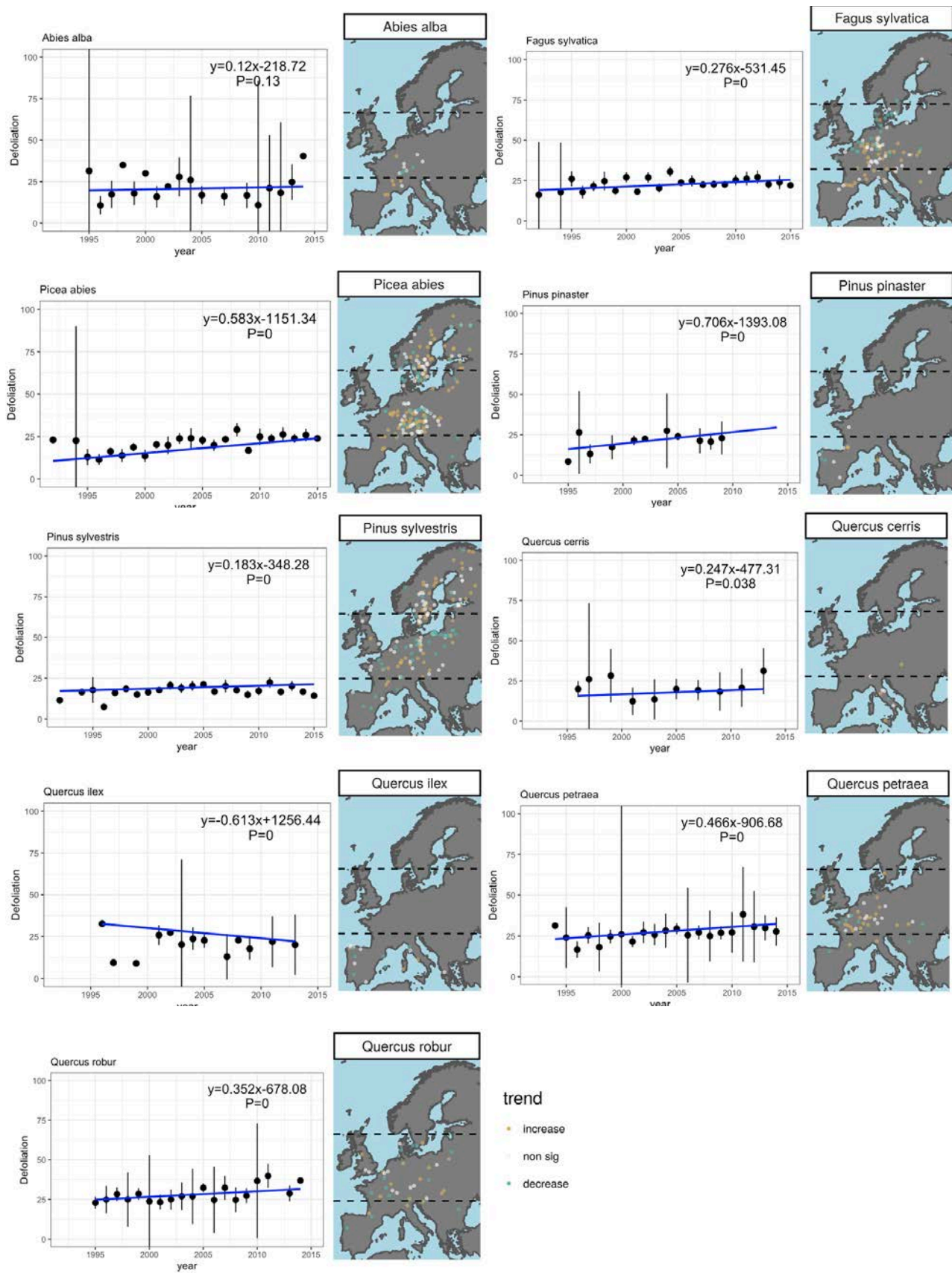
Figure 3.3. Trends and distribution of data by species, for each study species with n > 40 and plots > 5. Equation and p-value provided for each regression. Bars represent the point range for each year. P=0 means P<0.0009

Table 3.1. Summary of mixed model results for defoliation % across Europe, and separated by latitudinal fringe. Coefficients and p-values are shown for each variable.

All Europe (n=3,869)	Intercept	MAP spring	MAT spring	N dep. ox.	S ox.	SPEI12 AnnualAvg
Value	5.492	0.043	0.736	0.003	-0.002	-0.648
p-value	0.003	0.000	0.000	0.000	0.001	0.000

Nothern Europe (n=464)	Intercept	MAP spring	MAT spring ant.	foliar N		
Value	11.008	0.049	1.109	-0.772		
p-value	0.008	0.027	0.000	0.001		

Central Europe (n=2,720)	Intercept	MAP ant.	MAT ant.	MAT spring	S ox.	SPEI01 SummerAvg
Value	19.686	-0.040	-0.752	0.885	-0.005	0.521
p-value	0.000	0.000	0.000	0.000	0.000	0.000

Mediterranean latitudes (n=685)	Intercept	MAP spring				
Value	23.075	-0.038				
p-value	0.000	0.022				

3.3.3 Angiosperm and gymnosperm defoliation models

We found substantial differences in the variables that explained defoliation in angiosperms versus gymnosperms (Table 3.2). Defoliation in angiosperms was directly related to mean spring temperature, and by annual SPEI with a window of 3 months, and it was inversely correlated with mean winter precipitation, mean annual temperature of the previous year, sulfur oxide deposition, and SPEI annual average with a window of 12 months. In gymnosperms, defoliation was directly related to thermal amplitude, mean spring precipitation, mean spring temperature of the previous year, and N oxide deposition. It was negatively related to foliar N and annual SPEI with a 12-months window.

Table 3.2. Summary of mixed model results for defoliation % for angiosperms and gymnosperms. Coefficients and p-value are shown for each variable.

Angiosperms (n=1,401)	Intercept	MAP winter	MAT ant.	MAT spring	S ox.	SPEI 03 annual avg.	SPEI 12 annual avg.
Value	17.893	-0.033	-1.240	1.361	-0.002	1.109	-0.997
P-value	0.000	0.013	0.000	0.000	0.009	0.000	0.001

Gimnosperms (n=2,338)	Intercept	Thermal amplitude	MAP spring	MAT spring ant.	Foliar N	N dep. ox.	SPEI 12 annual avg.
Value	2.266	1.283	0.046	0.311	-0.305	0.004	-0.354
P-value	0.476	0.000	0.000	0.021	0.000	0.001	0.015

3.3.4 Defoliation trends by species

In species-specific models we found that defoliation in *Abies alba* was inversely correlated with the mean temperature of the previous year. In *Fagus sylvatica,* defoliation was inversely correlated

Table 3.3 Summary of mixed model results for defoliation % by species, where n > 40 individuals and the number of plots > 5. Variable coefficients and p-values are shown.

Abies alba (n=122)	Intercept	MAT ant.						
Value	32.823	-1.669						
p-value	0.000	0.029						

Fagus sylvatica (n=723)	Intercept	MAP winter	MAT ant	Foliar S				
Value	32.457	-0.037	-0.523	-2.615				
p-value	0.000	0.011	0.049	0.021				

Picea abies (n=1,162)	Intercept	Foliar Ca	MAP spring	MAT spring ant.	Foliar N	SPEI 12 annual avg.		
Value	13.116	-0.335	0.054	0.546	-0.288	-0.755		
p-value	0.000	0.050	0.000	0.008	0.045	0.001		

Pinus pinaster (n=67)	Intercept	MAT	Soil Org. C					
Value	72.990	-4.186	0.985					
p-value	0.000	0.000	0.004					

Pinus sylvestris (n=790)	Intercept	MAP spring	MAT ant.	MAT spring	Foliar P	pH difference	pH H2O	SPEI 12 annual avg.
Value	-6.060	0.054	-0.452	0.960	3.018	-3.535	3.375	-0.711
p-value	0.164	0.000	0.029	0.000	0.010	0.011	0.009	0.002

Quercus cerris (n=43)	Intercept	pH difference	Foliar S	SPEI 01 annual avg.				
Value	44.139	-0.519	5.224	1.541				
p-value	0.011	0.063	0.021	0.030				

Quercus ilex (n=85)	Intercept	Foliar S						
Value	10.600	10.783						
p-value	0.029	0.007						

Quercus petraea (n=275)	Intercept	MAT spring	MAT winter ant.	Foliar N	S ox.			
Value	8.432	2.151	-1.321	-0.356	-0.005			
p-value	0.433	0.000	0.002	0.072	0.003			

Quercus robur (n=216)	Intercept	MAT winter ant.						
Value	31.848	-0.893						
p-value	0.000	0.013						

with the mean precipitation in winter, mean temperature of the previous year, and foliar sulfur. Defoliation in *Picea abies* was directly correlated with the mean spring precipitation and mean spring temperature of the previous year, and was inversely correlated with foliar Ca, foliar N, and

yearly SPEI. In *Pinus pinaster,* defoliation was directly correlated with soil carbon and inversely correlated with mean annual temperature. Defoliation in *Pinus sylvestris* was directly correlated with mean spring precipitation, mean spring temperature, foliar P and soil pH, and inversely correlated with the mean annual temperature of previous year, the difference in pH calculated in water and in CaCl, which describes soil base saturation, and annual SPEI. *Quercus cerris* defoliation correlated directly with foliar sulfur and SPEI, and was inversely correlated with pH difference (pH in H2O - pH in CaCl). *Quercus ilex* correlated directly only with foliar S. Defoliation in *Quercus petraea* was directly correlated with the mean spring temperature, and was inversely correlated with the mean temperature of the previous winter, foliar N, and sulfur oxide deposition. Finally, *Quercus robur* defoliation was inversely correlated with the mean temperature of the previous winter (Table 3.3).

3.4 Discussion

3.4.1 Effects of foliar elemental composition on defoliation

Significant relationships between foliar elemental composition variables and defoliation in models for six from the nine analyzed species, and in the northern latitude fringe and in gymnosperms, highlight the importance of considering foliar elemental composition in attempts to understand and predict defoliation in European forests. According to our results for gymnosperms, *Picea abies* and *Quercus petraea* models*,* higher levels of foliar N were associated with low defoliation and could be considered an indicator of forest health in relative terms. Nitrogen is a crucial element for protein biosynthesis, light capture, and water-use efficiency, and thus it is directly linked to photosynthesis and foliar biomass (Field et al., 1983; Poorter et al., 2012; Evans and Clarke, 2019). N deficiency or decrease could contribute to the defoliation process. Furthermore, the detection of an inverse relationship between foliar N concentration and defoliation in the northern fringe is consistent with other studies showing that nitrogen supply limits plant production and growth in boreal forests (Högberg et al., 2017). In this case, foliar nitrogen is again suggested as a good indicator of forest health. In a nitrogen-deficient environment, an increase of nitrogen prevents defoliation in the whole fringe.

Another interesting finding was the relationship between defoliation and foliar S, which was surprising due to the different directions observed (i.e., it was directly correlated with defoliation in *Quercus ilex* and *Quercus cerris, but* and inversely correlated with defoliation in *Fagus sylvatica)*. S is an important basic protein constituent that is necessary for plant growth and development. Over the last few decades it has grown in importance because of a high amount of SO_x deposition in Europe, and associated plant toxicity (Pfanz and Beyschlag, 1993). However, due to environmental regulations, SO_x deposition started decreasing in the 1970s (Engardt et al., 2017). Our results suggest that defoliation increases with sulfur in the *Quercus* genera, which is

associated with sulfur toxicity by deposition that would have been absorbed. By contrast, *Fagus* appears to have a sulfur deficit, which causes a decrease in photosynthesis (Terry, 1976). This fact could be underlying the observed increase in defoliation in *F. sylvatica* over the study period.

P was present in the defoliation models for *Pinus sylvestris*. P is a basic requirement for plant growth because it is a component of genetic, metabolic, structural, and regulatory molecules, and frequently cannot be substituted by any other elements (White and Hammond, 2008). We would expect an inverse relationship between P and defoliation, given that *P. sylvestris* a fast-growing species that requires a large amount of P to rapidly build its structure. However, in our model, the relationship between foliar P and defoliation was direct. One possible explanation for this finding is seasonality. In other studies, seasonality has been shown to be related to higher levels of foliar P (Vallicrosa et al., 2021), where high seasonality and changing environmental conditions impair different tissues, leading to defoliation and favoring morphological and physiological adaptations. Consequently, in such changing environments the repair and generation of plant tissue may require higher levels of P. However, further research is needed to test this hypothesis. This explanation would also correspond with the extensive distribution range of *Pinus sylvestris* (Durrant et al., 2016), throughout which the species is subjected to very different pressures.

Defoliation in *Picea abies* had a negative relationship with foliar Ca, an essential plant nutrient that plays several structural roles in the cell wall and membranes. It is also a counter-cation for inorganic and organic anions in the vacuole. In addition, the Ca^{2+} concentration is also an obligate intracellular messenger, coordinating responses to numerous developmental cues and environmental challenges (White and Broadley, 2003). On this basis, it is reasonable to expect higher rates defoliation in the presence of a Ca^{2+} deficit.

The increase of atmospheric CO_2 has been related to a dilution process of foliar nutrients (Peñuelas et al., 2020). On this regard, if atmospheric CO_2 levels keep increasing as is predicted, the dilution of foliar nutrients would probably keep increasing proportionally. Consequently, the decrease of foliar nutrients such as N, P, Ca and S associated to climate change would also contribute to the defoliation increase of European forests.

3.4.2 Environmental effects on defoliation

Aside from foliar elemental composition, environmental conditions play a crucial role in understanding defoliation in European forests. We found relationships between defoliation, water availability, and deposition in the general model for all Europe. Precipitation in the spring increases defoliation, presumably due to nutrient leaching or/and light limitation due to clouds (Reinhardt and Smith, 2008). Aridity or 'less water availability' during the year also increases defoliation, possibly through embolisms in extreme conditions or simply poorer plant functioning. Thus,

defoliation associated with aridity could increase in the following years if the frequency and severity of droughts events increases in the Northern hemisphere, as is predicted with climate change (Meehk & Tebaldi, 2004; IPCC 2007; Van Oijen et al., 2014; Spinoni et al., 2017).

Nitrogen and sulfur deposition are associated with anthropic atmosphere contamination and are responsible for many ecological effects (Bobbink et al., 2013). Despite the fact that N and S deposition decreased in the recent decades (Engardt et al., 2017), nitrogen deposition still affects European forests, which can be seen as increasing defoliation in the general defoliation model (Table 3.1) and the gymnosperms defoliation model (Table 3.2). With respect to S deposition, this general negative correlation with deposition results from the coincidence of decreasing S deposition over recent decades with an almost generalized increase in defoliation.

The temporal range and homogeneity of precipitation also appeared to influence defoliation. In some of the models, such as the all-Europe model, central Europe model, and angiosperm, gymnosperm, *Picea abies,* and *Pinus sylvestris* models, we observed that although precipitation is crucial to avoid defoliation, intense periods of precipitation or torrential rains can provoke defoliation. Thus, a proper water income for the prevention of defoliation would be one that is continuous and homogenized. This hypothesis contrasts with current predictions of future precipitation patterns in Europe; models such as REMO 5.1 and the Intergovernmental Panel on Climate Change (IPCC) report predict increases in torrential rains in Europe due to climate change (Semmler and Jacob, 2004; IPCC, 2021). If these predictions are accurate, we would expect an increase in defoliation in Europe due to the concentration of precipitation. Indeed, this is already occurring.

The latitudinal fringe models (Figure 3.1) are important for accurately elucidating European defoliation responses to various pressures, because different pressures dominate in each latitudinal fringe that in turn differently affect forest defoliation and health. The most flagrant example is precipitation. In the northern and central fringes, precipitation in the spring increases defoliation but it decreases defoliation in the Mediterranean fringe. We attribute this result to the difference in water availability between latitudes, with water being more limiting in the Mediterranean. Where water is abundant, an increase of precipitation can decrease photosynthetic active radiation and favor leaching of the available nutrients, which hinders vegetal development. However, if water is limiting an increase in water income can be crucial to sprout. This effect has become even more important over recent decades, where droughts and aridity in the Mediterranean fringe have increased, with consequences for vegetation (Carnicer et al., 2011; Peñuelas et al., 2017).

The influence of short-term climatic legacies on defoliation is also remarkable. As seen in the latitudinal, angiosperm and gymnosperm, and species-specific models, the amount of

precipitation and temperature in the previous year commonly explains defoliation. The importance of climatic legacy on growth has been detected in the United States (Peltier et al., 2018) and Spain (Marqués et al., 2021). In this study, we also detected an effect of climatic legacy on defoliation at the European scale, by species and by latitudinal European region. Related to this finding, some seasons were found to be more crucial than others in determining defoliation, based on recurrence in the models. The most common seasons that appeared in our defoliation models were spring, followed by winter. In a seasonally changing climate such as that in Europe, which experiences four seasons over most of the continent, spring is the growing and sprout season for most species, especially deciduous ones. It seems reasonable that climatic variation in this season would determine yearly production.

3.4.3 Differences between defoliation rates

Previous studies using European defoliation record data from 1990 to 2007 showed an increase in defoliation only in the Mediterranean fringe, with the boreal and central fringes showing stable defoliation rates of approximately 15% and 20% respectively (Carnicer et al., 2011). In this study that includes data collected until 2015, we found that central Europe experienced higher rates of defoliation, followed by Mediterranean forests. It is likely that the drought effects of climate change are better tolerated in the Mediterranean fringe, which has historically been resistant to hot and dry conditions due to its climatic characteristics. By contrast, forests at central European latitudes, which evolved under more humid conditions, would suffer more severely during periods of drought. This hypothesis is consistent with the results of our central European latitude model (Table 3.1), which showed that reduced precipitation and the temperature of the last year increased defoliation.

The differences in defoliation between angiosperms and gymnosperms that we found were also remarkable. In all cases, defoliation levels in gymnosperms were higher. According to the presented models (Table 3.2), only water availability, expressed as SPEI12 annual Avg., affect both angiosperms and gymnosperms being more intense in angiosperms (-0.997) than in gymnosperms (-0.354). Despite the intensity of each factor affecting each group, differences between gymnosperms and angiosperms in defoliation reflect their physiology and internal functioning. The differences between gymnosperms and angiosperms have been of previous concern to the scientific community (Carnicer et al., 2013). Recently, it was concluded that angiosperms have a lower resistance to drought (low capacity to reduce the impact of initial drought) and gymnosperms show reduced recovery (to pre-drought level) (DeSoto et al., 2020). This difference is important because one extremely adverse year could have a serious effect on angiosperms defoliation, but only in that year. By contrast, the effects would last several years in gymnosperms, which could lead to the progressive accumulation of defoliation effects.

3.5 Final remarks

Defoliation has increased overall in European forests over the last decades but has decreased slightly at the northern latitudes. Defoliation in Europe is expected to keep increasing due to the predicted climate change conditions. Comparing between groups, gymnosperms showed higher rates of defoliation than angiosperms. Foliar elemental composition was related to defoliation in most European forest species, and was shown to be an informative complement to environmental variables and useful for assessing forest health. Nitrogen was the nutrient with the clearest effects, with the concentration of foliar nitrogen being inversely correlated with defoliation. However, other elements such as calcium, sulfur, and phosphorus also played a role in the defoliation of some European species. We emphasize the necessity of considering the different pressures that affect each climatic fringe, which in this case was perfectly exemplified by water availability at the Mediterranean latitudes versus the rest of Europe. Homogeneity of precipitation also appeared to be very important to reduce defoliation. The climatic legacy of the previous year also plays an important role in defoliation, as well as climatic variations in the spring. These findings should prove useful in the development of tools for assessing and understanding European forest health through defoliation data, which are broadly used and easily collected.

References

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J., Allard, G., Running, S.W., Semerci, A. & Cobb, N. (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For. Ecol. Manag.* **259(4)**, 660–684

Andreu, L., Gutiérrez, E., Macias, M., Ribas, M., Bosch, O. & Camarero, J.J. (2007), Climate increases regional tree-growth variability in Iberian pine forests. Global Change Biology, 13: 804-815. https://doi.org/10.1111/j.1365-2486.2007.01322.x

Bartoń, K. (2020) MuMIn: Multi-Model Inference. R package version 1.43.17. https://CRAN.R-project.org/package=MuMIn

Blanes, M. C., Viñegla, B., Salido, M. T. & Carreira, J. A. (2013). Coupled soil-availability and tree-limitation nutritional shifts induced by N deposition: insights from N to P relationships in *Abies pinsapo* forests. *Plant Soil* **366**, 67–81

Bobbink, Bal, D., Dobben, H.F., Jansen, A., Nijssen, M., Siepel, H., Schaminée, J., Smits, N., & Vries, W. (2013). The effects of nitrogen deposition on the structure and functioning of ecosystems.

Bussotti, F., Ferrini, F., Pollastrini, M. & Fini, A. (2014). The challenge of Mediterranean sclerophyllous vegetation under climate change: from acclimation to adaptation. *Environ. Exp. Bot.* **103**, 80–98 (2014).

Carnicer, J., Coll, M., Ninyerola, M., Pons, X., Sanchez, G. & Peñuelas, J. (2011). Widespread crown condition decline, food web disruption, and amplified tree mortality with increased climate change-type drought. Proceedings of the National Academy of Sciences. 108 (4): 1474-1478.

Carnicer, J. et al. (2013). Contrasting trait syndromes in angiosperms and conifers are associated with different responses of tree growth to temperature on a large scale . *Front. Pl. Sci.* **4,** 409.

Castagneri, D., Bottero, A., Motta, R. & Vacchiano, G. Repeated spring precipitation shortage alters individual growth patterns in Scots pine forests in the Western Alps. *Trees* **29**, 1699–1712 (2015).

De la Cruz, A. C. et al. (2014). Defoliation triggered by climate induced effects in Spanish ICP Forests monitoring plots. *Forest Ecology and Management* **331:** 245–255

De Marco, A. et al. Future impacts of nitrogen deposition and climate change scenarios on forest crown defoliation. *Environ. Pollut.* **194**, 171–180 (2014).

DeSoto, L., Cailleret, M., Sterck, F. *et al.* Low growth resilience to drought is related to future mortality risk in trees. *Nat Commun* **11**, 545 (2020). https://doi.org/10.1038/s41467-020-14300-5

De Vries, W., Dobbertin, M. H., Solberg, S., van Dobben, H. F. & Schaub, M. Impacts of acid deposition, ozone exposure and weather conditions on forest ecosystems in Europe: an overview. *Plant Soil* **380**, 1–45 (2014).

De Vries, W. & Posch, M. Modelling the impact of nitrogen deposition, climate change and nutrient limitations on tree carbon sequestration in Europe for the period 1900–2050. *Environ. Pollut.* **159**, 2289–2299 (2011).

Dewey Dunnington (2021). ggspatial: Spatial Data Framework for ggplot2. R package version 1.1.5. https://CRAN.R-project.org/package=ggspatial

Durrant, H. T., de Rigo, D. & Caudullo, G. *Pinus sylvestris* in Europe: distribution, habitat, usage and threats. In: San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Durrant, H. T. & Mauri, A. (eds) *European Atlas of Forest Tree Species* (Publ. Off. EU, 2016).

Eastaugh, C. S., Pötzelsberger, E. & Hasenauer H. Assessing the impacts of climate change and nitrogen deposition on Norway spruce (*Picea abies* L. Karst) growth in Austria with BIOME-BGC. *Tree Physiol.* **31**, 262–274 (2011).

Engardt, M., Simpson, D., Schwikowski, M. & Granat, L. Deposition of sulphur and nitrogen in Europe 1900–2050. Model calculations and comparison to historical observations. *Tellus B Chem. Phys. Meteorol.* **69**, 1328945 (2017).

European Environmental Agency. *Global and European temperatures.* https://www.eea.europa.eu/data-and-maps/indicators/global-and-european-temperature-10/assessment (2020).

Evans, J. R. & Clarke, V. C. (2019) The nitrogen cost of photosynthesis. *J. Exp. Bot.* **70**, 7–15.

Fernández-Martínez, M., Vicca, S., Janssens, I. *et al.* Nutrient availability as the key regulator of global forest carbon balance. *Nature Clim Change* **4,** 471–476 (2014). https://doi.org/10.1038/nclimate2177

Ferretti, M. Forest Health Assessment and Monitoring – Issues for Consideration. *Environ Monit Assess* **48,** 45–72 (1997). https://doi.org/10.1023/A:1005748702893

Ferretti, M. Potential and limitation of visual indices of tree condition. Chemosphere. **36**, 4–5, 1031-1036 (1998).

Ferretti, M., Fischer R. Forest monitoring, methods for terrestrial investigations in Europe with an overview of North America and Asia. Developments in Environmental Science. **12.** (2013)

Field, C., Merino, J. & Mooney, H. A. (1983). Compromises between water-use efficiency and nitrogen-use efficiency in five species of California evergreens. *Oecologia* **60**, 384–389

Gea-Izquierdo, G., Férriz, M., García-Garrido, S et al. Synergistic abiotic and biotic stressors explain widespread decline of Pinus pinaster in a mixed forest. Sci. Tot. Env. **1,** 685, 963-975 (2019).

Gottardini, E., Cristofolini, F., Cristofori, A et al. A multi-proxy approach reveals common and species-specific features associated with tree defoliation in broadleaved species. For. Eco. Man. **467,** 118151 (2020)

Hanewinkel, M., Cullmann, D. A., Schelhaas, M.-J., Nabuurs, G.-J. & Zimmermann, N. E. Climate change may cause severe loss in the economic value of European forest land. *Nat. Clim. Change* **3**, 203–207 (2013).

Harris, I., Osborn, T. J., Jones, P. & Lister, D. Version 4 of the CRU TS monthly high-resolution gridded multivariate climate dataset. *Sci. Data* **7**, 109 (2020).

Högberg, P., Näsholm, T., Franklin, O. & Högberg, M. N. Tamm Review: On the nature of the nitrogen limitation to plant growth in Fennoscandian boreal forests. *For. Ecol. Manag.* **403**, 161–185 (2017).

IPCC. *Climate Change 2007: Synthesis Report* (eds Core Writing Team, Pachauri, R. K. & Reisinger, A.) (IPCC 2007).

IPCC, 2021: Climate Change 2021: In press. The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. B. R. Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, R. Yu and B. Zhou (eds.)]. Cambridge University Press.

Jiang, Y., Rastetter, E.B., Shaver, G.R., Rocha, A.V., Zhuang, Q. and Kwiatkowski, B.L. Modeling long-term changes in tundra carbon balance following wildfire, climate change, and potential nutrient addition. Ecol Appl, **27**. 105-117. (2017)

Kauppi, P. E., Posch, M. & Pirinen, P. Large impacts of climate warming on the growth of Boreal forests since 1960. *PLoS One* **9**, e111340 (2014).

Komsta, L. mblm: Median-based linear models. R package version 0.12.1. https://CRAN.R-project.org/package=mblm (2019).

Lebourgeois, F., Rathgeber, C.B. and Ulrich, E. Sensitivity of French temperate coniferous forests to climate variability and extreme events (*Abies alba*, *Picea abies* and *Pinus sylvestris*). Journal of Vegetation Science, **21**: 364-376 (2010)

Linkosalo, T. Regularities and patterns in the spring phenology of some boreal trees. Silva Fennica 33(4): 237–245.(1999)

Loustau, D. et al. Modelling climate change effects on the potential production of French plains forest at the sub-regional level. *Tree Physiol.* **25**, 813–823 (2005).

Marqués, L. et al. Disentangling the legacies of climate and management on tree growth. *Ecosystems* https://doi.org/10.1007/s10021-021-00650-8 (2021).

Meehl, G. A. & Tebaldi, C. More intense, more frequent, and longer-lasting heat waves in the 21st century. *Science* **305**, 994–997 (2004).

Menzel, A., Dose, V. Analysis of long-term time series of the beginning of flowering by Bayesian function estimation. *Meteo. Zeit*. **14**, 3 (2005)

MENZEL, A., SPARKS, T.H., ESTRELLA, N., KOCH, E., AASA, A., AHAS, R., ALM-KÜBLER, K., BISSOLLI, P., BRASLAVSKÁ, O., BRIEDE, A., CHMIELEWSKI, F.M., CREPINSEK, Z., CURNEL, Y., DAHL, Å., DEFILA, C., DONNELLY, A., FILELLA, Y., JATCZAK, K., MÅGE, F., MESTRE, A., NORDLI, Ø., PEÑUELAS, J., PIRINEN, P., REMIŠOVÁ, V., SCHEIFINGER, H., STRIZ, M., SUSNIK, A., VAN VLIET, A.J.H., WIELGOLASKI, F.-E., ZACH, S. and ZUST, A. European phenological response to climate change matches the warming pattern. *Global Change Biology*, **12**: 1969-1976. (2006)

Mueller, R. C. et al. Differential tree mortality in response to severe drought: evidence for long-term vegetation shifts. *J. Ecol.* **93**, 1085–1093 (2005).

Peltier, D. M. P., Barber, J. J. & Ogle, K. Quantifying antecedent climatic drivers of tree growth in the Southwestern US. *Journal of Ecology.* **106**, 613–624 (2018).

Peñuelas, J., Gordon, C., Llorens, L. *et al.* Nonintrusive Field Experiments Show Different Plant Responses to Warming and Drought Among Sites, Seasons, and Species in a North–South European Gradient. *Ecosystems* **7,** 598–612 (2004). https://doi.org/10.1007/s10021-004-0179-7

Peñuelas, J., Ciais, P., Canadell, J.G. *et al.* Shifting from a fertilization-dominated to a warming-dominated period. *Nat Ecol Evol* **1,** 1438–1445 (2017). https://doi.org/10.1038/s41559-017-0274-8

Penuelas, J., Fernández-Martínez, M., Vallicrosa, H. *et al.* Increasing atmospheric CO2 concentrations correlate with declining nutritional status of European forests. *Commun Biol* **3,** 125 (2020). https://doi.org/10.1038/s42003-020-0839-y

Pfanz, H. & Beyschlag, W. Photosynthetic performance and nutrient status of Norway spruce (*Picea abies* L. Karst.) at forest sites in the Ore Mountains (Erzgebirge). *Trees* **7**, 115–122 (1993).

Phoenix, G. K. et al. Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments. *Glob. Change Biol.* **18**, 1197–1215 (2012).

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team nlme: Linear and nonlinear mixed effects models. R package version 3.1-144, https://CRAN.R-project.org/package=nlme> (2020).

Poorter, H. et al. Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *New Phytol.* **193**, 30–50 (2012).

Sardans, J. and Peñuelas, J. (2015), Potassium stoichiometry and global change. Global Ecology and Biogeography, 24: 261-275. https://doi.org/10.1111/geb.12259

Schmitz, A., Sanders, T.G.M., Bolte, A., Bussotti, F., Dirnböck, T., Johnson, J., Peñuelas, J., Pollastrini, M., Prescher, A-K., Sardans, J., Verstraeten, A., de VriesS, W. 2018. Responses of forest ecosystems in Europe to decreasing nitrogen deposition. Environmental Pollution 244 (2019)

Tausz M. (2007) Sulfur in forest ecosystems. In: Hawkesford M.J., De Kok L.J. (eds) Sulfur in Plants An Ecological Perspective. Plant Ecophysiology, vol 6. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-5887-5_3

Philip J. White, Martin R. Broadley. 2003. Calcium in plants. Annals of Botany 92: 487–511. https://doi.org/10.1093/aob/mcg164.

Reinhardt, K. & Smith, W. K. Impacts of cloud immersion on microclimate, photosynthesis and water relations of *Abies fraseri* (Pursh.) Poiret in a temperate mountain cloud forest. *Oecologia* **158**, 229–238 (2008).

Sardans, J. and Peñuelas, J. (2015), Potassium stoichiometry and global change. Global Ecology and Biogeography, 24: 261-275. https://doi.org/10.1111/geb.12259

Schmitz, A. et al. Responses of forest ecosystems in Europe to decreasing nitrogen deposition. *Environ. Pollut.* **244**, 980–994 (2019).

Semmler, T. & Jacob, D. Modeling extreme precipitation events—a climate change simulation for Europe. *Glob. Planet. Change* **44**, 119–127 (2004).

Shangguan, W., Dai, Y., Duan, Q., Liu, B. & Yuan, H., 2014. A Global Soil Data Set for Earth System Modeling. Journal of Advances in Modeling Earth Systems, 6: 249-263.

Slootweg, J., Posch, M., Hetteling, J.P., Mathijssen-Spiekman, E.A.M. Modelling nd mapping the impact of atmospheric deposition on plant species diversity in Europe. CEE status report.

Spinoni, J., Vogt, J. V., Naumann, G., Barbosa, P. & Dosio, A. Will drought events become more frequent and severe in Europe? *Int. J. Climatol.* **38**, 1718–1736 (2017).

Sun, L. K. et al. Leaf elemental stoichiometry of *Tamarix* Lour. Species in relation to geographic, climatic, soil, and genetic components in China. *Ecol. Eng.* **106**, 448–457 (2017).

Terrer, C., Jackson, R.B., Prentice, I.C. *et al.* Nitrogen and phosphorus constrain the CO2 fertilization of global plant biomass. *Nat. Clim. Chang.* **9,** 684–689 (2019). https://doi.org/10.1038/s41558-019-0545-2

Terry, N. Effects of Sulfur on the Photosynthesis of Intact Leaves and Isolated Chloroplasts of Sugar Beets , *Plant Physiology.* **57**, 4, 477–479 (1976)

Thomas, R. Q., Canham, C. D., Weathers, K. C. & Goodale, C. L. Increased tree carbon storage in response to nitrogen deposition in the US. *Nat. Geosci.* **3**, 13–17 (2010).

Vacek, S. et al. Effects of air pollution and climatic factors on Norway spruce forests in the Orlické hory Mts. (Czech Republic), 1979-2014. *Eur. J. For. Res.* **134**, 1127–1142 (2015).

Vallicrosa, H., Sardans, J., Maspons, J., Zuccarini, P., Fernández-Martínez, M., Bauters, M., Ciais, P., Obersteiner, M., Janssens, I. & Peñuelas, J. In press. Global maps and factors driving forest foliar elemental composition: the importance of genetic legacy.

Van Mantgem, P. J. et al. Widespread increase of tree mortality rates in the western United States. *Science* **323**, 521–524 (2009).

Van Oijen, M. et al. Impact of droughts on the carbon cycle in European vegetation: a probabilistic risk analysis using six vegetation models. *Biogeosciences* **11**, 6357–6375 (2014).

Vicca, S., Luyssaert, S., Peñuelas, J., Campioli, M., Chapin, F.S., III, Ciais, P., Heinemeyer, A., Högberg, P., Kutsch, W.L., Law, B.E., Malhi, Y., Papale, D., Piao, S.L., Reichstein, M., Schulze, E.D. and Janssens, I.A., Fertile forests produce biomass more efficiently. Ecology Letters, **15**: 520-526. (2012)

Vitasse, Y., Basler, D. What role for photoperiod in the bud burst phenology of European beech. *Eur J Forest Res* **132,** 1–8 (2013).

Vliet A. H., Groot R., Bellens Y., Braun P., Bruegger R. et al. The European Phenology Network. International Journal of Biometeorology, **4**, 202-212 (2003)

Wallace, Z. P., Lovett, G. M., Hart, J. E. & Machona, B. Effects of nitrogen saturation on tree growth and death in a mixed oak-forest. *For. Ecol. Manag.* **243**, 210–218 (2007).

Wang, R., Goll, D., Balkanski, Y., Hauglustaine, D., Boucher, O., Ciais, P., Janssens, I., Peñuelas, J., Guenet, B., Sardans, J., Bopp, L., Vuichard, N., Zhou, F., Li, B., Piao, S., Peng, S., Huang, Y., Tao, S. (2017) Global forest carbon uptake due to nitrogen and phosphorus deposition from 1850-2100. Global Change Biology 23, 4854-4872.

Wang, Y., Ciais, P., Goll, D., Huang, Y., Luo, Y., Wang, Y.-P., Bloom, A. A., Broquet, G., Hartmann, J., Peng, S., Penuelas, J., Piao, S., Sardans, J., Stocker, B. D., Wang, R., Zaehle, S., Zechmeister-Boltenstern, S. 2018. GOLUM-CNP v1.0: a data-driven modeling of carbon, nitrogen and phosphorus cycles in major terrestrial biomes. Geoscientific Model Development 11, 3903–3928

Wang, S., Zhang, Y., Ju, W. et al. Recent global decline of CO2 on vegetation photosynthesis. Science. **370**, 6522, 1295-1300. (2020)

White, P. J. & Broadley, M. R. Calcium in plants. *Ann. Bot.* **92**, 487–511 (2003).

White P. J. & Hammond J. P. Phosphorus nutrition of terrestrial plants. In: White P. J. & Hammond J. P. (eds) *The Ecophysiology of Plant-Phosphorus Interactions* (Springer, 2008).

Zang, C., Hartl-Meier, C., Dittmar, C., Rothe, A. & Menzel, A. Patterns of drought tolerance in major European temperate forest trees: climatic drivers and levels of variability. *Glob. Change Biol.* **20**, 3767–3779 (2014).

Zhao, J. H. gap: Genetic analysis package. R package version 1.2.3-1, https://CRAN.R-project.org/package=gap (2021).

Zhu, Z., Piao, S., Myneni, R. *et al.* Greening of the Earth and its drivers. *Nature Clim Change* **6,** 791–795 (2016). https://doi.org/10.1038/nclimate3004

Zimmermann, J., Hauck, M., Dulamsuren, C. & Leuschner, C. Climate warming-related growth decline affects *Fagus sylvatica*, but not other broad-leaved tree species in Central European mixed forests. *Ecosystems* **18**, 560–572 (2015).

4

Global maps and factors driving forest foliar elemental composition: the importance of evolutionary history

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Abstract

Consistent information on the current elemental composition of vegetation at global scale and the variables that determine it is lacking. To fill this gap, we gathered a total of 30912 georeferenced records on tree foliar concentrations of N, P, and K from published databases, and produced global maps of foliar N, P and K concentrations for woody plants using neural networks at a resolution of 1 km^2. We used data for climate, atmospheric deposition, soil, and morphoclimatic groups to train the neural networks. Foliar N, P and K do not follow clear global latitudinal patterns but are consistent with the hypothesis of soil substrate age. We additionally built generalized linear mixed models to investigate the evolutionary history effect together with the effects of environmental effects. In this comparison, evolutionary history effects explained most of the variability in all cases (mostly >60%). These results emphasize the determinant role of evolutionary history in foliar elemental composition, which should be incorporated in upcoming dynamic global vegetation models.

Keywords: Climate change; neural networks; global map; leaf; nitrogen; phosphorus; potassium

4.1 Introduction

In the Earth system, forests store close to 80% of all the biomass on earth, store 50 to 65% of terrestrial organic carbon and occupy a third of the terrestrial vegetated surface (Reichstein and Carvalhais, 2019). Despite its importance in carbon (C) cycles large uncertainties exist regarding stocks, turnover times and the carbon sink function in forest (Reichstein and Carvalhais, 2019). Recent studies show that accounting for stocks, fluxes, and availability of nitrogen (N), phosphorus (P) and potassium (K) in the plant-soil system can largely improve projections of

carbon cycles, especially when simulating global change impacts (Jiang et al., 2017; Sardans & Peñuelas, 2015; Peñuelas et al., 2017, 2020; Sun et al., 2017; Wang et al., 2018; 2019; Terrer et al., 2019). In fact, empirical evidence suggests that the availability of N, P and K limits the capacity of globally increasing C fixation by the rising atmospheric CO_2 concentrations (Vicca et al., 2012; Fernández-Martínez et al., 2014; Zhu et al., 2016; Bellassen et al., 2017, Wang et al 2020), which was recently addressed in Earth-system models (Meyerholt and Zaehle, 2015; Walker et al., 2015; Goll et al., 2017). Several studies have reported improvements in accuracy of the projections of the Earth-system models when information on N and P has been coupled to that of the C-cycle (Zaehle et al., 2010;2011;2015; Piao et al., 2013; Huang et al., 2016; Wang et al., 2017; Fernández-Martínez et al., 2019). The content/concentration of these elements in photosynthetic tissues is thus key for the functioning of terrestrial ecosystems, C-cycling, and for their response to current global changes, and can be further improved by the inclusion of other vegetation types in future studies.

The latitude-temperature theory supports a monotonic variation in foliar N and P concentrations with latitude due to latitude-induced shifts in temperature and precipitation. Nevertheless, this relationship remains unclear and sometimes opposite patterns have been reported (Reich and Oleksyn 2004; McGroddy et al. 2004; Kerkhoff et al. 2005; Yuan and Chen 2009; Ordoñez et al. 2009; Elser et al., 2010). The substrate age hypothesis, on the other hand, claims that older soils may be more N-abundant and P-limited than relatively younger soils (Walker and Syers 1976). Due to sustained P losses over geological timescales, P availability is expected to decrease with increasing soil age, implying a transition from N to P limitation when going from younger soils to older soils (Walker and Syers 1976; Chadwick et al. 1999; Vitousek et al., 2010). Although these global patterns might hold, foliar element concentrations can be highly heterogeneous on a regional scale. As such, foliar N and P concentrations have been shown to vary with mean annual precipitation within the tropics (Santiago et al., 2004) and within the temperate forest zone (Han et al., 2005). Additionally, soil type, temperature, water availability and even light intensity affect foliar nutrient concentrations (Ordoñez et al., 2009; Huxman et al., 2004; Voesenek and Pierik, 2008; Wang et al., 2012). This suggests that climatic and edaphic factors – should be included when trying to constrain variability in foliar nutrient concentrations. However, the consistency of these relations on a larger scale remains to be tested.

Most studies quantifying foliar elemental composition focus predominantly on foliar N and P, leaving foliar K largely understudied. Contrastingly, K is the most abundant nutrient in leaves after N and is considerably more abundant than P (Sardans et al., 2006; Zheng and Shangguan, 2007). Being a rock-derived nutrient, as P, its availability is mainly dependent on the parent material and weathering state (Catmak, 2005). Foliar K is important for water-use efficiency regulations in dry environments (Egilla et al., 2005; Graciano et al., 2005; Sardans et al., 2012a, 2012b) via its effects on stomatal function, cellular osmotic equilibrium and water fluxes (Babita et al., 2010; Laus et al., 2011; Sardans and Peñuelas 2015;2021). Importantly, K-limitation has been

reported across forests from different biomes (Olde Venterink et al., 2001; Tripler et al., 2006; Wright et al., 2011; Sardans and Peñuelas 2015; 2021), which further stresses the importance of including foliar K when trying to constrain foliar nutrient concentrations globally.

Evolutionary history effects are a long-term and complex product of the evolutionary processes resulting from a species' natural selection. This species adaptation to biotic and abiotic interactions with soil, climate and other species coexistence play a key role in determining the elemental foliar composition at continental (Sardans et al., 2015; Peñuelas et al., 2019) and global (Sardans et al., 2021) scales.

Bayesian models allow for the disentanglement of the species /taxonomical effects linked to long-term evolution (phylogeny) from those due to species/taxonomy linked to more recent convergent evolutionary processes, including strong selection within the phylogeny and recent epigenetic changes that are not directly due to and thus detected by strict use of phylogenetic distance matrix as covariable (Sardans et al., 2021). Apart from the evolutionary history effects, other abiotic (climate, soil type) and biotic (competition) factors play a significant role in explaining the foliar elemental composition variability found in large data sets of thousands of woody species growing in all continents (Sardans et al., 2015, 2021; Peñuelas et al., 2019).

Despite the important role of N, P and K in terrestrial ecosystems and their contribution to the global C-cycle models precision, only few studies have provided a global map approach to predict foliar N and P concentrations (Butler et al., 2017 and Moreno-Martínez et al., 2018). In the case of K, no gridded global map is available (Sardans et al. 2012a; Sardans and Peñuelas, 2015). Furthermore, a good understanding of the links between foliar nutrient concentrations and soil, climate and atmospheric deposition remains elusive while that evolutionary history effects on tree foliar nutrient concentrations at spatial scale remain unexplored as a whole.

Our first two aims were to (i) provide high resolution (1 km^2) grid maps of woody plants foliar N, P and K concentrations in woody communities at a global scale and (ii) identify global N, P and K patterns. We built models for foliar nutrient concentrations based only on environmental variables, without including the likely dominant evolutionary history effects, to provide relationships with the climate variables and thus allow to project changes of foliar composition in response to climate change. Our third aim was (iii) to investigate the evolutionary history effects in interaction with the effects of soil, climate and atmospheric deposition.

4.2 Results

4.2.1 High resolution (1 km^2) global maps of foliar N, P and K concentrations in woody communities based on environmental variables

The estimations of foliar N, P and K concentrations by neural network models showed precisions of 0.337, 0.040 and 0.181 normalized root mean squared error (RMSE) respectively and 0.63, 0.31
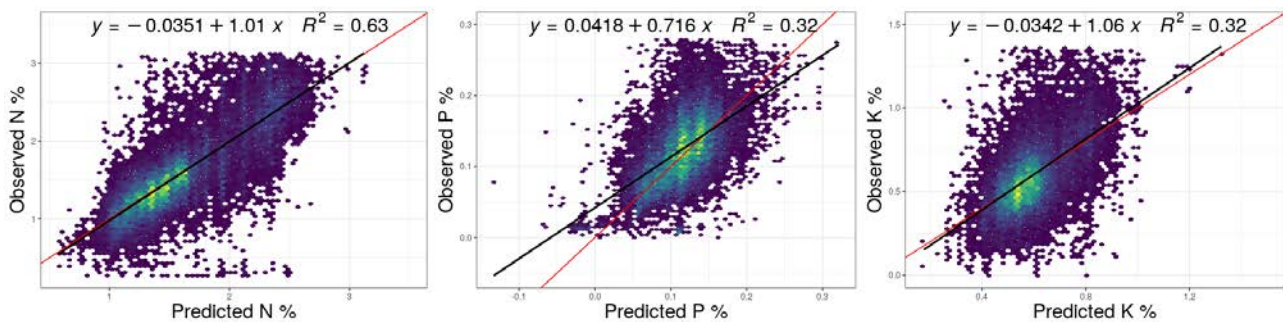
Figure 4.1 Observed versus predicted values for neural networks models predicting foliar N, P and K concentrations. Black line = fitted linear model between observed and predicted values. Red line = 1:1 line. RMSE = root mean squared error

and 0.32 R^2 respectively in predicted vs observed regressions (Figure 4.1).

Foliar N concentrations were predicted to be lowest in northwestern Eurasia and the north of North America. Foliar N concentrations were notably high in eastern Russia, central Africa, some regions in the Rocky Mountains and the Andes (Figure 4.2a) so to follow a latitudinal pattern at a glance is difficult. The map showing the standard error of the mean (SEM) estimation among replicates indicated that the predicted uncertainties were highest in mountainous areas, some regions in Siberia, the Labrador Peninsula, the Somali Peninsula and some sparse points (Figure S4.1a). The coefficient of variation (CV) for N in the neural-network models by morphoclimatic group ranged from 9.97 % in temperate coniferous to 33.46 % in Temperate coniferous, with a mean of 18.94 % CV for all forest types (Table S4.1).

The latitudinal pattern of foliar P concentrations is not clear either at a glance but lower levels are common in the tropical fringe. Foliar P concentrations were lowest in Northern Latitudes of North America, Florida, central Amazonia, some zones in central Africa, some zones in East Siberia, south-east of Asia and some zones in southern Australia. There were high foliar P concentrations in the Rocky Mountains and North America, the south of the Andes, central Europe, central parts of southern Africa and some spots in Siberia (Figure 4.2b). The high foliar P concentration in Siberia match with an uncertainty spot into the standard error of the mean map and also Labrador's Peninsula in an uncertain P zone. Other zones of uncertainty were in some parts of North America and in some isolated pixels in central Asia (Figure S4.1b). The CV for foliar P concentrations in the neural-network models ranged from 64.76% in temperate deciduous broadleaved to 109.30 % CV in tropical evergreen forests, with a mean of 98.26 % CV for all forest types (Table S4.1).

The predicted foliar K concentrations did not follow a global latitudinal pattern either. Some zones such as the Rocky Mountains, the southern Andes, the Somali Peninsula, and the North of Australia were predicted to have high foliar K concentrations. Foliar K concentrations were lowest
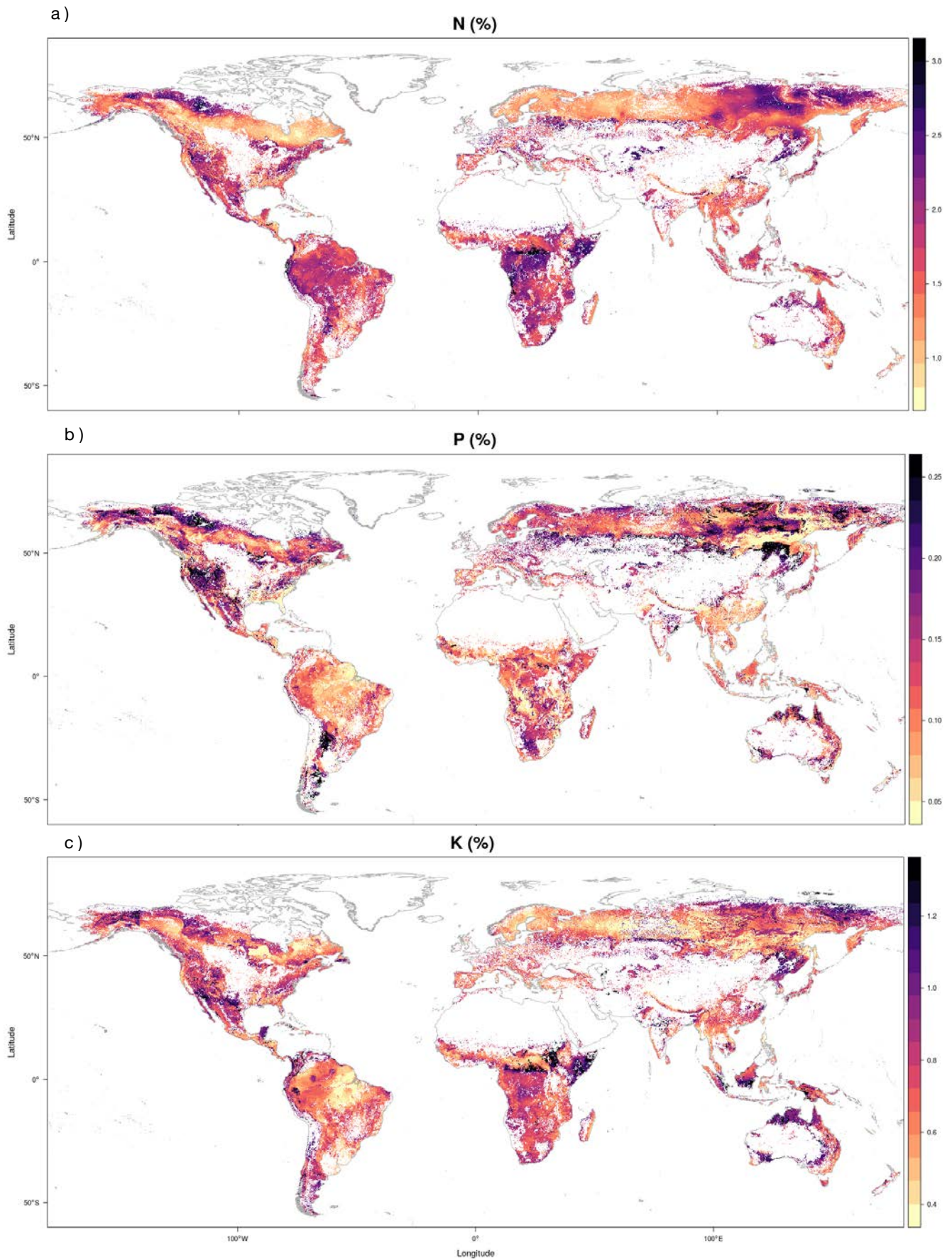
Figure 4.2 Neural Network predicted maps of foliar a) N, b) P and c) K concentration for woody plants. White areas indicate no woody vegetation.

at the mouth of the Amazonian River, in the Labrador Peninsula and central and west of Russia (Figure 4.2c). The zones with higher SEM are the south-east of Siberia, the south of Africa and the Rocky Mountains. The CV for K in the neural-network models ranged from 3.81 % in Temperate evergreen broadleaved forests to 27.64 % in Tropical evergreen, with a mean of 15.42 % CV for all forest types (Table S4.1).

4.2.2 Soil types

Spodosols and Histosols were the soil orders with lowest foliar N concentrations while Oxisols and Vertisols had the highest foliar N concentrations. The soil orders with the lowest foliar P concentrations were Oxisols and Ultisols, being Vertisols and Mollisols those with the highest foliar P concentrations. The lowest foliar K concentrations were found in Spodosols and Gelisols and the highest in Vertisols and Aridisols (Figure 4.3 and Table S4.2).

4.2.3 The environmental (climate and soil) versus the evolutionary history (phylogeny and species) effects in the different morphoclimatic types.

Nitrogen

The generalized linear models (glm) only with environmental variables conducted for each biome only explained from 5.5 % of the variability for temperate broadleaved evergreen to 38.8 % of the variability in N for boreal forests (Table S4.3). Soil pH, aridity index and solar radiation and precipitation seasonality correlated positively with foliar N concentrations in tropical evergreen forests. Soil gravel and evapotranspiration seasonality were positively correlated with foliar N concentrations while soil base saturation was negatively correlated in tropical deciduous forests. For temperate coniferous forest, foliar N concentrations correlated positively with soil pH and precipitation of the warmest quarter while soil base saturation evapotranspiration and soil pH were negatively correlated. In turn, foliar N concentrations in temperate broadleaved evergreens were correlated positively with aridity index, evapotranspiration and soil pH. In temperate broadleaved deciduous forests foliar N concentrations were correlated positively with annual precipitation and negatively with aridity index, soil pH and precipitation of the driest quarter. Finally, foliar N concentrations were positively correlated with minimum June, July and August precipitation and negatively correlated with maximum June, July and August precipitation and maximum temperature of the warmest month in boreal forests (Table S4.3).

The generalized mixed models (glmm), which besides environmental variables included the evolutionary history effects that comprise phylogeny and species, explained much more variance than glm. Evolutionary history effects (random variables in this case), also known as Pagel's lambda, explained 42-81% of the variability in foliar N concentrations while the exclusively environmental fixed variables explained only 1-58% (Table S4.4b). Phylogeny explained most of the variance in tropical evergreen, temperate coniferous, temperate broadleaved evergreen and boreal forests.
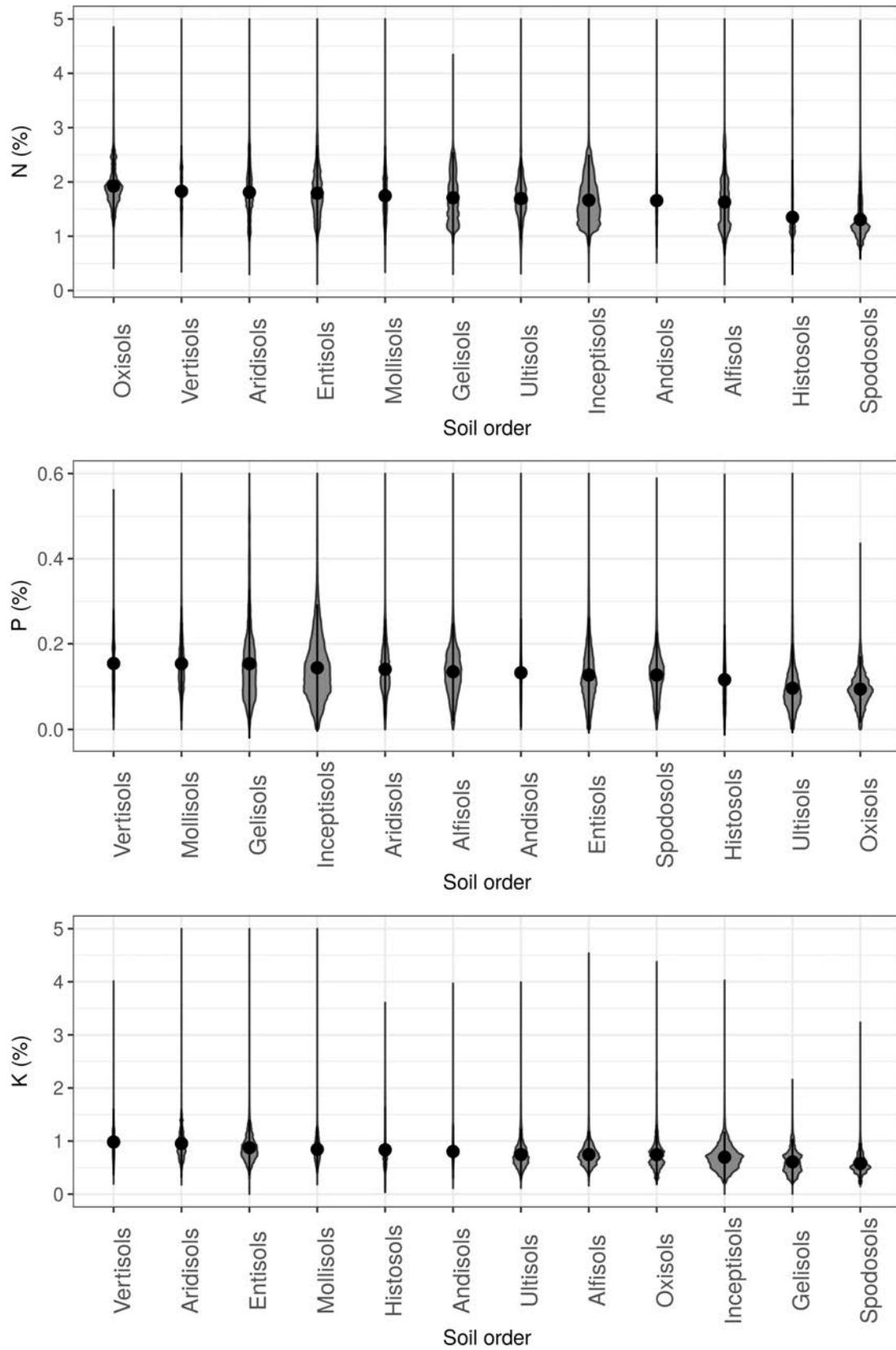
Figure 4.3 Violin plots of predicted values of foliar N, P and K concentrations grouped by soil order (USDA soil taxonomy). *The predicted values of foliar N, P and K concentrations differ significantly among soil types (P<0.05).*

Phosphorus

The glm models built entirely with environmental variables only explained from 5% of the total variance for tropical deciduous to 18% in temperate coniferous forests (Table S4.3), including: a positive relation of foliar P concentrations with evapotranspiration seasonality and a negative relation with inorganic N deposition, soil pH, isothermality and mean temperature of the driest quarter, in tropical evergreen forests; a positive relation of foliar P with exchangeable soil Ca and a negative relation with soil base saturation, soil pH and isothermality in tropical deciduous forests. Foliar P concentrations were positively related with aridity index and exchangeable soil K and negatively related with soil pH in temperate coniferous forests. In temperate broadleaved evergreen forests foliar P concentrations were negatively correlated with annual evapotranspiration, daily mean solar radiation, precipitation of the driest month and mean diurnal range. In temperate broadleaved deciduous forests a positive relation between evapotranspiration seasonality, solar radiation and precipitation of the driest month and foliar P concentrations has been found with also a negative relation with precipitation of the coldest quarter. Moreover, there

Without species and phylogeny
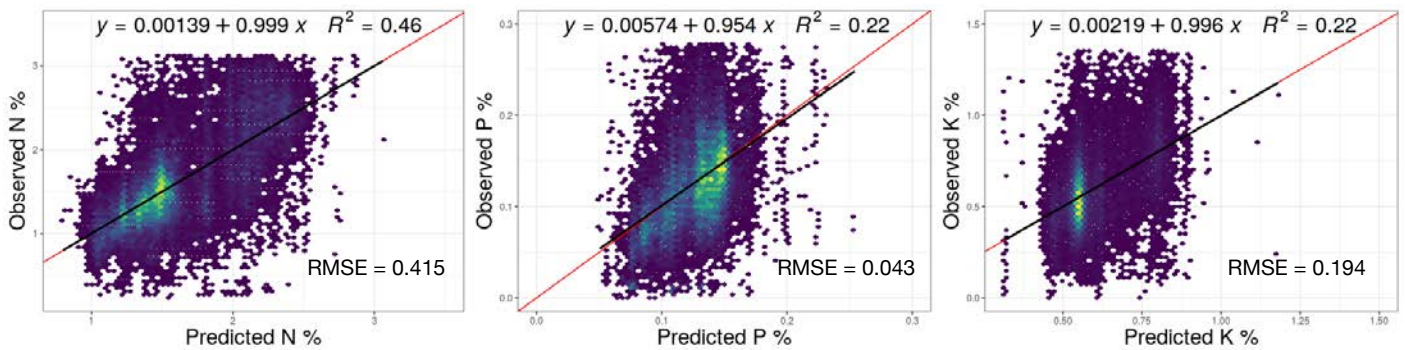
With species and phylogeny
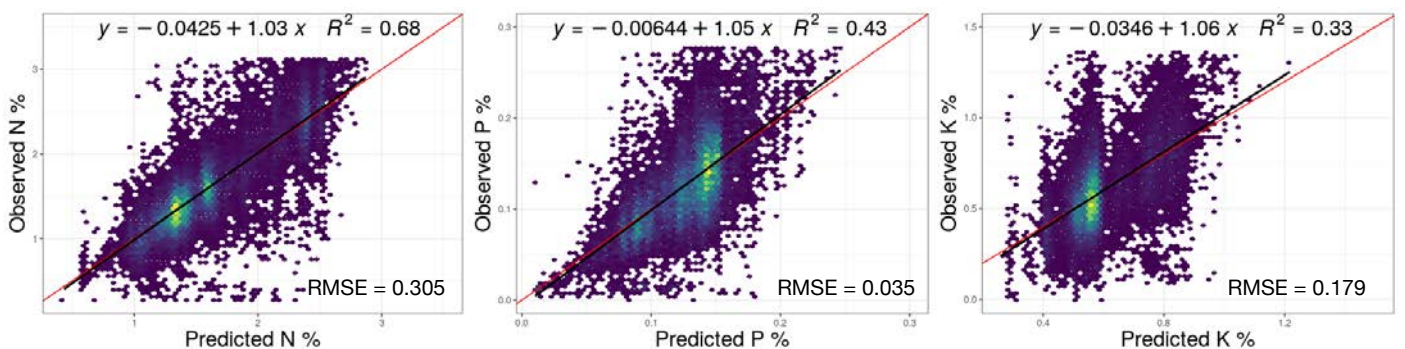
Figure 4.4 Observed versus predicted values for the generalized linear models (without legacy factors) and the generalized linear mixed models (with legacy factors) models predicting foliar N, P and K concentrations. Black line = fitted linear model between observed and predicted values. Red line = 1:1 line. Equation describing the black line. RMSE = root mean squared error.

was a positive relation between foliar P concentrations and maximum June, July and August

precipitation and mean annual temperature and a negative relation between foliar P concentrations and wet oxidate N deposition in boreal forest.

The glmm models, which besides environmental variables included the evolutionary history effects that comprise phylogeny and species, showed that these legacy effects explained most of the variance of foliar P concentrations, similar than in foliar N concentrations. The overall legacy effects thus explained 39-99% of total foliar P concentration variance in these biomes (Table S4.4b). Phylogeny explained most of the variance in tropical evergreen, temperate coniferous and boreal forests.

Potassium

The glm models built entirely with environmental variables explained total foliar K concentrations variance only between 3% for temperate broadleaved evergreen and temperate broadleaved deciduous and 97% for boreal forest types (Table S4.3). There was a positive relation between foliar K concentrations and soil exchangeable acidity and mean annual precipitation and a negative relation with dry N deposition in tropical evergreen. There was a negative relation between foliar K concentrations and evapotranspiration and wet reduced N deposition in tropical deciduous forests. Positive relationships between foliar K concentrations and aridity index, evapotranspiration seasonality, maximum June, July and August precipitation and mean annual temperature were found in temperate coniferous forest. There was a negative relation with solar radiation and precipitation of the driest quarter in temperate broadleaved evergreen forests. A positive relation between foliar K concentrations and soil pH and precipitation of the coldest quarter has been seen in temperate broadleaved deciduous. Finally, there was a positive relation between foliar K concentrations and mean temperature of coldest quarter, minimum temperature of the coldest month while there was a negative relation with dry N deposition and mean annual precipitation in boreal forests.

The glmm models, which besides environmental variables included the evolutionary history effects showed that these legacy effects explained most of the variance of the foliar K concentrations data, similar to foliar N and P concentrations. Legacy explained 22-98% of the variance, and the environmental fixed variables only explained from <1 to 8% of the variance (Table S4.4b).

The predictive power of the models for the whole dataset considering all the morphoclimatic types together increased highly when including evolutionary history effects in comparison with the models without them. The R^2 of the relationship between observed and predicted values increased from 46% to 68% for N, from 21% to 43% for P, and from 22% to 33% for K comparing the glm models with environmental variables against the glmm models considering also the evolutionary history (Figure 4.4).

4.3 Discussion

4.3.1 Global maps

We have produced raster maps with predicted foliar N, P and K concentrations with 1 km spatial resolution, by only considering environmental factors and no ecophysiological traits. Avoiding ecophysiological traits will allow for models to be easily adapted to future climate change scenarios, applying only the predicted environmental abiotic conditions. The addition of functional traits obtained by satellite remote sensing may offer slightly better performance (Moreno-Martínez et al. 2018), but these traits are difficult to predict and map for future scenarios. Our maps thus constitute the most reliable current proxy of global forest foliar elemental composition and can be easily used for modeling studies by providing the amount of foliar N, P and K concentrations in dry weight for each terrestrial location just from environmental variables.

The uncertainty of the predicted foliar concentrations was highest in zones with strong gradients of environmental variation and/or in zones with a low density of sampling points, such as the Rocky Mountains, the Himalayas and the Andes. Some of them also coincide with sparse vegetation points or big land extensions without experimental data available, like the Asian part of the boreal morphoclimatic type. The relationships between the observed foliar N, P and K concentrations and the expected concentrations in function of the neural network models used to build the maps have, however, proved to have reasonable goodness of fit and precision (Figure 4.1).

4.3.2 Environmental factors

The environmental drivers of foliar elemental composition were different across the six morphoclimatic types. This lack of common and consistent environmental global drivers is coherent with the divergence in results of more regional studies on foliar nutrients (e.g. Sandel et al., 2010 and Santiago et al., 2004). These diverging responses to the selected predictors reveal that plant sensitivity and determinant factors are dependent on morphoclimatic type emphasizing the differential pressures between latitudes and foliar type.

The correlations between foliar P concentrations and climatic variables seem to be partially ruled by instability of the system. In Tropical evergreen, Tropical deciduous and in Temperate broadleaved deciduous a higher seasonality or less isothermality is related with higher P levels. Foliar K concentrations were associated with water-dependent variables in Temperate coniferous, Temperate broadleaved evergreen and boreal, having higher K values in more arid conditions. These results are consistent with the narrow link between plant water-use efficiency and K concentrations (Sardans and Peñuelas, 2015) which acknowledges the osmotic use of K in water retention.

Foliar N and P concentrations under different soil types were consistent with the soil-age hypothesis at global scales, which describe young soils as N-limited and P-rich and old soils as N- rich and P -limited. Oxisols, which generally represent the oldest and more weathered surface soils, were sustaining woody plant communities with relatively more N and less P in their leaves, followed closely by Ultisoils which exhibited a similar pattern (Figure 4.4). This pattern, however, did not apply to forest communities on '*younger*' soils, where more P and less N is to be expected. Our corresponding explanation is at the level of the order of soil taxonomy's classification, and we must take into account that there is no family covering all the young soils. Vertisols is the soil order with simultaneously high levels of N, P and K . Those soils are characterized by high content of expansive clay and high cation exchange capacity (Soil Survey Staff. 1999).

4.3.3 Evolutionary history effects

The evolutionary history effects were found to explain most of the variability in foliar N, P and K concentrations, thus complementing and extending the prediction capacity of environmental factors. Most of the variance explained by the environmental factors in glm, was explained by evolutionary history effects in glmm. Species are associated with particular soil and climatic traits, so phylogeny incorporates climatic and soil conditions to some extent. This strong evolutionary history signal in foliar elemental composition has recently been reported in studies at local, regional and global scales (Castellanos et al., 2018; Peñuelas et al 2009, 2010, 2019; de la Riva et al., 2017; Hu et al., 2018; Reimann et al., 2018; Sardans and Peñuelas, 2015, Sardans et al, 2015, 2016b, 2020). For example, specific families with singular nutrient levels such as Fabaceae which are N-fixer have foliar N concentrations of 2.08 %, i.e. higher than the mean 1.62 % , whereas Proteaceae, which are typically from low-P environments (Hayes et al., 2021) have a mean of 0.066 % P, i.e. lower than the mean 0.126 %. Consequently, this key role of evolutionary history is important in order to determine the foliar elemental composition and is the most consistent explanation behind the lack of strong effect of climatic variables in similar studies of this topic (Reich and Oleksyn 2004; McGroddy et al. 2004; Kerkhoff et al. 2005; Yuan and Chen 2009; Ordoñez et al. 2009; Elser et al., 2010) that did not take evolutionary history effects into account.

Our results strongly resonate with the biogeochemical niche hypothesis (Peñuelas et al., 2008, 2019; Sardans et al., 2021), which predicts that different species, as evolutionary products, would have different optimal elemental compositions (elementomes). Phylogeny and species, as proxies of overall evolutionary history, explained on average more than 2/3 of the variability of the foliar concentrations of N, P and K. Phylogeny represents long-term evolutionary information, while species represent more short-term evolution, which underlie more recent evolution and coevolution of different and distant taxonomical clades by coinciding under similar environmental (climate, soil, competition) conditions. Several recent evolutionary events such as climate warming, species migration, shifts in species interactions (e.g. with herbivorous or parasitic species) and climatic convergence can all increase the rate of evolutionary convergence among

species from different clades or the evolutionary divergence within the same clade, and thus can also differentially increase the rate of evolution as observed in several characters in different species (Wright et al., 2006; Gillman et al., 2010; Kellner et al., 2011; Puurtinen et al., 2016; Jwa et al., 2017; Molina-Montenegro et al., 2018). All these factors cannot be "detected" by phylogeny but can be detected by taxonomy, in this case by the current species factor inside the evolutionary history effects.

The rate of species turnover in woody vegetation along spatial gradients, however, is expected to be outpaced by environmental change in short- and medium-term, as evidenced by projections of climatic, socio-economic and C-cycle models for the coming decades. The use of algorithms to predict foliar N, P and K concentrations in the near future has thus to be based on models including only climate, N deposition and soil traits that can effectively change in short time intervals of decades. Our study, though, identified the crucial explanatory role of evolutionary history effects as independent variables in global-scale models of foliar N, P and K concentrations. Evolutionary history effects were the main drivers controlling the foliar elemental composition under each environmental condition. Species is thus an important factor for plant functional traits present throughout the plant kingdom, for predicting the use of bioelements and thus for the biological control of biogeochemical cycles. The strong effects of evolutionary history suggest that the incorporation of dominant species into models, although difficult, would largely improve its predicting power. Species and their phylogeny, that represent long-term information stored in genes, together with more recent phenotypic/epigenetic shifts, determine the elemental composition of current plant cover.

The coexistence of species is another factor determining elemental composition. Considering coexistence in addition to environmental conditions and genetic legacies will favor even more the identification of the whole pool of variability of foliar elemental composition (Peñuelas et al. 2019). A possible approach with this purpose could come from combining our maps with GBIF (Global Biodiversity Information Facility) occurrences or improving the classification capacity of satellite imagery.

4.4 Final remarks and conclusions

We generated global maps for foliar N, P and K concentrations at resolutions of 1 km using neural-network machine learning, with mean R^2 values of 0.63, 0.32 and 0.32, and RMSE values of 0.34, 0.04 and 0.18 respectively, providing the best maps to date based only on environmental variables and without functional traits. The distribution of foliar N and P concentrations was consistent with the soil-age hypothesis. The pattern of foliar N, P and K concentrations and their relationships with environmental variables differed depending on the forest morphoclimatic type emphasizing different environmental pressures. Thus, the separation of global forest in the main morphoclimatic types is thus necessary to model forest canopy elemental composition. More than

that, adding evolutionary history effects through general linear mixed models to environmental variables largely increased the amount of explained variance emphasizing the role of evolutionary history in controlling foliar elemental composition, and encouraging the use of species in future models of foliar elemental composition.

4.5 Material and Methods

The methods summary is in figure 4.5.
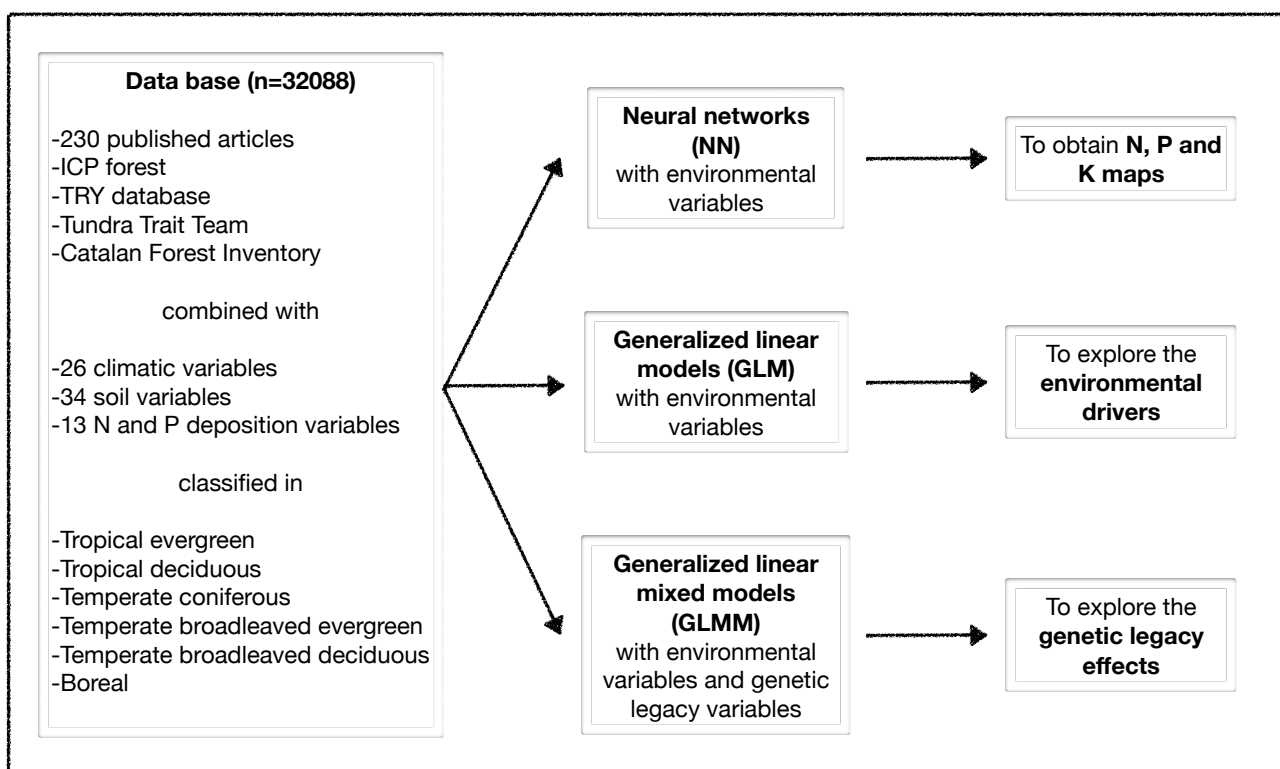
Figure 4.5 Summary of the methodology followed in this paper

4.5.1 Database generation

We combined 24631 inputs for N foliar concentrations in DW, 23726 for P foliar concentrations in DW and 18988 for K foliar concentrations in DW corresponding to woody plants around the world (Figure 4.6). The data were obtained from 257 published articles (Table S4.6), the TRY database (http://www.try-db.org/TryWeb/dp.php), the ICP Forest database (International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests, http://icp-forests.net/page/data-requests), the Tundra Trait Team and the Catalan Forest Inventory (Gracia et al., 2004). All foliar data were obtained using comparable analytical methods, mostly based on elemental analyzers for N, and on acid digestion coupled to optical spectrometry detection (in the majority of cases inductively coupled plasma to optical emission spectroscopy (ICP-OES) for P

and K). All foliar samples were mature leaves collected between 1990 and 2016. We only used data from georeferenced plots. The samples with values more than two times SD were considered outliers and removed in order to avoid analytical or transcription data errors. Final sample sizes (number of data points) and elements used in each model are shown in Table S4.5.
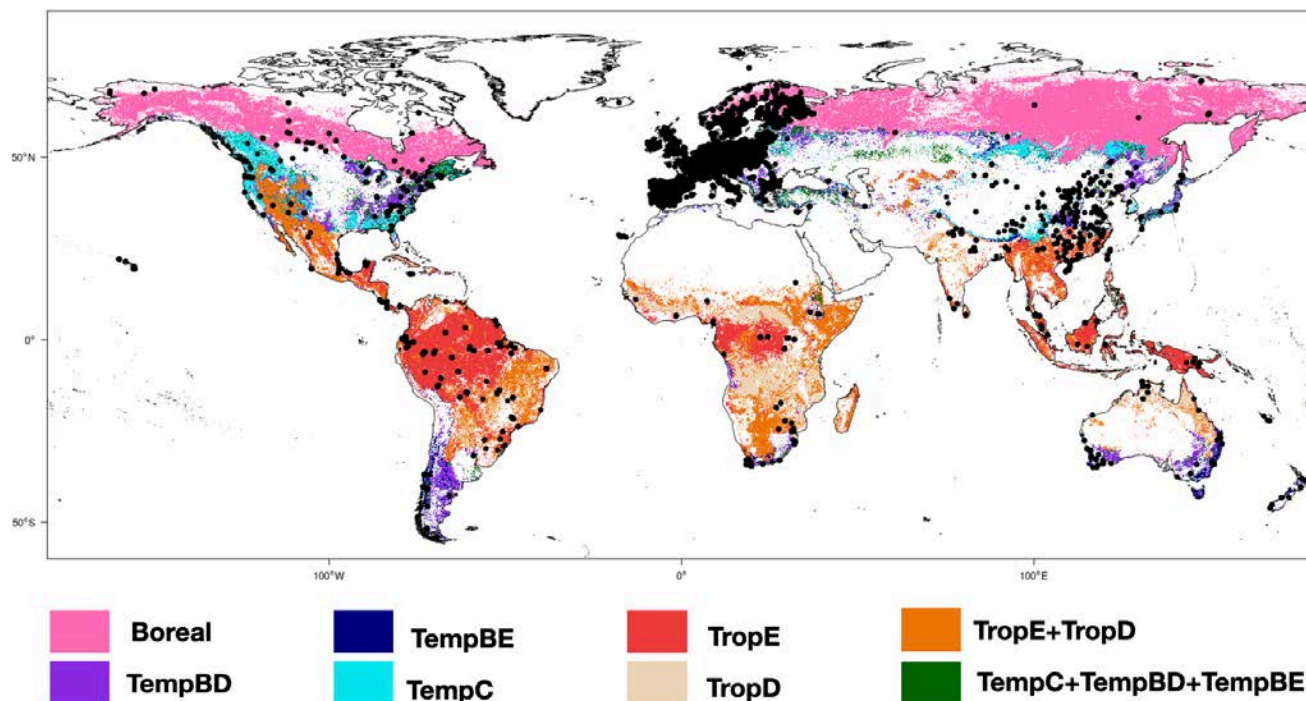
Figure 4.6 Sampling points for N, P and K. Colors corresponding to different morphoclimatic groups and its combinations in mixed forests. TempC = Temperate coniferous. TempEB= Temperate evergreen broadleaved. TempBD=Temperate broadleaved deciduous. TropE= Tropical evergreen. TropD= Tropical deciduous.

Data for soil, climate and N and P deposition were added to foliar concentrations using the *raster* (Hijmans, 2019) and *sf* (Pebesma, 2018) packages in R. Thirty-four soil variables from the Land-Atmosphere Interaction Research Group at Sun Yat-sen University were initially considered, at resolutions of 1 km at the equator (Shangguan et al., 2014). We initially considered 26 climatic variables, which were the standard bioclimatic variables from the WorldClim version 2 database (Fick and Hijmans, 2017) (Table S4.7), an aridity index and data for evapotranspiration from the CGIAR-CSI v2 database (Trabucco & Zomer, 2019) also at a resolution of 1 km2 at the equator. These climatic data have been estimated from long-term meteorological time series (1970-2000), based on interpolated climatic data provided by meteorological stations around the globe and adjusted to topography. Twelve variables of N deposition were extracted from Ackerman et al. (2018) (Table S4.7), which provided data for organic, inorganic, oxidate and reduced forms of N deposition at a resolution of 25 km at the equator The downscaling to 1 km was done with res function in raster R package (Hijmans et al., 2019). Data for P deposition were from Wang et al. (2017) at a resolution of 1 km and as a mean for 1997-2013 (Table S4.7).

This database of the foliar N, P and K concentrations and information for soil, climate and N and P deposition was classified in six morphoclimatic types of forest by geographical location and

species morphology. Those are tropical evergreen, tropical deciduous, temperate coniferous, temperate broadleaved evergreen, and temperate broadleaved deciduous and boreal. The tropical, temperate and boreal assignations were based on the WWF ecoregion map (Olson et al, 2001), where "Tundra" and "Boreal Forest/Taiga" account for boreal forests; "Temperate Broadleaf and Mixed forests", "Temperate grasslands, Savanas and Scrublands", "Montane grasslands and Scrublands", "Mediterranean Forests, woodlands and Scrub", "Temperate Conifer Forests" and "Deserts and xeric scrublands" higher than 30° latitude account for the temperate forests and "Tropical and subtropical Moist Broadleaved Forest", "Tropical and subtropical Dry Broadleaved Forest", "Tropical and subtropical grasslands, Savanas and Scrublands", "Tropical and subtropical Coniferous forests", "Mangroves", "Flooded Grasslands and Savannas" and "Deserts and xeric scrublands" lower than 30° latitude account for tropical forests. The evergreen, deciduous, coniferous and broadleaved designation was not assigned by map category but has been assigned by species characteristics according literature and specialist criterion in order to be more specific. In case some species could be deciduous or evergreen this species would be considered in both categories. Thus, the latitudinal designation is complemented by the foliar morphology in the different morphoclimatic type assignation. After, each morphoclimatic subset is the database used for each model training.

4.5.2 Neural networks and global maps of elemental composition

We built a total of 18 models, i.e. 6 groups of morphoclimatic types × 3 foliar variables (N, P and K concentrations), using neural networks in R with the package *keras* (Allaire & Chollet, 2019). The Kaiser-Meyer-Olkin method was applied to each subset of data for the 73 variables of soil, climate and deposition to automatically exclude the most strongly autocorrelated variables and avoid coliniality. We set the correlation threshold between 0.6 and 0.9 to obtain a set of the 19 remaining variables (Table S4.8). The datasets with remaining variables were then randomly divided into training, test and validation subsets, at a ratio of 60%, 20% and 20% (Lever et al., 2016), respectively and standardizing the data. A ponderation system based on weighting the samples by Europe and the rest of the World has been included to level the data origin and smooth the possible unbalancing effect from massive sampling in Europe. A neural network was constructed with one input layer, two hidden layers of 128 densely connected neurons and one output layer with one neuron, so we built independent networks for each nutrient and morphoclimatic forest type. The models were trained by optimizing mean squared errors on the test set. The validation data were used to cross-validate the accuracy of the model following the k-fold methodology and the root mean squared error (RMSE), the mean, the standard deviation, the coefficient of variation and R^2 was calculated for each model. Each model, was trained and calculated several times until stabilize the variation due to the random splitting of the data set. The mean of those values resulting of each repetition was calculated to obtain a final stable value with its corresponding standard error.

The variable importance function in the *Dalex* R package (Biececk, 2018) was used to identify the most influential predictor variables in the predicted outcome. This function calculates the loss function using the squared error of the model (_full_model_), randomizes each variable, calculates the loss in the absence of a predictive signal and calculates the loss with all variables randomized (_baseline_). We repeated this process 100 times per iteration in each model, for a mean total of 10 000 randomizations.

The land-cover map from ESA-CCI v2.0.7b at a resolution of 300 m was used to set up the model predictions for the six morphoclimatic forest types by element in three global raster maps. The resolution was modified to 1000 m using the aggregate function of the *raster* package with the mode as a gathering parameter. The land-cover map was then reclassified (Table S4.8) to our six morphoclimatic types to match the land-cover map with our prediction and use it to mask the vegetation for determining the distribution of each vegetation group. For mixed forests in the land cover map we calculated and plotted the corresponding mean values of each forests predictions for that region in order to better assess the reality. The neural network predictions were then plotted in three raster longitude/latitude WGS84 projection at a resolution of 1 km with the predicted percentages of foliar N, P and K concentrations in mature leaves of woody species. Uncertainty maps were also provided for each element using the mean standard error of the mean (SEM).

4.5.3 Generalized linear models

We selected the 10 most important variables based on their importance in the neural-network model to identify significant correlations with N, P, K concentrations. We then created a subset of the six least strongly correlated variables and built saturated glm models using these six variables and their first-degree interactions following gamma family distribution with an inverse link. We then applied the dredge function in the MuMIn R package (Barton, 2019) to select the final model based on the lowest Akaike information criterion and a maximum of six variables and interactions per model. We also determined the proportion of deviance explained by the glm or a pseudo-R^2 value with the Dsquared function into modEvA (Barbosa et al., 2015) R package.

4.5.4 Generalized linear mixed models

We used Bayesian generalized linear mixed models in the *MCMCglmm* R package (Hadfield 2010) to understand the combined role of phylogeny and species compared to the predictors used for the neural network (variables for soil, climate and deposition). We again selected the 10 most important variables for each biome and element based on the importance of the neural-network variables and also created a subset of the six least strongly correlated fixed variables for running the models. We also used the phylogenetic tree provided by Qian and Jin, 2015 which matches with 1580 of our species. Those corresponding species conformed to the random part of the model as "phylogeny" and the species group as "species". We ultimately built 6 × 3 models with

the form: element~var1+var2+var3+var4+var5+var6, random=phylogeny+species. The random variance explained by phylogeny and by species was thus also specified.

4.5.5 Soil age

We used the USDA classification of soil taxonomy when no map of soil age was available for determining whether and how the age of the substrate also influenced the foliar elemental compositions. The USDA classification partially and categorically considers soil age in their order divisions and allowed us to infer the effect of the soil age into elemental composition. We grouped our predicted N, P and K concentrations by their corresponding soil orders. Then we compared the orders using an ANOVA and a Tukey's post hoc test with the HSD.test from *agricolae* (Mendiburu, 2020) R package. Finally, we represented the results in violin plots by soil order.

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References

Ackerman, Daniel E; Chen, Xin; Millet, Dylan B. (2018). Global nitrogen deposition (2°×2.5° grid resolution) simulated with GEOS-Chem for 1984-1986, 1994-1996, 2004-2006, and 2014-2016. Retrieved from the Data Repository for the University of Minnesota, https://doi.org/10.13020/D6KX2R.

Allaire, J.J., François Chollet, F. (2019). keras: R Interface to 'Keras'. R package version 2.2.5.0. https://CRAN.R-project.org/package=kerasBabita, M., Maheswari, M., Rao, L.M., Shanker, A.K., Rao, D.G., 2010. Osmotic adjustment, drought tolerance and yield in castor (*Ricinus communis* L.) hybrids. Environ. Exp. Bot. 69, 243-249.

Babita, M., Maheswari, M., Rao, L.M., Arun K. Shanker, Gangadhar-Rao, D. Osmotic adjustment, drought tolerance and yield in Castor (*Ricinius communis L.*) hybrids (2010). Environmental and Experimental Botany, 69, 3, 243-249.

Barbosa, A.M., Real, R., Munoz, A.R. & Brown, J.A. (2015). New measures for assessing model equilibrium and prediction mismatch in species distribution models. Diversity and Distributions, 19(10), 1333-1338

Kamil Barton (2019). MuMIn: Multi-Model Inference. R package version 1.43.15. https://CRAN.R-project.org/package=MuMIn

Bellassen, V., Viovy, N., Luyssaert, S., Maire, G.m, Schelhaas, M.J., Ciais, P. (2017) Reconstruction and attribution of the carbon sink of European forest between 1950-2000. Global Change Biology 17, 3274-3292.

Biececk P (2018). "DALEX: Explainers for Complex Predictive Models in R." _Journal of Machine Learning Research_, *19*(84), 1-5. <URL:http://jmlr.org/papers/v19/18-416.html>.

Butler, E.E., Datta, A., Flores-Morenno, H., Chen, M., Wythers, K.R., Fazayeli, F., Banerjee, A., Atkin, O.K., Kattge, J., Amiaud, B., Blonder, B., Boenisch, G., Bond-Lamberty, B., Brown, K.A., Byun C., Campetella, G., Cerbolini, B.E.L., Cornelissen, J., H.C., Craine, J.M., Craven, D., de Vries, F.T., Díaz, S., Domingues, T.F., Forey, E., Gonazàlez-Melo, A., Gross, N., Han, W., Hattich, W.N., Hickler, T., Jansen, S., Kramer,

K., Kraft, N.J.B., Kurokawa, H., Laughlin, D.C., Meir, P., Minden, V., Niinemets, Ü., Onoda, Y., Peñuelas, J., Read, Q., Sack, L., Schamp, B., Soudzilovskaia, N.A., Spasojevic, M.J., Sosinski, E., Thorton, P.E., Valladares, F., van Bodegon, P.M., Williams M., Wirth, C., Reich, P.B. (2017) Mapping local and global variability in plant trait distributions. Proceedings of the National Academy of Sciences, USA, E10937-E10946.

Castellanos, A.E., Llano-Sotelo, J.M., Machado-Encinas, L.I., Lopez-Pina, J.E., Romo-Leon, J.R., Sardans, J., Peñuelas, J., 2018. Foliar C, N and P stoichiometry characterize successful plant ecological strategies in the Sonoran Desert. Plant Ecol. 219, 775-788.

Catmak, I., (2005) The role of potassium in alleviating detrimental effects of abiotic stresses in plants. J. Plant Nutrit. Soil Sci. 168, 521-530.

Chadwick OA, Derry LA, Vitousek PM, Huebert BJ, Hedin LO (1999) Changing sources of nutrients during four million years of ecosystem development. Nature 397:491–497

de la Riva, E.G., Maranon, T., Violle, C., Villar, R., Perez-Ramos, I.M., 2017. Biogeochemical and Ecomorphological Niche segregation of Mediterranean Woody species along a local gradient. Front Plant Sci. 8, art num 1242.

Egilla, J.N., Davies, F.T., Boutton, T.W., 2005. Drought stress influences leaf water content, photosynthesis, and water-use efficiency of *Hibiscus rosa-sinensis* at three potassium concentrations. Photosynthetica 43, 135-140.

Elser, J.J., Fagan, W.F., Kerkhoff, A.J., Swensson, N.G., Enquist, B.J. (2010) Biological Stoichiometry of plant production: metabolism, scaling and ecological response to global change. New Phytologist 186, 593-608.

Fick, S.E., Hijmans, R. (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology. 37, 12, 4302-4315. https://doi.org/10.1002/joc.5086.

Fernández-Martínez, M., Vicca, S., Janssens, I. *et al.* Nutrient availability as the key regulator of global forest carbon balance. *Nature Clim Change* **4,** 471–476 (2014). https://doi.org/10.1038/nclimate2177

Fernández-Martínez, M., Sardans, J., Chevallier, F. *et al.* Global trends in carbon sinks and their

relationships with CO2 and temperature. *Nature Clim Change* **9,** 73–79 (2019). https://doi.org/10.1038/s41558-018-0367-7

Gillman, L. N., Keeling, D. J., Gardner, R.C., Wright, S.D. (2010) Faster evolution of highly conserved in tropical plants. Journal of Evolution and Biology 23:1327-1330.

Goll, D.S., Vuichard, N., Maignan, F., Jornet-Puig, A., Sardans, J., Violette, A., Peng, S., Sun, Y., Kvakic, M., Guimberteau, M., Guenet, B., Zaehle, S., Peñuelas, J., Janssens, I.A., Ciais, P. (2017) A representation of the phosphorus cycle for ORCHIDEE (revision 4520). Geosciences Model Development 10, 3745-3770.

Gracia C., Ibàñez J.J., Burriel J.A., Mata T., Vayreda J. (2000-2004). *Inventari Ecològic i Forestal de Catalunya*. CREAF, Bellaterra. ISBN de la sèrie: 84-931323-0-6

Graciano, C., Guiamet, J.J., Goya, J.F., 2005. Impact of nitrogen and phosphorus fertilization on drought responses in *Eucalyptus grandis* seedlings. For. Ecol. Manag. 212, 40-49.

Hadfield, JD. (2010). MCMC Methods for Multi-Response Generalized Linear Mixed Models: The
MCMCglmm R Package. Journal of Statistical Software, 33(2), 1-22. URL
https://www.jstatsoft.org/v33/i02/.

Han W, Fang J, Guo D, Zhang Y (2005) Leaf nitrogen phosphorus stoichiometry across 753 terrestrial plant species in China. New Phytol 168:377–385

Hayes, P.E., Nge, F.J., Cramer, M.D. *et al.* Traits related to efficient acquisition and use of phosphorus promote diversification in Proteaceae in phosphorus-impoverished landscapes. *Plant Soil* **462,** 67–88 (2021). https://doi.org/10.1007/s11104-021-04886-0

Hijmans, R. J. (2019). raster: Geographic Data Analysis and Modeling. R package version 2.8-7. https://CRAN.R-project.org/package=raster

Hu, M.J., Peñuelas, J., Sardans, J., Sun, Z.G., Wilson, B., Huang, J.F., Zhu, Q.L., Tong, C., 2018. Stoichiometry patterns of plant organ N and P in coastal herbaceous wetlands along the East China Sea: implications for biogeochemical niche. Plant Soil 431, 273-288.

Huang, S., Bartlett, P., Arain, M.A. (2016) An analysis of global terrestrial carbon, water and energy dynamics using the carbon-nitrogen coupled CLASS-CTEM model. Ecological Modelling 336, 36-56.

Huxman, TE, Smith, MD, Fay, PA, Knapp, AK, Shaw, MR, Loik, ME, Smith, SD, Tissue, DT, Zak, JC, Weltzin, JF, et al. 2004 Convergence across biomes to a common rain-use efficiency. Nature 429, 651-654.

Jiang, Y., Rastetter, E.B., Shaver, G.R., Rocha, A.V., Zhuang, Q., Kwiatkowski, B.L. (2017) Modeling long-term changes in tundra carbon balance following wildfire, climate change, and potential nutrient addition. Ecological Applications 27, 105-117.

Jwa, N. S., Hwang, B. K. (2017) Convergent evolution of pathogen effectors toward reactive oxygen species signaling networks in plants. *Frontiers of Plant Sci*ence 8:1687.

Kellner, A., Ritz, C. M., Schlittenhaedt, P., Hellwig, F. H. (2011) Genetic differentiation in the genus Lithps L. (Ruschoideae, Aizoaceae) reveals a high level of convergent evolution and reflects geographic distribution. Plant Biology 13:368-380.

Kerkhoff AJ, Enquist BJ, Elser JJ, Fagan WF (2005) Plant allometry, stoichiometry and the temperature-dependence of primary productivity. Glob Ecol Biogeogr 14:585–598

Laus, M.N., Soccio, M., Trono, D., Liberatore, M.T., Pastore, D., 2011. Activation of the plant mitochondrial potassium channel by free fatty acids and acyl-CoA esters: a possible defense mechanism in the response to hyperosmotic stress. J. Exp. Bot. 62, 141-154.

Lever, J., Krzywinski, M. & Altman, N. Model selection and overfitting. *Nat Methods* **13,** 703–704 (2016). https://doi.org/10.1038/nmeth.3968

Liu, Y., Wang, T., Huang, M., Yao, Y., Ciais, P., Piao, S. (2016) Changes in interannual climate sensitivities of terrestrial cabon fluxes during the 21st century predicted by CMIP5 earth system models. Journal of Geophysical Research: Biogeosciences 121, 903-918.

McGroddy ME, Daufresne T, Hedin LO (2004) Scaling of C/N/P stoichiometry in forest worldwide: implications of terrestrial Redfield-type ratios. Ecology 85:2390–2401

Felipe de Mendiburu (2020). agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-2

Metzger, M.J.; Bunce, R.G.H.; Jongman, R.H.G.; Sayre, R.; Trabucco, A.; Zomer, R. A high-resolution bioclimate map of the world: A unifying framework for global biodiversity research and monitoring. Glob. Ecol. Biogeogr. 2013, 22, 630–638.

Meyerholt, J., Zaehle, S. (2015) The role of stoichiometry flexibility in modelling forest ecosystem responses to nitrogen fertilization. New Phytologist 208, 1042-1055.

Molina-Montenegro, M.A Acuña-Rodríguez, I. S., Flores, T. S. M., Hereme, R., Lafon, A., Atala, C., Torres-Díaz, C. (2018) Is the causes of plant invasions the result of rapid adaptive evolution in seed traits? evidence a latitudinal rainfall gradient? *Frontieres in Plant Science* 9:208.

Moreno-Martínez, A; Camps-Valls, G; Kattge, J; Robinson, N; Reichestein, M; van Bodegom, P; Kramer, K; Hans, J; Cornelissen, C; Reich, P; Bahn, M; Niinemets, U; Peñuelas, J; Craine, JM; Cerabolini, BEL; Minden, V; Laughlin, DC; Sack, L; Allred, B; Baraloto, C; Byun, C; Soudzilovskaia, NA; Running, SW. (2018). A methodology to derive global maps of leaf traits using remote sensing and climate data. Remote sensing of Environment. 218. 69-88.

Neirynck, J., Maddelein, D., Keersmaeker, L., Lust, N., Muys, B. Biomass and nutrient cycling of a highly productive Corsician pine stand on former heathland in northern Belgium. Ann For. Sci. 55 (1998) 389-405. DOI: 10.1051/forest:19980401.

Olde Venterink H, van der Vliet RE, Wassen MJ (2001) Nutrient limitation along a productivity gradient in wet meadows. Plant Soil 234:171–179

Olson, DM; Eric Dinerstein, Eric D. Wikramanayake, Neil D. Burgess, George V. N. Powell, Emma C. Underwood, Jennifer A. D'amico, Illanga Itoua, Holly E. Strand, John C. Morrison, Colby J. Loucks, Thomas F. Allnutt, Taylor H. Ricketts, Yumiko Kura, John F. Lamoreux, Wesley W. Wettengel, Prashant Hedao, Kenneth R. Kassem, Terrestrial Ecoregions of the World: A New Map of Life on Earth: A new global map of terrestrial

ecoregions provides an innovative tool for conserving biodiversity, *BioScience*, Volume 51, Issue 11, November 2001, Pages 933–938, https://doi.org/10.1641/0006-3568(2001)051[0933:TEOTWA]2.0.CO;2

Ordoñez JC, van Bodegom PM, Witte JPM, Wright IJ, Reich PB, Aerts R (2009) A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Glob Ecol Biogeogr 18:137–149.

Pebesma, E., 2018. Simple Features for R: Standardized Support for Spatial Vector Data. The R
Journal 10 (1), 439-446, https://doi.org/10.32614/RJ-2018-009

Peñuelas J, Sardans J, Ogaya R, Estiarte M (2008) Nutrient stoichiometric relations and biogeochemical niche in coexisting plant species: effect of simulated climate change. Polish Journal of Ecology 56: 613-622.

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Welde, M., Bopp, L., Boucher, O., Godderis, Y., Hinsinger, P., Llusia, J., Nardin, E., Vicca, S., Obersteiner, M., Janssens, I.A. (2013)Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. Nature Communications 4, 2934.

Peñuelas, J., Ciais, P., Canadell, JG., Janssens, I., Fernandez-Martinez, M., Carnicer, J., Obersteiner, M., Piao S., Vautard, R., Sardans, J. (2017). Shifting from a fertilization-dominated to a warming-dominated period. Nature Ecology and Evolution 1, 1438–1445.

Peñuelas, J., Fernández-Martínez, M., Ciais, P., Jou, D., Piao, S., Obersteiner, M., Vicca, S., Janssens, I., Sardans, J. (2019). The bioelements, the elementome, and the biogeochemical niche. Ecology. doi: 10.1002/ecy.2652

Peñuelas, J., Jannssens, I., Ciais, P., Obersteiner, M., Sardans, J. 2020. Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity, food security, and human health. Global Change Biology, doi: 10.1111/GCB.14981

Piao, S., Sitch, S., Ciais, P., Friedlingstein, P., Peylin, P., Wang, X., Ahlström, A., Anav, A.,

Canadell, J. G., Cong, N., Hun tingford, C., Jung, M., Levis, S., Levy, P.E., Li, J., Lin, X., Lomas, M.R., Lu, M., Luo, Y., Ma, Y., Myneni, R.B., Poulter, B., Sun, Z., Wang, T., Viovy, N., Zaehle, S., Zeng, N. (2013) Evaluation of terrestrial carbon cycle models for their response to climate variability and to CO_2 trends. Global Change Biology 19, 2117-2132.

Puurtinen, M. Elo, M., Jalasvuori, M., Kahilainen, A., Ketola, T., Kotiaho, J. S., Mönkkönen, M., Pentikäinen, O. T. (2016) Temperature-dependent mutational robustness can explain faster molecular evolution at warm temperatures, affecting speciation rate and global patterns of species diversity. Ecography 39: 1025-1033.

Qian, H. and Jin, Y. An updated megaphylogeny of plants, a tool for generating plant phylogenies and an analysis of phylogenetic community structure (2015). Journal of Plant Ecology. 1-7. doi:10.1093/jpe/rtv047

Reich, P.B. and Oleksyn, J. (2004) Global patterns of plant leaf N and P in relation to temperature and latitude. Proceedings of the National Academy of Sciences, USA. 101, 11001-11006.

Reichstein, M., Carvalhais, N. Aspects of Forest Biomass in the Earth System: Its Role and Major Unknowns. *Surv Geophys* **40,** 693–707 (2019). https://doi.org/10.1007/s10712-019-09551-x

Reimann, C., Fabian, K., Flem, B., Andersson, M., Filzmoser, P., Englmaier, P., 2018. Geosphere-biosphere circulation of chemical elements in soil and plant systems from a 100 km transect from southern central Norway. Sci. Total Env. 639, 129-145. DOI:10.1016/j.scitotenv.2018.05.070.

Sandel, B., Goldstein, L.J., Kraft, N.J.B., Okie, J.G., Shuldman, M.I., Ackerly, D.D., Cleland, E.E., Suding, K.N., 2010. Contrasting trait responses in plant communities to experimental and geographic variation in precipitation. New Phytol. 188, 565-575.

Santiago, L.S., Kitajima, K, Wright, S.J., Mulkey, S.S., 2004. Coordinated changes in photosynthesis, water relations and leaf nutritional traits of canopy trees along a precipitation gradient in lowland tropical forest. Oecologia 139, 495-502.

Sardans, J., Rodà, F., Peñuelas, J., 2006. Effects of a nutrient pulse supply on nutrient status of the Mediterranean trees *Quercus ilex* subsp. *ballota* and *Pinus halepensis* on different soils and under different competitive pressure. Trees 20, 619-632.

Sardans, J., Rivas-Ubach, A., Peñuelas, J., 2012a. The elemental stoichiometry of aquatic and terrestrial ecosystems and its relationships with organism life style and ecosystem structure and function: a review. Biogeochemistry 111, 1-39.

Sardans, J., Rivas-Ubach, A., Peñuelas, J., 2012b. The C:N:P stoichiometry of organisms and ecosystems in a changing world: a review and perspectives. Persp. Plant Ecol. Evol. Syst. 14, 33-47.

Sardans J., Peñuelas J. 2015. Potassium: a neglected nutrient in global change. Global Ecology and Biogeography 24: 261-275.

Sardans J, Janssens IA, Alonso R, Veresoglou SD, Rillig MC, Sanders TGM, Carnicer J, Filella I, Farre-Armengol G, Peñuelas J (2015) Foliar elemental composition of European forest tree species associated with evolutionary traits and present environmental and competitive conditions. Glob Ecol Biogeogr 24:240-255.

Sardans J, Alonso R, Janssens I, Carnicer J, Vereseglou S, Rillig MC, Fernández-Martínez M, Sanders TGM, Peñuelas J (2016a) Foliar and soil concentrations and stoichiometry of N and P across European *Pinus sylvestris* forests: relationships with climate, N deposition and tree growth. Funct Ecol 30:676–689, doi: 10.1111/1365-2435.12541

Sardans J, Alonso R, Carnicer J, Fernandez-Martinez M, Vivanco MG, Peñuelas J (2016b) Factors influencing the foliar elemental composition and stoichiometry in forest trees in Spain. Persp Plant Ecol Evol Syst 18:52–69.

Sardans J, Vallicrosa H, Zuccarini P, Farré-Armengol G, Fernandez-Martinez M, Pueguero G, Gargallo-Garriga A, Ciais P, Janssens IA, Obersteiner M, Richter A, Peñuelas J (2021) Can elemental composition define tree species? The biogeochemical niche hypothesis. Nature Ecol Evol. 5.2.184. DOI: 10.1038/s41559-020-01348-1

Sardans, J., Peñuelas, J. 2021. Potassium Control of Plant Functions: Ecological and Agricultural Implications. Plants. In Press.

Shangguan, W., Dai, Y., Duan, Q., Liu, B. and Yuan, H., 2014. A Global Soil Data Set for Earth System Modeling. Journal of Advances in Modeling Earth Systems, 6: 249-263.

Soil Survey Staff (1999) Soil taxonomy. A basic system of soil classification for making and interpreting soil surveys, 2nd edn. US Department of Agriculture Handbook 436. Natural Resources Conservation Service.

Sun, Y., Peng, S., Goll, D.S., Ciais, P., Guenet, B., Guimberteau, M., Hinsinger, P., Janssens, I.A., Peñuelas, J., Piao, S., Poulter, B., Violette, A., Yang, X., Yin, Y., Zang, H. (2017) Diagnosing phosphorus limitations in natural terrestrial ecosystems in carbon cycle models. Earth's Future 5, 730-749.

Terrer, C., Jackson, R., Prentice, i.C., Keenan, T., Kaiser, C., Vicca, S., Fisher, J., Reich, P., Stocker, B., Hungate, B., Peñuelas, J., McCallum, I., Soudzilovskaia, N., Cernusak, L., Talhelm, A., Van Sundert, K., Piao, S., Newton, P., Hovenden, M., Blumenthal, D., Liu, Y., Müller, C., Winter, K., Field, C., Viechtbauer, W., Van Lissa, C., Hoosbeek, M., Watanabe, M., Koike, T., Leshyk, V., Polley, H., Franklin, O. 2019. Nitrogen and phosphorus constrain the CO_2 fertilization of global plant biomass. Nature Climate Change 9, 684-689.

Trabucco, Antonio; Zomer, Robert (2019): Global Aridity Index and Potential Evapotranspiration (ET0) Climate Database v2. figshare. Fileset. https://doi.org/10.6084/m9.figshare.7504448.v3

Tripler, C.E., Kaushal, S.S., Likens, G.E., Walter, M.T., 2006. Patterns in potassium dynamics in forest ecosystems. Ecol. Lett. 9, 451-466.

Olson, D. M., Dinerstein, E., Wikramanayake, E. D., Burgess, N. D., Powell, G. V. N., Underwood, E. C., D'Amico, J. A., Itoua, I., Strand, H. E., Morrison, J. C., Loucks, C. J., Allnutt, T. F., Ricketts, T. H., Kura, Y., Lamoreux, J. F., Wettengel, W. W., Hedao, P., Kassem, K. R. 2001. Terrestrial ecoregions of the world: a new map of life on Earth. Bioscience 51(11):933-938.

Vergutz, L., Manzoni, S., Porporato, A., Novais, R.F. & Jackson, R.B. (2012) Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. Ecological Monographs, 82, 205–220.

Vicca, S; Luyssaert, S; Peñuelas, J; Campioli, M; Chapin III, FS; Ciais, P; Heinemeyer, A; Högberg, P; Kutsch, WL; Law BE; Malhi Y; Papale, D; Piao, SL; Reichestein, M; Schulze, ED; Janssens IA. (2012). Fertile forests produce biomass more efficiently. Ecology letters. 15 (6) 520-526.

Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications and nitrogen-phosphorus interactions. Ecol Appl 20:5–15

Voesenek, L.A.C.J., Pierik, R., 2008. Plant science – Plant stress profiles. Science 320, 880-881.

Walker, A.P., Zaehle, S., Medlyn, B.E., De kauwe, M.G., Asao, S., Hickler, T., Norby, R.J. (2015. Predicting long-term carbon sequestration in response to CO2 enrichment: how and why do current ecosystem models differ? Global Bioceochemistry Cycles 5, 1-20.

Walker TW, Syers JK (1976) The fate of phosphorus during pedogenesis. Geoderma 15:1–19

Wang, D., Heckathorn, S.A., Wang, X.Z., Philpott, S.M., 2012. A meta-analysis of plant physiological and growth responses to temperature and elevated CO2. Ocologia 169, 1-13.

Wang, R., Goll, D., Balkanski, Y., Hauglustaine, D., Boucher, O., Ciais, P., Janssens, I., Peñuelas, J., Guenet, B., Sardans, J., Bopp, L., Vuichard, N., Zhou, F., Li, B., Piao, S., Peng, S., Huang, Y., Tao, S. (2017) Global forest carbon uptake due to nitrogen and phosphorus deposition from 1850-2100. Global Change Biology 23, 4854-4872.

Wang, Y., Ciais, P., Goll, D., Huang, Y., Luo, Y., Wang, Y.-P., Bloom, A. A., Broquet, G., Hartmann, J., Peng, S., Penuelas, J., Piao, S., Sardans, J., Stocker, B. D., Wang, R., Zaehle, S., Zechmeister-Boltenstern, S. 2018. GOLUM-CNP v1.0: a data-driven modeling of carbon, nitrogen and phosphorus cycles in major terrestrial biomes. Geoscientific Model Development 11, 3903–3928

Wright, J. W., Davies, K. F., Lau, J. A., McCall, A. C., McKay, J. K. (2006) Experimental verification of ecological niche modelling in a heterogeneous environment. Ecology 87:2433-2439.

Songhan Wang, Yongguang Zhang, Weimin Ju, Jing M. Chen, Philippe Ciais, Alessandro Cescatti, Jordi Sardans, Ivan A. Janssens, Mousong Wu, Joseph A. Berry, Elliott Campbell, Marcos Fernández-Martínez, Ramdane Alkama, Stephen Sitch, Pierre Friedlingstein, William K. Smith, Wenping Yuan, Wei He, Danica Lombardozzi, Markus Kautz, Dan Zhu, Sebastian Lienert, Etsushi Kato, Benjamin Poulter, Tanja GM Sanders, Inken Krüger, Rong Wang, Ning Zeng, Hanqin Tian, Nicolas Vuichard, Atul K. Jain, Andy Wiltshire, Vanessa Haverd, Daniel S. Goll, Josep Peñuelas. Recent global decline of CO2 fertilization effects on vegetation photosynthesis. Science (in press)

Wright, S.J., Yavitt, J.B., Wurzburger, N., Turner, B.L., Tanner, E.V.L., Sayer, E.J., Santiago, L.S., Kaspari, M., Hedin, L.O., Harms, K.E., Garcia, M.N., Corre, M.D., 2011. Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. Ecology 92, 1616-1625.

Yuan ZY, Chen HYH (2009) Global trends in senesced-leaf nitrogen and phosphorus. Glob Ecol Biogeogr 18:532–542

Zaehle, S., Friedlingstein, P., Friend, A. D. 2010. Terrestrial nitrogen feedbacks may accelerate future climate change. Geophysical Research Latters 37, L01401.

Zaehle, S., Ciais, P., Friend, A. D., Prieur, V. 2011. Carbon benefits of anthropogenic reactive nitrogen offset nitrous oxide emissions. Nature Geosciences 4, 601-605.

Zaehle, S., Jones, C. D., Houlton, B., Lamarque, J. F., Boberston, E. 2015. Nitrogen availability reduces CMIP5 projections of twenty-first-century land carbón uptake. Journal of Climate 28:2494-2511.

Zheng S, Shangguan Z (2007) Soil patterns of leaf nutrient traits of the plants in the loess plateau of China. Trees 21:357–370

Zhu, Z., Piao, S., Myneni, R.B., Huang, M., Zeng, Z., Canadell, J.G., Ciais, P., Sitch, S., Friedlingstein, P., Arneth, A., Cao, C., Cheng, L., Kato, E., Koven, C., Li, Y., Lian, X., Liu, Y., Liu, R., Mao, J., Pan, Y., Peng, S., Peñuelas, J., Poulter, B., Pugh, T.A.M., Stocker, B.D., Viovy, N., Wang, X., Wang, Y., Xiao, Z., Yang, H., Zaehle, S., Zeng, N. (2016) Greening of the

earth and its drivers. Nature Climate Change 6, 791-795.162

5

Global distribution and drivers of forest biome foliar N:P ratios

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Abstract

Detailed understanding of patterns and drivers of terrestrial N:P ratios is essential for the accurate prediction of impacts of global change on ecosystem biogeochemical cycling and function. However, confirmation of the global distribution and drivers of foliar N:P ratios across contrasting terrestrial ecosystems remains lacking. In this study, we used neural network predictions to create a global map of woody plant foliar N:P ratios for four contrasting forest biomes (boreal, temperature coniferous and broadleaf, and tropical) based on a database comprising 24347 records. We then tested impacts on the N:P ratios of potential environmental drivers using generalized linear models and genetic drivers using generalized linear mixed models. We found that foliar N:P ratios are negatively associated with latitude, with higher N:P ratios occurring in tropical forests and lower N:P ratios occurring in boreal forests; globally, N:P ratios indicate greater levels of P limitation than N limitation. The influence of environmental factors varied among the four forest biomes, likely due to contrasting combined environmental conditions; this finding would have been obscured had we conducted a single "forest biome" analysis. Genetic legacy explained significant variation in woody plant foliar N:P ratios and we suggest its inclusion in future studies to improve N:P ratio predictions. Overall, our study will be useful to improve prediction of effects of global change on biogeochemical cycles and ecosystem functioning.

5.1 Introduction

In terrestrial systems, the ratio of nitrogen (N) to phosphorus (P) (N:P) content of plant foliar tissue is an indicator of spatio-temporal variations in plant physiological and ecosystem biogeochemical function, including N and/or P limitation or co-limitation (Schreeg et al., 2014), species niche occupation (Peñuelas et al., 2019), and vegetation carbon (C) retention capacity (Güsewell 2004; Ågren 2008; Tian et al., 2018). For example, in natural and agricultural ecosystems, biomass

growth is constrained by N:P leaf ratios (Vitousek & Howarth, 1991; Van Duren & Pegtel 2000; Das et al. 2006).

The relationship between foliar N:P content and terrestrial ecosystem biogeochemical function may be described by the growth rate hypothesis (GRH) that states that fast growing species require rapid protein synthesis, which in turn, requires large amounts of P-rich RNA. When essential resources, such as N or light, are at high levels of availability, increased growth rate is dependent on increases in P-rich RNA, as indicated by research that shows N:P ratios, particularly of leaf tissue, is negatively correlated with plant growth rate (Peng et al., 2011a; Rivas-Ubach et al., 2012; Yu et al., 2012; Yan et al., 2015; Jing et al., 2017). Under non-limiting conditions, balanced foliar N:P ratios of mature plants may be 14-16 on a mass basis (Elser et al., 2000; Knecht & Göransson, 2004; Zhang & Elser, 2017), although further research is needed for confirmation (Sardans et al., 2021), because Güsewell (2004) and Greenwood et al. (2008) reported ratios of N:P on a mass basis of <10 indicate limited N and ratios >20 indicate limited P, in contrast to Koerselman & Meuleman (1996), who reported ratios of <14 indicate limited N and ratios >16 indicate limited P. In terrestrial plants N:P increase relatively at low growth rates, given its relationship with amino acid limitation in protein synthesis and reaches a maximum. N:P decreases in high growth rate when the ensemble of amino acids rates is related to P-rich RNA (Ågren, 2004; Yu et al., 2012). In this relationships, though, N concentrations scales slower than P concentrations in leaves and its correlation is not linear (Ågren, 2004, 2008; Reich and Oleksyn, 2004; Kerkhoff and Enquist, 2005; Niklas et al., 2005; Niklas and Cobb, 2005; Sardans et al., 2012; Sardans et al., 2016, b; Tian et al., 2018).

These links between plant growth rate and overall N:P ratios also vary with ontogeny and climate conditions. At initial stages of development, N:P ratios are negatively correlated with plant growth, due to the allocation of the nutrients to biomass production (Zhang et al., 2019), whereas at the mature growth stage, when plants invest greater proportions of N and P to non-growth functions, this relation becomes weaker (Kerkhoff and Enquist, 2005; Peñuelas and Sardans, 2009; Rivas-Ubach et al., 2012; Zhang et al., 2019). Temperature and water availability have been shown to increase community level N:P ratios (Fan et al., 2016), due to more favorable growth conditions (Sardans et al., 2011; Sun et al., 2017), while under drought conditions, N:P ratios may shift to improve water use efficiency (Rivas-Ubach et al., 2012; Qiao et al., 2018; Sun et al., 2019; Peñuelas et al., 2020; Sardans et al., 2021).

Quantification of plant N:P ratios may be used as a tool to understand plant community structure and function (Zhao et al., 2019), including across successional stages within biomes, where they are expected to be lower in rapidly growing pioneer plant species, such as grasses, than in slow growing, late-successional species, such as tree species (Peñuelas et al., 2013; Sardans and Peñuelas, 2013; Busch et al., 2018). For example, N:P ratios increase with successional stage in African savanna vegetation (grasses: 8.6; broad-leaf trees: 13.7; and, fine-leaf trees: 18) (Ratnam et al., 2008) and in eastern China (grassland: 7.38; early successional mixed forest: 14-16; late successional mixed forest: 18-20) (Yan et al., 2008). These associations

are related to plant life history strategies that tend to vary across several stages of succession, as indicated by the associations between high N:P ratios and stress-tolerators, low N:P ratios and fast-growing, ruderal species, and intermediate N:P ratios and competitor species (Yan et al., 2008; Busch et al., 2018; Peñuelas et al., 2019). Changes in N:P ratios are known to affect the structure of terrestrial food webs (Fanin et al., 2013; et al., 2014; Zechmeister-Boltenstern et al., 2015; Paseka and Grunberg, 2019), while imbalances between N and P decrease ecosystem C retention capacity (Carnicer et al., 2015; Peñuelas et al., 2013) and affect ecosystem species composition, structure, diversity, and function (Sterner and Elser, 2002; Peñuelas et al., 2019;2020; Sardans et al., 2021).

In addition to local and regional scale variations in terrestrial foliar N and P concentrations and ratios, global-scale studies of their patterns and drivers have revealed monotonic decreases in N:P ratios from tropical to polar latitudes (McGroddy et al., 2004, Reich and Oleksyn, 2004; Kerkhoff et al., 2005; Yuan and Chen, 2009; Zhang et al. 2018, 2019b) that are consistent with latitudinal increases in N:P ratios with mean annual precipitation and temperature and the soil-age hypothesis (Walker and Sayers, 1976) that states older tropical soils may be more N-abundant and P-limited than relatively younger soils. However, these patterns and drivers of N:P ratios are inconsistent with findings of regional scale studies that have reported latitudinal increases in N:P (De Frenne et al., 2013; Sardans et al., 2016a,b; Zhang et al., 2019b). Our objectives were to a) use three approaches to create global maps of foliar N:P ratios across ecosystems, based on modeled climate, soil, and N and P deposition data; b) identify global drivers of woody vegetation N:P ratios; and, c) explore the role of genetic legacy (phylogenetics) in foliar N:P ratios of woody plants.

5.2 Materials and Methods

5.2.1 Database compilation

We compiled 25761 and 23815 globally distributed georeferenced records of woody plant N and P foliar concentrations, respectively, from the TRY (http://www.try-db.org) and ICP Forests (http://icp-forests.net) databases, the Catalan Forest Inventory (Gracia et al., 2004) and 230 published articles (Table S5.1). Data comprised comparable analytical methodologies, based on elemental analyses for N and sample acid digestion coupled to optical detection methods for P, for foliar samples collected between 1990 and 2016. Data with 2× standard deviation (SD) were considered outliers and removed to avoid mistakes in data collation or transcription; N:P ratios were then calculated from the remaining values, before data with 2× SD were again removed. Following outlier removal, our database comprised 20851 records.

Soil, climate, and N and P deposition data for locations of the georeferenced foliar N:P ratio data were input to the database using *raster* (Hijmans, 2020) and *sf* (Pebesma, 2018) R packages. Initially, we considered 34 soil variables from the Land-Atmosphere Interaction

Research Group at Sun Yat-sen University (Shangguan et al., 2014), 26 climate variables, comprising the standard bioclimate variables from the WorldClim version 2 database (Fick and Hijmans, 2017), and evapotranspiration and aridity index data from the CGIAR-CSI v2 database (Trabucco & Zomer, 2019) at 1-km resolution at the equator. The climate data comprise estimations interpolated from a globally distributed, meteorological time series (1970-2000) and adjusted for topography. Twelve N deposition variables, based on reduced, oxidized, inorganic, and organic forms of N deposition, at 25-km resolution at the equator from 1984 to 2016 were extracted from Ackerman et al. (2018); data were downscaled to 1-km using the *res* function in the R *raster* package (Hijmans, 2020). Mean P deposition data for the period 1997–2013 were derived from Wang et al. (2017) at a resolution of 1 km. Following exclusion of some variables, due to redundancy, the final database comprised 73 soil, climate, and N and P deposition variables as drivers of woody plant foliar N:P concentrations (Table S5.2).

The database was divided into four types of forest biome, comprising tropical, temperate coniferous, temperate broadleaved and boreal, based on the WWF ecoregion map (Olson et al., 2001) that classifies "tundra" and "boreal forest/taiga" as boreal forests, "temperate broadleaf and mixed forests", "temperate grasslands, savannas and scrublands", "montane grasslands and scrublands", "Mediterranean forests, woodlands and scrub", "temperate conifer forests" and "deserts and xeric scrublands" from >30° latitude as temperate forests, and "tropical and subtropical moist broadleaved forest", "tropical and subtropical dry broadleaved forest", "tropical and subtropical grasslands, savannas and scrublands", "tropical and subtropical coniferous forests", "mangroves", "flooded grasslands and savannas", and "deserts and xeric scrublands" <30° latitude as tropical forests (Figure S5.3). The distinction between temperate coniferous and broadleaf forests was based on species taxonomy.

5.2.2 Global models of woody plant foliar N:P ratios

Neural Networks

One model per forest biome was built based on neural networks (NN) using the *keras* package in R (Allaire & Chollet, 2019). The Kaiser-Meyer-Olkin method was applied to each of the four subsets of data for the 53 climate, soil, and deposition variables to exclude those that were most strongly autocorrelated and to avoid overparameterization; the correlation threshold was settled according to result in 19 remaining variables (Table S5.4). Then, the datasets with remaining variables were randomly divided into training (60%), test (20%), and validation (20%) subsets (Lever et al., 2016), and data were standardized using *scale* function in R (R core team, 2020). Due to the greater number of data records derived from Europe, data collected from outside the region were weighted to avoid any potential European bias. We built independent NNs for the four forest biomes, where they were constructed with one input layer, two hidden layers of 128 densely connected neurons and one output layer comprising one neuron. The validation data were used to cross-validate the accuracy of the model following the K-fold methodology and the root mean

squared error (RMSE), mean, SD, coefficient of variation, and R^2 were calculated for each model; the mean of values (±SE) from each repetition was calculated to obtain a final stable value.

The model predictions were extrapolated to the ESA-CCI land cover map v2.0.7b (ESA, 2017), at 300-m resolution, for the four forest biomes by element in three global raster maps; the resolution was modified to 1000-m using the aggregate function of the *raster* package in R (Hijmans, 2020), with mode as a gathering parameter. Then, the land-cover map was reclassified (Table S5.4) to the four forest biomes to match it with our predictions and use it to mask vegetation to determine the distribution of each forest type. The NN predictions were plotted at 1-km resolution in three raster longitude/latitude WGS 84 (World Geodesic System, 1984) projections, with the predicted woody plant foliar N:P ratios for mature leaves. Uncertainty maps were also provided for each element using the mean SE.

Generalized linear models

To test for associations between the soil, climate, and N and P deposition variables and N:P ratios, we selected the 10 most important variables, based on their NN variable importance, among which, a subset of the six least correlated variables was created and used to build generalized linear models (GLMs), with first-degree interactions following the gamma family distribution with inverse link. We then applied the dredge function in the *MuMIn* R package (Barton, 2019) to select the final models, based on the lowest Akaike information criterion, that comprised a maximum of six variables and interactions per model. The determined proportion of deviance explained by the GLM, or a pseudo-R^2 value, was calculated using the Dsquared function in the *modEvA* (Barbosa et al., 2015) R package.

Generalized linear mixed models

We used Bayesian generalized linear mixed models (GLMMs) in the *MCMCglmm* R package to understand the combined role of phylogeny and species compared with the roles of the soil,

Table 5.1 Neural network performance for all morphoclimatic groups. RMSE = Root mean squared error. MAE = Mean absolute error. SD = Standard deviation. CV = Coefficient of variation

	Tropical	Temperate coniferous	Temperate broadleaves	Boreal
RMSE (%)	7.077	3.622	5.619	1.918
MAE (%)	5.504	2.614	4.181	1.370
Mean (%)	20.698	14.442	18.827	12.346
SD (%)	1.972	1.453	1.677	1.730
CV (%)	0.095	0.101	0.089	0.140

climate, and deposition predictors used for the NN. For the NN predictors, we again selected the 10 most important variables for each forest type and element, based on their importance in the

NN, and created a subset of the six least strongly correlated fixed variables. For phylogeny, we used the phylogenetic tree provided by Qian and Jin (2015) that contained 744 of the species in our database; the species and their phylogenetic relations conformed to the random part of the models. The four models followed the form: element ~ var1+var2+var3+var4+var5+var6, random=phylogeny+species. The random variables, comprising species and phylogeny, describe the long-term evolutionary effects on genotypes, where phylogeny targets the response magnitude, due to phylogenetic distance, and species targets interspecific variability not directly related to phylogenetic distance.

5.2.3 Soil age

We used the USDA classification (Soil survey staff, 2014) of soil taxonomy at the order level to determine the influence of substrate age on woody plant foliar N:P ratios in the four forest types. We grouped the predicted N:P ratios from the NNs by their corresponding soil order and then compared the orders using ANOVA and Tukey's post hoc test with the HSD.test function in the *agricolae* (Mendiburu, 2020) R package; the results were visualized as violin plots by soil order, using ggplot2 (Wickham, 2016) by mean descending order.

5.3 Results

5.3.1 Global distribution of woody plant foliar N:P ratios

Of the three approaches used to predict woody plant foliar N:P ratios, greatest predictive power, based on predicted vs observed R^2, was provided by NNs ($R^2 = 0.69$; RMSE = 3.80) (Figure 5.1). The NNs predicted mean N:P ratios for the forest types were greatest in tropical forests (20.7±2.0), followed by temperate broadleaved forests (18.8±1.7), temperature coniferous forests (14.4±1.5), and boreal forests (12.4±1.7) (Table 5.1).
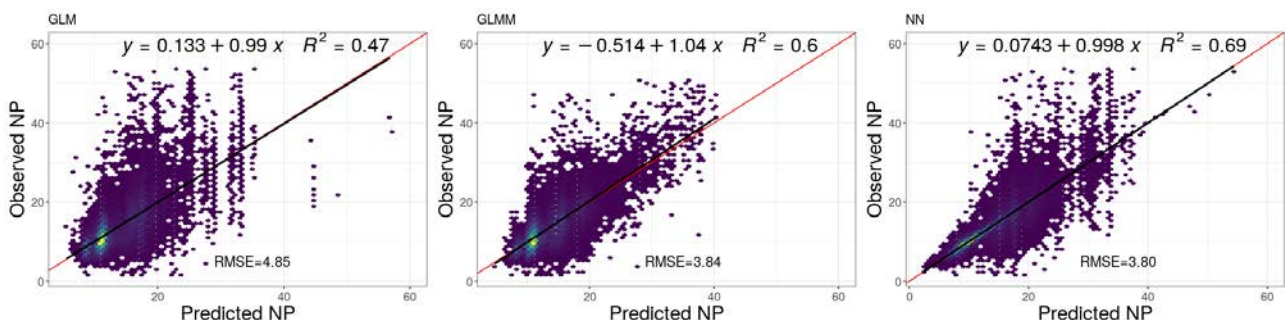
Figure 5.1 Predicted versus observed foliar N:P values across the four forest biomes estimated by generalized linear modelling (GLM), generalized linear mixed modelling (GLMM), and neural networks (NN). Black line is the fitted linear model between predicted and expected values; red line is line of unity.

The global distribution of woody plant foliar N:P ratios, based on NN predictions (Figure 5.2), confirmed lowest ratios are largely concentrated in boreal regions, particularly in northern Europe, as well as other regions, including India, northern Sub-Saharan Africa, and Patagonia. In contrast, regions with higher N:P ratios are generally located in the tropics and subtropics,

Table 5.2 Summary of the generalized linear model per morphoclimatic group.

		Estimate	Pr(>|t|)
Tropical	(Intercept)	0.050	0.000
	Soil base saturation	0.000	0.002
	Soil cation exchange capacity	0.001	0.000
	Potential evapotranspiration seasonality	-0.001	0.000
	Maximum December, January, February precipitation	-0.000	0.000
	Soil base saturation:soil cation exchange capacity	-0.000	0.000
	Soil base saturation:Potential evapotranspiration seasonality	0.000	0.000
	AIC / pR^2	25536.497	0.230
Temperate coniferous	(Intercept)	0.045	0.000
	Aridity index	-0.000	0.000
	Potential evapotranspiration seasonality	0.001	0.000
	Maximum June, July, August precipitation	0.001	0.000
	Mean temperature of the driest quarter	-0.002	0.000
	Aridity index:Mean temperature of the driest quarter	0.000	0.000
	Potential evapotranspiration seasonality:Maximum June, July, August precipitation	-0.000	0.000
	AIC / pR^2	61842.197	0.115
Temperate broadleaved	(Intercept)	0.087	0.000
	Minimum June, July, August precipitation	0.001	0.000
	Daily mean solar radiation	-0.000	0.000
	Precipitation of the driest month	-0.001	0.000
	Precipitation of the driest quarter	-0.000	0.000
	Minimum June, July, August precipitation:Daily mean solar radiation	0.000	0.000
	Daily mean solar radiation:Precipitation of the driest quarter	0.000	0.000
	AIC / pR^2	35556.667	0.098
Boreal	(Intercept)	0.186	0.000
	Maximum June, July, August precipitation	-0.006	0.000
	Mean temperature of warmest quarter	-0.008	0.012
	Precipitation of wettest quarter	0.002	0.000
	Maximum June, July, August precipitation:Mean temperature of warmest quarter	0.000	0.000
	Maximum June, July, August precipitation:Precipitation of wettest quarter	-0.000	0.000
	Mean temperature of warmest quarter:Precipitation of wettest quarter	-0.000	0.000
	AIC / pR^2	8655.749	0.103

including Amazonia, southeast Asia, southern Sub-Saharan Africa, northeast China, and north, south west Australia.

N:P
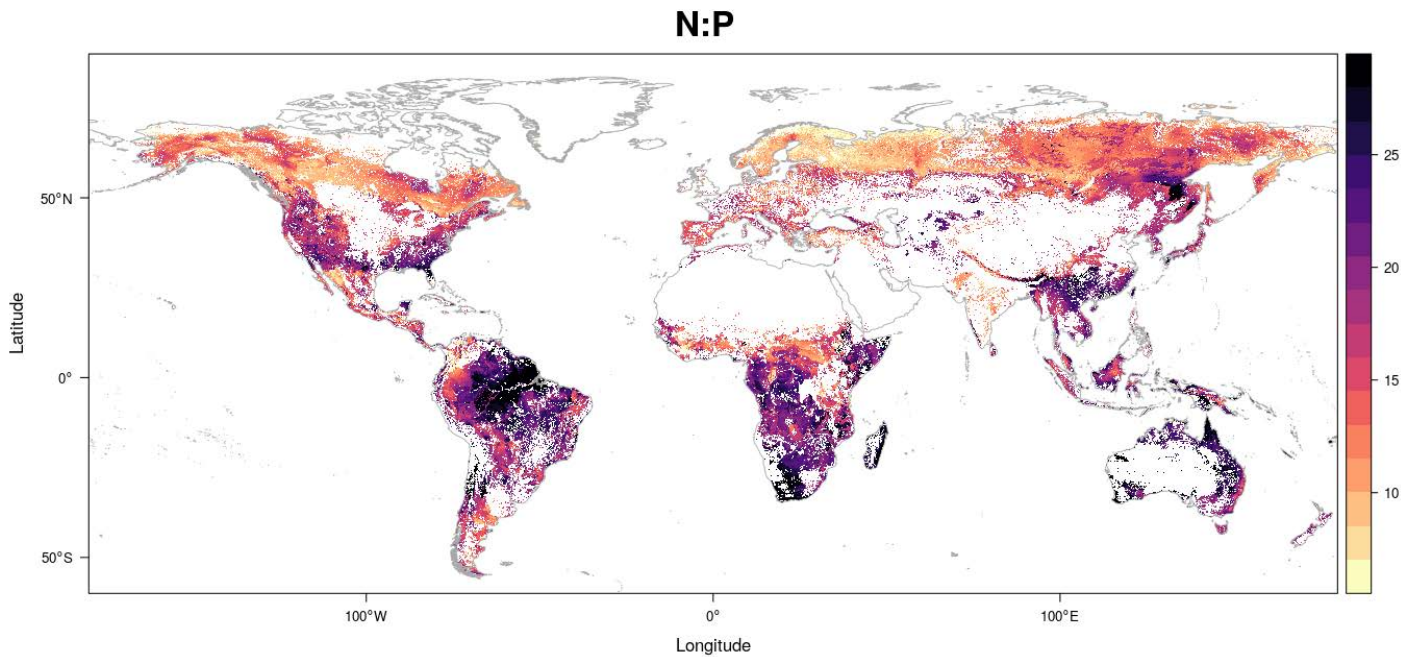
Figure 5.2 Predicted woody plant foliar N:P ratios across global forest biomes; unshaded areas indicate lack of data.

5.3.2 Environmental drivers of N:P ratios

The GLM used to test impacts of potential environmental drivers on woody plant foliar N:P ratios (Table 5.2) (global R^2 = 0.47 and RMSE = 4.85; Figure 5.1) showed positive relations with soil base saturation and soil cation exchange capacity in tropical forests and negative relations with potential evapotranspiration seasonality and maximum December, January and February precipitation (forest type R^2 = 0.23). In temperate coniferous forests (forest type R^2 = 0.11), there are positive relations with potential evapotranspiration seasonality and maximum June, July and August precipitation and negative relations with aridity index and mean temperature of the driest quarter, while in temperate broadleaf forests (forest type R^2 = 0.10), minimum June, July and August precipitation is positively related to foliar N:P ratios and daily mean solar radiation, precipitation of the driest month and precipitation of the driest quarter are negatively related to foliar N:P ratios; in boreal forests (forest type R^2 = 0.10), there is a positive relation with precipitation of the wettest quarter and negative relations with maximum June, July and August precipitation and mean temperature of the warmest quarter (Table 5.2).

5.3.3 Phylogenetic drivers of N:P ratios

The GLMM used to test impacts of phylogenetic drivers on woody plant foliar N:P ratios (global R^2 = 0.6 and RMSE = 3.84; Figure 5.1) showed that random factor variability (phylogeny and species) in N:P ratios is greater than for that of the fixed environmental factors. The variability in N:P ratios explained by phylogeny + species ranges from 40.2% for tropical forests to 82.7% for boreal forests, while variability explained by phylogeny ranges from 6.8% for tropical forests to 50.0% for

Table 5.3 Summary of generalized linear mixed model. Variability explained by for the whole model and by random and fixed part. Phylogeny, species and units belongs to random variability explained. R2c = R2m + R2

	R²c	R²m (fixed)	R² (random)	Phylogeny	Species	Units
Tropical	0.527	0.125	0.402	0.068	0.392	0.540
Temperate coniferous	0.832	0.005	0.827	0.500	0.331	0.169
Temperate broadleaved	0.692	0.019	0.672	0.387	0.299	0.315
Boreal	0.851	0.022	0.829	0.206	0.641	0.153

temperate coniferous and variability explained by species ranged from 29.9% for temperate broadleaf forests to 64.1% for boreal forests (Table 5.3).

5.3.4 Soil age as a driver of N:P ratios

Woody plant foliar N:P ratios vary among soil orders (Figure 5.3) and tend to be higher from Oxisols (mean = 19.1) that occur in tropical wet forests of south America and central Africa, followed by Ultisols (mean = 16.3) that occur in South-eastern Asia and in sparse spots in the tropical fringe. In contrast, lowest N:P ratios are found from Spodosols (mean = 11.4) that typically occur in cold and humid regions, such as Atlantic Europe, Scandinavia, and Canada, followed by
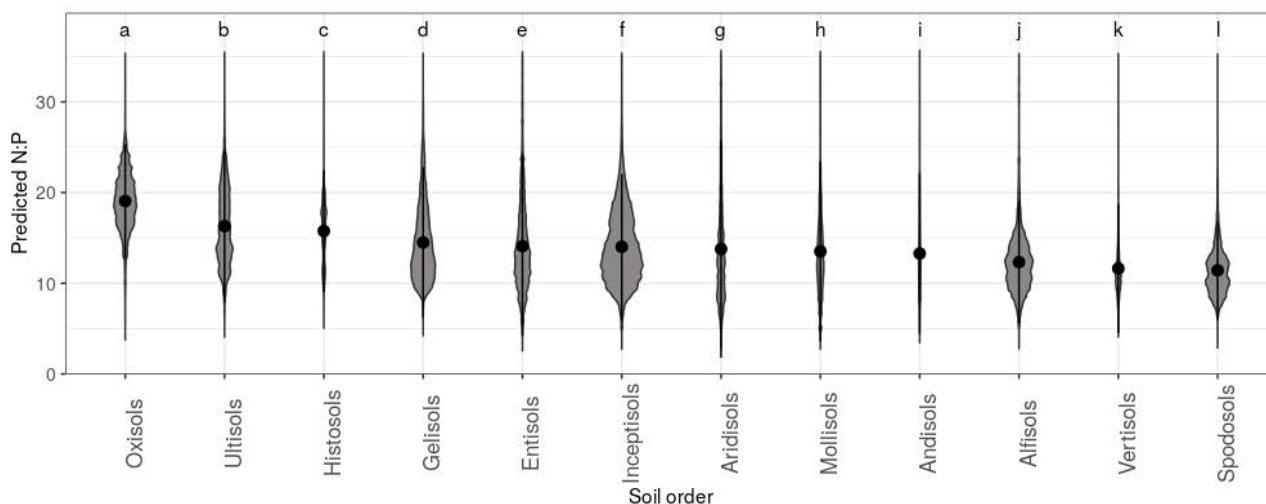
Figure 5.3 Variation in predicted global woody plant foliar N:P ratios among USDA soil taxonomy orders. Predicted values differ significantly among soil types (P< 0.05).

Vertisols (mean = 11.6), known to be conformed by expansive clay with high nutrient retention that occur in isolated zones with exchanging floods and drought periods such as east Australia, south India, Sudan between others.

5.4 Discussion

5.4.1 Global distribution maps of foliar N:P ratios

We constructed a high resolution global map of N:P ratios using NNs trained with environmental variables that identifies forest regions with N and P limitation, that play a role in the biogeochemical function of ecosystems. Although global foliar N and P maps are available (Butler et al., 2017; Moreno-Martínez et al., 2018; Vallicrosa et al., 2021), this is the first gridded N:P ratio map at the global scale constructed from an extensive N:P database using machine learning tools. We chose not to calculate global distribution of wood plant foliar N:P based on currently available N and P maps, because this approach would aggregate errors for each element and assume drivers of foliar N and P are the same as drivers of N:P ratios. Thus, we trained specific N:P ratio models to test for their drivers.

We found that our model predictions follow the substrate age hypothesis (Walker and Syers 1976), where foliar N:P ratios are high in the tropics and low at boreal latitudes (Chadwick et al., 1999; Vitousek et al., 2010) (Table 5.1); indeed, our results showed that woody plant foliar N:P ratios range from 12.34 in boreal forests to 20.70 in tropical forests. Given limitations of N and P limitation occur when N:P ratios are <10 and >20, respectively (Güsewell, 2004; Greenwood et al. 2008), our results indicate that woody vegetation tends to be more P limited than N limited, supporting our predictions and concurring with results reported by Du et al. (2020) that showed 18% of the global terrestrial surface, excluding agricultural, urban, and glacial areas, was under N limitation while 43% was P limited, predominantly in the tropics.

At the regional scale, our model predictions support the old, climatically buffered and infertile landscapes theory (Hopper, 2009), which states these types of landscape, such as the Cape floristic region in South Africa and the Southwest Floristic Region of Australia, are characterized by high levels of P limitation (Lambers et al., 2008, 2010; Oliveira et al., 2015), as indicated by the high N:P ratios for southern Africa and southwest Australia. We also found differences in N:P ratios between temperate coniferous (mean = 14.4) and broadleaf forests (mean = 18.8) (Table 5.1) that may indicate successional differences, as many coniferous   trees are typically fast-growing, early successional species, which require higher proportions of P than broadleaf species that tend to be slower-growing, late successional species, with lower requirements for P (Peñuelas et al., 2013; Sardans and Peñuelas, 2013; Busch et al., 2018).

5.4.2 Environmental drivers

Our decision to model environmental drivers of woody plant foliar N:P ratio by forest biome at the global scale was justified, because impacts of the drivers varied among biomes and with latitude, with apparently contrasting effects. For example, potential evapotranspiration seasonality was negatively related to foliar N:P ratios in tropical forests and positively related to those in temperate coniferous forests, while in temperate coniferous and broadleaf forests, low water availability, expressed in aridity index or precipitation levels, was negatively related with foliar N:P ratios;

these results may indicate that foliar N decreases in temperate latitudes under low levels of water availability (Sardans et al., 2008). In contrast to at temperate latitudes, greater levels of precipitation in boreal forests lead to lower N:P ratios, indicating that mineral leaching, which tends to affect the more mobile N than P, may be more of a constraint than water availability, particularly at these high latitudes where N is frequently more limiting than P. Foliar N:P ratios were related to soil properties only in tropical forests, supporting previous studies that have shown key links between soil properties and tropical foliar elemental composition (Both et al., 2018, Hernández-Vargas et al., 2019).

5.4.3 Genetic drivers

Phylogeny and species explained more variability in foliar N:P ratios than environmental factors for each of the forest biomes (Table 5.3), supporting previous studies in which genetic legacy effects were found to explain most of the variability in woody plant foliar N, P, and potassium concentrations at the global scale (Sardans et al., 2021; Vallicrosa et al., 2021). Genetic legacy effects are a product of long- and short-term evolutionary processes (represented by phylogeny and species, respectively), during which species adapt to shifts in abiotic and biotic stressors, caused by climate, soil, and interactions with other species. While phylogeny include ancient adaptation and differentiation from other clades, species include more epigenetic factors which, through evolutive convergence could generate similar N:P relations with different clades.

Most of the variance explained by the environmental factors in glm, was explained by genetic legacy effects in glmm. Genotypes are associated with particular soil and climatic traits, so phylogeny incorporates climatic and soil conditions to some extent. Indeed, strong genetic legacy signals for foliar elemental composition have recently been reported at local, regional, and global scales (Castellanos et al., 2018; de la Riva et al., 2017; Hu et al., 2018; Reimann et al., 2018; Sardans and Peñuelas, 2015, Sardans et al, 2015, 2016a), supporting studies of the biogeochemical niche hypothesis in plants (Peñuelas et al., 2008, 2010, 2019; Sardans et al., 2021) that have reported phylogeny and species, as proxies of overall genetic legacy, explain >75% of the variability in foliar elemental composition.

Disentangling single genetic legacy effects is problematic and highlights the large proportion of variability in boreal forest woody species N:P ratios that is explained by species (Table 5.3). Globally, boreal ecosystems contain some of the lowest levels of vascular plant diversity (Brummitt et al., 2020) and forests in these climate zones tend to be dominated by low numbers of coniferous species (Freedman, 1999). Thus, this high level of woody species homogeneity and associated low levels of species coexistence and interspecific competition are likely to lead to low levels of phenotypic plasticity and greater stability of foliar N:P ratios. In the other forest biomes, where levels of species diversity and heterogeneity are greater, it is likely that species foliar N:P ratios reflect levels of species coexistence (Peñuelas et al. 2019; Sardans et al., 2021), thus limiting the predictive power of phylogeny and species, as supported by our results that showed tropical forest foliar N:P ratios were least explained by these random factors.

Our study shows that forest species diversity and heterogeneity are key drivers of woody plant foliar N:P proportions, so we recommend their inclusion, along with environmental conditions in future studies of foliar N:P. To facilitate the inclusion of species in global-scale studies of N:P ratios, the Global Biodiversity Information Facility offers free, globally georeferenced species data that may be used in niche modeling of remotely sensed airborne imaging spectroscopy and laser imaging detection and ranging (LiDAR) data added to a clustering algorithm (Yi et al., 2020).

5.5 Conclusions

Our study provides the first global distribution maps of woody plant foliar N:P ratios and confirmation of their negative relation with latitude as a principal driver. Globally, forest biomes are more P limited than N limited, as suggested by previous studies. We found variation in effects of environmental drivers on N:P ratios among forest biomes, due to contrasting associated environmental pressures; these variations would have been masked in a single global "forest biome" analysis. Genetic legacy was a key driver of N:P variability and its inclusion in future studies of N:P ratios will lead to improved prediction of effects of global change on biogeochemical cycles and ecosystem function, including forest primary production, respiration, and C-cycling.

References

Ackerman, Daniel E; Chen, Xin; Millet, Dylan B. (2018). Global nitrogen deposition (2°×2.5° grid resolution) simulated with GEOS-Chem for 1984-1986, 1994-1996, 2004-2006, and 2014-2016. Retrieved from the Data Repository for the University of Minnesota, https://doi.org/10.13020/D6KX2R.

Ågren, G.I., 2008. Stoichiometry and nutrition of plant growth in natural communities. Annu. Rev. Ecol. Evol. Syst. 39, 153e170.

Allaire, J.J., François Chollet, F. (2019). keras: R Interface to 'Keras'. R package version 2.2.5.0. https://CRAN.R-project.org/package=kerasBabita, M., Maheswari, M., Rao, L.M., Shanker, A.K., Rao, D.G., 2010. Osmotic adjustment, drought tolerance and yield in castor (*Ricinus communis* L.) hybrids. Environ. Exp. Bot. 69, 243-249.

Barbosa, A.M., Real, R., Munoz, A.R. & Brown, J.A. (2015). New measures for assessing model equilibrium and prediction mismatch in species distribution models. Diversity and Distributions, 19(10), 1333-1338

Barton, K. (2019). MuMIn: Multi-Model Inference. R package version 1.43.15. https://CRAN.R-project.org/package=MuMIn

Both, S., Riutta, T., Paine, C.E.T., Elias, D.M.O., Cruz, R.S., Jain, A., Johnson, D., Kritzler, U.H., Kuntz, M., Majalap-Lee, N., Mielke, N., Montoya Pillco, M.X., Ostle, N.J., Arn Teh, Y., Malhi, Y. and Burslem, D.F.R.P. (2019), Logging and soil nutrients independently explain plant trait expression in tropical forests. New Phytol, 221: 1853-1865. https://doi.org/10.1111/nph.15444

Brummitt, N, Araújo, AC, Harris, T. Areas of plant diversity—What do we know? Plants, People, Planet. 2020; 3: 33– 44. https://doi.org/10.1002/ppp3.10110

Busch, V., Klaus, V.H., Penone, C., Schafer, D., Boch, S., Prati, D., Muller, J., Socher, S.A., Niinemets, Ü, Peñuelas, J., Hölzel, N., Fischer, M. and Kleinebecker, T. (2018) Nutrient stoichiometry and land use rather than species richness determine plant

functional diversity. Ecol. Evol., 8, pp. 601-616, 10.1002/ece3.3609

Butler, E.E., Datta, A., Flores-Morenno, H., Chen, M., Wythers, K.R., Fazayeli, F., Banerjee, A., Atkin, O.K., Kattge, J., Amiaud, B., Blonder, B., Boenisch, G., Bond-Lamberty, B., Brown, K.A., Byun C., Campetella, G., Cerbolini, B.E.L., Cornelissen, J., H.C., Craine, J.M., Craven, D., de Vries, F.T., Díaz, S., Domingues, T.F., Forey, E., Gonazàlez-Melo, A., Gross, N., Han, W., Hattich, W.N., Hickler, T., Jansen, S., Kramer, K., Kraft, N.J.B., Kurokawa, H., Laughlin, D.C., Meir, P., Minden, V., Niinemets, Ü., Onoda, Y., Peñuelas, J., Read, Q., Sack, L., Schamp, B., Soudzilovskaia, N.A., Spasojevic, M.J., Sosinski, E., Thorton, P.E., Valladares, F., van Bodegon, P.M., Williams M., Wirth, C., Reich, P.B. (2017) Mapping local and global variability in plant trait distributions. Proceedings of the National Academy of Sciences, USA, E10937-E10946.

Carnicer, J., Sardans, J., Stefanescu, C., Ubach, A., Bartrons, M., Asensio, D., & Penuelas, J. (2015). Global biodiversity, stoichiometry and eco- system function responses to human-induced C-N-P imbalances. Journal of Plant Physiology, 172, 82–91. https ://doi.org/ 10.1016/j. jplph.2014.07.022

Castellanos, A.E., Llano-Sotelo, J.M., Machado-Encinas, L.I., Lopez-Pina, J.E., Romo-Leon, J.R., Sardans, J., Peñuelas, J., 2018. Foliar C, N and P stoichiometry characterize successful plant ecological strategies in the Sonoran Desert. Plant Ecol. 219, 775-788.

Chadwick, O., Derry, L., Vitousek, P. *et al.* Changing sources of nutrients during four million years of ecosystem development. *Nature* 397, 491–497 (1999). https://doi.org/10.1038/17276

Das K, Dang R, Shivananda TN (2006) Effect of N, P and K fertilizers on their availability in soil in relation to the Stevia plant (Stevia rebaudiana Bert.). Arch Agron Soil Sci 52:679–685

De Frenne, P., Graae, B.J., Rodríguez-Sánchez, F., Kolb, A., Chabrerie, O., Decocq, G., De Kort, H., De Schrijver, A., Diekmann, M., Eriksson, O., Gruwez, R., Hermy, M., Lenoir, J., Plue, J., Coomes, D.A. and Verheyen, K. (2013), Latitudinal gradients as natural laboratories to infer species' responses to temperature. J Ecol, 101: 784-795. https://doi.org/ 10.1111/1365-2745.12074

de la Riva, E.G., Maranon, T., Violle, C., Villar, R., Perez-Ramos, I.M., 2017. Biogeochemical and Ecomorphological Niche segregation of Mediterranean Woody species along a local gradient. Front Plant Sci. 8, art num 1242.

Du, E., Terrer, C., Pellegrini, A.F.A., Ahlström, A., van Lissa, C.J., Zhao, X., Xia, N., Wu, X., Jackson, R.B. (2020). Global patterns of terrestrial nitrogen and phosphorus limitation. Nature geoscience 13(3), 221-226.

Elser JJ, Fagan WF, Denno RF, Dobberfuhl DR, Folarin A, Huberty A, Interlandi S, Kilham SS, McCauley E, Schulz KL, Siemann H, Sterner RW (2000) Nutritional con- strains in terrestrial and freshwater food webs. Nature 408:578–580

ESA. Land Cover CCI Product User Guide Version 2. Tech. Rep. (2017). Available at: maps.elie.ucl.ac.be/CCI/viewer/download/ESACCI-LC-Ph2-PUGv2_2.0.pdf

Fan, J.W., Harris, W., Zhong, H.P. Stoichiometry of leaf nitrogen and phosphorus of grassland on the inner Mongolian and Qinghai-Tibet Plateaus in relation to climatic variables and vegetation organization levels. Ecol. Res., 31 (2016), pp. 821-829, 10.1007/s11284-016-1392-5

Fanin, N., Fromin, N., Buatois, B. and Hättenschwiler, S. (2013). An experimental test of the hypothesis on non-homeostatic consumer stoichiometry in a plant litter-microbe system. Ecology letters, 16: 764-772.

Fick, S.E., Hijmans, R. (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology. 37, 12, 4302-4315. https://doi.org/10.1002/joc.5086.

Freedman B. (1999) Boreal forest (taiga). In: Environmental Geology. Encyclopedia of Earth Science. Springer, Dordrecht. https://doi.org/10.1007/1-4020-4494-1_43

Gracia C., Ibàñez J.J., Burriel J.A., Mata T., Vayreda J. (2000-2004). *Inventari Ecològic i Forestal de Catalunya*. CREAF, Bellaterra. ISBN de la sèrie: 84-931323-0-6

Greenwood, D.J., Karpinets,T.V., Zhang, K., Bosh-Serra, A., Boldrini, A. and Karawulova, L. (2008). A Unifying Concept for the Dependence of Whole-crop N : P Ratio on Biomass: Theory and Experiment, Annals of Botany, 102(6), 967–977, https://doi.org/10.1093/aob/mcn188

Güsewell, S., 2004. N:P ratios in terrestrial plants: variation and functional significance. New Phytol. 164, 243e266

Hernández-Vargas, G, Sánchez-Velásquez, LR, López-Acosta, JC, Noa-Carrazana, JC, Perroni, Y. Relationship between soil properties and leaf functional traits in early secondary succession of tropical montane cloud forest. Ecol. Res. 2019; 34: 213–224. https://doi.org/10.1111/1440-1703.1267

Hijmans, R. J. (2020). raster: Geographic Data Analysis and Modeling. R package version 3.4-5. https://CRAN.R-project.org/package=raster

Hopper, S.D. OCBIL theory: towards an integrated understanding of the evolution, ecology and conservation of biodiversity on old, climatically buffered, infertile landscapes. Plant Soil 322, 49–86 (2009). https://doi.org/10.1007/s11104-009-0068-0

Hu, M.J., Peñuelas, J., Sardans, J., Sun, Z.G., Wilson, B., Huang, J.F., Zhu, Q.L., Tong, C., 2018. Stoichiometry patterns of plant organ N and P in coastal herbaceous wetlands along the East China Sea: implications for biogeochemical niche. Plant Soil 431, 273-288.

Jing, H., Zhou, H., Wang, G., Xue, S., Liu, G. and Duan, M. (2017). Nitrogen addition changes the stoichiometry and growth rate of different organs in Pinus tabuliformis seedlings. Frontiers in plans science. https://doi.org/10.3389/fpls.2017.01922

Kerkhoff, A.J., Enquist, B.J., Elser, J.J. and Fagan, W.F. (2005), Plant allometry, stoichiometry and the temperature-dependence of primary productivity. Global Ecology and Biogeography, 14: 585-598. https://doi.org/10.1111/j.1466-822X.2005.00187.x

Knecht M.F. and Göransson, A. (2004) Terrestrial plants require nutrients in similar proportions. Tree Physiol 24:447–460

Koerselman W. and Meuleman A.F.M. (1996) The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. Journal of Applied Ecology, 33, 1441–1450.

Lambers H, Raven JA, Shaver GR, Smith SE. 2008. Plant nutrient-acquisition strategies change with soil age. Trends in Ecology & Evolution 23: 95–103

Lambers H, Brundrett MC, Raven JA, Hopper SD. 2010. Plant mineral nutrition in ancient landscapes: high plant species diversity on infertile soils is linked to functional diversity for nutritional strategies. Plant and Soil 334: 11–31

Lever, J., Krzywinski, M. & Altman, N. Model selection and overfitting. Nat Methods 13, 703–704 (2016). https://doi.org/10.1038/nmeth.3968

McGroddy, M.E., Daufresne, T. and Hedin, L.O. (2004), SCALING OF C:N:P STOICHIOMETRY IN FORESTS WORLDWIDE: IMPLICATIONS OF TERRESTRIAL REDFIELD-TYPE RATIOS. Ecology, 85: 2390-2401. https://doi.org/10.1890/03-0351

Mendiburu, F. (2020). agricolae: Statistical Procedures for Agricultural. Research. R package version 1.3-2

Moreno-Martínez, A; Camps-Valls, G; Kattge, J; Robinson, N; Reichestein, M; van Bodegom, P; Kramer, K; Hans, J; Cornelissen, C; Reich, P; Bahn, M; Niinemets, U; Peñuelas, J; Craine, JM; Cerabolini, BEL; Minden, V; Laughlin, DC; Sack, L; Allred, B; Baraloto, C; Byun, C; Soudzilovskaia, NA; Run- ning, SW. (2018). A methodology to derive global maps of leaf traits using remote sensing and climate data. Remote sensing of Environment. 218. 69-88.

Niklas, K.J. and Cobb, E.D. (2005), N, P, and C stoichiometry of Eranthis hyemalis (Ranunculaceae) and the allometry of plant growth. Am. J. Bot., 92: 1256-1263. https://doi.org/10.3732/ajb.92.8.1256

Niklas, K.J., Owens, T., Reich, P.B. and Cobb, E.D. (2005), Nitrogen/phosphorus leaf stoichiometry and the scaling of plant growth. Ecology Letters, 8: 636-642. https://doi.org/10.1111/j.1461-0248.2005.00759.x

Oliveira RS, Galvão HC, de Campos MCR, Eller CB, Pearse SJ, Lambers H. 2015. Mineral nutrition of campos rupestres plant species on contrasting nutrient-impoverished soil types. New Phytologist, 205: 1183–1194.

Olson, D. M., Dinerstein, E., Wikramanayake, E. D., Burgess, N. D., Powell, G. V. N., Underwood, E. C., D'Amico, J. A., Itoua, I., Strand, H. E., Morrison, J. C., Loucks, C. J., Allnutt, T. F., Ricketts, T. H., Kura, Y., Lamoreux, J. F., Wettengel, W. W., Hedao, P., Kassem, K. R. 2001. Terrestrial ecoregions of the world: a new

map of life on Earth. Bioscience 51(11):933-938.

Paseka, R.E. and Grunberg, R.L. (2019), Allometric and trait-based patterns in parasite stoichiometry. Oikos, 128: 102-112. https://doi.org/10.1111/oik.05339

Pebesma, E., 2018. Simple Features for R: Standardized Support for Spatial Vector Data. The R Journal 10 (1), 439-446, https://doi.org/10.32614/RJ-2018-009

Peng, Y., Karl J. Niklas, Shucun Sun. (2011). The relationship between relative growth rate and whole-plant C : N : P stoichiometry in plant seedlings grown under nutrient-enriched conditions, Journal of Plant Ecology, Volume 4, Issue 3, September 2011, Pages 147–156, https://doi.org/10.1093/jpe/rtq026

Peñuelas J, Sardans J, Ogaya R, Estiarte M (2008) Nutrient stoichiometric relations and biogeochemical niche in coexisting plant species: effect of simulated climate change. Polish Journal of Ecology 56: 613-622.

J. Peñuelas, J. Sardans, J. Llusia, S.M. Owen, J. Carnicer, T.W. Giambelluca, E.L. Rezende, M. Waite, U. Niinemets. Fast returns on "leaf economics" and different biogeochemical niche in invasive compared with native plant species. Glob. Change Biol. Bioenergy, 16 (2010), pp. 2171-2185

Penuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velde, M., Bopp, L., … Janssens, I. A. (2013). Human-induced nitrogen-phosphorus imbal- ances alter natural and managed ecosystems across the globe. Nature Communications, 4, 2934. https ://doi.org/10.1038/ncomm s3934

Peñuelas, J., Fernández-Martínez, M., Ciais, P., Jou, D., Piao, S., Obersteiner, M., Vicca, S., Janssens, I. A., and Sardans, J.. 2019. The bioelements, the elementome, and the biogeochemical niche. Ecology 100(5):e02652. 10.1002/ecy.2652

Peñuelas, J., Janssens, I., Ciais, P., Obersteiner, M., Sardans, J. 2020. Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity, food security, and human health. Global Change Biology 2020;26:1962–1985. doi: 10.1111/GCB.14981.

Peñuelas, J. and Sardans, J. Elementary factors. Nature 460, 803–804 (2009). https://doi.org/10.1038/460803a

Qian, H. and Jin, Y. An updated megaphylogeny of plants, a tool for generating plant phylogenies and an analysis of phylogenetic community structure (2015). Journal of Plant Ecology. 1-7. doi:10.1093/jpe/rtv047

Qiao, Y.J., Yang, W., Zhao, Y.X., Jeelani, N., Xu, L.Q., Zhao, H., Zhang, Y.A., An, S.Q., Leng, X., 2018. How Spartina alterniflora adapts to a new environment created by embankment reclamation through C-N-P stoichiometry in the coastal wetlands of eastern China. Mar. Fresh. Res. 69, 823-832. DOI:10.1071/MF17374.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ratnam, J., Sankaran, M., Hanan, N.P. et al. Nutrient resorption patterns of plant functional groups in a tropical savanna: variation and functional significance. Oecologia 157, 141–151 (2008). https://doi.org/10.1007/s00442-008-1047-5

Reich, P.B. and Oleksyn, J. (2004) Global patterns of plant leaf N and P in relation to temperature and latitude. Proceedings of the National Academy of Sciences, USA. 101, 11001-11006.

Reimann, C., Fabian, K., Flem, B., Andersson, M., Filzmoser, P., Englmaier, P., 2018. Geosphere-biosphere circulation of chemical elements in soil and plant systems from a 100 km transect from southern central Norway. Sci. Total Env. 639, 129-145. DOI:10.1016/j.scitotenv.2018.05.070.

Rivas-Ubach, A., Sardans, J., Perez-Trujillo, M., Estiarte, M., Peñuelas, J., 2012. Strong relationship between elemental stoichiometry and metabolome in plants. Proc. Natl. Acad. Sci. USA 109, 4181–4186.

Sardans, J., Peñuelas, J., Estiarte, M. and Prieto, P. (2008). Warming and drought alter C and N concentration, allocation and accumulation in a Mediterranean shrubland. Global Change Biology, 14: 2304-2316. https://doi.org/10.1111/j.1365-2486.2008.01656.x

Sardans, J., Rivas-Ubach, A., Peñuelas, J. (2011). Factors affecting nutrient concentration and

stoichiometry of forest trees in Catalonia (NE Spain). Forest Ecology and Management. 262(11), 2024-2034. https://doi.org/10.1016/j.foreco.2011.08.019

Sardans, J., Rivas-Ubach, A., Peñuelas, J. (2012). The C:N:P stoichiometry of organisms and ecosystems in a changing world: A review and perspectives. Perspectives in Plant Ecology, Evolution and Systematics. 14(1), 33-47. https://doi.org/10.1016/j.ppees.2011.08.002

Sardans J, Janssens IA, Alonso R, Veresoglou SD, Rillig MC, Sanders TGM, Carnicer J, Filella I, Farre-Armengol G, Peñuelas J (2015) Foliar elemental composition of European forest tree species associated with evolutionary traits and present environmental and competitive conditions. Glob Ecol Biogeogr 24:240-255.

Sardans, J., Alonso, R., Janssens, I.A., Carnicer, J., Vereseglou, S., Rillig, M.C., Fernández-Martínez, M., Sanders, T.G.M. and Peñuelas, J. (2016a), Foliar and soil concentrations and stoichiometry of nitrogen and phosphorous across European Pinus sylvestris forests: relationships with climate, N deposition and tree growth. Funct Ecol, 30: 676-689. https://doi.org/10.1111/1365-2435.12541

Sardans, J., Alonso, R., Carnicer, J., Fernández-Martínez, M., Vivanco, M.G. and Peñuelas, J. (2016b). Factors influencing the foliar elemental composition and stoichiometry in forest trees in Spain. Perspectives in Plant Ecology, Evolution and Systematics. 18, 52-69.

Sardans, J., Janssens, I.A., Ciais, P., Obersteiner, M., Peñuelas, J. (2021). Recent advances and future research in ecological stoichiometry. Perspectives in Plant ecology, evolution and systematics. 50, 12561.

Sardans, J., Peñuelas, J. Plant-soil interactions in Mediterranean forest and shrublands: impacts of climatic change. Plant Soil 365, 1–33 (2013). https://doi.org/10.1007/s11104-013-1591-6

Sardans J., Peñuelas J. 2015. Potassium: a neglected nutrient in global change. Global Ecology and Biogeography 24: 261-275.

Schreeg, L. A., Santiago, L. S., Wright, S. J., Turner, B. L. 2014. Stem, root, and older leaf N:P ratios are more responsive indicators of soil nutrient availability than new foliage. Ecology 95:2062-2068.

Shangguan, W., Dai, Y., Duan, Q., Liu, B. and Yuan, H., 2014. A Global Soil Data Set for Earth System Modeling. Journal of Advances in Modeling Earth Systems, 6: 249-263.

Soil Survey Staff. 2014. Keys to Soil Taxonomy, 12th ed. USDA -Natural Resources Conservation Service, Washington, DC.

Sterner, R., Elser J.J. 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to Biosphere. Princenton University Press. Princenton, New Jersey. pp. 439.

Sun,Y., Peng, S., Goll, D.S., Ciais, P., Guenet, B., Guimberteau,M., Hinsinger, P., Janssens, I.A., Peñuelas, J., Piao, S., Poulter, B., Violette, A., Yang, X., Yin, Y., Zeng, H. Diagnosing phosphorus limitations in natural terrestrial ecosystems in carbon cycle models. Earth's Fut.,5(2017),pp. 730-749, 10.1002/2016EF000472

Sun, H.F., Li, Q., Lei, Z.F., Zhang, J.B., Song, X.Z., Song, X.Z., 2019. Ecological stoichiometry of nitrogen and phosphorus in Moso bamboo (Phyllostachys edulis) during the explosive growth period of new emergent shoots. J. Plant Res. 132, 107-115. DOI:10.1007/s10265-018-1070.5.

Tian, D., Yan, Z., Niklas, K.J., Han, W., Kattge, J., Reich, P.B., Luo, Y., Chen, Y., Tang, Z., Hu, H., Wright, I.J., Schmid, B., Fang, J., 2018. Global leaf nitrogen and phosphorus stoichiometry and their scaling exponent. Natl. Sci. Rev. https://doi.org/ 10.1093/nsr/nwx142/4750788.

Trabucco, Antonio; Zomer, Robert (2019): Global Aridity Index and Potential Evapotranspiration (ET0) Climate Database v2. figshare. Fileset. https://doi.org/10.6084/m9.figshare.7504448.v3

Vallicrosa, H., Sardans, J., Maspons, J., Zuccarini, P., Fernández-Martínez, M., Bauters, M., Ciais, P., Goll, D., Obersteiner, M., Janssens, I.A. and Peñuelas, J. In press. Global maps and factors driving forest foliar elemental composition: the importance of genetic legacy.

Van Duren IC, Pegtel DM (2000) Nutrient limitations in wet, drained and rewetted fen meadows: evaluation of methods and results. Plant Soil 220:35–47

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and sea: how can it occur? Biogeochemistry 13:87–115

Vitousek, P.M., Porder, S., Houlton, B.Z. and Chadwick, O.A. (2010), Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. Ecological Applications, 20: 5-15. https://doi.org/10.1890/08-0127.1

Walker, T.W. and Syers, J.K. The fate of phosphorus during pedogenesis. (1976). Geoderma 15(1), 1-19.

Wang, R., Goll, D., Balkanski, Y., Hauglustaine, D., Boucher, O., Ciais, P., Janssens, I., Peñuelas, J., Guenet, B., Sardans, J., Bopp, L., Vuichard, N., Zhou, F., Li, B., Piao, S., Peng, S., Huang, Y., Tao, S. (2017) Global forest carbon uptake due to nitrogen and phosphorus deposition from 1850-2100. Global Change Biology 23, 4854-4872.

Wickham, H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.

YAN En-Rong, WANG Xi-Hua, ZHOU Wu. N:P STOICHIOMETRY IN SECONDARY SUCCESSION IN EVERGREEN BROAD-LEAVED FOREST, TIA NTONG, EAST CHINA[J]. Chin J Plan Ecolo, 2008, 32(1): 13-22.

Yan, Z., Kim, N., Han, W. et al. Effects of nitrogen and phosphorus supply on growth rate, leaf stoichiometry, and nutrient resorption of Arabidopsis thaliana . Plant Soil 388, 147–155 (2015). https://doi.org/10.1007/s11104-014-2316-1

Yi H.Y., ZENG Yuan, ZHAO Yu-Jin, ZHENG Zhao-Ju, XIONG Jie, ZHAO Dan. Forest species diversity mapping based on clustering algorithm (2020). Chinese Journal of Plant ecology. 44 (6). 598-615. DOI: 10.17521/cjpe.2019.0347

Yu, Q., Wu, H., He, N., Lü, X., Wang, Z., Elser, J.J., Wu, J. and Han, X. (2012). Testing the Growth Rate Hypothesis in Vascular Plants with above- and below-grownd biomass. PLoS ONE 7(3): e32162. https://doi.org/10.1371/journal.pone.0032162

Yuan, Z. and Chen, H.Y.H. (2009), Global trends in senesced-leaf nitrogen and phosphorus. Global Ecology and Biogeography, 18: 532-542. https://doi.org/10.1111/j.1466-8238.2009.00474.x

Zechmeister-Boltenstern, S., Keiblinger, K.M., Mooshammer, M., Peñuelas, J., Richter, A., Sardans, J. and Wanek, W. (2015), The application of ecological stoichiometry to plant–microbial–soil organic matter transformations. Ecological Monographs, 85: 133-155. https://doi.org/10.1890/14-0777.1

Zhao Y.D., He, X.,D., Chen L., Ding, X.F., Li M.Q., Xu, P.Y., Gao Y.B. Features on N/P ratio of plants with different functional groups between two types of steppe in semi-arid area[J].Sciences in Cold and Arid Regions, 2019, 11(5): 371-381.

Zhang, J. and Elser, J.J., 2017. Carbon:nitrogen:phosphorus in fungi: a meta-analysis. Front. Microbiol. 8, art num 1281. DOI:10.3389/fmicb.2017.01281.

W.P. Zhang, D. Fornara, G.C. Liu, J. Peñuelas, J. Sardans, J.H. Sun, L.Z. Zhang, L. Li Ontogenic shift in the relationship between the rate of aboveground growth and N:P stoichiometry in higher plants. Funct Ecol. Submitted. (2019a)

X. Zhang, X. He, J. Gao, L. Wang. Latitudinal and climate effects on key plant traits in Chinese forest ecosystems Glob. Ecol. Conser., 17 (2019b), Article e00527, 10.1016/j.gecco.2019.e00527

6

Empirical support for the biogeochemical niche hypothesis in forest trees

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Abstract

The possibility of using the elemental compositions of species as a tool to identify species/genotype niche remains to be tested at a global scale. We investigated relationships between the foliar elemental compositions (elementomes) of trees at a global scale with phylogeny, climate, N deposition and soil traits. We analysed foliar N, P, K, Ca, Mg and S concentrations in 23,962 trees of 227 species. Shared ancestry explained 60–94% of the total variance in foliar nutrient concentrations and ratios whereas current climate, atmospheric N deposition and soil type together explained 1–7%, consistent with the biogeochemical niche hypothesis which predicts that each species will have a specific need for and use of each bio-element. The remaining variance was explained by the avoidance of nutritional competition with other species and natural variability within species. The biogeochemical niche hypothesis is thus able to quantify species-specific tree niches and their shifts in response to environmental changes.

6.1 Main

The 'niche' theory is fundamental to ecology, because niches are both drivers and consequences of evolutionary processes[1,2,3,4]. The concept is easy to understand theoretically: each species tends to occupy a particular position along the gradients of all abiotic and biotic variables that define and determine fitness. This view is consistent with the niche concepts defined by Tracy and Christian[1], Wright et al.[5] and Swanson et al.[6], among many others, where the niche is directly associated with a multivariate space. Accurate measurement of the exact parameters of a niche, however, is challenging due to the large number of variables that affect organisms within ecosystems. Several approaches have been developed in recent decades to more easily manage this complex and multivariate concept—for example, niche regeneration[7] and functional

niche[8],[9],[10]. A more general and easier method to define and measure species niches using field data, however, is needed.

The recently proposed biogeochemical niche (BN) hypothesis[11],[12],[13] incorporates most, if not all, niche parameters using species-specific elemental composition and stoichiometry. The assumptions underlying this hypothesis are based on the idea that each species is a unique genetic pool of individuals and a product of long-term evolutionary processes, so that each species should have a specific morphological structure and functionality (from gene expression to physiological processes). Fundamental biological processes (for example, growth, secondary metabolism, reproduction and storage of bio-elements) have distinct rates in different species depending on selection pressures, so different species must differentially allocate elements to various traits of tissues and organs. Each species should thus tend to have its own elemental composition and stoichiometry (homeostatic component of BN). The changing circumstances during the lives of organisms, however, should also determine a necessary phenotypical plasticity to allow the individuals of each species to adapt its functionality and morphology during their lives (plasticity component). This ability differs in extent and quality among species. BN plasticity depends on the current genotypic and thus genetic variability of a population, and also on the phenotypical plasticity of individuals to respond to environmental shifts throughout their lives. The BN hypothesis allows us to detect plasticity at two levels: (1) within populations due to the intraspecific variability in elemental composition of a set of individuals of the same species living under the same environmental conditions at a specific time (for example, due to individual genotypic differences or different ontogenetic stages), and (2) at the individual level (phenotypic plasticity), by indicating how each individual of a population varies its elemental composition when environmental conditions shift[13].

The BN hypothesis is useful for representing the ecological niche of each species in a hyperdimensional volume generated by different bio-elemental concentrations and stoichiometric relationships, which could be simply and practically tested by a combined chemical and mathematical approach using multivariate and phylogenetic analyses[13]. The position of each species in the hyperdimensional volume can shift with time and changing environmental conditions, depending on the degree of species-specific stoichiometric plasticity (plasticity component), but the BN hypothesis should also tend to maintain its own identity relative to the BNs of other species (homeostatic component)[13]. BN space at a specific time should therefore be a consequence of historical and current trends toward maximization of fitness in response to abiotic and biotic circumstances such as trophic relationships and water, light or nutrient availabilities and fluxes[13],[14],[15]. The various levels of plasticity among species can be detected because homeostatic species will occupy a smaller volume in multidimensional space, and plastic species will occupy a larger volume[13]. For example, comparing the movement/expansion/contraction of the BNs of two populations of different species or genotypes submitted to the same environmental shifts will thus provide information about their levels of BN plasticity[13]. The temporal shifts of the BN of a species or population can also be calculated, so we can follow the

signatures of evolution on the BN. BNs also allow us to describe and quantify the expansion, contraction and extinction of niches and the appearance of new niches (for example, when new species colonize an ecosystem)[13]. The BN hypothesis, however, has been experimentally tested only at small spatial and phylogenetic scales[11,12,13,14,15,16,17].

We aimed to test the BN hypothesis in forest trees across all forest biomes and continents (Extended Data Fig. 1). Our study is based on the elementome of leaves, the plant organ where most compounds (from those allocated to growth or reproduction to those allocated to energy metabolism, defence or storage) are synthesized and where photosynthesis, the most crucial plant function, occurs. Leaves thus constitute a key organ in plant functioning, and we can define the 'species biogeochemical niche' for each species in its environmental circumstances by analysis of changes in foliar elemental composition. We built a global data set to test the extent to which (1) shared ancestry, (2) abiotic factors (for example, climate, N deposition and soil traits) and (3) biotic factors (for example, composition of the community inhabited by a tree) affect the BN. We hypothesized that each species would have a different need and use for each bio-element to optimize function, and thus that shared ancestry would account for a large part of the foliar elemental composition in a wide set of data from a broad spectrum of species. Species, however, also evolve to some degree during fluctuating environmental conditions, so some of the variability in elemental composition should be due to the phenotypic component and thus to current abiotic and biotic conditions such as climate, soil type, atmospheric N deposition and competition, which should account for another part of the variability of elemental composition. We thus expected to demonstrate the suitability of using the elemental compositions of organisms, the elementome[13], to define species-specific 'niche differences' in a tangible and measurable way, providing a valuable tool for establishing and identifying species niches.

6.2 Results

6.2.1 Phylogeny and BN size

The analysis of Pagel's λ identified significant phylogenetic signals in foliar N, P, K, S, Ca and Mg concentrations, N/Ca, N/Mg, P/Ca, P/Mg, P/S, K/Ca and K/S ratios and the scores of the first six principal component analysis (PCA) axes (Supplementary Table 1, Figures 6.1–6.4, Extended Data Figs. 2–5 and Supplementary Figs. 1–9), with Pagel's λ values in several variables >0.5. These variables significantly tended to be more similar in the clades of more recently separated species than in those of more phylogenetically distant species, thus demonstrating that the divergence of the values of the variables among clades over time was largely and significantly driven by evolutionary processes. For example, foliar N concentrations were significantly more similar among Pinaceae species than between Pinaceae and Fagaceae species, with Pinaceae species generally having lower values than Fagaceae species (Figure 6.1). The phylogenetic signals using Pagel's λ were generally more similar in the subset of the database that also contained information for foliar C concentrations (7,479 datapoints representing 138 species) than in the

general database without foliar C concentrations (Supplementary Tables 2 and 3 and Supplementary Figs. 10–14). Of the 33 variables studied, only the foliar C/K ratio did not have a significant λ. Furthermore, these Pagel's λ values were high (>0.6 for most of the nutrient variables) (Supplementary Table 2).

0.642   Trait value   4.287

Length = 176.162
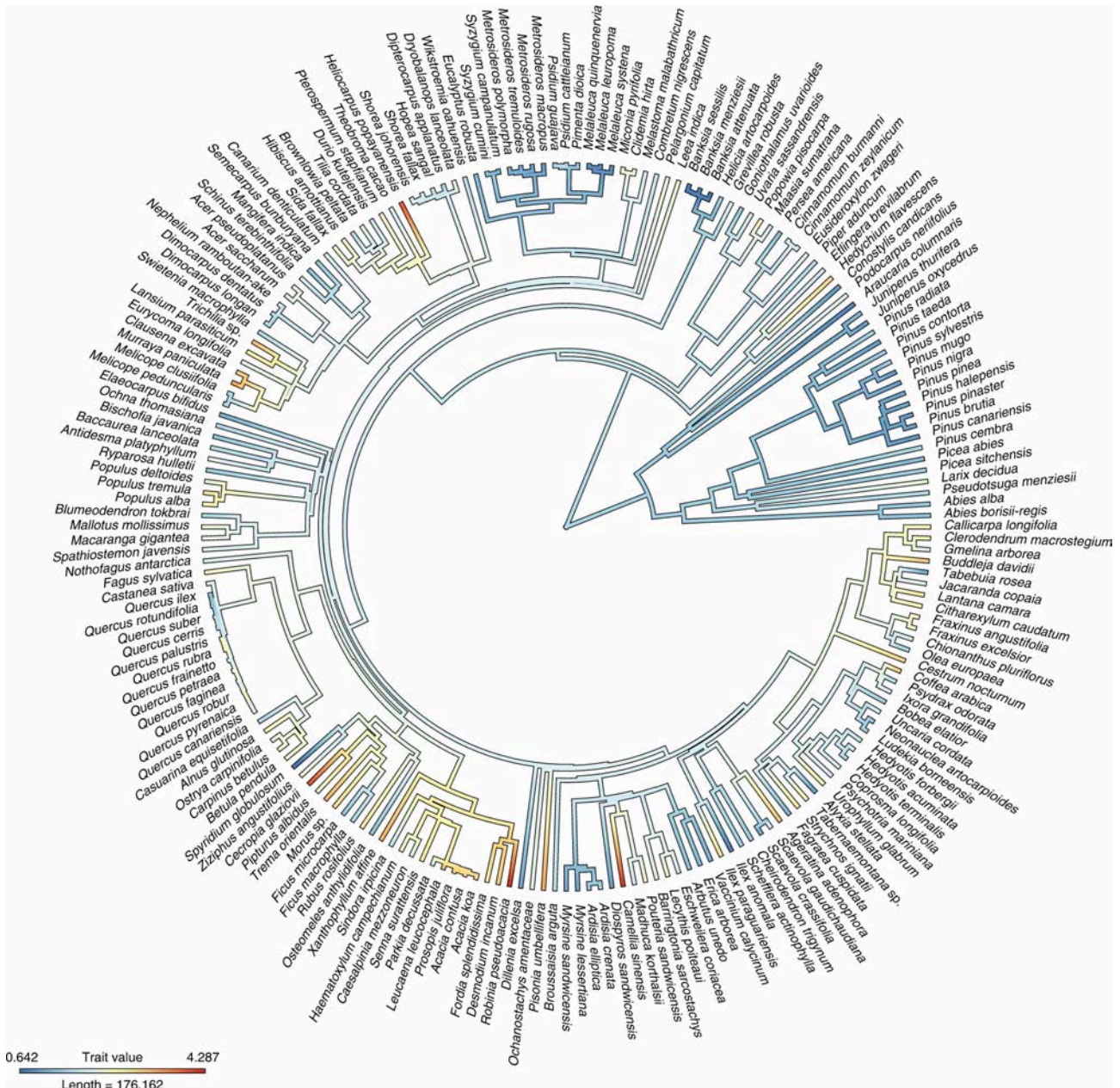
Figure 6.1 Phylogenetic diagram of foliar N concentration (percentage of dry weight) (25,112 datapoints) in the phylogenetic tree.

The range of values in a functional discriminant analysis (FDA) (mainly along root 1, which explained 90.8% of the total variance) that represented the sizes of species-specific BNs was significantly larger for the species subjected to lower climatic stress (*Quercus robur* and *Quercus petraea*) than for Mediterranean species adapted to drought (*Pinus halepensis*, *P.*

pinaster and *Quercus ilex*) (Figure 6.5 and Supplementary Tables 4 and 5). These five species were the most abundant in our survey and were clearly separated in the multivariate space by significant distances (squared Mahalanobis distances; Supplementary Table 4) and in all foliar variables that significantly contributed to the separation of all species (Supplementary Table 5). We also detected a strong phylogenetic effect in the distribution of scores along the first three root axes of the FDA (Supplementary Table 6).
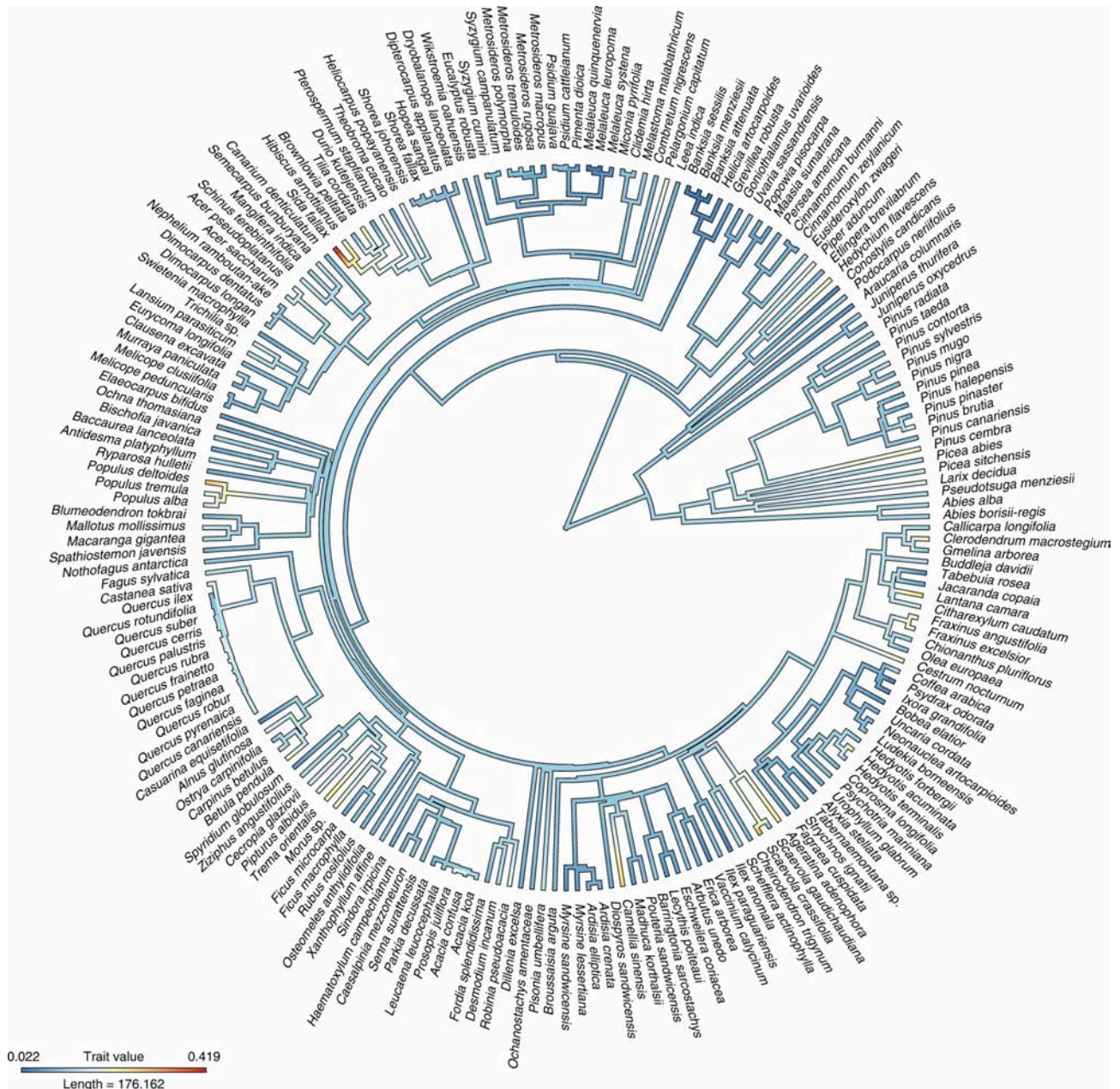
Figure 6.2 Phylogenetic diagram of foliar P concentration (percentage of dry weight) (25,112 datapoints) in the phylogenetic tree.

6.2.2 Abiotic factors

Climate, N deposition and soil type

Phylogeny explained significantly most of the variance in foliar elemental composition: 58.7–91.7% (mean 80.9%) of the variance in foliar concentration of the six bio-elements, 39.0–94.1% (mean 68.8%) of the variance in their pairwise ratios and 43.2–89.6% (mean 74.0%) of the variance in the scores of the first three axes of a PCA (Supplementary Table 7). Species significantly explained 1.4–14.6% (mean 5.58%) of the variance in foliar concentrations of the six bio-elements, 0.4–28.1% (mean 8.1%) of the variance in pairwise ratios and 0.6–13.8% (mean 5.07%) of the variance in the scores of the first three PC scores. Inheritance thus significantly
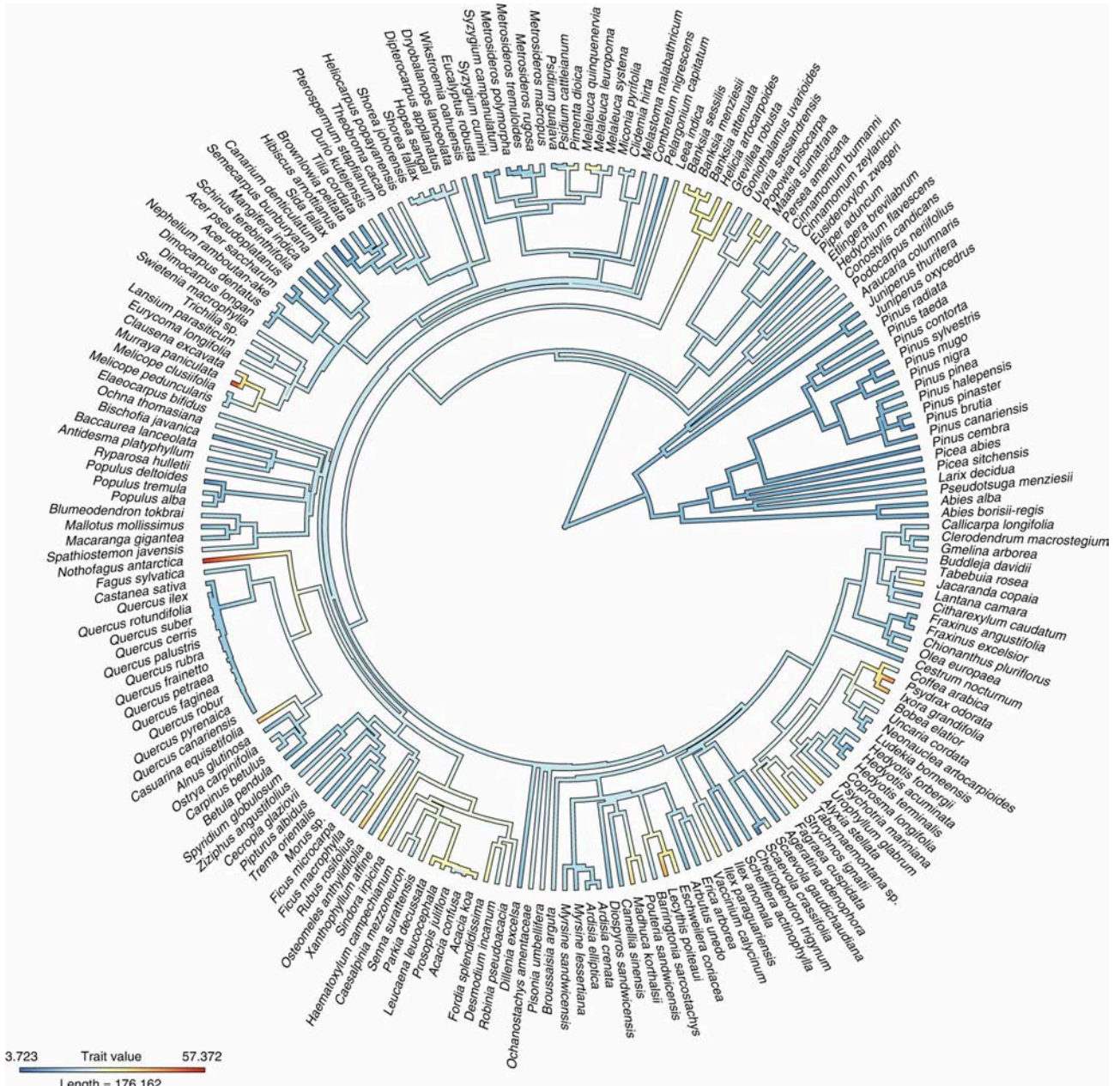
Figure 6.3 Phylogenetic diagram of foliar N/P ratio (25,112 datapoints) in the phylogenetic tree.

explained 73.3–93.6% (mean 86.5%) of the variance in foliar concentration of the six bio-elements, 67.1–94.5% (mean 81.7%) of the variance in their pairwise ratios and 57–90.2% (mean 79.1%) of the variance in the scores of the first three PCs.

The climatic variables and N deposition, independently from their effect on current species distribution, significantly explained 0.43–4.2% (mean 2.26%) of the variance in foliar concentration of the six bio-elements, 0.27–7.4% (mean 5.05%) of the variance in their ratios and 0.35–11.0% (mean 4.23%) of the variance in the scores of the first three PCs (Supplementary Table 7). Mean annual precipitation (MAP) and mean annual temperature (MAT) were the most

Figure 6.4 Phylogenetic diagram of PC1 scores (25,112 datapoints) in the phylogenetic tree.

important climatic variables explaining the variance in elemental concentrations, with some relevant exceptions. For example, the variability in foliar N concentration was partially due to positive correlations with MAP, MAT, N deposition and mean annual solar radiation (Supplementary Table 6). Interestingly, MAT and mean solar radiation had contrasting relationships

Figure 6.5 Plot of the first two roots of functional discriminant analysis using *P. pinaster*, *P. halepensis*, *Q. ilex*, *Q. petraea* and *Q. robur* as dependent categorical grouping factors, and foliar N, P, K, S, Ca and Mg concentrations and pairwise ratios as continuous independent variables.

with MAP and N deposition on overall foliar elemental composition, as indicated by the PC1 scores (Supplementary Table 7).

Higher precipitation was significantly associated with lower foliar concentrations of metal elements (K, Ca and Mg) and P and was significantly and positively correlated with foliar N and S concentrations (Supplementary Table 7). MAT was correlated significantly and positively with foliar N and metal (K, Ca and Mg) concentrations and N/P ratio, and negatively with foliar P concentrations. N deposition was correlated positively with foliar N, P and S concentrations and negatively with foliar Ca, Mg and K concentrations.

Soil type explained a low but significant percentage of the variance in elemental composition and ratios (Supplementary Table 8), ranging between 0.1% for the foliar P/S ratio to 2.0% for foliar K concentrations. A PCA, however, indicated that trees growing in different soil types occupied significantly different areas of the two-dimensional plot of the first two PC axes (Figure 6.6) and that this distribution was mainly explained by phylogeny ($R2=0.72$, 0.84 and 0.87

for PC1, PC2 and PC3 scores, respectively) (Supplementary Table 8). Trees growing on Inceptisols and Alfisols, typical of temperate forests, occupied a central position in this space. Trees growing in Alfisols, typical of wet and mesic temperate forests, had intermediate foliar N and K concentrations and the second highest foliar P concentration (Extended Data Fig. 6), with intermediate foliar N/P, N/K and P/K ratios (Extended Data Fig. 7). Trees growing in Spodosols, very common in boreal and alpine coniferous forests, had the highest P and lowest K foliar concentrations (Extended Data Fig. 6) and thus the highest foliar P/K ratios (Extended Data Fig. 7). Trees growing in Oxisols, typical of wet tropical forests, had the second highest foliar N concentrations and the lowest foliar P concentrations (Extended Data Fig. 6), the highest foliar N/P and N/K ratios and the lowest foliar P/K ratio (Extended Data Fig. 7).
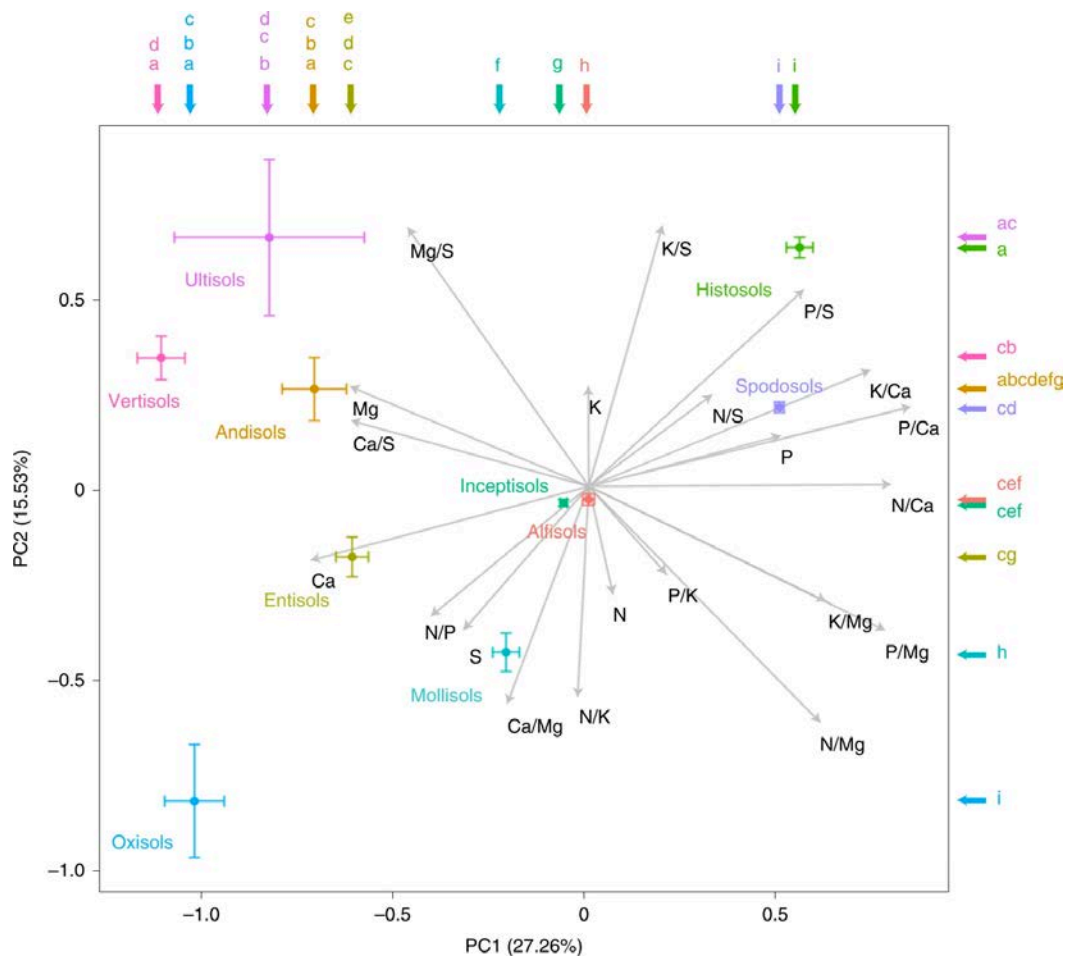
Figure 6.6 Plot of PCA cases and variables superimposed, defined by the first two components of the PCA, with foliar N, P, K, Ca, Mg and S concentrations as variables and with soil orders as cases.

6.2.3 Biotic factors

Competition of foliar elementomes among coexisting species

Foliar N and P concentrations, the N/P ratio and the scores for the first two PCA axes for species frequently shifted significantly when the distributions of two species overlapped. These

differences usually explained little of the variance in foliar variables, but they were statistically significant (Supplementary Tables 9–15). For example, foliar N and P concentrations, the N/P ratio and the scores for the first two PCA axes differed significantly when *Pinus abies* grew in areas with and without *Q. robur* (Extended Data Fig. 8a), although the percentage of explained variance was ≤3.5% (Supplementary Table 9). These shifts were reciprocal. Foliar P concentrations, N/P ratios and PC1 scores also differed significantly between subsets of *Q. robur* growing in areas with and without *P. abies* (Extended Data Fig. 8a and Supplementary Table 10), explaining a maximum of only 5% of total variance. Foliar P concentrations, N/P ratios and PC1 scores differed significantly between *Abies alba* coexisting or not with *Q. petraea* (Extended Data Fig. 8b and Supplementary Table 11), explaining a maximum of 5% of total variance, and foliar N and P concentrations, N/P ratios and PC1 scores differed significantly between *Q. petraea* coexisting or not with *A. alba* (Supplementary Table 12), explaining a maximum of 4% of total variance. Foliar N concentrations and PC1 scores differed significantly between *Fagus sylvatica* growing in areas with and without *Pinus sylvestris* (Extended Data Fig. 8c and Supplementary Table 13), explaining a maximum of 2% of total variance. Foliar N and P concentrations, N/P ratios and PC1 scores differed significantly between *P. sylvestris* growing in areas with and without *F. sylvatica* (Extended Data Fig. 8c and Supplementary Table 14), explaining a maximum of 1% of total variance. The percentage of variance explained for some variables was much higher in some cases—for example, 17% for the foliar N concentration of *P. sylvestris* growing with or without *Q. robur* (Supplementary Table 14) and 35% for the foliar P concentration of *Larix decidua* growing with or without *Q. robur* (Supplementary Table 15).

6.3 Discussion

6.3.1 BN size and phylogeny

Use of the foliar concentrations of several bio-elements significantly separated the species in the hypervolume generated by the corresponding multivariate analyses, as previously reported only in smaller studies[11,12,13,14,15,16,17,18]. The FDA plot (showing 95.8% of total explained variance) clearly separated the BNs of species, with *Pinus* species having positive values on root 1 and *Quercus* species having more negative values, also consistent with the positive link between phylogenetic distance and species-specific BN identity among species.

The elements N, P, K, S, Ca and Mg contribute differentially to plant metabolic and physiological functions and to cells, tissues and organs. We therefore expected that different species, as evolutionary products, would have different optimal elementomes[11,12,13,14]. The results of our analysis on the global set of 227 of the most representative tree species worldwide (163, 58 and 6 from tropical, temperate and boreal biomes, respectively) strongly support this hypothesis. The results also indicated that foliar BNs of the species became more similar as their

phylogenetic distance decreased. These results are fully consistent with Kerkhoff et al.[19], who also found a consistent and significant phylogenetic signal in N and P concentrations in plant organs in a set of 1,287 plant species. A small number of published studies of BNs, all including fewer species, has also reported significant organ or body stoichiometric dependence on taxonomy and/or phylogeny of plant and animal species, although not all studies detected links between species phylogeny and N/P ratios[13,14,15,20]. The great majority of the studies nevertheless found significant relationships between species' elemental composition and taxonomic and phylogenetic distance[13,14,15,20]. Similar results have also been obtained in ionomic studies[21].

Phylogeny and species, as proxies of overall genomic difference, however, did not explain 100% of variability in the elementome. Some of the phylogenetic lines of distant clades may have been exposed to similar environmental conditions that would have driven parallel selection of the characteristics that determine elemental concentrations, consequently eliciting convergence to more similar elementomes than would be expected from their phylogenetic distance. In other words, species that are phylogenetically distant (for example, those that have developed on different continents but under current similar environmental conditions) may occupy a similar BN. For example, a change toward a warmer climate can increase the speed of evolution of several characters differently in different species[5,22,23]. Several other factors, such as species migration, changes in species interactions (for example, with herbivorous or parasitic species) and climatic convergence, can increase the speed of evolutionary convergence among species in different clades[24,25,26]. Distant clades could thus evolve under new, more similar, environmental conditions, favouring a trend toward convergence in functionality and thus in elementome. The results nevertheless indicated that evolutionary processes have significantly contributed to differences in foliar elementomes that originated during species diversification, directly explaining 57–94.5% (average 85.7%) of the variance in foliar concentrations and ratios. Anacker and Strauss[27] also reported that niche differences among species increased with phylogenetic distance, again consistent with our results. Part of the inheritability factor that differs from phylogeny explained an average of about 7% of variance in the variables studied, perhaps due to the recent divergent evolution of more proximal taxonomical species adapting to distinct and divergent environmental shifts in their respective distribution areas.

6.3.2 Climate and N deposition

Several studies have reported trends in foliar N and P concentrations and N/P ratios in trees growing along climatic and latitudinal gradients[28,29,30,31], but their results have not always fully agreed. Most studies have observed a general trend toward decreasing foliar and litter P concentrations and increasing N/P ratios as latitude decreased and MAT, MAP and length of growing season increased[28,29,30,31,32,33,34]. Not all studies, however, have detected clear patterns of N/P ratios among or within climatic areas[33]. Townsend et al.[34] and Lovelock et al.[35],

for example, found no relationship between N/P ratio and either latitude or MAP in tropical areas. Even when a significant relationship was detected in these studies, climatic variables explained only a small fraction of the variation in foliar elements—for example, 16–25 and 5–35% reported by Yuan and Chen[31] and Reich and Oleksyn[28], respectively, depending on the variable. These studies used linear models that considered only climatic variables and N deposition without phylogeny and species as random factors. The variance explained by climatic variables in our study decreased in our Bayesian analyses when we added species as a random factor (0.3–11.0%, with a mean of 4.3% among all variables) (Supplementary Table 6).

Our results thus indicate significant relationships of foliar nutrient composition with N deposition and basic climatic traits such as MAP and MAT at a global scale. A decrease in foliar P concentrations and an increase in foliar N concentrations and N/P ratios with increasing MAT are consistent with the higher N and lower P concentrations in plants frequently observed toward equatorial latitudes. We also identified a general and significant relationship of foliar concentrations of the main macronutrients with N deposition. Lower foliar metal concentrations with higher levels of N deposition are totally consistent with the higher leaching of soil bases associated with N deposition and with competition among bases for plant absorption with ammonium[36]. Higher foliar N and S concentrations with increased N deposition are also due to the consequent higher availability of soil N and S[37,38]. Interestingly, we also identified a globally positive correlation between higher levels of N deposition and higher foliar P concentrations when local studies reported all types of results, from increases to decreases in foliar P concentrations. Increases in P concentrations have been associated with higher capacities of plants and microbes to mobilize and take up more P due to higher N availability[39,40]. Lower foliar P concentrations under higher N loads, however, have been associated with stronger P limitation[41,42,43]. Our results thus indicated that N deposition in forests at the global scale significantly tends to increase foliar P concentrations but also N/P ratios, thus generally trending toward more P-limited forests.

6.3.3 Soil type

Soil type explained a modest but significant amount of variance (0.1–2%) in tree foliar composition and stoichiometry. In fact, soil type and its capacity to supply some of the most important bio-elements to plants is partially due to historic and current climatic conditions. Species foliar elementomes were consistent with the traits of the various soil types (Figure 6.6). Trees growing in Inceptisols and Alfisols, typical of temperate forests, occupied a central position in the PCA space, suggesting a more balanced elemental composition than trees growing in other soil types. Trees growing in Andisols and Vertisols, two soil types rich in readily weathered minerals such as Ca^{2+} and Mg^{2+} (refs. 44,45), typically had higher than average foliar Ca and Mg concentrations. Andisols are volcanic soils that are frequently rich in Fe-Mg silicates and in anorthite, a Ca feldspar. Ertisols are characterized by high concentrations of expandable clays such as vermiculite and montmorillonite that are also rich in Mg and Ca, respectively. Trees

growing in Spodosols (typical of sandy soils) had the lowest concentrations of Ca, Mg and K, which could be linked to the high leaching of basic cations in these acidic soils and, consequently, the low content of exchangeable complexes and slow mineralization. Trees growing in Spodosols also had the highest foliar P concentrations (Supplementary Fig. 10) and thus the highest foliar P/K ratios. Trees growing in Oxisols (wet tropical forests) had the second highest foliar N concentrations, the lowest foliar P concentrations, the highest foliar N/P and N/K ratios and the lowest foliar P/K ratio (Supplementary Fig. 11). These results for Oxisols are consistent with recent observations of low foliar P concentrations and high foliar N/P ratios in wet tropical forests[28,32]. Our global study thus associates high foliar N/K and low foliar P/K ratios with wet tropical forests. Relationships between foliar BN and soil type along natural gradients have recently been observed[46], but these relationships may not be as strong as expected and may not necessarily be universal. Ordoñez et al.[29] observed that the concentrations of some elements and ratios were correlated between soil type and photosynthetic tissues but that others were not.

6.3.4 Competition among coexisting species

Foliar N and P concentrations, N/P ratio and scores for the first two PCA axes for species shifted significantly when the distributions of two species overlapped. These differences usually explained little of the variance in foliar elemental concentrations and ratios but were significant (Supplementary Tables 8–14). For example, foliar N and P concentrations, N/P ratio and scores for the first two PCA axes differed significantly when *P. abies* grew in areas with and without *Q. robur*, although the percentage of explained variance was ≤3.5% (Supplementary Table 8). These shifts were reciprocal and occurred for pairs of tree species that were the most dominant in Europe (Supplementary Tables 8–14). These results from field analyses are consistent with those of an experiment in seminatural grasslands where the target species shifted their elemental compositions depending on neighbouring species[17].

6.3.5 Homeostasis versus plasticity

Intraspecific variability explained a significant amount (2–20%) of the total random variability of all elemental concentrations and ratios (Supplementary Tables 6 and 7). Species that have evolved in highly fluctuating environments are expected to have a greater capacity of functional and/or morphological shifts and thus require a more plastic stoichiometry than species that have evolved in a more stable environment[13,39]. Our results confirmed these expectations: the range of values in the FDA that represented the sizes of species-specific BNs was higher ($P<0.001$ along roots 1 and 2) for species subjected to less climatic stress (*Q. robur* and *Q. petraea*) than for the Mediterranean species adapted to drought (*P. halepensis*, *P. pinaster* and *Q. ilex*) (Figure 6.5 and Supplementary Tables 5 and 6). These results indicated a trade-off between adaptation to being competitive in a stable environment versus being successful in a more fluctuating environment.

Different levels of environmental stress cause a continuum of strategies between homeostasis and plasticity. Species growing in more stressful environments and with poor resource availability have less BN plasticity than those growing in less stressful and richer environments[47,48].

6.4 Conclusions

The results of this study provide clear support for the BN hypothesis[13]. First, each species had a different BN, with a significant trend of larger differences in BNs as phylogenetic distance and evolutionary time increased. Recent evolutionary convergence due, for example, to recent adaptation of distant clades to similar soil or climatic environments, however, indicated that the differences in BN among species could not be fully resolved by phylogenetic analyses alone. Second, environmental factors such as climate and soil type also explained an important part of the intraspecific variance in BN. These effects were moderate but significant and independent of taxonomy. Each species could be represented by its specific space in the hypervolume generated by multivariate analysis of its foliar elemental composition and stoichiometry (elementome), so its specific plasticity was observed in the shift of its space in response to environmental changes. Third, coexisting, competing species tended to have distinct BNs to minimize competitive pressure. Fourth, a trade-off between adaptations to being competitive in a stable environment versus being successful in a more fluctuating environment generated a continuum of strategies between homeostasis and plasticity.

6.5 Methods

6.5.1 Data acquisition

Foliar data

We gathered 23,962 datapoints of foliar N, P, K, Ca, Mg and S concentrations, expressed as percentage dry weight. These data corresponded to 227 tree species at a global scale, including all latitudes and ecosystems. We considered only those tree species with more than three locations. The data were obtained from 192 publications (Supplementary Table 1) and inventories such as the Catalan Forest Inventory[49]. We also gathered and used a subset of 7,479 datapoints with 138 species that included information on foliar C concentration, in addition to foliar N, P, K, Mg, Ca and S concentrations, for identification of possible differences in the analyses with or without C concentrations. All data were obtained from leaves using comparable and homologated analytical methods (see the ICP forests manual, *Sampling and Analysis of Needles and Leaves*, http://icp-forests.net/page/icp-forests-manual). The N, P, K, S, Ca and Mg pairwise ratios were calculated on a mass basis. Nutrient concentrations for the same species from different databases were analysed using mixed models, with database as a fixed factor and country as a random factor; no significant differences were found. All foliar samples were collected between

1990 and 2015. We used data derived from georeferenced plots only; Supplementary Fig. 1 shows the distribution of those plots.

Data for climate, soil and N and P deposition

Climatic and soil data were added to the foliar stoichiometric data using the raster package in R (v.2.6–7). These data were obtained from the WorldClim 2.0 database[50], with a resolution of 1 km2 at the equator: minimum average temperature, maximum average temperature, average solar radiation, maximum wind speed, average wind speed, mean water vapour pressure, MAT, mean diurnal range, isothermality, temperature seasonality, maximum temperature of the warmest month, minimum temperature of the coldest month, annual temperature range, mean temperature of the wettest quarter, mean temperature of the driest quarter, mean temperature of the coldest quarter, MAP, mean precipitation of the wettest month, mean precipitation of the driest month, mean precipitation seasonality, mean precipitation of the wettest quarter, mean precipitation of the driest quarter, precipitation of the warmest quarter and precipitation of the coldest quarter. This climatic model was calculated for a long meteorological time series (1970–2000) based on interpolated values of climatic data provided by meteorological stations throughout the territory and adjusted to the observed topography. Five aridity indices were calculated using the climatic data[51,52,53,54].

The data for the deposition of atmospheric N and P were obtained from Global Threats to Human Water Security and River Biodiversity[55], with a resolution of 1 km2 at the equator. Soil taxonomies (order and suborder) were obtained from the USDA Global Soils Region Map (https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/use/?cid=nrcs142p2_054013), which provides a resolution of 1km2 at the equator.

6.5.2 Phylogenetic and statistical analyses

Phylogenetic signal

We prepared a phylogenetic tree containing the species in our database to test for phylogenetic signals using R statistical software[56]. We thereby obtained a phylogenetic tree containing a selection of species from PhytoPhylo, an available megaphylogeny of vascular plants[57]. We used the read.tree and drop.tip functions from the R ape package[58] to load the PhytoPhylo tree and removed all species that were not in our database.

We used the phylosig function from the R phytools package[59] to test for phylogenetic signals in the foliar elemental compositions of species and therefore to determine the extent to which foliar N, P, K, S, Ca and Mg concentrations, pairwise ratios and PCA scores had phylogenetic signals. The phylosig function calculates statistics of a phylogenetic signal (Pagel's λ) and *P* values based on the variance in phylogenetically independent contrasts relative

to tip shuffling randomization[60]. We chose to analyse phylogenetic signals in the data using Pagel's λ assumption, based on a study by Münkemüller et al.[61] comparing the advantages and disadvantages of various methods for estimation of phylogenetic signals. Pagel's λ method can provide reliable measurements of effect size and discriminate between more complex models of trait evolution (such as polygenic organismic traits)[62]. Mean λ in Pagel's method does not change as the number of species in a phylogeny increases, and is recommended for large phylogenies with >50 species (or taxa)[63], unlike other methods.

We also used the contMap function of the phytools package to graphically reconstruct the values of traits with a phylogenetic signal across our phylogeny. We used the ape package[50] to load the phylogenetic tree and select the species included in it. The contMap function estimates the ancestral characters at internal nodes using maximum likelihood and assuming Brownian motion as a model for trait evolution[55], and then interpolates the ancestral condition along the branches of the tree[64]

BN size

Another interesting trait when comparing taxa is BN size. We thus conducted FDA to determine whether different but closely related species typical of different environments (from more to less climatic stress) tended to have different BN sizes. We compared five of the most important forest species in Europe: *Q. petraea*, *Q. robur*, *Q. ilex*, *P. pinaster* and *P. halepensis*. All five species were represented at 600–800 sites in our database across their distributions. FDA is a multivariate analysis that derives optimal separation between groups (in this case, the different sets of individuals of each species) by maximizing between-group variance and minimizing within-group variance of the set of independent continuous variables used in the analysis (in this case, foliar N, P, K, S, Ca and Mg concentrations and their pairwise ratios)[65]. We compared the range of scores in the first two roots of the FDA as a measure of the size of the 'niche space' of each species. The first two roots of the FDA explained 95.8% of variance in the foliar elemental composition of the various species. We then analysed the roles of phylogeny and species in the dispersion of canonical scores on the root axes of the FDA using Bayesian phylogenetic linear mixed models and the MCMCglmm package[66] in R. Phylogeny and species were included as random factors: the phylogenetic term accounted for variability in the shared ancestry while the species term accounted for species-specific traits independent of shared ancestry.

Analysis of the relationships of foliar elemental composition with climatic variables and N deposition

We tested the effects of climate and N deposition on the foliar concentrations of bio-elements, their ratios and PC scores (from the PCA of all elemental foliar concentrations and their pairwise ratios) using Bayesian phylogenetic linear mixed models and the MCMCglmm package[66] in R.

We used MAT, MAP, annual radiation, mean annual vapour pressure deficit, range of diurnal temperatures and N deposition as fixed effects. Phylogeny and species were included as random factors: the phylogenetic term accounted for variability in shared ancestry while the species term accounted for species-specific traits independent of the shared ancestry. Both random factors together thus accounted for variance explained by heritability. We repeated these analyses using soil type rather than climate and N deposition as fixed effects.

Analysis of the relationships between foliar elemental composition and soil type

We analysed differences in foliar variables among the various soil types (taxonomic orders). A soil map was generated using the R packages raster and rgdal to obtain soil classification for each sample location. We chose soil type (orders of soil taxonomy) as the most accurate taxon at the pixel scale in the USDA Global Soils Region Map. No data were found for Gelisols or Aridisols. We tested the effect of soil order on the foliar concentration of bio-elements, their ratios and PC scores (from the PCA of all elemental foliar concentrations and their pairwise ratios) using Bayesian phylogenetic linear mixed models and the MCMCglmm package[66] in R. We used soil orders as fixed effects. Phylogeny and species were included as random factors: the phylogenetic and species terms were introduced as random factors accounting for variance explained by heritability, as described previously.

We performed PCAs for foliar N, P, K, Ca, Mg and S concentrations and N/P ratios to further explore the relationships between trees growing under different soil types and their overall elemental compositions. We then analysed the scores of the PC1 and PC2 axes to detect differences in overall foliar elemental composition depending on the order of the soil in which they grew, using Bayesian phylogenetic linear mixed models and the MCMCglmm package[66] in R, in which the first three PCA axes were the response variables and soil order was the fixed predictor. Phylogeny and species were included as random factors, as in the previous analysis of FDA scores. Model parameters (soil types) with non-overlapping 95% credible intervals were considered to differ significantly.

Analysis of differences in species foliar elemental composition and stoichiometry between populations growing in different communities with different species compositions

We used the map of species distribution in the European Information System on Forest Genetic database, http://portal.eufgis.org/data/. We compared the foliar N, P and K concentrations of pairwise species with comparable co-occurring and non-co-occurring surfaces with an overlapping distribution of 25–75%. We established sets of individuals in the overlapping area between the compared species and both areas where only one of the species was present. The data were analysed in R using the packages raster (v.3.4.3), rgeos (v.3.4.4), maptools (v.3.4.3), maps (v.3.4.3), rworldmap (v.3.4.4), ggmap (v.3.4.3) and rworldxtra (v.3.4.4). We used these tools

to select species with large distributions and many datapoints in our database (600–800) and combined species in several possible pairs. We compared the two portions of global distributions for each species of each pair that overlapped or not with the distribution of the other species. We used analysis of variance to compare N, P and K concentrations and PC1 and PC2 scores (from the PCA of the six bio-elements and their pairwise ratios) for each species inside and outside the overlapping zone (with or without competition between the two species, respectively).

Supplementary material

All supplementary figures and tables are available online at: https://www.nature.com/articles/s41559-020-01348-1

Data availability

All data are available at the Global Ecology Unit CREAF-CSIC-UAB (glonuteco.creaf.cat/data/).

Code availability

All code is available at the Global Ecology Unit CREAF-CSIC-UAB (glonuteco.creaf.cat/data/).

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Contributions

J.S. and J.P. conceived, designed and supervised the project. J.S., H.V., I.A.J., J.P. and P.Z. searched the bibliography and gathered data. J.S., H.V., P.Z., M.F.-M., A.R., P.C., M.O., I.A.J. and J.P. checked the data and contributed to data accuracy selection. J.S., H.V., G.F.-A., J.P. and M.F.-M. developed the statistical analyses. J.S., J.P., M.F.-M., H.V. and G.F.-A. created the tables and figures. J.S. wrote the paper. J.P., H.V., G.P., A.G.-G., M.F.-M., G.F.-A., A.R., P.C., M.O. and I.A.J. revised the manuscript. All authors read and revised the final version of the manuscript.

References

1. Tracy, C. R. & Christian, K. A. Ecological relations among space, time, and thermal niche axes. *Ecology* **67**, 609–615 (1986).

2. Peterson, A. T., Soberon, J. & Sanchez-Cordero, V. Conservatism of ecological niches in evolutionary time. *Science* **285**, 1265–1267 (1999).

3. Hirzel, A. H., Hausser, J., Chessel, D. & Perrin, N. Ecological-niche factor analysis: how to compute habitat-suitability maps without absence data? *Ecology* **83**, 2017–2036 (2002).

4. Sexton, J. P., Montiel, J., Shay, J. E., Stephens, M. R. & Slatyer, R. A. Evolution of ecological niche breadth. *Rev. Ecol. Evol. Syst.* **48**, 183–206 (2017).

5. Wright, J. W., Davies, K. F., Lau, J. A., McCall, A. C. & McKay, J. K. Experimental verification of ecological niche modelling in a heterogeneous environment. *Ecology* **87**, 2433–2439 (2006).

6. Swanson, H. K. et al. A new probabilistic method for quantifying n-dimensional ecological niches and niche overlap. *Ecology* **96**, 318–324 (2015).

7. Grubb, P. J. The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biol. Rev.* **52**, 107–145 (1977).

8. Herrel, A., Spithoven, L., Van Damme, V. & De Vree, F. Sexual dimorphism of head size in *Gallotia galloti*: testing the divergence hypothesis by functional analyses. *Funct. Ecol.* **13**, 289–297 (1999).

9. Mouillot, D. et al. Niche overlap estimates based on quantitative functional traits: a new family of non-parametric indices. *Oecologia* **145**, 345–353 (2005).

10. Kraft, N., Valencia, R. & Ackerly, D. D. Functional traits and niche based tree community assembly in an Amazonian forest. *Science* **322**, 580–582 (2008).

11. Peñuelas, J., Sardans, J., Ogaya, R. & Estiarte, M. Nutrient stoichiometric relations and biogeochemical niche in coexisting plant species: effect of simulated climate change. *Pol. J. Ecol.* **56**, 613–622 (2008).

12. Peñuelas, J. et al. Faster returns on "leaf economics" and different biogeochemical niche in invasive compared with native plant species. *Glob. Change Biol.* **16**, 2171–2185 (2010).

13. Peñuelas, J. et al. The bioelements, the elementome and the "biogeochemical niche". *Ecology* **100**, e02652 (2019).

14. Sardans, J. et al. Factors influencing the foliar elemental composition and stoichiometry in forest trees in Spain. *Persp. Plant Ecol. Evol. Syst.* **18**, 52–69 (2016).

15. Sardans, J. et al. Foliar elemental composition of European forest tree species associated with evolutionary traits and present environmental and competitive conditions. *Glob. Ecol. Biogeogr.* **24**, 240–255 (2015).

16. Urbina, I. et al. Shifts in the elemental composition of plants during a very severe drought. *Environ. Exp. Bot.* **111**, 63–73 (2015).

17. Urbina, I. et al. Plant community composition affects the species biogeochemical niche. *Ecosphere* **8**, e01801 (2017).

18. White, P. J. et al. Testing distinctness of shoot ionomes of angiosperm families using the Rothamsted Park grass continuous hay experiment. *N. Phytol.* **196**, 101–109 (2012).

19. Kerkhoff, A. J., Fagan, W. F., Elser, J. J. & Enquist, B. J. Phylogenetic and growth form variation in the scaling of nitrogen and phosphorus in the seed plants. *Am. Nat.* **168**, E103–E122 (2006).

20. Sun, L. K. et al. Leaf elemental stoichiometry of *Tamarix* Lour. Species in relation to geographic, climatic, soil, and genetic components in China. *Ecol. Eng.* **106**, 448–457 (2017).

21. Neugebauer, K. et al. Variation in the angiosperm ionome. *Physiol. Plant.* **163**, 306–322 (2018).

22. Gillman, L. N., Keeling, D. J., Gardner, R. C. & Wright, S. D. Faster evolution of highly conserved in tropical plants. *J. Evol. Biol.* **23**, 1327–1330 (2010).

23. Puurtinen, M. et al. Temperature-dependent mutational robustness can explain faster molecular evolution at warm temperatires, affecting speciation rate and global patterns of species diversity. *Ecography* **39**, 1025–1033 (2016).

24. Kellner, A., Ritz, C. M., Schlittenhaedt, P. & Hellwig, F. H. Genetic differentiation in the genus *Lithops* L. (Ruschoideae, Aizoaceae) reveals a high level of convergent evolution and reflects geographic distribution. *Plant Biol.* **13**, 368–380 (2011).

25. Jwa, N. S. & Hwang, B. K. Convergent evolution of pathogen effectors toward reactive oxygen species signaling networks in plants. *Front. Plant Sci.* **8**, 1687 (2017).

26. Molina-Montenegro, M. A. et al. Is the success of plant invasions the result of rapid adaptive evolution in seed traits? Evidence from a latitudinal rainfall gradient. *Front. Plant Sci.* **9**, 208 (2018).

27. Anacker, B. L. & Strauss, S. Y. Ecological similarity is related to phylogenetic distance between species in a cross-niche field transplant experiment. *Ecology* **97**, 1807–1818 (2016).

28. Reich, P. B. & Oleksyn, J. Global patterns of plant leaf N and P in relation to temperature and latitude. *Proc. Natl Acad. Sci. USA* **101**, 11001–11106 (2004).

29. Ordoñez, J. C. et al. A global study of relationships between leaf traits, climate and

soil measures of nutrient fertility. *Glob. Ecol. Biogeogr.* **18**, 137–149 (2009).

30. Kerkhoff, A. J., Enquist, B. J., Elser, J. J. & Fagan, W. F. Plantallometry, stoichiometry and the temperature-dependence of primary productivity. *Glob. Ecol. Biogeogr.* **14**, 585–598 (2005).

31. Yuan, Z. Y. & Chen, H. Y. H. Global trends in senesced-leaf nitrogen and phosphorus. *Glob. Ecol. Biogeogr.* **18**, 532–542 (2009).

32. Vitousek, P. M., Porder, S., Houlton, B. Z. & Chadwick, O. A. Terrestrial phosphorus limitation: mechanisms, implications and nitrogen–phosphorus interactions. *Ecol. Appl.* **20**, 5–15 (2010).

33. McGroddy, M. E., Daufresne, T. & Hedin, L. O. Scaling of C/N/P stoichiometry in forest worldwide: implications of terrestrial Redfield-type ratios. *Ecology* **85**, 2390–2401 (2004).

34. Townsend, A. R., Cleveland, C. C., Asner, G. P. & Bustamante, M. M. C. Controls over foliar N:P ratios in tropical rainforest. *Ecology* **88**, 107–118 (2007).

35. Lovelock, C. E., Feller, I. C., Ball, M. C., Ellis, J. & Sorell, B. Testing the growth rate vs. geochemical hypothesis for latitudinal variation in plant nutrients. *Ecol. Lett.* **10**, 1154–1163 (2007).

36. Marschner, H. *Mineral Nutrition of Higher Plants* (Academic Press, 1995).

37. Zhang, Y. et al. Log-term trends in total inorganic nitrogen and sulfur deposition in US from 1990 to 2010. *Atmos. Chem. Phys.* **18**, 9091–9106 (2018).

38. Horn, K. J. et al. Growth and survival relationships of 71 tree species with nitrogen and sulfur deposition across the conterminous U.S. *PLoS ONE* **14**, e0212984 (2019).

39. Papanikolaou, N., Britton, A. J., Helliwell, R. C. & Johnson, D. Nitrogen deposition, vegetation burning and climate warming act independently on microbial community structure and enzyme activity associated with decomposing litter in low-alpine heath. *Glob. Change Biol.* **16**, 3120–3132 (2010).

40. Marklein, A. R. & Houlton, B. Z. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *N. Phytol.* **193**, 696–704 (2012).

41. Sardans, J. et al. Foliar and soil concentrations and stoichiometry of nitrogen and phosphorus across European *Pinus sylvestris* forests: relationships with climate, N deposition and tree growth. *Funct. Ecol.* **30**, 676–689 (2016).

42. Peñuelas, J. et al. Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. *Nat. Commun.* **4**, 2934 (2013).

43. Penuelas, J. et al. Increasing atmospheric CO2 concentrations correlate with declining nutritional status of European forests. *Commun. Biol.* **3**, 125 (2020).

44. Ahmad, N. & Mermut, A. *Vertisols and Technologies for their Development* 1st edn, Vol. 24 (Elsevier, 1996).

45. Nishiue, A., Nanzyo, M., Kanno, H. & Takahashi, T. Properties and classification of volcanic ash soils around Lake Kuwanuma on the eastern footslope of Mt. Funagata in Miyagi prefecture, northeastern Japan. *Soil Sci. Plant Nutr.* **60**, 848–862 (2014).

46. De la Riva, E. G. et al. Biogeochemical and ecomorphological niche segregation of Mediterranean woody species along a local gradient. *Fron. Plant Sci.* **8**, 1242 (2017).

47. Yu, Q. et al. Stoichiometry homeostasis of vascular plants in the inner Mongolia grassland. *Oecologia* **166**, 1–10 (2011).

48. Sardans, J., Albert Rivas-Ubach, A. & Peñuelas, J. The elemental stoichiometry of aquatic and terrestrial ecosystems and its relationships with organismic lifestyle and ecosystem structure and function: a review. *Biogeochemistry* **111**, 1–39 (2012).

49. Gracia, C., Burriel, J. A., Ibàñez, J. J., Mata, T. & Vayreda, J. *Inventari ecològic i forestal de Catalunya: regió forestal V* (CREAF, 2004).

50. Fick, A. E. & Hijmans, R. J. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.* **37**, 4302–4315 (2017).

51. Lang, R. *Verwitterung und Bodenbildung als Einfuehrung in die Bodenkunde* (Schweizerbart Science Publishers, 1920).

52. Köppen, W. Klassification der Klimate nach Tempertur, Niederschlag and Jahreslauf. *Petermanns Geog. Mitt.* **64**, 243–248 (1918).

53. De Martonne, E. Nouvelle carte mondiale de l'indece d'aridité. *Ann. Géogr.* **51**, 242–250 (1942).

54. Emberger, L. La vegetation de la región Mèditeranéenne, essai d'une classification des groupements vegetaux. *Rev. Gén. Bot.* **42**, 641–662, 705–721 (1930).

55. Vorösmarty, C. J. et al. Global threats to human water security and river biodiversity. *Nature* **467**, 555–561 (2010).

56. R Development Core Team *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2011).

57. Qian, H. & Jin, Y. An updated megaphylogeny of plants, a tool for generating plant phylogenies, and an analysis of phylogenetic community structure. *J. Plant Ecol.* **9**, 233–239 (2016).

58. Paradis, E., Claude, J. & Strimmer, K. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* **20**, 289–290 (2004).

59. Revell, L. J. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* **3**, 217–223 (2012).

60. Blomberg, S. P., Garland, T. & Ives, A. R. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**, 717–745 (2003).

61. Münkemüller, T. et al. How to measure and test phylogenetic signal. *Methods Ecol. Evol.* **3**, 743–756 (2012).

62. Pagel, M. Inferring the historical patterns of biological evolution. *Nature* **401**, 877–884 (1999).

63. Felsenstein, J. Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15 (1985).

64. Revell, L. J. Two new graphical methods for mapping trait evolution on phylogenies. *Methods Ecol. Evol.* **4**, 754–759 (2013).

65. Raamsdonk, L. M. et al. A functional genomics strategy that uses metabolome data to reveal the phenotype of silent mutations. *Nat. Biotechnol.* **19**, 45–50 (2001).

66. Hadfield, J. D. MCMC methods for multi-response generalised linear mixed models: the MCMCglmm R package. *J. Stat. Softw*. **33**, 2 (2010).

7

General conclusions

In this thesis I have worked around the elemental composition and stoichiometry in leaves of woody plants, and I have established and/or determined their relations with environmental drivers. I started exploring the effect of N deposition on foliar elemental composition and stoichiometry of dominant species in a Mediterranean forest under drought conditions through an experimental design. I obtained evidence of the capability of Mediterranean species to incorporate rapidly this N addition under drought conditions even though its magnitude differs between species. Thus, we observed that the addition of N can partially counteract the decrease of N as a response of drought. Despite of these results we cannot consider the addition of N as a good drought effect palliative because its addition could generate nutrient imbalances and modify the competition capacity of the species.

In a continental context of Europe, I wanted to describe the defoliation tendencies, determine the relationship of defoliation with environmental conditions and establish the relation between defoliation and foliar elemental composition, which is a new approach. All these relations intended to be useful in terms of determining the health of European forests relating higher defoliation rates with worse forest health state. According to the results, the defoliation is increasing in Europe the last decades and it is expected to keep increasing due to climate change conditions. Also, gymnosperms showed higher defoliation rates than angiosperms and most of the studied species showed an increasing defoliation. We emphasize the necessity of considering the different pressures that affect different latitudes. Homogeneity of precipitation, spring conditions and climatic legacy of the previous year also appeared to be very important in defoliation. Furthermore, I want to highlight the established relation of defoliation with foliar elemental composition where foliar N has shown to be strongly related with defoliation, with an inverse correlation. Foliar S, Ca and P were also important in some European species.

The increasing attention of foliar elemental composition in the scientific community generated a big amount of global data in the last years that, in conjunction with new computational resources and techniques allows to conduct an accurate analysis at global scale. In this thesis I used these resources to provide high resolution global maps of N, P, K and N:P foliar elemental composition which, from my point of view, are a promising tool to increase the

accuracy of global C-cycle modeling. In all the cases the foliar elemental composition responded differently to climatic pressures depending on its morphoclimatic group. The global distribution of N, P and K did not have a strong latitudinal component but N:P ratio clearly showed an inverse correlation with latitude. Furthermore, N, P and N:P generally followed the soil-age hypothesis.

Most of the chapters in this thesis discuss around the biogeochemical niche hypothesis and in all the cases the importance of species identity or the genetic pool is highlighted. The most specific chapter around this topic is the 6th, where a clear and specific empirical support confirming the biogeochemical niche theory is provided. According to the results, from 60% to 94% of chemical variability depends on phylogenetic differences between species, from 1% to 7% depends on environmental conditions and the rest vary depending on biotic competitions. In the global models of N, P, K and N:P also a big part of explained variability depended on the evolutionary history of the studied species and in the 2nd chapter the different studied species had an independent multi-space of chemical conditions and reacted differently to the different conditions. So, with all this evidence gathered, genetic legacy and species identity should be considered in future investigations regarding foliar elemental composition.

Appendix

Appendix III: Foliar elemental composition and weather as drivers of defoliation rate in European forests

Table S3.1. Studied variables summary including variable name, origin and brief description

Variable name	Origin	Description
SPEI 01 annual avg.	https://spei.csic.es/	Annual average SPEI of 1 month window
SPEI 01 spring avg.	https://spei.csic.es/	Spring average SPEI of 1 month window
SPEI 01 summer avg.	https://spei.csic.es/	Summer average SPEI of 1 month window
SPEI 03 annual avg.	https://spei.csic.es/	Annual average SPEI of 3 month window
SPEI 03 spring avg.	https://spei.csic.es/	Spring average SPEI of 3 month window
SPEI 03 summer avg.	https://spei.csic.es/	Summer average SPEI of 3 month window
SPEI 06 annual avg.	https://spei.csic.es/	Annual average SPEI of 6 month window
SPEI 06 spring avg.	https://spei.csic.es/	Spring average SPEI of 6 month window
SPEI 06 summer avg.	https://spei.csic.es/	Summer average SPEI of 6 month window
SPEI 12 annual avg.	https://spei.csic.es/	Annual average SPEI of 12 month window
SPEI 12 spring avg.	https://spei.csic.es/	Spring average SPEI of 12 month window
SPEI 12 summer avg.	https://spei.csic.es/	Summer average SPEI of 12 month window
MAP	CRU (Harris et al., 2020)	Mean annual precipitation
MAP spring	CRU (Harris et al., 2020)	Mean spring precipitation
MAP winter	CRU (Harris et al., 2020)	Mean winter precipitation
MAT	CRU (Harris et al., 2020)	Mean annual temperature
MAT spring	CRU (Harris et al., 2020)	Mean spring temperature
MAT winter	CRU (Harris et al., 2020)	Mean winter temperature
MAP ant.	Transformation	Mean annual precipitation of previous year
MAP spring ant.	Transformation	Mean annual precipitation of previous spring
MAP winter ant.	Transformation	Mean annual precipitation of previous winter
MAT ant.	Transformation	Mean annual temperature of previous year
MAT spring ant.	Transformation	Mean annual temperature of previous spring
MAT winter ant.	Transformation	Mean annual temperature of previous winter
Foliar N	ICP forests	In dry weight (mg/g)
Foliar S	ICP forests	In dry weight (mg/g)
Foliar P	ICP forests	In dry weight (mg/g)

Table S3.1 Continuation

Foliar P	ICP forests	In dry weight (mg/g)
Foliar Ca	ICP forests	In dry weight (mg/g)
Foliar mg	ICP forests	In dry weight (mg/g)
Foliar K	ICP forests	In dry weight (mg/g)
Soil Org. C	Sun Yat-sen Univ. (Shangguan et al., 2014)	
Soil pH H_2O	Sun Yat-sen Univ. (Shangguan et al., 2014)	
N dep. ox.	Sun Yat-sen Univ. (Shangguan et al., 2014)	
N dep. red.	Sun Yat-sen Univ. (Shangguan et al., 2014)	
S ox.	Sun Yat-sen Univ. (Shangguan et al., 2014)	
Soil pH difference	Transformation	Soil pH H_2O - Soil pH $CaCl_2$
Thermal amplitude	Transformation	Annual maximum temperature - Annual minimum temperature

Table S3.2 Summary of Chow test results between angiosperms and gymnosperms in al-Europe and all three latitudinal fringes.

Angiosperms vs gymnosperms	F-value	P-value
General	3476.237	0.000
North	704.863	0.000
Central	2821.822	0.000
Mediterranean	265.789	0.000

Appendix IV: Global maps and factors driving forest foliar elemental composition: the importance of evolutionary history
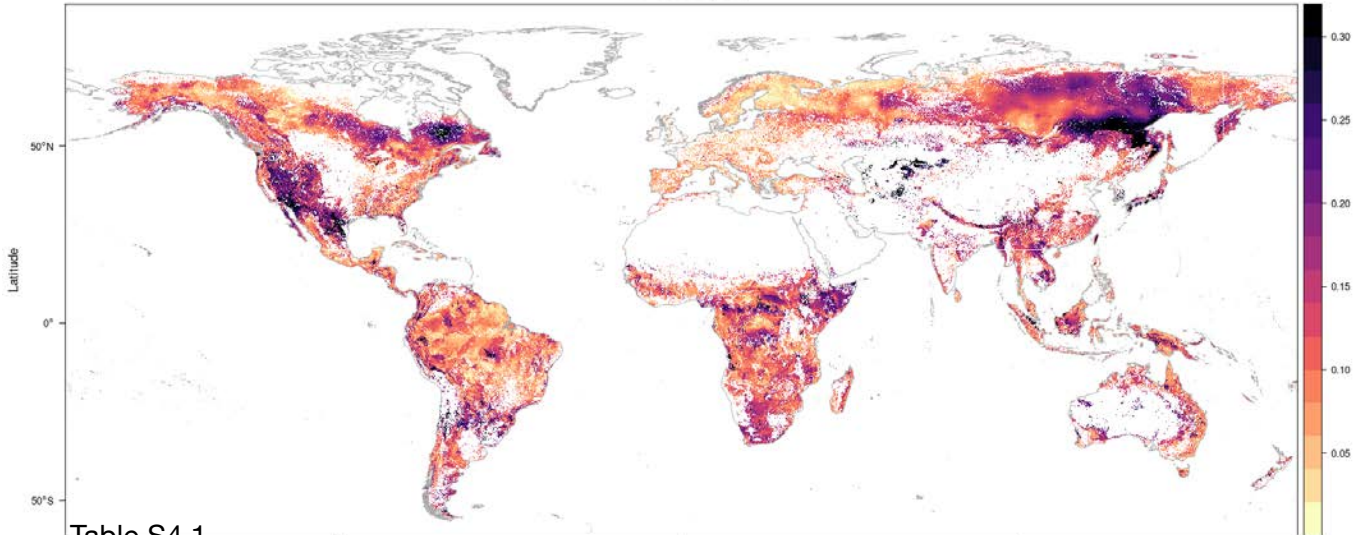
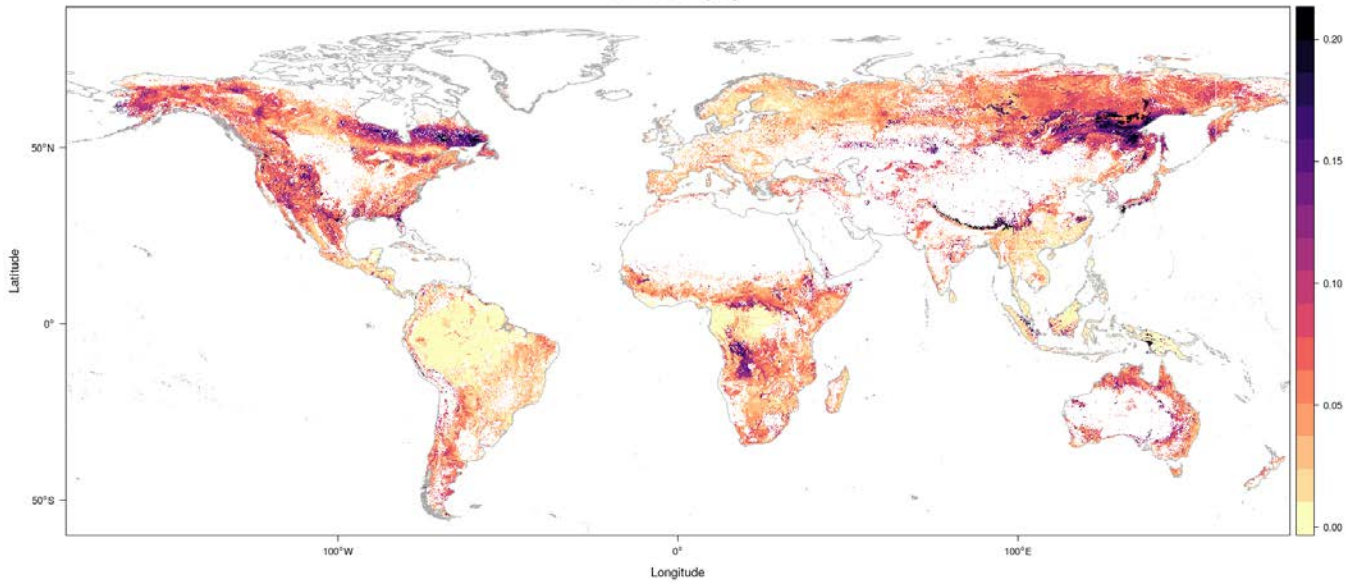**SE of N (%)**

Table S4.1

SE of P (%)

Figure S4.1 Continue
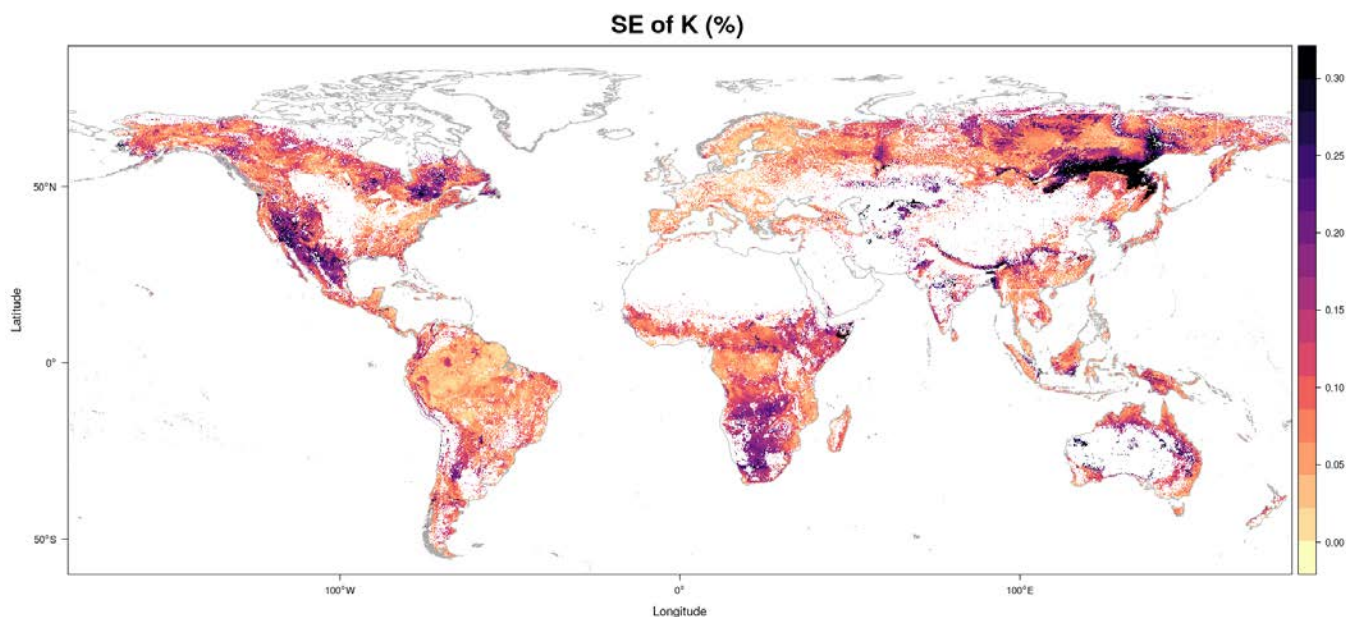
Figure S4.1 Neural network standard error (SE) maps of a) N, b) P, and c) K for tree covered zones. White areas indicate no tree vegetation cover.

Table S4.1 Root Mean Squared Error (RMSE), Mean Absolut Error (MAE), Mean, Standard Deviation of the predictions (SD) and Coefficient of Variation (CV) of neural network prediction for each forest morfoclimatic type.

	Nutrient	Tropical evergreen	Tropical deciduous	Temperate coniferous	Temperate evergreen broadleaves	Temperate deciduous broadleaves	Boreal	Mean
RMSE (%)	N	0.483	0.548	0.251	0.335	0.446	0.310	0.395
MAE (%)		0.377	0.426	0.185	0.242	0.312	0.214	0.293
Mean (%)		1.793	1.916	1.264	1.660	2.187	1.528	1.725
SD (%)		0.333	0.191	0.423	0.257	0.254	0.375	0.306
CV (%)		18.572	9.969	33.465	15.482	11.614	24.542	18.941
RMSE (%)	P	0.061	0.072	0.055	0.057	0.057	0.057	0.060
MAE (%)		0.047	0.058	0.042	0.044	0.043	0.042	0.046
Mean (%)		0.109	0.139	0.140	0.160	0.169	0.135	0.142
SD (%)		0.119	0.149	0.148	0.165	0.110	0.135	0.137
CV (%)		109.298	107.013	105.697	102.726	64.761	100.087	98.264
RMSE (%)	K	0.266	0.268	0.161	0.268	0.224	0.161	0.225
MAE (%)		0.208	0.204	0.121	0.194	0.168	0.120	0.169
Mean (%)		0.738	0.825	0.641	0.814	0.918	0.678	0.769
SD (%)		0.204	0.184	0.089	0.031	0.078	0.111	0.116
CV (%)		27.642	22.303	13.885	3.808	8.497	16.372	15.418

Table S4.2 Post-hoc results of predicted foliar N, P and K concentrations for the different soil orders. Q25, Q50 and Q75 are the quartiles.

	Element	mean	std	r	Q25	Q50	Q75
Oxisols		1.925	0.380	9747759	1.696	1.877	2.067
Vertisols		1.827	0.421	1474053	1.518	1.840	2.159
Aridisols		1.809	0.451	5965666	1.540	1.773	2.079
Entisols		1.792	0.442	10399889	1.491	1.761	2.062
Mollisols		1.745	0.456	3835740	1.440	1.732	2.042
Gelisols	N	1.708	0.423	12555849	1.355	1.668	2.024
Ultisols		1.695	0.377	9532959	1.465	1.689	1.924
Inceptisols		1.665	0.419	23939807	1.334	1.610	1.947
Andisols		1.656	0.437	992731	1.357	1.603	1.896
Alfisols		1.629	0.492	11362246	1.225	1.577	1.949
Histosols		1.349	0.531	2068271	1.072	1.181	1.387
Spodosols		1.305	0.360	8112938	1.077	1.199	1.449
Vertisols		0.154	0.063	1468477	0.106	0.152	0.196
Mollisols		0.153	0.066	3717903	0.110	0.146	0.185
Gelisols		0.153	0.087	12438420	0.092	0.140	0.192
Inceptisols		0.144	0.075	23093731	0.091	0.134	0.186
Aridisols		0.140	0.059	5861452	0.101	0.136	0.175
Alfisols	P	0.134	0.057	11325446	0.096	0.133	0.170
Andisols		0.132	0.063	961789	0.087	0.127	0.167
Entisols		0.127	0.068	10135525	0.082	0.118	0.163
Spodosols		0.127	0.051	7996245	0.093	0.128	0.157
Histosols		0.116	0.065	1839547	0.068	0.108	0.150
Ultisols		0.096	0.052	8855638	0.062	0.088	0.121
Oxisols		0.094	0.039	9510712	0.069	0.092	0.116
Vertisols		0.977	0.288	1478594	0.764	0.981	1.157
Aridisols		0.938	0.255	5844462	0.768	0.909	1.065
Entisols		0.875	0.255	11351275	0.707	0.844	1.004
Mollisols	K	0.843	0.216	4005397	0.690	0.824	0.974
Histosols		0.829	0.385	2101576	0.607	0.727	0.858
Andisols		0.804	0.255	990715	0.642	0.768	0.923
Ultisols		0.746	0.239	9789001	0.586	0.711	0.863
Alfisols		0.745	0.209	11510110	0.608	0.722	0.850

TS4.2 Continuation

Oxisols		0.743	0.286	9777929	0.565	0.714	0.852
Inceptisols	K	0.697	0.240	24365193	0.530	0.679	0.827
Gelisols		0.608	0.218	12520339	0.444	0.587	0.737
Spodosols		0.575	0.193	8053901	0.457	0.540	0.665

Table S4.3 Generalized Mixed Model (GLM) results for foliar N, P and K concentrations. It includes variable slope and p value, model Akaike Information Criterion (AIC) and model R2. See Table S7 for description of variable abbreviations.

		N		P			K		
		Estimate	Pr(>|t|)		Estimate	Pr(>|t|)		Estimate	Pr(>|t|)
	(Intercept)	-2.896	0.000	(Intercept)	44.934	0.000	(Intercept)	1.132	0.000
	Ai_et0	0.000	0.000	Inorganic_Ndeptot	-0.005	0.000	EXH	0.172	0.000
	PHH2O	0.062	0.000	PETseasonality	0.085	0.000	Inorganic_Ndepdry	-0.000	0.048
	Var41	0.000	0.000	PHCA	-0.188	0.000	Bio_12	0.000	0.000
Tropical evergreen	Bio_15	0.000	0.010	Bio_3	-0.374	0.000	EXH:inorganic_Ndepdry	-0.000	0.000
	Ai_et0:PHH2O	-0.000	0.000	Bio_9	-1.038	0.000	EXH:Bio_12	-0.000	0.000
	PHH2O:var41	-0.000	0.000	Bio_3:Bio_9	0.016	0.000	Inorganic_Ndepdry:Bio_12	0.000	0.011
	AIC / pR²	7264.805	0.101	AIC / pR²	-13489.126	0.116	AIC / pR²	658.477	0.054
	(Intercept)	0.455	0.000	(Intercept)	22.451	0.000	(Intercept)	2.189	0.000
	BS	-0.002	0.000	BS	-0.118	0.000	Et0_yr	-0.000	0.000
	CEC	-0.002	0.051	EXCA	0.091	0.021	Oxred_Ndepwetred	-0.003	0.000
	GRAV	0.005	0.001	PHH2O	-0.199	0.000	Bio_7	-0.006	0.453
Tropical deciduous	PETseasonality	0.005	0.000	Bio_3	-0.054	0.000	Oxred_Ndepwetred:Bio_7	0.000	0.001
	BS:CEC	0.000	0.000	BS:PHH2O	0.002	0.000			
	CEC:GRAV	-0.000	0.000						
	AIC / pR²	2844.228	0.060	AIC / pR²	-4219.656	0.049	AIC / pR²	170.243	0.044
	(Intercept)	0.668	0.000	(Intercept)	13.730	0.000	(Intercept)	-0.086	0.495
	BS	-0.001	0.000	Ai_et0	0.000	0.000	Ai_et0	0.000	0.000
	Et0_yr	-0.000	0.000	EXK	9.917	0.000	PETseasonality	0.008	0.000
	PHCA	0.005	0.000	PHH2O	-0.144	0.000	Var29	0.006	0.000
Temperate coniferous	PHK	-0.109	0.000	PHK	-2.859	0.000	Bio_1	0.109	0.000
	Bio_18	0.000	0.000	Ai_et0:EXK	-0.001	0.000	Ai_et0:var29	-0.000	0.000
	Et0_yr:PHK	0.000	0.000	PHH2O:PHK	0.044	0.000	Ai_et0:Bio_1	-0.000	0.000
	AIC / pR²	4056.611	0.249	AIC / pR²	-44732.147	0.184	AIC / pR²	-9733.303	0.043

Table S4.3 Continuation

Temperate broadleaved evergreen	(Intercept)	-0.442	0.020	(Intercept)	14.905	0.000	(Intercept)	3.552	0.000
	Ai_et0	0.000	0.000	Ai_et0	-0.000	0.000	Et0_yr	-0.000	0.404
	Et0_yr	0.000	0.001	PETseasonality	-0.065	0.000	Var41	-0.000	0.019
	PHH2O	0.018	0.000	Bio_14	0.146	0.000	Bio_17	-0.014	0.000
	Ai_et0:et0_yr	0.000	0.000	Bio_17	-0.096	0.000	Et0_yr:Bio_17	-0.000	0.081
	Ai_et0:PHH2O	-0.000	0.000	Ai_et0:Bio_17	0.000	0.000	Var41:Bio_17	0.000	0.000
	Et0_yr:PHH2O	-0.000	0.000	PETseasonality:Bio_17	0.001	0.000			
	AIC / pR^2	2924.360	0.055	AIC / pR^2	-7082.053	0.049	AIC / pR^2	194.543	0.028
Temperate broadleaved deciduous	(Intercept)	0.505	0.000	(Intercept)	5.925	0.000	(Intercept)	0.999	0.000
	Ai_et0	-0.000	0.000	Organic_Ndepdry	0.378	0.000	Ai_et01	-0.000	0.490
	PHH2O	-0.000	0.048	Oxred_Ndeptotox	-0.004	0.002	PHCA	0.003	0.000
	Bio_12	0.000	0.000	PNZ	-0.001	0.000	Bio_14	0.001	0.256
	Bio_17	-0.000	0.000	Var31	0.084	0.000	Bio_19	0.001	0.002
	Ai_et0:Bio_17	0.000	0.000	Bio_1	0.604	0.000	Ai_et0:Bio_14	0.000	0.000
				Var31:Bio_1	-0.007	0.000	Bio_14:Bio_19	-0.000	0.000
	AIC / pR^2	7441.211	0.076	AIC / pR^2	-6455.099	0.098	AIC / pR^2	-226.012	0.026
Boreal	(Intercept)	1.016	0.000	(Intercept)	6.326	0.000	(Intercept)	3.460	0.000
	var28	0.086	0.000	Oxred_Ndepwetox	-0.006	0.013	Inorganic_Ndepdry	-0.002	0.000
	var29	-0.060	0.000	Var29	0.019	0.000	Bio_11	0.124	0.001
	Bio_5	-0.029	0.000	Bio_1	0.364	0.000	Bio_12	-0.001	0.000
	var28:var29	-0.000	0.000	Bio_10	-0.039	0.191	Bio_6	0.060	0.021
	var28:Bio_5	-0.004	0.000	Oxred_Ndepwetox:Bio_	0.005	0.000	Bio_11:Bio_6	0.004	0.000
	var29:Bio_5	0.003	0.000	Bio_1:Bio_10	-0.056	0.000	Bio_12:Bio_6	-0.000	0.000
	AIC / pR^2	3206.911	0.388	AIC / pR^2	-9105.834	0.079	AIC / pR^2	-1562.190	0.070

Table S4.4 (a and b): Generalized linear mixed model (GLMM) fixed effects with significant variables in bold (<0.05). Description of abbreviations in Table S7 (a). Glmm performance and % of variance explained by each random factor: R2 (random) = R2c - R2m (b).

		Nitrogen fixed effects		Phosphorus fixed effects			Potassium fixed effects		
		post mean	p MCMC		post mean	p MCMC		post mean	p MCMC
Tropical evergreen	**(Intercept)**	2.222	0.001	**(Intercept)**	0.062	0.012	**(Intercept)**	0.488	0.001
	Bio_15	0.001	0.407	**Bio_3**	0.000	0.199	**Bio_4**	-0.000	0.464
	Ai_et0	0.000	0.253	**Bio_9**	-0.001	0.088	**Bio_6**	0.003	0.563
	Var41	-0.000	0.001	**PETseasonality**	-0.001	0.003	**Bio_7**	0.010	0.047
	BS	-0.000	0.880	**PHCA**	0.001	0.001	**Bio_12**	0.000	0.484
	PHH2O	-0.003	0.199	**Inorganic_Ndepdry**	0.000	0.015	**EXH**	-0.006	0.084
	PHK	0.039	0.189	**Inorganic_Ndeptot**	-0.000	0.764	**Inorganic_Ndepdry**	0.000	0.169
Tropical deciduous	**(Intercept)**	2.281	0.001	**(Intercept)**	0.010	0.853	**(Intercept)**	0.370	0.360
	PETseasonality	-0.012	0.001	**Bio_3**	0.000	0.024	**Bio_1**	-0.003	0.821
	BS	-0.002	0.483	**Bio_17**	0.000	0.061	**Bio_7**	0.000	0.993
	CEC	-0.002	0.676	**BS**	-0.000	0.713	**Et0_yr**	0.000	0.032
	EXH	-0.007	0.547	**EXCA**	-0.002	0.012	**Var30**	0.000	0.700
	GRAV	0.002	0.433	**PHCA**	0.000	0.677	**Oxred_Ndepdryred**	0.000	0.617
	PHH2O	0.003	0.631	**PHH2O**	0.001	0.089	**Oxred_Ndepwetred**	0.000	0.805
Temperate coniferous	**(Intercept)**	2.048	0.001	**(Intercept)**	0.350	0.891	**(Intercept)**	0.607	0.708
	Bio_9	0.004	0.001	**Ai_et0**	-0.000	0.077	**Bio_11**	-0.001	0.492
	Bio_18	0.000	0.080	**BS**	0.000	0.001	**Bio_13**	0.001	0.001
	Et0_yr	-0.001	0.001	**EXK**	-0.002	0.929	**Ai_et0**	-0.000	0.001
	BS	0.003	0.001	**PHCA**	-0.000	0.924	**PETseasonality**	0.000	0.880
	PHCA	-0.010	0.001	**PHH2O**	-0.001	0.001	**Var28**	0.000	0.132
	PHK	0.032	0.001	**PHK**	-0.007	0.204	**Var29**	0.000	0.717

Table S4.4 a. Continuation

Temperate broadleaved evergreen	(Intercept)	1.857	0.001	(Intercept)	0.129	0.001	(Intercept)	0.501	0.001		
	Et0_yr	-0.000	0.004	Bio_1	-0.000	0.372	Bio_14	0.004	0.001		
	Ai_et0	-0.000	0.181	Var31	0.000	0.003	Bio_17	-0.001	0.059		
	BS	-0.001	0.011	PNZ	0.000	0.424	Et0_yr	0.000	0.004		
	PHCA	0.007	0.001	Inorganic_Ndepwet	0.000	0.001	Var41	-0.000	0.311		
	PHH2O	-0.002	0.056	Organic_Ndepdry	-0.001	0.205	Inorganic_Ndeptot	0.000	0.001		
	PHK	-0.067	0.001	Oxred_Ndeptotox	-0.000	0.001	Oxred_Ndepwetox	-0.002	0.001		
Temperate broadleaved deciduous	(Intercept)	1.939	0.001	(Intercept)	0.146	0.001	(Intercept)	0.877	0.001		
	Bio_12	-0.000	0.003	Bio_14	-0.001	0.001	Bio_12	0.000	0.380		
	Bio_17	0.000	0.057	Bio_17	0.000	0.001	Bio_13	-0.000	0.929		
	Bio_19	-0.000	0.149	Ai_et0	-0.000	0.713	Bio_14	-0.000	0.552		
	Ai_et0	0.000	0.001	PETseasonality	-0.000	0.005	Bio_19	0.000	0.023		
	Var28	0.000	0.479	EXCA	-0.000	0.017	Ai_et0	-0.000	0.001		
	PHH2O	0.001	0.300	Inorganic_Ndeptot	-0.000	0.583	PHCA	-0.001	0.001		
Boreal	(Intercept)	1.529	0.001	(Intercept)	0.236	0.001	(Intercept)	0.643	0.001		
	Bio_1	0.001	0.887	Bio_1	0.002	0.003	Bio_6	0.017	0.025		
	Bio_5	0.009	0.032	Bio_10	0.004	0.001	Bio_11	-0.029	0.003		
	Bio_9	0.001	0.683	Et0_yr	-0.000	0.001	Bio_12	0.000	0.753		
	Var28	-0.009	0.001	Var29	-0.000	0.001	Bio_13	0.000	0.939		
	Var29	0.007	0.001	Oxred_Ndeptotox	0.000	0.519	Var29	-0.000	0.948		
	Inorganic_Ndepw	-0.000	0.397	Oxred_Ndepwetox	-0.000	0.124	Inorganic_Ndepdry	0.000	0.001		

Table S4.4 b.

		R^2c	R^2m (fixed)	R^2 (random)	phylo	species	units
Nitrogen	Tropical evergreen	0.742	0.018	0.723	0.555	0.182	0.263
	Tropical deciduous	0.551	0.036	0.515	0.406	0.128	0.466
	Temperate coniferous	0.720	0.082	0.638	0.319	0.372	0.309
	Temperate broadleaved evergreen	0.826	0.008	0.817	0.500	0.324	0.176
	Temperate broadleaved deciduous	0.436	0.010	0.426	0.144	0.287	0.570
	Boreal	0.769	0.012	0.758	0.548	0.218	0.234
Phosphorus	Tropical evergreen	0.662	0.024	0.639	0.435	0.219	0.346
	Tropical deciduous	0.424	0.030	0.394	0.220	0.186	0.594
	Temperate coniferous	0.988	0.000	0.988	0.552	0.435	0.012
	Temperate broadleaved evergreen	0.802	0.009	0.793	0.296	0.505	0.200
	Temperate broadleaved deciduous	0.795	0.006	0.789	0.247	0.546	0.206
	Boreal	0.752	0.017	0.736	0.420	0.328	0.252
Potassium	Tropical evergreen	0.494	0.037	0.456	0.176	0.298	0.526
	Tropical deciduous	0.337	0.082	0.254	0.194	0.082	0.724
	Temperate coniferous	0.980	0.000	0.980	0.559	0.421	0.020
	Temperate broadleaved evergreen	0.388	0.022	0.366	0.116	0.258	0.626
	Temperate broadleaved deciduous	0.239	0.016	0.223	0.134	0.092	0.774
	Boreal	0.816	0.011	0.805	0.603	0.210	0.187

Table S4.5 Final number of samples used in each morphoclimatic forest type model

	N	P	K
Tropical evergreen	3862	3761	1946
Tropical deciduous	1341	1368	366
Temperate coniferous	11152	11165	10720
Temperate broadleaved evergreen	2267	1799	1486
Temperate broadleaved deciduous	3813	3791	2825
Boreal	2196	1842	1645
sum	24631	23726	18988

Table S4.6 Published articles used as a source for data assembling.

Author	Journal	Title	Authors	Vol	Num	Pags	DOI
Achat et al 2018	Ecological Monographs	Nutrient remobilization in tree foliage as affected by soil nutrients and leaf life span	Achat, DL (Achat, David Ludovick)[1]; Pousse, N (Pousse, Noemie)[2]; Nicolas, M (Nicolas, Manuel)[3]; Augusto, L (Augusto, Laurent)[4]	88	3	408-428	
Aerts et al. 2009	Functional Ecology	Seasonal climate manipulations result in species-specific changes in leaf nutrient levels and isotopic composition in a sub-arctic bog	Aerts, R (Aerts, Rien)[1]; Callaghan, TV (Callaghan, Terry V.)[2,3]; Dorrepaal, E (Dorrepaal, Ellen)[1]; van Logtestijn, RSP (van Logtestijn, Richard S. P.)[1]; Cornelissen, JHC (Cornelissen, Johannes H. C.)[1]	23	4	680-688	10.1111/ j.1365-2435.2009.01566.x
Albaugh et al. 2010	Southern Journal of Applied Forestry	Characterization of Foliar Macro- and Micronutrient Concentrations and Ratios in Loblolly Pine Plantations in the Southeastern United States	Albaugh, JM (Albaugh, Janine M.)[1]; Blevins, L (Blevins, Leandra)[1]; Allen, HL (Allen, H. Lee)[1]; Albaugh, TJ (Albaugh, Timothy J.)[1]; Fox, TR (Fox, Thomas R.)[2]; Stape, JL (Stape, Jose L.)[1]; Rubilar, RA (Rubilar, Rafael A.)[3]	34	2	53-64	10.1093/sjaf/34.2.53
Almeida et al. 2014	Ciências Agrárias	Nutrient cycling in mango trees	Cinara Xavier de Almeida1*; José Luiz Pita Junior2; Danilo Eduardo Rozane3; Henrique Antunes de Souza4; Amanda Hernandes5; William Natale6; Antonio Sergio Ferraudo	35	1	259-266	10.5433/1679-0359.2014 v35n1p259
Alongi et al. 2003	Trees-structure and function	Nutrient partitioning and storage in arid-zone forests of the mangroves Rhizophora stylosa and Avicennia marina	Alongi, DM (Alongi, DM); Clough, BF (Clough, BF); Dixon, P (Dixon, P); Tirendi, F (Tirendi, F)	17	1	51-60	10.1007/ s00468-002-0206-2

Ameztegui et al 2017	Functional Ecology	Shade tolerance and the functional trait: demography relationship in temperate and boreal forests	Ameztegui, A (Ameztegui, Aitor)[1,2,3]; Paquette, A (Paquette, Alain)[1]; Shipley, B (Shipley, Bill)[4]; Heym, M (Heym, Michael)[5]; Messier, C (Messier, Christian)[1,6]; Gravel, D (Gravel, Dominique)[4,7,8]	31	4	821-830	10.1111/1365-2435.12804
Amores et al. 2006	Water, Air and Soil Pollution	Nutritional status of Northern Spain beech forests	Amores, G (Amores, G.); Bermejo, R (Bermejo, R.); Elustondo, D (Elustondo, D.); Lasheras, E (Lasheras, E.); Santamaria, JM (Santamaria, J. M.)	177	(1-4)	227-238	10.1007/s11270-006-9157-3
Andivia et al. 2010	European Journal Forest Research	Nutrients return from leaves and litterfall in a mediterranean cork oak (Quercus suber L.) forest in southwestern Spain	Andivia, E (Andivia, Enrique)[1]; Fernandez, M (Fernandez, Manuel)[1]; Vazquez-Pique, J (Vazquez-Pique, Javier)[1]; Gonzalez-Perez, A (Gonzalez-Perez, Aranzazu)[1]; Tapias, R (Tapias, Raul)[1]	129	1	(5-12)	10.1007/s10342-009-0274-6
Annan-Afful et al. 2004	Soil Science and Plant Nutrition	Nutrient and bulk density characteristics of soil profiles in six land use systems along topo-sequences in inland valley watersheds of Ashanti region, Ghana	Annan-Afful, E (Annan-Afful, E); Iwashima, N (Iwashima, N); Otoo, E (Otoo, E); Asubonteng, KO (Asubonteng, KO); Kubota, D (Kubota, D); Kamidohzono, A (Kamidohzono, A); Masunaga, T (Masunaga, T); Wakatsuki, T (Wakatsuki, T)	50	5	649-664	10.1080/00380768.2004.10408522
Artemkina et al., 2016	Russian Journal of Ecology	Chemical Composition of Juniperus Sibirica Needles (Cupressaceae) in the Forest-Tundra Ecotone, the Khibiny Mountains	Artemkina, N.A., Orlova, M.A., Lukina, N. V.	4		243-250	0.1134/S106741361604007X
Bai et al. 2012	Journal of Applied Ecology	Grazing alters ecosystem functioning and C:N:P stoichiometry of grasslands along a regional precipitation gradient	Bai, YF (Bai, Yongfei)[1]; Wu, JG (Wu, Jianguo)[1,2,3]; Clark, CM (Clark, Chris M.)[2,3]; Pan, QM (Pan, Qingmin)[1]; Zhang, LX (Zhang, Lixia)[1]; Chen, SP (Chen, Shiping)[1]; Wang, QB (Wang, Qibing)[1]; Han, XG (Han, Xingguo)[1]	49	6	1204-1215	10.1111/j.1365-2664.2012.02205.x
Baruch&Goldstein_Hawaii_Med_Old	International journal of plant science	Responses to light and water availability of four invasive Melastomataceae in the Hawaiian islands	Baruch, Z (Baruch, Z); Pattison, RR (Pattison, RR); Goldstein, G (Goldstein, G)	161	1	107-118	10.1086/314233
Bassow&Bazzaz_PETERSHAM__MA	Ecology	How environmental conditions affect canopy leaf-level photosynthesis in four deciduous tree species	Bassow, SL (Bassow, SL); Bazzaz, FA (Bazzaz, FA)	79	8	2660-2675	10.1890/0012-9658(1998)079[2660:HECACL]2.0.CO;2
Bauer et al. 1997	Tree physiology	Nutrient contents and concentrations in relation to growth of Picea abies and Fagus sylvatica along a European transect	Bauer, G (Bauer, G); Schulze, ED (Schulze, ED); Mund, M (Mund, M)	17	12	777-786	
Bauters et al. 2017a	Biogeosciences	Parallel functional and stoichiometric trait shifts in South American and African forest communities with elevation	Bauters, M., H. Verbeeck, M. Demol, S. Bruneel, C. Taveirne, D. Van der Heyden, L. Cizungu, and P. Boeckx	14		5313–5321	
Bauters et al. 2017b	Ecosystems	Functional Composition of Tree Communities Changed Topsoil Properties in an Old Experimental Tropical Plantation	Bauters, M., H. Verbeeck, S. Doetterl, E. Ampoorter, G. Baert, P. Vermeir, K. Verheyen, and P. Boeckx	20		861–871	

Bauters et al. 2019	Biotropica	Long-term recovery of the functional community assembly and carbon pools in an African tropical forest succession	Bauters, M., O. Vercleyen, B. Vanlauwe, J. Six, B. Bonyoma, H. Badjoko, W. Hubau, A. Hoyt, M. Boudin, H. Verbeeck, and P. Boeckx	51		319–329	
Bombonato et al.2010	Plant Ecol	Variations in the foliar nutrient content of mire plants: effects of growth-form based grouping and habitat	Bombonato, L (Bombonato, Laura)[1]; Siffi, C (Siffi, Chiara)[1]; Gerdol, R (Gerdol, Renato)[1]	211	2	235-251	10.1007/s11258-010-9786-x
Bond et al 1999. Oecol 120: 183-192,	Oecologia	Foliage physiology and biochemistry in response to light gradients in conifers with varying shade tolerance	Bond, BJ (Bond, BJ); Farnsworth, BT (Farnsworth, BT); Coulombe, RA (Coulombe, RA); Winner, WE (Winner, WE)	120	2	183-192	10.1007/s004420050847
Bongers_et_al_Los_Tuxtlas	Botanical gazette	Leaf characteristics of the tropical rain-forest flora of Los-Tuxtlas, Mexico	BONGERS, F (BONGERS, F); POPMA, J (POPMA, J)	151	3	354-365	10.1086/337836
Boni et al. 2016	Ciência florestal	Comparative assessment of the nutritional status for baru (Dipterix alata) seedlings	Thais Soto Boni, Kátia Luciene Maltoni, Salatier Buzetti, Ana Maria Rodrigues Cassiolato	26	1	109-121	10.5902/1980509821095
Bormann et al. 1990	Forest ecology and management						
Bubier et al. 2011	Oecologia	Effects of nutrient addition on leaf chemistry, morphology, and photosynthetic capacity of three bog shrubs	Bubier, JL (Bubier, Jill L.)[1]; Smith, R (Smith, Rose)[1]; Juutinen, S (Juutinen, Sari)[1]; Moore, TR (Moore, Tim R.)[2]; Minocha, R (Minocha, Rakesh)[3]; Long, S (Long, Stephanie)[3]; Minocha, S (Minocha, Subhash)[4]	167	2	355-368	10.1007/ s00442-011-1998-9
Bündchen et al 2013	Scientia Forestalis	Nutritional status and nutrient use efficiency in tree species of subtropical forest in southern Brazil	Bundchen, M (Buendchen, Marcia)[1]; Boeger, MRT (Tones Boeger, Maria Regina)[2]; Reissmann, CB (Reissmann, Carlos Bruno)[3]; da Silva, SLC (Couto da Silva, Sabrina Leticia)[1]	41	98	227-236	
Bunderson, E. D. & Weber, D.J.1986	Forest Science	Foliar nutrient composition of juniperus-osteosperma and environmental interactions	BUNDERSON, ED (BUNDERSON, ED); WEBER, DJ (WEBER, DJ)	32	1	149-156	
Burton et al. 1993	Soil Science Society Am. J.	Foliar nutrients in sugar maple forests along a regional pollution-climate gradient	BURTON, AJ (BURTON, AJ); PREGITZER, KS (PREGITZER, KS); MACDONALD, NW (MACDONALD, NW)	57	6	1619-1628	10.2136/ sssaj1993.036159950057 00060036x
Bussotti et al. 2000	Trees-structure and function	Leaf morphology and macronutrients in broadleaved trees in central Italy	Bussotti, F (Bussotti, F); Borghini, F (Borghini, F); Celesti, C (Celesti, C); Leonzio, C (Leonzio, C); Bruschi, P (Bruschi, P)	14	7	361-368	10.1007/s004680000056
Bussotti et al. 2005	Tree physiology	Leaf morphology and chemistry in Fagus sylvatica (beech) trees as affected by site factors and ozone: results from CONECOFOR permanent monitoring plots in Italy	Bussotti, F (Bussotti, F); Pancrazi, M (Pancrazi, M); Matteucci, G (Matteucci, G); Gerosa, G (Gerosa, G)	25	2	211-219	10.1093/treephys/ 25.2.211
Caldeira et al. 2002	Annals of Forest Science	Quantification of nutrient content in above-ground biomass of young Acacia mearnsii De Wild., provenance Bodalla	Caldeira, MVW (Caldeira, MVW); Schumacher, MV (Schumacher, MV); Spathelf, P (Spathelf, P)	59	8	833-838	10.1051/forest:2002081

Caldeira et al. 2017	Scientia Forestalis	Levels and redistribution of nutrientes in the leaves of Bixa arborea Huber. and Joannesia princeps Vell. in a florest restoration area	Caldeira, MVW (Winckler Caldeira, Marcos Vinicius) [1] ; Moreira, FL (Moreira, Fagner Luciano)[2] ; Goncalves, ED (Goncalves, Elzimar de Oliveira)[1] ; Campanharo, IF (Campanharo, Italo Favoreto)[3] ; Castro, KC (Castro, Kallil Chaves)	45	114	305-317	10.18671/ scifor.v45n114.07
Campo & Dirzo, 2003	Journal of Tropical Ecology	Leaf Quality and Herbivory Responses to Soil Nutrient Addition in Secondary Tropical Dry Forests of Yucatán	Campo, J., Dirzo, R.	19	5	525-530	
Canadell et al. 1992	Vegetatio	Variation in tissue element concentrations in Quercus-ilex L over a range of different soils	CANADELL, J (CANADELL, J); VILA, M (VILA, M)	100		273-282	
Cekstere et al. 2010	Baltic Forestry	Macronutrient Status in the Different-Aged Lime Trees (Tilia x vulgaris H.) in Riga Streets	Cekstere, G (Cekstere, Gunta)[1,2] ; Osvalde, A (Osvalde, Anita)[1]	16	1	16-22	
Chaturvedi et al. 2011	Journal of vegetation science	Leaf attributes and tree growth in a tropical dry forest	Chaturvedi, RK (Chaturvedi, R. K.)[1] ; Raghubanshi, AS (Raghubanshi, A. S.)[1] ; Singh, JS (Singh, J. S.)[1]	22	5	917-931	10.1111/ j.1654-1103.2011.01299.x
Chen et al. 2009	Journal of Plant Nutrition and Soil Science	Mulberry nutrient management for silk production in Hubei Province of China	Chen, F (Chen, Fang)[1] ; Lu, JW (Lu, Jianwei)[2] ; Zhang, MC (Zhang, Mingchu)[3] ; Wan, KY (Wan, Kaiyuan)[1] ; Liu, DB (Liu, Dongbi)[4]	172	2	245-253	10.1002/jpln.200800093
Cheng XB et al 2012. Photosynthetic a	Photosynthetica	Photosynthesis, leaf morphology and chemistry of Pinus koraiensis and Quercus mongolica in broadleaved Korean pine mixed forest	Cheng, XB (Cheng, X. B.) [1,2] ; Wu, J (Wu, J.)[3] ; Han, SJ (Han, S. J.)[1] ; Zhou, YM (Zhou, Y. M.)[1] ; Wang, XX (Wang, X. X.)[4] ; Wang, CG (Wang, C. G.)[1,2] ; Zhao, J (Zhao, J.)[1,2] ; Hu, QH (Hu, Q. H.)[5]	50	1	56-66	10.1007/ s11099-012-0005-y
Choula et al. 2017	Applied Ecology and Environmental Research	Regeneration, growth and nutrient partitioning of three woody species on degraded tropical rainforest land	Choula, F (Choula, F.)[1] ; Taffouo, VD (Taffouo, V. D.) [1] ; Priso, RJ (Priso, R. J.)[1] ; Etame, J (Etame, J.)[1] ; Zapfack, L (Zapfack, L.)[2] ; Ntsomboh, NG (Ntsomboh, Ntsefong G.)[2,3] ; Ngane, KB (Ngane, K. B.)[4]	15	1	363-378	10.15666/aeer/ 1501_363378
Clancy et al. 2004	Canadian Journal of Forest Research	Foliar nutrients and induced susceptibility: genetic mechanisms of Douglas-fir resistance to western spruce budworm defoliation	Clancy, KM (Clancy, KM); Chen, Z (Chen, Z); Kolb, TE (Kolb, TE)	34	4	939-949	10.1139/X03-264
Colin-Belgrand et al. 1990	Annals of Forest Science						

Corcioli et al. 2016	Cerne	Macro and micronutrient deficiency in mature seedlings of khaya ivorensis studied in nursery	Corcioli, G (Corcioli, Graciella)[1]; Borges, JD (Borges, Jacomo Divino)[1]; de Jesus, RP (de Jesus, Roberta Paula)[1]	22	1	121-128	10.1590/01047760201622012085
Corrêa et al. 2013	Scientia Forestalis	Litterfall and macronutrients in a Eucalyptus dunnii stand in a degraded pasture in Pampa Biome, South Brazil	Correa, RS (Correa, Robson Schaff)[1]; Schumacher, MV (Schumacher, Mauro Valdir)[2]; Momolli, DR (Momolli, Dione Richer)[3]	41	97	65-74	
Courtois et al. 1998	Annals of Forestal Sciences						
Dale et al. 2015	Plant and Soil	Isolating the effects of precipitation, soil conditions, and litter quality on leaf litter decomposition in lowland tropical forests	Dale, SE (Dale, Sarah E.)[4,1]; Turner, BL (Turner, Benjamin L.)[2]; Bardgett, RD (Bardgett, Richard D.)[3]	394	(1-2)	225-238	10.1007/ s11104-015-2511-8
Dalling 1994	Association for Tropical Biology						
de Cassia-Silva C et al 2017. Plant Ecology	Plant Ecology	When the same is not the same: phenotypic variation reveals different plant ecological strategies within species occurring in distinct Neotropical savanna habitats	de Cassia-Silva, C (de Cassia-Silva, Cibele)[1,2]; Cianciaruso, MV (Cianciaruso, Marcus V.)[2]; Maracahipes, L (Maracahipes, Leandro)[2]; Collevatti, RG (Collevatti, Rosane G.)[1]	218	10	1221-1231	10.1007/ s11258-017-0765-3
De la Riva EG et al, 2018	Trees-structure and function	Relationships between leaf mass per area and nutrient concentrations in 98 Mediterranean woody species are determined by phylogeny, habitat and leaf habit	de la Riva, EG (de la Riva, Enrique G.)[1,2]; Villar, R (Villar, Rafael)[1]; Perez-Ramos, IM (Perez-Ramos, Ignacio M.)[3]; Quero, JL (Luis Quero, Jose)[4]; Matias, L (Matias, Luis)[3]; Poorter, L (Poorter, Lourens)[5]; Maranon, T (Maranon, Teodoro)[3]	32	2	497-510	10.1007/ s00468-017-1646-z
Deans et al. 1999	Forest Ecology and Management	Nutrient and organic-matter accumulation in Acacia senegal fallows over 18 years	Deans, JD (Deans, JD); Diagne, O (Diagne, O); Lindley, DK (Lindley, DK); Dione, M (Dione, M); Parkinson, JA (Parkinson, JA)	124	(2-3)	153-167	10.1016/ S0378-1127(99)00063-8
DeLucia91Ecol	Ecology	Resource-use efficiency and drought tolerance in adjacent great-basin and sierran plants	DELUCIA, EH (DELUCIA, EH); SCHLESINGER, WH (SCHLESINGER, WH)	72	1	51-58	10.2307/1938901
Derroire G et al 2018	Scientific Report	Contrasting patterns of leaf trait variation among and within species during tropical dry forest succession in Costa Rica	Derroire, G (Derroire, Geraldine)[1,2]; Powers, JS (Powers, Jennifer S.)[3,4]; Hulshof, CM (Hulshof, Catherine M.)[5]; Varela, LEC (Cardenas Varela, Luis E.)[6]; Healey, JR (Healey, John R.)[1]	8	285		10.1038/ s41598-017-18525-1
Dominguez et al. 2015	Spanish Journal of Agricultural Research	Leaf blade and petiole nutritional evolution and variability throughout the crop season for Vitis vinifera L. cv. Graciano	Dominguez, N (Dominguez, Natalia)[1]; Garcia-Escudero, E (Garcia-Escudero, Enrique)[1]; Romero, I (Romero, Izaskun)[1]; Benito, A (Benito, Ana)[1]; Martin, I (Martin, Ignacio)[1]	13	3	e0801	10.5424/sjar/ 2015133-5142

Dordel et al. 2011	Canadian Journal of Forest Research	Effects of nurse-tree crop species and density on nutrient and water availability to underplanted Toona ciliata in northeastern Argentina	Dordel, J (Dordel, Julia)[1]; Simard, SW (Simard, Suzanne W.)[1]; Bauhus, J (Bauhus, Juergen)[2]; Guy, RD (Guy, Robert D.)[1]; Prescott, CE (Prescott, Cindy E.)[1]; Seely, B (Seely, Brad)[1]; Hampel, H (Hampel, Hermann); Pozas, LJ (Pozas, Luciano J.)[3]	41	9	1754-1768	10.1139/X11-093
Dos Santos et al. 2006	Forest Ecology and Management	Growth, leaf nutrient concentration and photosynthetic nutrient use efficiency in tropical tree species planted in degraded areas in central Amazonia	dos Santos, UM (dos Santos, UM); Goncalves, JFD (Goncalves, JFD); Feldpausch, TR (Feldpausch, TR)	226	(1-3)	299-309	10.1016/j.foreco.2006.01.042
Duan and Chang, 2015	Ecological Engineering	Responses of lodgepole pine (Pinus contorta) and white spruce (Picea glauca) to fertilization in some reconstructed boreal forest soils in the oil sands region.	Duan, M., Chang, S.X.	84		354-361	10.1016/j.ecoleng.2015.09.046
Duquesnay A. et al 2000	Tree Phsiology	Spatial and temporal variability of foliar mineral concentration in beech (Fagus sylvatica) stands in northeastern France	Duquesnay, A (Duquesnay, A); Dupouey, JL (Dupouey, JL); Clement, A (Clement, A); Ulrich, E (Ulrich, E); Le Tacon, F (Le Tacon, F)	20	1	13-22	
El-Jendoubi et al. 2012	Plant and Soil	Prognosis of iron chlorosis in pear (Pyrus communis L.) and peach (Prunus persica L. Batsch) trees using bud, flower and leaf mineral concentrations	El-Jendoubi, H (El-Jendoubi, Hamdi)[1]; Igartua, E (Igartua, Ernesto)[2]; Abadia, J (Abadia, Javier)[1]; Abadia, A (Abadia, Anunciacion)[1]	354	(1-2)	121-139	10.1007/s11104-011-1049-7
Ellison et al., 2019	Ecology	Poor nutrition as a potential cause of divergent tree growth near the Arctic treeline in northern Alaska	Ellison, S.B.Z., Sullivan, P.F., Cahoon, S.M.P., Hewitt, R.E.	100	12		
Faheem siddiqui et al. 2014	Pakistan Journal Botany	Foliar and soil nutrient distribution in conifer forests of moist temperate areas of himalayan and hindukush region of Pakistan: a multivariate approach	Siddiqui, MF (Siddiqui, Muhammad Faheem)[1]; Shaukat, SS (Shaukat, Syed Shahid)[2]; Ahmed, M (Ahmed, Moinuddin)[2]; Khan, IA (Khan, Imran Ahmed)[3]; Khan, N (Khan, Nasrullah)[4]	46	5	1811-1827	
Fajardo & Siefert 2016	Oecologia	Phenological variation of leaf functional traits within species	Fajardo, A (Fajardo, Alex)[1]; Siefert, A (Siefert, Andrew)[2]	180	4	951-959	10.1007/s00442-016-3545-1
Fajardo et al 2017	Journal of Ecology	An assessment of carbon and nutrient limitations in the formation of the southern Andes tree line	Fajardo, A (Fajardo, Alex)[1]; Piper, FI (Piper, Frida I.)[1]	105	2	517-527	10.1111/1365-2745.12697
Ferreira et al. 2016	Forest ecology and management	Nutrient release from decomposing Eucalyptus harvest residues following simulated management practices in multiple sites in Brazil	Ferreira, GWD (Ferreira, Gabriel W. D.)[1]; Soares, EMB (Soares, Emanuelle M. B.)[1]; Oliveira, FCC (Oliveira, Fernanda C. C.)[1]; Silva, IR (Silva, Ivo R.)[1]; Dungait, JAJ (Dungait, Jennifer A. J.)[2]; Souza, IF (Souza, Ivan F.)[1]; Vergutz, L (Verguetz, Leonardus)[1]	370		(1-11)	10.1016/j.foreco.2016.03.047
Fiala et al. 2017	Zprávy Lesnického Výzkumu						
Finer 1992	Scandinavian Journal of Forest Research	Nutrient Concentrations in Pinus sylvestris Growing on an Ombrotrophic Pine Bog, and the Effects of PK and NPK Fertilization	Finer, L (Finer, Leena)	7	(1-4)	205-218	10.1080/02827589209382713

Galuszka et al. 2011	Environ. Monit. Assess.	The influence of chloride deicers on mineral nutrition and the health status of roadside trees in the city of Kielce, Poland	Galuszka, A (Galuszka, Agnieszka)[1]; Migaszewski, ZM (Migaszewski, Zdzislaw M.)[1]; Podlaski, R (Podlaski, Rafal)[2]; Dolegowska, S (Dolegowska, Sabina)[1]; Michalik, A (Michalik, Artur)[1]	176	(1-4)	451-464	10.1007/ s10661-010-1596-z
Gatto et al. 2014	Revista Brazileira da Ciência do solo	Nutrient cycling and balance in the Eucalyptus sp soil-plant system in the Distrito Federal, Brazil	Gatto, A (Gatto, Alcides)[1]; Bussinguer, AP (Bussinguer, Angela Pereira)[2]; Ribeiro, FC (Ribeiro, Fabiana Campos)[2]; de Azevedo, GB (de Azevedo, Gileno Brito)[2]; Bueno, MC (Bueno, Maria Cristina)[3]; Monteiro, MM (Monteiro, Marina Morais)[2]; de Souza, PF (de Souza, Pierre Farias)[2]	38	3	879-887	10.1590/ S0100-06832014000300019
Giachetti et al. 2007	Plant Biosystems	Effects of tannery waste on growth dynamics and metal uptake in Salix Alba L.	Giachetti, G (Giachetti, G.); Sebastiani, L (Sebastiani, L.)	141	1	22-30	10.1080/11263500601153511
Giertych et al. 1997	Tree physiology	Distribution of elements along the length of Scots pine needles in a heavily polluted and a control environment	Giertych, MJ (Giertych, MJ); DeTemmerman, LO (DeTemmerman, LO); Rachwal, L (Rachwal, L)	17	11	697-703	
Gonçalves-Alvim SJ, et al 2006. Plant Ecology	Plant ecology	Sclerophylly in Qualea parviflora (Vochysiaceae): influence of herbivory, mineral nutrients, and water status	Goncalves-Alvim, SJ (Goncalves-Alvim, S. J.); Korndorf, G (Korndorf, Gaspar); Fernandes, GW (Fernandes, G. Wilson)	187	2	153-162	10.1007/ s11258-004-3286-9
Gong et al. 2016							
González-García et al. 2016	Iforest Biogeosciences and Forestry	Nutritional, carbon and energy evaluation of Eucalyptus nitens short rotation bioenergy plantations in northwestern Spain	Gonzalez-Garcia, M (Gonzalez-Garcia, Marta)[1]; Hevia, A (Hevia, Andrea)[1]; Majada, J (Majada, Juan)[1]; Rubiera, F (Rubiera, Fernando)[2]; Barrio-Anta, M (Barrio-Anta, Marcos)[3]	9		303-310	10.3832/ifor1505-008
Grootemaat et al. (2017), Oikos	Oikos	Scaling up flammability from individual leaves to fuel beds	Grootemaat, S (Grootemaat, Saskia)[1]; Wright, IJ (Wright, Ian J.)[1]; van Bodegom, PM (van Bodegom, Peter M.)[2]; Cornelissen, JHC (Cornelissen, Johannes H. C.)[3]	126	10	1428-1438	10.1111/oik.03886
Grubb et al. 1994	Journal of Tropical Ecology	Mineral nutrient status of coastal hill dipterocarp forest and adinandra belukar in Singapore - Analysis of soil, leaves and litter	GRUBB, PJ (GRUBB, PJ); TURNER, IM (TURNER, IM); BURSLEM, DFRP (BURSLEM, DFRP)	10	4	559-577	10.1017/ S0266467400008233
Guan ZJ et al 2011. J Plant Res	Journal of plant research	Leaf anatomical structures of Paphiopedilum and Cypripedium and their adaptive significance	Guan, ZJ (Guan, Zhi-Jie)[1,2]; Zhang, SB (Zhang, Shi-Bao)[3]; Guan, KY (Guan, Kai-Yun)[1]; Li, SY (Li, Shu-Yun)[1]; Hu, H (Hu, Hong)[1]	124	2	289-298	10.1007/ s10265-010-0372-z
Guimaraes et al. 2015	Revista Árvore	Biomass and nutrients of Eucalyptus dunnii Maiden stand in Pampa Gaucho	Guimaraes, CC (Guimaraes, Claudiney Couto)[1]; Schumacher, MV (Schumacher, Mauro Valdir)[2]; Witshoreck, R (Witshoreck, Rudi)[3]; Souza, HP (Souza, Huan Pablo)[1]; Santo, JC (Santo, Joel Carvalho)[3]	39	5	873-882	10.1590/0100-67622015000500010

Güswell & Koerselman2002	Perspectives in Plant Ecology, Evolution and Systematics	Variation in nitrogen and phosphorus concentrations of wetland plants	Gusewell, S (Gusewell, S); Koerselman, M (Koerselman, M)	5	1	37-61	10.1078/1433-8319-00000 22
Hall et al. 1973	Phytopathological notes						
Han et al. 2005	New Phytologist	Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China	Han, WX (Han, WX); Fang, JY (Fang, JY); Guo, DL (Guo, DL); Zhang, Y (Zhang, Y)	168	2	377-385	10.1111/ j.1469-8137.2005.01530.x
Han et al. 2012	Ecology Letters						
Han et al. 2016	Forest Science and Technology	The effects of organic manure and chemical fertilizer on the growth and nutrient concentrations of yellow poplar (Liriodendron tulipifera Lin.) in a nursery system	Han, SH (Han, Si Ho)[1]; An, JY (An, Ji Young)[2]; Hwang, J (Hwang, Jaehong)[3]; Kim, SB (Kim, Se Bin)[1]; Park, BB (Park, Byung Bae)[1]	12	3	137-143	10.1080/21580103.2015.1 135827
Han, 2006							
Harrington et al. 1995	Oecologia	Forest growth along a rainfall gradient in Hawaii - Acacia-koa stand structure, productivity, foliar nutrients, and water-use and nutrient-use efficiencies	HARRINGTON, RA (HARRINGTON, RA); FOWNES, JH (FOWNES, JH); MEINZER, FC (MEINZER, FC); SCOWCROFT, PG (SCOWCROFT, PG)	102	3	277-284	10.1007/BF00329794
Hart et al. 2003	Forest Ecology and Management	Biomass and macro-nutrients (above- and below-ground) in a New Zealand beech (Nothofagus) forest ecosystem: implications for carbon storage and sustainable forest management	Hart, PBS (Hart, PBS); Clinton, PW (Clinton, PW); Allen, RB (Allen, RB); Nordmeyer, AH (Nordmeyer, AH); Evans, G (Evans, G)	174	(1-3)	281-294	10.1016/ S0378-1127(02)00039-7
Hartemink 2001	Forest ecology and management	Biomass and nutrient accumulation of Piper aduncum and Imperata cylindrica fallows in the humid lowlands of Papua New Guinea	Hartemink, AE (Hartemink, AE)	144	(1-3)	19-32	10.1016/ S0378-1127(00)00655-1
Harvey et al. 1999	Canadian Journal of Forest Research						
He JS et al 2006	Oecologia	Stoichiometry and large-scale patterns of leaf carbon and nitrogen in the grassland biomes of China	He, JS (He, Jin-Sheng); Fang, JY (Fang, Jingyun); Wang, ZH (Wang, Zhiheng); Guo, DL (Guo, Dali); Flynn, DFB (Flynn, Dan F. B.); Geng, Z (Geng, Zhi)	149	1	115-122	10.1007/ s00442-006-0425-0
Hejcman et al. 2016	Grass and Forage Science	Forage quality of leaf fodder from the main woody species in Iceland and its potential use for livestock in the past and present	Hejcman, M (Hejcman, M.)[1,2]; Hejcmanova, P (Hejcmanova, P.)[3]; Pavlu, V (Pavlu, V.)[1]; Thorhallsdottir, AG (Thorhallsdottir, A. G.)[4]	71	4	649-658	10.1111/gfs.12224
Helmisaari et al. 1990	Scandinavian Journal of Forest Research	Temporal Variation in Nutrient Concentrations of Pinus sylvestris Needles	Helmisaari, HS (Helmisaari, Helja-Sisko)	5	(1-4)	177-193	10.1080/02827589009382 604
Hobbie SE et al 2002. Oecologia	Oecologia	Foliar and soil nutrients in tundra on glacial landscapes of contrasting ages in northern Alaska	Hobbie, SE (Hobbie, SE); Gough, L (Gough, L)	131	3	453-462	10.1007/ s00442-002-0892-x
Hofmeister et al.2002	Forest Ecology and Managment	Eutrophication of deciduous forests in the Bohemian Karst (Czech Republic): the role of nitrogen and phosphorus	Hofmeister, J (Hofmeister, J); Mihaljevic, M (Mihaljevic, M); Hosek, J (Hosek, J); Sadlo, J (Sadlo, J)	169	3	213-230	10.1016/ S0378-1127(01)00756-3

Hopmans et al. 2009	Forest ecology and managem ent	Changes in total carbon and nutrients in soil profiles and accumulation in biomass after a 30-year rotation of Pinus radiata on podzolized sands: Impacts of intensive harvesting on soil resources	Hopmans, P (Hopmans, Peter)[1]; Elms, SR (Elms, Stephen R.)[2]	258	10	2183-21 93	10.1016/ j.foreco.2009.02.010
Hosseini et al. 2010	Polish Journal of Ecology	Growth and nutrition of restored Quercus castaneifolia ca mey. Versus native Celtis australis L. tree species: conservation perspectives of hyrcanian forest (Iran)	Hosseini, SM (Hosseini, Seyed Mohsen)[1]; Raouhi-Moghaddam, E (Raouhi-Moghaddam, Einollah)[1,2,3]; Ebrahimi, E (Ebrahimi, Ezzatollah)[4]; Rahmani, A (Rahmani, Ahmad)[4]; Tabari, M (Tabari, Masoud) [1]	58	1	41-54	
Houle & Moore, 2019	Forest Ecology and Managem ent	Soil solution, foliar concentrations and tree growth response to 8 years of ammonium-nitrate additions in two boreal forests of Quebec, Canada	Houle, D., Moore, J.D.	437		263-271	10.1016/ j.foreco.2019.01.024
Hrdlicka et al. 1998	Trees-structure and function	Element content in leaves of birch (Betula verrucosa Ehrh.) in an air polluted area	Hrdlicka, P (Hrdlicka, P); Kula, E (Kula, E)	13	2	68-73	10.1007/s004680050188
Hrdlicka et al. 2011	Polish Journal of Environm ental Studies	Changes in Element Content of Birch Leaves (Betula pendula Roth) in Polluted Air	Hrdlicka, P (Hrdlicka, Petr)[1]; Kula, E (Kula, Emanuel)[2]	20	3	661-670	
Hunt, F. J. Et al 2008	Soil, Biology & Biochemis try	Influence of foliar phosphorus and nitrogen contents on chemical properties of water extractable organic matter derived from fresh and decomposed sugar maple leaves	Hunt, JF (Hunt, James F.)[1]; Ohno, T (Ohno, Tsutomu)[1]; Fernandez, IJ (Fernandez, Ivan J.)[1]	40	7	1931-19 39	10.1016/ j.soilbio.2008.04.004
Jama et al. 2000	Agrofores try Systems	Tithonia diversifolia as a green manure for soil fertility improvement in western Kenya: A review	Jama, B (Jama, B); Palm, CA (Palm, CA); Buresh, RJ (Buresh, RJ); Niang, A (Niang, A); Gachengo, C (Gachengo, C); Nziguheba, G (Nziguheba, G); Amadalo, B (Amadalo, B)	49	2	201-221	10.1023/ A:1006339025728
Jiménez et al. 2015	Forest Ecology and Managem ent	Monthly foliar-nutrient pattern in a semiarid Aleppo pine plantation five years after thinning	Jimenez, MN (Jimenez, M. N.)[1]; Navarro, FB (Navarro, F. B.)[1]	347		63-72	10.1016/ j.foreco.2015.01.032
Jonard et al 2009	Annals of Forest Science	Forest floor contribution to phosphorus nutrition: experimental data	Jonard, M (Jonard, Mathieu)[1]; Augusto, L (Augusto, Laurent)[2]; Morel, C (Morel, Christian)[2]; Achat, DL (Achat, David L.)[3]; Saur, E (Saur, Etienne)[4]	66	5	510	10.1051/forest/2009039
Jose et al. 1996	Canadian Journal of Forest Research	Aboveground production efficiency and canopy nutrient contents of mixed-hardwood forest communities along a moisture gradient in the central United States	Jose, S (Jose, S); Gillespie, AR (Gillespie, AR)	26	12	2214-22 23	10.1139/x26-250
Kadeba 1991	Forest Ecology and Managem ent	Aboveground biomass production and nutrient accumulation in an age sequence of Pinus caribaea stands	KADEBA, O (KADEBA, O)	41	(3-4)	237-248	10.1016/0378-1127(91)90 106-6

Citation	Journal	Title	Authors	Vol	Issue	Pages	DOI
Kanowski 2001	Austral Ecology	Effects of elevated CO2 on the foliar chemistry of seedlings of two rainforest trees from north-east Australia: Implications for folivorous marsupials	Kanowski, J (Kanowski, J)	26	2	165-172	10.1046/ j.1442-9993.2001.01103.x
Karlsson et al. 2002	Forest Ecology and Managem ent	Mineral nutrients in needles of Pinus sylvestris seed trees after release cutting and their correlations with cone production and seed weight	Karlsson, C (Karlsson, C); Orlander, G (Orlander, G)	166	(1-3)	183-191	10.1016/ S0378-1127(01)00684-3
Kayama et al. 2009	Landscap e and Ecological Engineeri ng	Nutrient dynamics and carbon partitioning in larch seedlings (Larix kaempferi) regenerated on serpentine soil in northern Japan	Kayama, M (Kayama, Masazumi)[1]; Makoto, K (Makoto, Kobayashi)[2]; Nomura, M (Nomura, Mutsumi)[3]; Satoh, F (Satoh, Fuyuki)[3]; Koike, T (Koike, Takayoshi)[2]	5	2	125-135	10.1007/ s11355-009-0069-4
Kayama et al. 2009b	Trees-structure and function	Growth characteristics of Sakhalin spruce (Picea glehnii) planted on the northern Japanese hillsides exposed to strong winds	Kayama, M (Kayama, Masazumi)[2]; Makoto, K (Makoto, Kobayashi)[3]; Nomura, M (Nomura, Mutsumi)[2]; Sasa, K (Sasa, Kaichiro)[2]; Koike, T (Koike, Takayoshi)[1,3]	23	1	145-157	10.1007/ s00468-008-0263-2
Kikamägi et al. 2014	Trees-structure and function	The growth and nutrients status of conifers on ash-treated cutaway peatland	Kikamagi, K (Kikamaegi, Karin)[1]; Ots, K (Ots, Katri) [1]; Kuznetsova, T (Kuznetsova, Tatjana)[1]; Pototski, A (Pototski, Aleksander)[2]	28	1	53-64	10.1007/ s00468-013-0929-2
Klemmedson 1992	Forest ecology and managem ent	Decomposition and nutrient release from mixtures of gambel oak and ponderosa pine leaf litter	KLEMMEDSON, JO (KLEMMEDSON, JO)	47	14	349-361	10.1016/0378-1127(92)90 284-G
Klumpp et al. 2002	Water, Air and Soil Pollution	Foliar nutrient contents in tree species of the Atlantic rain forest as influenced by air pollution from the industrial complex of Cubatao, SE-Brazil	Klumpp, A (Klumpp, A); Domingos, M (Domingos, M); Klumpp, G (Klumpp, G)	133	(1-4)	315-333	10.1023/ A:1012914720934
Knops JMH & Koenig WD 1997. Plant Ecology	Plant ecology	Site fertility and leaf nutrients of sympatric evergreen and deciduous species of Quercus in central coastal California	Knops, JMH (Knops, JMH); Koenig, WD (Koenig, WD)	130	2	121-131	10.1023/ A:1009798327200
Kranabetter et al., 2020		Contrasting conifer species productivity in relation to soil carbon, nitrogen and phosphorus stoichiometry of British Columbia perhumid rainforests	Kranabetter, J.M., Sholinder, A., de Montigny, L.				https://doi.org/10.5061/ dryad.0gb5mkkwr
Kuan Ching et al2003	Taiwan Journal of Forest Science						
Kurczynska et al. 1997	Environm ental Pollution	The influence of air pollutants on needles and stems of Scots pine (Pinus sylvestris L.) trees	Kurczynska, EU (Kurczynska, EU); Dmuchowski, W (Dmuchowski, W); Wloch, W (Wloch, W); Bytnerowicz, A (Bytnerowicz, A)	98	3	325-334	10.1016/ S0269-7491(97)00141-3
Kutbay et al. 2005	Belgian journal of botany	Seasonal nutrient levels and foliar resorption in Juniperus phoenicea	Kutbay, HG (Kutbay, HG); Ok, T (Ok, T); Bilgin, A (Bilgin, A); Yalcin, E (Yalcin, E)	138	1	67-75	
Kuznetsova et al. 2009	Baltic Forestry	The Growth of Lodgepole Pine (Pinus contorta var. latifolia Engelm.) in a Reclaimed Oil Shale Mining Area, Abandoned Agricultural Land and Forestland	Kuznetsova, T (Kuznetsova, Tatjana)[1]; Tilk, M (Tilk, Mari)[1]; Ots, K (Ots, Katri)[1]; Lukjanova, A (Lukjanova, Aljona)[1]; Parn, H (Parn, Henn)[1]	15	2	186-194	

Lal_etal_Inceptisol	Canadian Journal of Botany	Effect of leaf habit and soil type on nutrient resorption and conservation in woody species of a dry tropical environment	Lal, CB (Lal, CB); Annapurna, C (Annapurna, C); Raghubanshi, AS (Raghubanshi, AS); Singh, JS (Singh, JS)	79	9	1066-1075	10.1139/b01-077
Lamont_S_Africa_10_Kylemore	Functional Ecology	High leaf mass per area of related species assemblages may reflect low rainfall and carbon isotope discrimination rather than low phosphorus and nitrogen concentrations	Lamont, BB (Lamont, BB); Groom, PK (Groom, PK); Cowling, RM (Cowling, RM)	16	3	403-412	10.1046/ j.1365-2435.2002.00631.x
Lee_NZ_Murchison_Mtns	New Zeland Journal of Botany	Effect of artificial defoliation of midribbed snow tussock, chionochloa-pallens, in the Murchison Mountains, Fjordland, New Zeland	LEE, WG (LEE, WG); MILLS, JA (MILLS, JA); LAVERS, RB (LAVERS, RB)	26	4	511-523	10.1080/0028825X.1988.10410658
Lehmann et al. 1995	Agroforestry Systems	Decomposition and nutrient release from leaves, twigs and roots of 3 alley-cropped tree legumes in central Togo	LEHMANN, J (LEHMANN, J); SCHROTH, G (SCHROTH, G); ZECH, W (ZECH, W)	29	1	21-36	10.1007/BF00711279
Lemma 2012	Journal of Plant Nutrition and Soil Science	Soil chemical properties and nutritional status of trees in pure and mixed-species stands in south Ethiopia	Lemma, B (Lemma, Bekele)	175	5	769-774	10.1002/jpln.201100221
Lemma et al. 2007	Soil Biology & Biochemitry	Decomposition and substrate quality of leaf litters and fine roots from three exotic plantations and a native forest in the southwestern highlands of Ethiopia	Lemma, B (Lemma, Bekele); Nilsson, I (Nilsson, Ingvar); Kleja, DB (Kleja, Dan Berggren); Olsson, M (Olsson, Mats); Knicker, H (Knicker, Heike)	39	9	2317-2328	10.1016/ j.soilbio.2007.03.032
Li & Peng, 2001	Plant and soil	Litter decomposition and nitrogen mineralization of soils in subtropical plantation forests of southern China, with special attention to comparisons between legumes and non-legumes	Li, ZA (Li, ZA); Peng, SK (Peng, SK); Rae, DJ (Rae, DJ); Zhou, GY (Zhou, GY)	229	1	105-116	10.1023/ A:1004832013143
Li et al. 2014	Ecol Res	Variation in leaf nitrogen and phosphorus stoichiometry in the nitrogen-fixing Chinese sea-buckthorn (Hippophae rhamnoides L. subsp sinensis Rousi) across northern China	Li, XW (Li, Xiaowei)[2,1]; Sun, K (Sun, Kun)[1]; Li, FY (Li, Frank Yonghong)[3]	29	4	723-731	10.1007/ s11284-014-1165-y
Li et al. 2015							
Lodhiyal et al. 2003	Forest Ecology and Management	Aspects of nutrient cycling and nutrient use pattern of Bhabar Shisham forests in central Himalaya, India	Lodhiyal, N (Lodhiyal, N); Lodhiyal, LS (Lodhiyal, LS)	176	(1-3)	237-252	10.1016/ S0378-1127(02)00268-2
Londero et al. 2011	Ciência florestal	Export and nutritional replacement in the first thinning of Pinus taeda L. in a second rotation area	Londero, EK (Londero, Eduardo Kneipp)[1]; Schumacher, MV (Schumacher, Mauro Valdir); Szymczak, DA (Szymczak, Denise Andreia); Viera, M (Viera, Marcio)	21	3	487-497	
Long JR et al 2015	Functional Ecology						
López-Serrano et al. 2005	Forest Ecology and Management	Effects of silvicultural treatments and seasonal patterns on foliar nutrients in young post-fire Pinus halepensis forest stands	Lopez-Serrano, FR (Lopez-Serrano, FR); de las Heras, J (de las Heras, J); Gonazalez-Ochoa, AI (Gonazalez-Ochoa, AI); Garcia-Morote, FA (Garcia-Morote, FA)	210	(1-3)	321-336	10.1016/ j.foreco.2005.02.042
Luo et al., 2005							

Macek, P et al2012	Arctic, Antarctic, and Alpine Research.	Plant Nutrient Content Does Not Simply Increase with Elevation under the Extreme Environmental Conditions of Ladakh, NW Himalaya	Macek, P (Macek, Petr)[1,2]; Klimes, L (Klimes, Leos)[1]; Adamec, L (Adamec, Lubomir)[1]; Dolezal, J (Dolezal, Jiri)[1,2]; Chlumska, Z (Chlumska, Zuzana)[1,2]; de Bello, F (de Bello, Francesco)[1]; Dvorskry, M (Dvorskry, Miroslav)[1,2]; Rehakova, K (Rehakova, Klara)[1]	44	1	62-66	10.1657/1938-4246-44.1.62
Mahadeva et al. 2015	Journal of Plant Nutrition	Evaluation of macro and micro nutritive elemental levels in the mealy-bug (Maconellicoccus hirsutus)- Infested leaves of mulberry (Morus sp.)	Mahadeva, A (Mahadeva, A.)[1]; Shree, MP (Shree, M.P.)[2]	38	1	96-107	10.1080/01904167.2014.920381
Majer et al. 1992	Australian Journal of Ecology	Variation in foliar nutrients in Eucalyptus trees in eastern and western-Australia	MAJER, JD (MAJER, JD); RECHER, HF (RECHER, HF); GANESHANANDAM, S (GANESHANANDAM, S)	17	4	383-393	10.1111/j.1442-9993.1992.tb00821.x
Mankovska et al. 2004	Environmental Pollution	Chemical and morphological characteristics of key tree species of the Carpathian Mountains	Mankovska, B (Mankovska, B); Godzik, B (Godzik, B); Badea, O (Badea, O); Shparyk, Y (Shparyk, Y); Moravcik, P (Moravcik, P)	130	1	41-54	10.1016/j.envpol.2003.10.020
Marcos et al. 2002	Forest ecology and management	Atmospheric deposition in oligotrophic Quercus pyrenaica forests: implications for forest nutrition	Marcos, GM (Marcos, GM); Lancho, JFG (Lancho, JFG)	171	(1-2)	17-29	10.1016/S0378-1127(02)00458-9
Marmiroli et al. 2012	Biomass & bioenergy	Effect of dairy effluent on the biomass, transpiration, and elemental composition of Salix kinuyanagi Kimura	Marmiroli, M (Marmiroli, Marta)[1]; Robinson, BH (Robinson, Brett H.)[2]; Clothier, BE (Clothier, Brent E.)[3]; Bolan, NS (Bolan, Nanthi S.)[4]; Marmiroli, N (Marmiroli, Nelson)[1]; Schulin, R (Schulin, Rainer)[5]	37		282-288	10.1016/j.biombioe.2011.12.001
Martín-García et al. 2012	European Journal Forest Research	Relating visual crown conditions to nutritional status and site quality in monoclonal poplar plantations (Populus x euramericana)	Martin-Garcia, J (Martin-Garcia, Jorge)[1,2]; Merino, A (Merino, Agustin)[3]; Diez, JJ (Javier Diez, Julio)[1]	131	4	1185-1198	10.1007/s10342-011-0590-5
Mason et al 2015, Evolution	Evolution	Evolution of the leaf economics spectrum in herbs: Evidence from environmental divergences in leaf physiology across Helianthus (Asteraceae)	Mason, CM (Mason, Chase M.)[1]; Donovan, LA (Donovan, Lisa A.)[1]	69	10	2705-2720	10.1111/evo.12768
Maynard and Fairbarns, 1994	Northern Forestry Centre	Boreal ecosystem dynamics of arnew plots: Base line studies in the prairie provinces.	Maynard, D.G., Fairbarns, M.D.				
Mediavilla et al. 2011	Trees-structure and function	Morphological and chemical leaf composition of Mediterranean evergreen tree species according to leaf age	Mediavilla, S (Mediavilla, Sonia)[1]; Gonzalez-Zurdo, P (Gonzalez-Zurdo, Patricia)[1]; Garcia-Ciudad, A (Garcia-Ciudad, Antonia)[2]; Escudero, A (Escudero, Alfonso)[1]	25	4	669-677	10.1007/s00468-011-0544-z
Mellert et al. 2014	European Journal Forest Research	Nutrient limitation and site-related growth potential of Norway spruce (Picea abies [L.] Karst) in the Bavarian Alps	Mellert, KH (Mellert, K. H.)[1]; Ewald, J (Ewald, J.)[1]	133	3	433-451	10.1007/s10342-013-0775-1

Mendoza et al. 2014	Bosque	Nutrient resorption in dominant species of forests in the 'Espinal Mesopotamico' (Argentina)	Mendoza, CA (Mendoza, Carlos A.)[1]; Turrion, MB (Belen Turrion, M.)[2,3]; Acenolaza, PG (Acenolaza, Pablo G.)[4,5]; Gallardo, JF (Gallardo, Juan F.)[6]; Pando, V (Pando, Valentin)[7]	35	2	185-193	10.4067/ S0717-92002014000200006
Merilä et al. 2008	Boreal environment research	Relationships between needle nutrient composition in Scots pine and Norway spruce stands and the respective concentrations in the organic layer and in percolation water	Merila, P (Merila, Paivi)[1]; Derome, J (Derome, John)[2]	13		35-47	
Mesanza et al. 1992	Ann Sci For						
Metali et al 2015	New Phytologist	Controls on foliar nutrient and aluminium concentrations in a tropical tree flora: phylogeny, soil chemistry and interactions among elements	Metali, F (Metali, Faizah)[1]; Abu Salim, K (Abu Salim, Kamariah)[1]; Tennakoon, K (Tennakoon, Kushan)[1]; Burslem, DFRP (Burslem, David F. R. P.)[2]	205	1	280-292	10.1111/nph.12987
Michopoulos et al. 2007	Plant and soil	Nutrient cycling and foliar status in an urban pine forest in Athens, Greece	Michopoulos, P (Michopoulos, Panagiotis); Baloutsos, G (Baloutsos, George); Economou, A (Economou, Anastasios); Samara, C (Samara, Constantini); Thomaidis, NS (Thomaidis, Nikolaos S.); Grigoratos, T (Grigoratos, Theodoros)	294	(1-2)	31-39	10.1007/ s11104-007-9224-6
Millner et al. 2012	New Forests	Foliar nutrients in Eucalyptus species in New Zealand	Millner, JP (Millner, J. P.)[1]; Kemp, PD (Kemp, P. D.)[1]	43	2	255-266	10.1007/ s11056-011-9279-3
Mitchell1936	Black Rock Forest Papers						
Moore et al. 2004	Western Journal of Applied Forestry	Foliar nutrient characteristics of four conifer species in the interior Northwest United States	Moore, JA (Moore, JA); Mika, PG (Mika, PG); Shaw, TM (Shaw, TM); Garrison-Johnston, MI (Garrison-Johnston, MI)	19	1	13-24	10.1093/wjaf/19.1.13
Moreira et al2006	Forest Ecology and Management						
Mubarak et al. 2008	Communications in Soil Science and Plant Analysis	Decomposition and nutrient release from litter fall in the semi-arid tropics of Sudan	Mubarak, AR (Mubarak, A. R.)[1]; Elbashir, AA (Elbashir, A. A.)[1]; Elamin, LA (Elamin, L. A.)[2]; Daldoum, DMA (Daldoum, D. M. A.)[1]; Steffens, D (Steffens, D.)[3]; Benckiser, G (Benckiser, G.)[4]	39	15-16	2359-2377	10.1080/00103620802292574
Murovhi et al. 2012	Agroforestry systems	Seasonal changes in litter fall and its quality from three sub-tropical fruit tree species at Nelspruit, South Africa	Murovhi, NR (Murovhi, Nndamuleleni Romeo)[1]; Materechera, SA (Materechera, Simeon Albert)[2]; Mulugeta, SD (Mulugeta, Sendros D.)[2]	86	1	61-71	10.1007/ s10457-012-9508-6
Nayak et al. 2011	Journal of Plant Nutrition	Diagnosis and recommendation integrated system approach for nitrogen, phosphorus, potassium, and zinc foliar diagnostic norms for aonla in central indo-gangetic plains	Nayak, AK (Nayak, A. K.)[1]; Sharma, DK (Sharma, D. K.)[1]; Singh, CS (Singh, C. S.)[1]; Mishra, VK (Mishra, V. K.)[1]; Singh, G (Singh, Gurbachan)[1]; Swarup, A (Swarup, Anand)[1]	34	4	547-556	10.1080/01904167.2011.538116

Neves et al. 2007	Estuaries and Coasts	Primary production and nutrient content in two salt marsh species, Atriplex portulacoides L. and Limoniastrum monopetalum L., in Southern Portugal	Neves, JP (Neves, J. P.); Ferreira, LF (Ferreira, L. F.); Simoes, MP (Simoes, M. P.); Gazarini, LC (Gazarini, L. C.)	30	3	459-468	10.1007/BF02819392
Nieminen et al. 2016	European Journal Forest Research	Whole-tree, stem-only, and stump harvesting impacts on site nutrient capital of a Norway spruce-dominated peatland forest	Nieminen, M (Nieminen, Mika)[1]; Laiho, R (Laiho, Raija)[1]; Sarkkola, S (Sarkkola, Sakari)[1]; Penttila, T (Penttila, Timo)[1]	135	3	531-538	10.1007/s10342-016-0951-1
Niinemets et al. 2005	Tree physiology	Species differences in timing of leaf fall and foliage chemistry modify nutrient resorption efficiency in deciduous temperate forest stands	Niinemets, U (Niinemets, U); Tamm, U (Tamm, U)	25	8	1001-1014	10.1093/treephys/25.8.1001
Niu et al, 2016. Journal of Applied Ecology	Journal of Applied Ecology	Grazing-induced shifts in community functional composition and soil nutrient availability in Tibetan alpine meadows	Niu, KC (Niu, Kechang)[1,2,3]; He, JS (He, Jin-Sheng)[2]; Lechowicz, MJ (Lechowicz, Martin J.)[3]	53	5	1554-1564	10.1111/1365-2664.12727
Oleksyn et al 2000. Trees 14: 271-281.	Trees-structure and function	Ontogenetic patterns of leaf CO2 exchange, morphology and chemistry in Betula pendula trees	Oleksyn, J (Oleksyn, J); Zytkowiak, R (Zytkowiak, R); Reich, PB (Reich, PB); Tjoelker, MG (Tjoelker, MG); Karolewski, P (Karolewski, P)	14	4	271-281	
Oleksyn et al. 2002	Ann For Sci	Needle nutrients in geographically diverse Pinus sylvestris L. populations	Oleksyn, J (Oleksyn, J); Reich, PB (Reich, PB); Zytkowiak, R (Zytkowiak, R); Karolewski, P (Karolewski, P); Tjoelker, MG (Tjoelker, MG)	59	1	(1-18)	10.1051/forest:2001001
Oliva et al. 2014	Ciência florestal	Nutritional composition of provenances and progeny of mate (Ilex paraguariensis St. Hil.) cultivaded in distrofic red oxisoils	Oliva, EV (Oliva, Elaine Vivian)[1]; Reissmann, CB (Reissmann, Carlos Bruno)[2]; Gaiad, S (Gaiad, Sergio)[3]; de Oliveira, EB (de Oliveira, Edilson Batista)[3]; Sturion, JA (Sturion, Jose Alfredo)[3]	24	4	793-805	
Oliveira et al 2014	Plos One	Stress Tolerance and Ecophysiological Ability of an Invader and a Native Species in a Seasonally Dry Tropical Forest	Oliveira, MT (Oliveira, Marciel Teixeira)[1]; Matzek, V (Matzek, Virginia)[2]; Medeiros, CD (Medeiros, Camila Dias)[1]; Rivas, R (Rivas, Rebeca)[1]; Falcao, HM (Falcao, Hiram Marinho)[1]; Santos, MG (Santos, Mauro Guida)[1]	9	8	e105514	10.1371/journal.pone.0105514
Olsson et al. 2000	Plant and soil	Nutrient status in needles of Norway spruce and Scots pine following harvesting of logging residues	Olsson, BA (Olsson, BA); Lundkvist, H (Lundkvist, H); Staaf, H (Staaf, H)	223	(1-2)	163-175	10.1023/A:1004892109615
Osaki et al. 1998	Plant and soil	Nutritional characteristics in leaves of native plants grown in acid sulfate, peat, sandy podzolic, and saline soils distributed in Peninsular Thailand	Osaki, M (Osaki, M); Watanabe, T (Watanabe, T); Ishizawa, T (Ishizawa, T); Nilnond, C (Nilnond, C); Nuyim, T (Nuyim, T); Sittibush, C (Sittibush, C); Tadano, T (Tadano, T)	201	2	175-182	10.1023/A:1004389331808
Osaki et al. 2003	Plant Foods for Human Nutrition	Mineral characteristics of the leaves of 166 plant species with different phylogeny in the temperate region	Osaki, M (Osaki, M); Yamada, S (Yamada, S); Ishizawa, T (Ishizawa, T); Watanabe, T (Watanabe, T); Shinano, T (Shinano, T); Tuah, SJ (Tuah, SJ); Urayama, M (Urayama, M)	58	2	139-152	10.1023/A:1024474815392
Ouimet et al. 1992	Canadian Journal of Forest Research	Growth and foliar nutrient status of sugar maple - incidence of forest decline and reaction to fertilization	OUIMET, R (OUIMET, R); FORTIN, JM (FORTIN, JM)	22	5	699-706	10.1139/x92-093

Palm et al. 1990	Biotropica	Decomposition and nutrient release patterns of the leaves of 3 tropical legumes	PALM, CA (PALM, CA); SANCHEZ, PA (SANCHEZ, PA)	22	4	330-338	10.2307/2388550
Papatheodorou et al. 2004	Journal of Arid Environments	Nutrient attributes of tissues in relation to grazing in an evergreen sclerophyllous shrub (Quercus coccifera L.) dominating vegetation in Mediterranean-type ecosystems	Papatheodorou, EM (Papatheodorou, EM); Stamou, GP (Stamou, GP)	59	2	217-227	10.1016/ j.jaridenv.2003.12.012
Pâques et al. 1994	Forest Ecology and Management	Relationship between foliar nutrient concentrations and growth of hybrid larch (Larix x Eurolepis henry)	PAQUES, LE (PAQUES, LE)	63	(2-3)	153-167	10.1016/0378-1127(94)90 108-2
Peri et al. 2006	Forest ecology and management	Dynamics of above- and below-ground biomass and nutrient accumulation in an age sequence of Nothofagus antarctica forest of Southern Patagonia	Peri, PL (Peri, Pablo L.); Gargaglione, V (Gargaglione, Veronica); Pastur, GM (Pastur, Guillermo Martinez)	233	1	85-99	10.1016/ j.foreco.2006.06.009
Peri et al. 2008	Forest ecology and management	Above- and belowground nutrients storage and biomass accumulation in marginal Nothofagus antarctica forests in Southern Patagonia	Peri, PL (Peri, Pablo L.)[1,3]; Gargaglione, V (Gargaglione, Veronica)[1]; Pastur, GM (Pastur, Guillermo Martinez)[2,3]	255	7	2502-25 11	10.1016/ j.foreco.2008.01.014
Piatek et al. 2017	Canadian Journal of Forest Research	Thinning effects on foliar elements in eastern hemlock: implications for managing the spread of the hemlock woolly adelgid	Piatek, KB (Piatek, Kathryn B.)[1,4]; Fajvan, MA (Fajvan, Mary Ann)[2]; Turcotte, RM (Turcotte, Richard M.)[3]	47	1	81-88	10.1139/cjfr-2016-0260
Pietrzykowski et al. 2013	Environ Monit Assess	Scots pine needles macronutrient (N, P, K, CA, MG, and S) supply at different reclaimed mine soil substrates-as an indicator of the stability of developed forest ecosystems	Pietrzykowski, M (Pietrzykowski, Marcin)[1]; Wos, B (Wos, Bartlomiej)[1]; Haus, N (Haus, Nicholas)[2]	185	9	7445-74 57	10.1007/ s10661-013-3111-9
Potocic et al. 2003	Periodicu m biologoru m	Recovery of black pine (Pinus nigra Arn.) needles in response to lowered SO2 in the air - a case study of Plomin thermal power plant (Croatia)	Potocic, N (Potocic, N); Seletkovic, I (Seletkovic, I); Lepedus, H (Lepedus, H); Cesar, V (Cesar, V)	105	3	319-323	
Prajjwal et al. 2011	Current Science						
Price et al, 2014. Functional Ecology	Functiona l Ecology	The functional assembly of experimental grasslands in relation to fertility and resource heterogeneity	Price, JN (Price, Jodi N.)[1,2]; Gazol, A (Gazol, Antonio)[1]; Tamme, R (Tamme, Riin)[1]; Hiiesalu, I (Hiiesalu, Inga)[1,3]; Partel, M (Paertel, Meelis) [1]	28	2	509-519	10.1111/1365-2435.12186
Prieto et al 2018, Functional Ecology	Functiona l Ecology	Leaf carbon and oxygen isotopes are coordinated with the leaf economics spectrum in Mediterranean rangeland species	Prieto, I (Prieto, Ivan)[1,2]; Querejeta, JI (Querejeta, Jose I.)[2]; Segrestin, J (Segrestin, Jules)[1]; Volaire, F (Volaire, Florence)[3]; Roumet, C (Roumet, Catherine)[1]	32	3	612-625	10.1111/1365-2435.13025
Proemse et al., 2016	Forests	Foliage chemistry of Pinus banksiana in the Athabasca Oil Sands Region, Alberta, Canada	Proemse, B. C., Maynard, D.G., Mayer, B	7		312	10.3390/f7120312
Raitio et al. 2000	Plant and soil	Effect of provenance on free amino acid and chemical composition of Scots pine needles	Raitio, H (Raitio, H); Sarjala, T (Sarjala, T)	221	2	231-238	10.1023/ A:1004745122911
Rathfon et al. 1993	Forest Ecology and Management	Temporal variation in foliar nutrient concentrations of pitch pine, loblolly-pine and the pitch x loblolly hybrid	RATHFON, RA (RATHFON, RA); JOHNSON, JE (JOHNSON, JE); BURGER, JA (BURGER, JA); KREH, RE (KREH, RE); FERET, PP (FERET, PP)	58	(1-2)	137-151	10.1016/0378-1127(93)90 136-B

Raveh 2013	Journal of Plant Nutrition and Soil Science	Citrus leaf nutrient status: A critical evaluation of guidelines for optimal yield in Israel	Raveh, E (Raveh, Eran)	176	3	420-428	10.1002/jpln.201200411
Read et al., 2002	Australian Journal of Botany	Foliar mineral content of Nothofagus species on ultramafic soils in New Caledonia and non-ultramafic soils in Papua New Guinea	Read, J., Ferris, J.M., Jaffré, T.	50		607-617	
Renteria and Jaramillo 2011	Oecologia	Rainfall drives leaf traits and leaf nutrient resorption in a tropical dry forest in Mexico	Renteria, LY (Renteria, Lyliana Y.)[1]; Jaramillo, VJ (Jaramillo, Victor J.)[1]	165	1	201-211	10.1007/s00442-010-1704-3
Rezende et al. 2016	Acta Scientiarum						
Ribeiro et al. 2008	Cerne	Nutrition of mate-tree with ammonium sulfate fertilizer	Ribeiro, MM (Ribeiro, Marcia Marzagao)[1]; Reissmann, CB (Reissmann, Carlos Bruno)[1]; Correa, DR (Correa, Daniel Resende)	14	3	204-211	
Richardson et al 2008	Functional Ecology	Shifts in leaf N : P ratio during resorption reflect soil P in temperate rainforest	Richardson, SJ (Richardson, Sarah J.)[1]; Allen, RB (Allen, Robert B.)[1]; Doherty, JE (Doherty, James E.)[2]	22	4	738-745	10.1111/j.1365-2435.2008.01426.x
Roberntz et al. 1999	Trees-structure and function	Effects of long-term CO2 enrichment and nutrient availability in Norway spruce. II. Foliar chemistry	Roberntz, P (Roberntz, P); Linder, S (Linder, S)	14	1	17-27	10.1007/s004680050003
Romanyà et al. 1996	Forest Science	Nutritional status and deficiency diagnosis of Pinus radiata plantations in Spain	Romanya, J (Romanya, J); Vallejo, VR (Vallejo, VR)	42	2	192-197	
Rosa et al. 2014	Cerne	Nutrients concentration and accumulation in teak stands in the state of Mato Grosso, Brasil	Rosa, TDD (de Deus Rosa, Tania de Fatima)[1]; Scaramuzzal, WLMP (Matos Pereira Scaramuzzal, Walcylene Lacerda)[1]; da Silva, RG (da Silva, Renata Goncalves)[1]	21	1	51-57	10.1590/01047760201521011274
Rosberg et al. 2006	Forest Ecology and Management	Effects of liming and fertilization on tree growth and nutrient cycling in a Scots pine ecosystem in Norway	Rosberg, I (Rosberg, Ingvald); Frank, J (Frank, Jon); Stuanes, AO (Stuanes, Arne O.)	237	(1-3)	191-207	10.1016/j.foreco.2006.09.045
Rothe et al. 2003	Forest Ecology and Management	Do admixed broadleaves improve foliar nutrient status of conifer tree crops?	Rothe, A (Rothe, A); Ewald, J (Ewald, J); Hibbs, DE (Hibbs, DE)	172	(2-3)	327-338	10.1016/S0378-1127(01)00800-3
Rouhi-Moghaddam et al. 2008	Forest Ecology and Management	Comparison of growth, nutrition and soil properties of pure stands of Quercus castaneifolia and mixed with Zelkova carpinifolia in the Hyrcanian forests of Iran	Rouhi-Moghaddam, E (Rouhi-Moghaddam, Einollah)[1,2]; Hosseini, SM (Hosseini, Seyed Mohsen)[1]; Ebrahimi, E (Ebrahimi, Ezzatollah)[3]; Tabari, M (Tabari, Masoud)[1]; Rahmani, A (Rahmani, Ahmad)[3]	255	(3-4)	1149-1160	10.1016/j.foreco.2007.10.048
Rytter et al. 2010	New Forests	Root preparation technique and storage affect results of seedling quality evaluation in Norway spruce	Rytter, RM (Rytter, Rose-Marie)[1,2]; Rytter, L (Rytter, Lars)[2]	39	3	355-368	10.1007/s11056-009-9176-1
Safou-Matondo et al. 2005	Forest Ecology and Management	Hybrid and clonal variability of nutrient content and nutrient use efficiency in Eucalyptus stands in Congo	Safou-Matondo, R (Safou-Matondo, R); Deleporte, P (Deleporte, P); Laclau, JP (Laclau, JP); Bouillet, JP (Bouillet, JP)	210	(1-3)	193-204	10.1016/j.foreco.2005.02.049

Samecka-Cymerman et al. 2009	Trees-structure and function	Short shoots of Betula pendula Roth. as bioindicators of urban environmental pollution in Wroclaw (Poland)	Samecka-Cymerman, A (Samecka-Cymerman, Aleksandra)[1]; Kolon, K (Kolon, K.)[1]; Kempers, AJ (Kempers, A. J.)[2]	23	5	923-929	10.1007/s00468-009-0334-z
Santa Regina 2000	Forest Ecology and Management	Biomass estimation and nutrient pools in four Quercus pyrenaica in Sierra de Gata Mountains, Salamanca, Spain	Santa-Regina, I (Santa-Regina, I)	132	(2-3)	127-141	10.1016/S0378-1127(99)00219-4
Santamaría et al. 1998	Chemosphere	Influence of air pollution on the nutritional status of Navarra's forests, Spain	Santamaria, JM (Santamaria, JM); Martin, A (Martin, A)	36	(4-5)	943-948	10.1016/S0045-6535(97)10152-7
Santiago et al. 2007	Functional Ecology	Leaf functional traits of tropical forest plants in relation to growth form	Santiago, LS (Santiago, L. S.); Wright, SJ (Wright, S. J.)	21	1	19-27	10.1111/j.1365-2435.2006.01218.x
Sariyildiz et al. 2005	Forest Ecology and Management	Variation in the chemical composition of green leaves and leaf litters from three deciduous tree species growing on different soil types	Sariyildiz, T (Sariyildiz, T); Anderson, JM (Anderson, JM)	210	(1-3)	303-319	10.1016/j.foreco.2005.02.043
Scalon et al 2017, Oikos	Oikos	To recycle or steal? Nutrient resorption in Australian and Brazilian mistletoes from three low-phosphorus sites	Scalon, MC (Scalon, Marina C.)[1,2]; Wright, IJ (Wright, Ian J.)[1]; Franco, AC (Franco, Augusto C.)[2]	126	1	32-39	10.1111/oik.03455
Scalon MC et al 2017. Plant soil	Plant and soil	Influence of long-term nutrient manipulation on specific leaf area and leaf nutrient concentrations in savanna woody species of contrasting leaf phenologies	Scalon, MC (Scalon, Marina Correa)[1,3]; Haridasan, M (Haridasan, Mundayatan)[2]; Franco, AC (Franco, Augusto Cesar)[1]	421	(1-2)	233-244	10.1007/s11104-017-3437-0
Schumacher et al. 2008	Ciência florestal	Biomass and nutrient in an Hovenia dulcis Thunb. Stand at Fepagro Florestas, Santa Maria, RS	Schumacher, MV (Schumacher, Mauro Valdir)[1]; Brun, EJ (Brun, Eleandro Jose)[2,3]; Illana, VB (Illana, Vinicius Borges); Dissiuta, SI (Dissiuta, Stefano Ilha); Agne, TL (Agne, Tiago Leal)	18	(1-2)	27-37	10.5902/19805098519
Scott et al. 2012	Forest Ecology and Management	Short-term effects of firebreaks on seedling growth, nutrient concentrations and soil strength in southern Australian wet eucalypt forests	Scott, RE (Scott, R. E.)[1,2,3]; Hovenden, MJ (Hovenden, M. J.)[2]; Neyland, MG (Neyland, M. G.)[1,2]; Mitchell, SJ (Mitchell, S. J.)[4]; Adams, PR (Adams, P. R.)[1]; Wood, MJ (Wood, M. J.)[1]	278		110-117	10.1016/j.foreco.2012.05.004
Siddiqui et al. 2009	Pakistan Journal Botany	Chronosequence and crown strata effects on foliar nutrient concentrations in teak (Tectona grandis L.f) plantations	Siddiqui, MT (Siddiqui, M. Tahir)[1]; Shah, AH (Shah, A. H.)[1]; Yaqoob, S (Yaqoob, S.)[1]	41	6	3023-3034	
Sikström 2002	Canadian Journal of Forest Research	Effects of liming and fertilization (N, PK) on stem growth, crown transparency, and needle element concentrations of Picea abies stands in southwestern Sweden	Sikstrom, U (Sikstrom, U)	32	10	1717-1727	10.1139/X02-094
Singh 1996	Forest ecology and management	Effect of site preparation techniques on Prosopis juliflora in an alkali soil	Singh, G (Singh, G)	80	(1-3)	267-278	10.1016/0378-1127(95)03543-5
Singh et al. 2010	Indian Journal of Horticulture	Influence of pruning intensities on leaf nutrient composition in some mango cultivars planted under high density	Singh, SK (Singh, Sanjay Kumar)[1]; Singh, SK (Singh, S. K.)[1]; Sharma, RR (Sharma, R. R.)[1]; Srivastav, M (Srivastav, Manish)[1]; Patel, VB (Patel, V. B.)[1]	67	1	16-20	

Sirois et al. 1998	Agroforestry Systems	Influence of remnant trees on nutrients and fallow biomass in slash and burn agroecosystems in Guinea	Sirois, MC (Sirois, MC); Margolis, HA (Margolis, HA); Camire, C (Camire, C)	40	3	227-246	10.1023/A:1006093329468
Skonieczna et al. 2014	Drewno	Element content of Scots pine (Pinus sylvestris L.) stands of different densities	Skonieczna, J (Skonieczna, Joanna)[1]; Malek, S (Malek, Stanislaw)[2]; Polowy, K (Polowy, Krzysztof)[1]; Wegiel, A (Wegiel, Andrzej)[1]	57	192	77-87	10.12841/wood.1644-3985.S13.05
Specht et al. 2006	Forest Ecology and Management	Foliar nutrient concentrations in mixed-species plantations of subtropical cabinet timber species and their potential as a management tool	Specht, A (Specht, A.); Turner, J (Turner, J.)	233	(2-3)	324-337	10.1016/j.foreco.2006.05.029
Sridhar et al. 2013	Journal of Forestry Research	Nutrient composition of pill millipede manure of the Western Ghats, India	Sridhar, KR (Sridhar, Kandikere R.)[1]; Kadamannaya, BS (Kadamannaya, Bombrana S.)[2]; Karamchand, KS (Karamchand, Kishore S.)[1]	24	3	539-545	10.1007/s11676-013-0385-2
Stanley et al. 1999	Forest Ecology and Management	Biomass and nutrient accumulation in pure and mixed plantations of indigenous tree species grown on poor soils in the humid tropics of Costa Rica	Stanley, WG (Stanley, WG); Montagnini, F (Montagnini, F)	113	1	91-103	10.1016/S0378-1127(98)00417-4
Stock and Verboom 2012	Global Ecology and Biogeography	Phylogenetic ecology of foliar N and P concentrations and N:P ratios across mediterranean-type ecosystems	Stock, WD (Stock, William D.)[1]; Verboom, GA (Verboom, G. Anthony)[2]	21	12	1147-1156	10.1111/j.1466-8238.2011.00752.x
Stuhrmann et al. 1994	Forest ecology and management	Mineral-nutrition, soil factors and growth rates of Gmelina arborea plantations in the humid lowlands of northern Costa Rica	STUHRMANN, M (STUHRMANN, M); BERGMANN, C (BERGMANN, C); ZECH, W (ZECH, W)	70	(1-3)	135-145	10.1016/0378-1127(94)90081-7
Sun et al. 2015	Canadian Journal of Forest Research	Biogeographic patterns of multi-element stoichiometry of Quercus variabilis leaves across China	Sun, X (Sun, Xiao)[1,2]; Kang, HZ (Kang, Hongzhang)[1]; Kattge, J (Kattge, Jens)[3,4]; Gao, Y (Gao, Yue)[2]; Liu, CJ (Liu, Chunjiang)[1,5]	45	12	1827-1834	10.1139/cjfr-2015-0110
Tan X et al 2017	Trees-structure and function	Invasive Rhus typhina invests more in height growth and traits associated with light acquisition than do native and non-invasive alien shrub species	Tan, XF (Tan, Xiangfeng)[1,2,3]; Guo, X (Guo, Xiao)[1,4]; Guo, WH (Guo, Weihua)[1,2]; Liu, SN (Liu, Shuna)[1,2]; Du, N (Du, Ning)[1,2]	32	4	1103-1112	10.1007/s00468-018-1698-8
Tausz et al 2004	Forest Ecology and Management	A survey of foliar mineral nutrient concentrations of Pinus canariensis at field plots in Tenerife	Tausz, M (Tausz, M); Trummer, W (Trummer, W); Wonisch, A (Wonisch, A); Goessler, W (Goessler, W); Grill, D (Grill, D); Jimenez, MS (Jimenez, MS); Morales, D (Morales, D)	189	(1-3)	49-55	10.1016/j.foreco.2003.07.034
Teklay 2004	Plant and soil	Seasonal dynamics in the concentrations of macronutrients and organic constituents in green and senesced leaves of three agroforestry species in southern Ethiopia	Teklay, T (Teklay, T)	267	(1-2)	297-307	10.1007/s11104-005-0124-3
Teng et al., 2003	The forestry chronicle	Response of advance regeneration to intensity of harvest and fertilization on boreal wetlands	Teng, Y., Bailey, S.E., Foster, N.W., Hazlett, P.W.	79	1		
Thelin et al 2002	Forest Ecology and Management	The nutrient status of Norway spruce in pure and in mixed-species stands	Thelin, G (Thelin, G); Rosengren, U (Rosengren, U); Callesen, I (Callesen, I); Ingerslev, M (Ingerslev, M)	160	(1-3)	115-125	10.1016/S0378-1127(01)00464-9

Tiedemann et al. 1996	Great Basin Naturalist	Nutrient distribution in Quercus gambelii stands in central Utah	Tiedemann, AR (Tiedemann, AR); Clary, WP (Clary, WP)	56	2	119-128	
Toky et al. 1994	Journal of Arid Environments	Interaction between Rhizobium inoculation and nitrogen fertilizer application on growth and nodulation of Acacia nilotica subsp indica	TOKY, OP (TOKY, OP); BENIWAL, RS (BENIWAL, RS); SHARMA, PK (SHARMA, PK)	27	1	49-54	10.1006/jare.1994.1044
Toplu et al. 2009	Journal of Plant Nutrition	Leaf Mineral Composition of Olive Varieties and Their Relation to Yield and Adaptation Ability	Toplu, C (Toplu, Celil)[2]; Uygur, V (Uygur, Veli)[1]; Yildiz, E (Yildiz, Ercan)[2]	32	9	1560-1573	10.1080/01904160903094321
Townsend et al. 2007	Ecology	Controls over foliar N : P ratios in tropical rain forests	Townsend, AR (Townsend, Alan R.); Cleveland, CC (Cleveland, Cory C.); Asner, GP (Asner, Gregory P.); Bustamante, MMC (Bustamante, Mercedes M. C.)	88	1	107-118	10.1890/0012-9658(2007)88[107:COFNRI]2.0.CO;2
Trueman et al. 2013	Journal of Soil Science and Plant Nutrition	Nutrient partitioning among the roots, hedge and cuttings of Corymbia citriodora stock plants	Trueman, SJ (Trueman, S. J.)[1]; McMahon, TV (McMahon, T. V.)[2]; Bristow, M (Bristow, M.)[3]	13	4	977-989	
Tuohy_etal_Zimbabwe_CHID	Oecologia	Photosynthesis in relation to leaf nitrogen and phosphorus content in Zimbabwean trees	TUOHY, JM (TUOHY, JM); PRIOR, JAB (PRIOR, JAB); STEWART, GR (STEWART, GR)	88	3	378-382	10.1007/BF00317582
Ukonmaanaho et al. 2008	Boreal environment research	Litterfall production and nutrient return to the forest floor in Scots pine and Norway spruce stands in Finland	Ukonmaanaho, L (Ukonmaanaho, Liisa)[1]; Merila, P (Merila, Paivi)[2]; Nojd, P (Nojd, Pekka)[1]; Nieminen, TM (Nieminen, Tina M.)[1]	13		67-91	
Ülo Niinemets & Kalevi Kull2003	Acta Oecologica - International journal of ecology	Leaf structure vs. nutrient relationships vary with soil conditions in temperate shrubs and trees	Niinemets, U (Niinemets, U); Kull, K (Kull, K)	24	4	209-219	10.1016/S1146-609X(03)00094-8
Umaña MN et al 2016	American Naturalist	Interspecific Functional Convergence and Divergence and Intraspecific Negative Density Dependence Underlie the Seed-to-Seedling Transition in Tropical Trees	Umana, MN (Umana, Maria Natalia)[1]; Forero-Montana, J (Forero-Montana, Jimena)[2]; Muscarella, R (Muscarella, Robert)[3]; Nytch, CJ (Nytch, Christopher J.)[2]; Thompson, J (Thompson, Jill)[4,5]; Uriarte, M (Uriarte, Maria)[6]; Zimmerman, J (Zimmerman, Jess)[2,5]; Swenson, NG (Swenson, Nathan G.)[1,7]	187	1	99-109	10.1086/684174
Uozumi et al. 2012	Soil Science and Plant Nutrition	Adaptive strategy of six native herbaceous species growing over the whole region of Mt. Daisen: characteristics of N, P, K, Ca, and Mg accumulation in leaves	Uozumi, Y (Uozumi, Yasuyuki)[1]; Yamada, S (Yamada, Satoshi)[2]; Masunaga, T (Masunaga, Tsugiyuki)[3]; Hioki, Y (Hioki, Yoshiyuki)[2]; Fujiyama, H (Fujiyama, Hideyasu)[2]	58	5	583-594	10.1080/00380768.2012.717502
Van Wesemael 1992	Forest ecology and management						

Vanguelova et al. 2010	Biogeoch emistry	Long term effects of whole tree harvesting on soil carbon and nutrient sustainability in the UK	Vanguelova, E (Vanguelova, Elena)[1]; Pitman, R (Pitman, Rona)[1]; Luiro, J (Luiro, Jukka)[2]; Helmisaari, HS (Helmisaari, Helja-Sisko)[2]	101	(1-3)	43-59	10.1007/ s10533-010-9511-9
Varnagiryte-Kabasinskiene et al. 2015	Iforest - Biogeosci ences and Forestry	Vertical position of dry mass and elemental concentrations in Pinus sylvestris L. canopy under the different ash-nitrogen treatments	Varnagiryte-Kabasinskiene, I (Varnagiryte-Kabasinskiene, Iveta)[1,2]; Stakenas, V (Stakenas, Vidas)[1]; Miksys, V (Miksys, Virgilijus)[1]; Kabasinskas, A (Kabasinskas, Audrius)[3]	8		838-845	10.3832/ifor1342-007
Viera et al. 2009	Ciência florestal	Nutrients concentration and retranslocation in the Pinus taeda L. needles	Viera, M (Viera, Marcio)[1]; Schumacher, MV (Schumacher, Mauro Valdir) [2]	19	(3-4)	375-382	
Viera et al. 2011	Revista Árvore	Biomass and removed nutrients in the first thinning of Pinus taeda L. stand in Cambara do Sul, RS	Viera, M (Viera, Marcio)[1]; Schumacher, MV (Schumacher, Mauro Valdir) [2]; Bonacina, DM (Bonacina, Darlan Michel)	35	3	371-379	10.1590/ S0100-6762201100030000 1
Vitousek et al 1995	Ecology	Foliar nutrients during long-term soil development in Hawaiian montane Rain-forest	VITOUSEK, PM (VITOUSEK, PM); TURNER, DR (TURNER, DR); KITAYAMA, K (KITAYAMA, K)	76	3	712-720	10.2307/1939338
Wallin et al. 1998	Canadian Journal of Forest Research	Association of within-tree jack pine budworm feeding patterns with canopy level and within-needle variation of water, nutrient, and monoterpene concentrations	Wallin, KF (Wallin, KF); Raffa, KF (Raffa, KF)	28	2	228-233	10.1139/cjfr-28-2-228
Wan et al. 2009	Journal of Plant Nutrition	Nutrient Elements in Leaves of Rare and Endangered Species in Wuhan Botanical Garden, China	Wan, KY (Wan, Kai-yuan) [1,2]; Chen, F (Chen, Fang)[1] ; Tao, Y (Tao, Yong)[1,2]; Chen, SS (Chen, Shu-sen)[1]	32	11	1914-19 40	10.1080/01904160903242 391
Wang et al. 1995	Forest Ecology and Managem ent	Aboveground biomass and nutrient accumulation in an age sequence of aspen (Populus tremuloides) stands in the boreal white and black spruce zone, British Columbia	WANG, JR (WANG, JR); ZHONG, AL (ZHONG, AL); COMEAU, P (COMEAU, P); TSZE, M (TSZE, M); KIMMINS, JP (KIMMINS, JP)	78	(1-3)	127-138	10.1016/0378-1127(95)03 590-0
Wang et al. 1997	Forest ecology and managem ent	White spruce foliar nutrient concentrations in relation to tree growth and soil nutrient amounts	Wang, GG (Wang, GG); Klinka, K (Klinka, K)	98	1	89-99	10.1016/ S0378-1127(97)00048-0
Wang et al. 2003	Plant and soil	Seasonal changes in element contents in mangrove element retranslocation during leaf senescene	Wang, WQ (Wang, WQ); Wang, M (Wang, M); Lin, P (Lin, P)	252	2	187-193	10.1023/ A:1024704204037
Wang et al. 2007	Scandinav ian Journal of Forest Research	Effects of wood ash, vitality fertilizer and logging residues on needle and root chemistry in a young Norway spruce stand	Wang, P (Wang, Pei); Olsson, BA (Olsson, Bengt A.); Lundkvist, H (Lundkvist, Helene)	22	2	136-148	10.1080/02827580701231 480
Wang W & Lin P 1999	Mangrove s and salt marshes						
Wang, 1987b							
Wiersma et al. 2007	Environm ental Monitorin g Assessme nt	Forest vegetation monitoring and foliar chemistry of red spruce and red maple at Acadia National Park in Maine	Wiersma, GB (Wiersma, G. Bruce); Elvir, JA (Elvir, Jose Alexander); Eckhoff, JD (Eckhoff, Janet D.)	126	(1-3)	27-37	10.1007/ s10661-006-9329-z

Wigley et al 2016	Journal of Ecology	Leaf traits of African woody savanna species across climate and soil fertility gradients: evidence for conservative versus acquisitive resource-use strategies	Wigley, BJ (Wigley, Benjamin J.)[1,2,3]; Slingsby, JA (Slingsby, Jasper A.)[4,5]; Diaz, S (Diaz, Sandra)[6,7]; Bond, WJ (Bond, William J.)[4,8]; Fritz, H (Fritz, Herve)[3]; Coetsee, C (Coetsee, Corli)[2,9]	104	5	1357-1369	10.1111/1365-2745.12598
Wu et al. 2007	Forest Ecology and Management	Mineral nutrient status of tree species in relation to environmental factors in the subtropical rain forest of Taiwan	Wu, CC (Wu, Chia-Chi); Tsui, CC (Tsui, Chun-Chih); Hseih, CF (Hseih, Chang-Fu); Asio, VB (Asio, Victor B.); Chen, ZS (Chen, Zueng-Sang)	239	(1-3)	81-91	10.1016/ j.foreco.2006.11.008
Wu et al. 2011	Silvae Genetica	Variations in biomass, nutrient contents and nutrient use efficiency among Chinese fir provenances	Wu, PF (Wu, Pengfei)[1]; Tigabu, M (Tigabu, Mulualem)[2]; Ma, XQ (Ma, Xiangqing)[1]; Oden, PC (Oden, Per Christer)[2]; He, YL (He, Youlan)[1]; Yu, XT (Yu, Xintuo)[1]; He, ZY (He, Zhiying)[1]	60	(3-4)	95-105	10.1515/sg-2011-0014
Wu et al. 2012	Forest Ecology and Management	Leaf nitrogen and phosphorus stoichiometry of Quercus species across China	Wu, TG (Wu, Tonggui)[1]; Dong, Y (Dong, Yi)[1]; Yu, MK (Yu, Mukui)[1]; Wang, GG (Wang, G. Geoff)[2]; Zeng, DH (Zeng, De-Hui)[3]	284		116-123	10.1016/ j.foreco.2012.07.025
Wu et al. 2014	Ecological Complexity	Patterns of leaf nitrogen and phosphorus stoichiometry among Quercus acutissima provenances across China	Wu, TG (Wu, Tonggui)[1]; Wang, GG (Wang, G. Geoff)[2]; Wu, QT (Wu, Qianting)[1,3]; Cheng, XR (Cheng, Xiangrong)[1]; Yu, MK (Yu, Mukui)[1]; Wang, W (Wang, Wan)[1]; Yu, XB (Yu, Xuebiao)[3]	17		32-39	10.1016/ j.ecocom.2013.07.003
Xu 2006	Ann. For. Sci.	Nutrient dynamics in decomposing needles of Pinus luchuensis after typhoon disturbance in a subtropical environment	Xu, XN (Xu, Xiaoniu)	63	7	707-713	10.1051/forest:2006051
Xu et al., 2003							
Xue et al. 1996	Forest Ecology and Management	Nutrient cycling in a Chinese-fir (Cunninghamia lanceolata) stand on a poor site in Yishan, Guangxi	Xue, L (Xue, L)	89	(1-3)	115-123	
Yan et al. 2016	Journal of Plant Ecology	Leaf nutrient dynamics and nutrient resorption: a comparison between larch plantations and adjacent secondary forests in Northeast China	Yan, T (Yan, Tao)[1,2,3]; Lu, XT (Lu, Xiaotao)[1]; Yang, K (Yang, Kai)[1,2]; Zhu, JJ (Zhu, Jiaojun)[1,2]	9	2	165-173	10.1093/jpe/rtv034
Yang et al 2015	Journal of Ecology	C:N:P stoichiometry of Artemisia species and close relatives across northern China: unravelling effects of climate, soil and taxonomy	Yang, XJ (Yang, Xuejun)[1]; Huang, ZY (Huang, Zhenying)[1]; Zhang, KL (Zhang, Keliang)[1]; Cornelissen, JHC (Cornelissen, Johannes H. C.)[2]	103	4	1020-1031	10.1111/1365-2745.12409
Yang et al. 2016	Canadian Journal of Forest Research	Sources of variability in tissue chemistry in northern hardwood species	Yang, Y (Yang, Yang)[1]; Yanai, RD (Yanai, Ruth D.)[1]; Fatemi, FR (Fatemi, Farrah R.)[2]; Levine, CR (Levine, Carrie R.)[3]; Lilly, PJ (Lilly, Paul J.)[4]; Briggs, RD (Briggs, Russell D.)[1]	46	3	285-296	10.1139/cjfr-2015-0302
Zas, R. & Serrada, R. 2003	Forest Ecology and Management	Foliar nutrient status and nutritional relationships of young Pinus radiata D. Don plantations in northwest Spain	Zas, R (Zas, R); Serrada, R (Serrada, R)	174	(1-3)	167-176	10.1016/ S0378-1127(02)00027-0

Zatylny et al. 2006	Journal of Plant Nutrition	Development of standard concentrations of foliar nutrients for saskatoon	Zatylny, AM (Zatylny, AM); St-Pierre, RG (St-Pierre, RG)	29	2	195-207	10.1080/01904160500468662
Zech et al., 1991	Turrialba	Mineral deficiencies of forest trees in Yucatan (Mexico) and consequences for land-use	Zech, W., Drechsel, P., Neugebauer, B.	41	2	230-236	
Zhai et al., 1987							
Zhou et al. 2016	Journal of forestal research						

Table S4.7 Abbreviations, complete names and units for the variables used to build the models.

Nick	Complete names	Units	Source
Var28	Minimum June July August precipitation	mm	Metzger et al., 2013
Var29	Maximum June July August precipitation	mm	Metzger et al., 2013
Var30	Minimum December January February precipitation	mm	Metzger et al., 2013
Var31	Maximum December January February precipitation	mm	Metzger et al., 2013
Var41	Daily mean solar radiation	kJ m-2 day-1	Fick and Hijmans, 2017
Var42	Altitude	m	Fick and Hijmans, 2017
BD	Bulk density	g/cm3	Shangguan et al., 2014
BS	Base saturation	%	Shangguan et al., 2014
CACO3	CaCo3	% of weight	Shangguan et al., 2014
CEC	Cation exchange capacity	cmol/kg	Shangguan et al., 2014
CLAY	Clay content	% of weight	Shangguan et al., 2014
ECE	Electrical conductivity	ds/m	Shangguan et al., 2014
ESP	Exchangeable Na	cmol/kg	Shangguan et al., 2014
EXAL	Exchangeable Al	cmol/kg	Shangguan et al., 2014
EXCA	Exchangeable Ca	cmol/kg	Shangguan et al., 2014
EXH	Exchangeable acidity	cmol/kg	Shangguan et al., 2014
EXK	Exchangeable K	cmol/kg	Shangguan et al., 2014
EXMG	Exchangeable Mg	cmol/kg	Shangguan et al., 2014
EXNA	Exchangeable Na	cmol/kg	Shangguan et al., 2014
GRAV	Gravel content	% of volume	Shangguan et al., 2014
GYP	Gypsum	% of weight	Shangguan et al., 2014
OC	Organic carbon	% of weight	Shangguan et al., 2014

PBR	P Bray1 method	ppm of weight	Shangguan et al., 2014
PHCA	Ph(CaCl2)		Shangguan et al., 2014
PHH2O	Ph(H2o)		Shangguan et al., 2014
PHK	Ph(KCl)		Shangguan et al., 2014
PNZ	P retention New Zeland method	% of weight	Shangguan et al., 2014
SAND	Sand content	% of weight	Shangguan et al., 2014
SILT	Silt content	% of weight	Shangguan et al., 2014
TC	Total carbon	% of weight	Shangguan et al., 2014
TK	Total K	% of weight	Shangguan et al., 2014
TN	Total N	% of weight	Shangguan et al., 2014
TP	Total P	% of weight	Shangguan et al., 2014
TS	Total S	% of weight	Shangguan et al., 2014
VMC1	Volumetric water content at -10 KPa	% of volume	Shangguan et al., 2014
VMC2	Volumetric water content at -33 KPa	% of volume	Shangguan et al., 2014
VMC3	Volumetric water content at -1500 KPa	% of volume	Shangguan et al., 2014
Modelslat	Latitude	°	-
Inorganic_Ndepdry	Dry inorganic N deposition	kgN·km−2·year−1	Ackerman et al. (2018)
Inorganic_Ndeptot	Total inorganic N deposition	kgN·km−2·year−1	Ackerman et al. (2018)
Inorganic_Ndepwet	Wet inorganic N deposition	kgN·km−2·year−1	Ackerman et al. (2018)
Organic_Ndepdry	Dry organic N deposition	kgN·km−2·year−1	Ackerman et al. (2018)
Organic_Ndeptot	Total organic N deposition	kgN·km−2·year−1	Ackerman et al. (2018)
Organic_Ndepwet	Wet organic N deposition	kgN·km−2·year−1	Ackerman et al. (2018)
Oxred_Ndepdryox	Dry oxidate N deposition	kgN·km−2·year−1	Ackerman et al. (2018)
Oxred_Ndepdryred	Dry reduced N deposition	kgN·km−2·year−1	Ackerman et al. (2018)
Oxred_Ndeptotox	Total oxidate N deposition	kgN·km−2·year−1	Ackerman et al. (2018)
Oxred_Ndeptotred	Total reduced N deposition	kgN·km−2·year−1	Ackerman et al. (2018)
Oxred_Ndepwetox	Wet oxidate N deposition	kgN·km−2·year−1	Ackerman et al. (2018)

Oxred_Nd epwetred	Wet reduced N deposition	kgN·km−2·year−1	Ackerman et al. (2018)
Pdep	P deposition	mg m-2 year-1	Wang et al. (2017)
Bio_1	Annual mean temperature	°C	Fick and Hijmans, 2017
Bio_2	Mean diurnal range	°C	Fick and Hijmans, 2017
Bio_3	Isothermality		Fick and Hijmans, 2017
Bio_4	Temperature seasonality		Fick and Hijmans, 2017
Bio_5	Maximum temperature of the warmest month	°C	Fick and Hijmans, 2017
Bio_6	Minimum temperature of the coldest month	°C	Fick and Hijmans, 2017
Bio_7	Annual temperature range	°C	Fick and Hijmans, 2017
Bio_8	Mean temperature of the wettest quarter	°C	Fick and Hijmans, 2017
Bio_9	Mean temperature of the driest quarter	°C	Fick and Hijmans, 2017
Bio_10	Mean temperature of the warmest quarter	°C	Fick and Hijmans, 2017
Bio_11	Mean temperature of the coldest quarter	°C	Fick and Hijmans, 2017
Bio_12	Annual precipitation	mm	Fick and Hijmans, 2017
Bio_13	Precipitation of the wettest month	mm	Fick and Hijmans, 2017
Bio_14	Precipitation of the driest month	mm	Fick and Hijmans, 2017
Bio_15	Precipitation seasonality		Fick and Hijmans, 2017
Bio_16	Precipitation of wettest quarter	mm	Fick and Hijmans, 2017
Bio_17	Precipitation of driest quarter	mm	Fick and Hijmans, 2017
Bio_18	Precipitation of warmest quarter	mm	Fick and Hijmans, 2017
Bio_19	Precipitation of coldest quarter	mm	Fick and Hijmans, 2017
Et0_yr	Annual evapotranspiration	mm/day	Trabucco & Zomer, 2019
Ai_et0	Aridity Index		Trabucco & Zomer, 2019
PETseasonality	Evapotranspiration seasonality		Trabucco & Zomer, 2019

TS8. a) Reclassification table from the European Space Agency - Climate Change Iniciative - Land Cover (ESA-CCI-LC) map (ESA. Land Cover CCI Product User Guide Version 2. Tech. Rep. 2017. Available at: maps.elie.ucl.ac.be/CCI/viewer/download/ESACCI-LC-Ph2-PUGv2_2.0.pdf) to our morphoclimatic types. b) Equivalent categories from ESA-CCI-LC map

Vegetation group	ESA-CCI-LC correspondence
Tropical evergreen	12,40,50,70,71,72,90,100,120,121,160,170
Tropical deciduous	12,40,60,61,62,90,100,120,122,160,170
Temperate coniferous	12,40,70,71,72,80,81,82,90,100
Temperate broadleaved evergren	12,40,50,90,100,120,121,160,170
Temperate broadleaved deciduous	12,40,60,61,62,90,100,120,122,160,170
Boreal	40,60,61,62,70,71,72,80,81,82,90,100,120,121,122,160,170
No data	10,11,20,30,110,130,140,150,151,152,153,180,190,200,201,202,210,220

TS8. b

Legend of the global CCI-LC maps, based on LCCS

Value	Label	Color
0	No Data	
10	Cropland, rainfed	
11	Herbaceous cover	
12	Tree or shrub cover	
20	Cropland, irrigated or post-flooding	
30	Mosaic cropland (>50%) / natural vegetation (tree, shrub, herbaceous cover) (<50%)	
40	Mosaic natural vegetation (tree, shrub, herbaceous cover) (>50%) / cropland (<50%)	
50	Tree cover, broadleaved, evergreen, closed to open (>15%)	
60	Tree cover, broadleaved, deciduous, closed to open (>15%)	
61	Tree cover, broadleaved, deciduous, closed (>40%)	
62	Tree cover, broadleaved, deciduous, open (15-40%)	
70	Tree cover, needleleaved, evergreen, closed to open (>15%)	
71	Tree cover, needleleaved, evergreen, closed (>40%)	
72	Tree cover, needleleaved, evergreen, open (15-40%)	
80	Tree cover, needleleaved, deciduous, closed to open (>15%)	
81	Tree cover, needleleaved, deciduous, closed (>40%)	
82	Tree cover, needleleaved, deciduous, open (15-40%)	
90	Tree cover, mixed leaf type (broadleaved and needleleaved)	
100	Mosaic tree and shrub (>50%) / herbaceous cover (<50%)	
110	Mosaic herbaceous cover (>50%) / tree and shrub (<50%)	
120	Shrubland	
121	Evergreen shrubland	
122	Deciduous shrubland	
130	Grassland	
140	Lichens and mosses	
150	Sparse vegetation (tree, shrub, herbaceous cover) (<15%)	
151	Sparse tree (<15%)	
152	Sparse shrub (<15%)	
153	Sparse herbaceous cover (<15%)	
160	Tree cover, flooded, fresh or brakish water	
170	Tree cover, flooded, saline water	
180	Shrub or herbaceous cover, flooded, fresh/saline/brakish water	
190	Urban areas	
200	Bare areas	
201	Consolidated bare areas	
202	Unconsolidated bare areas	
210	Water bodies	
220	Permanent snow and ice	

Table S4.9 Final variables selected per each model using the Kaiser-Meyer-Olkin method. The 19 least correlated per model. TropE = Tropical evergreen, TropD = Tropical deciduous, TempC = Temperate coniferous, TempBE = Temperate broadleaved evergreen, TempBD = Temperate broadleaved deciduous, Bor = Boreal. Complete variable names are shown in table S7.

vars	N_TropE	N_TropD	N_TempC	N_TempEB	N_TempDB	N_Bor	P_TropE	P_TropD	P_TempC	P_TempEB	P_TempDB	P_Bor	K_TropE	K_TropD	K_TempC	K_TempEB	K_TempDB	K_Bor
var28		x		x	x				x		x	x			x			
var29		x			x				x			x			x		x	x
var30									x					x				
var31	x													x				x
var41	x	x	x	x	x			x	x	x	x					x	x	
var42																		
BD																		x
BS	x	x	x	x	x			x	x	x	x				x		x	
CACO3		x							x						x			
CEC	x	x					x	x		x			x	x	x			
CLAY																		
ECE		x								x					x			
ESP														x				
EXAL		x		x			x									x		
EXCA			x	x				x	x	x				x	x	x	x	
EXH	x	x					x						x					
EXK	x	x	x	x	x			x	x	x	x	x	x		x		x	x
EXMG														x				
EXNA																		
GRAV		x					x											
GYP													x					
OC																		
PBR																		
PHCA	x	x	x	x	x			x	x	x	x			x	x		x	
PHH2O	x	x	x	x	x			x	x	x		x		x	x	x	x	
PHK	x	x	x	x	x			x	x	x	x			x	x	x	x	
PNZ																		
SAND																		
SILT																		

TC				x											
TK		x		x		x			x						
TN															
TP															
TS															
VMC1									x						
VMC2															
VMC3							x								
modelslat						x									
inorganic_Ndepdry					x	x				x	x	x	x		x
inorganic_Ndeptot					x	x		x		x	x	x	x		x
inorganic_Ndepwet					x	x		x		x	x	x	x		x
organic_Ndepdry															
organic_Ndeptot															
organic_Ndepwet															
oxred_Ndepdryox	x					x	x								
oxred_Ndepdryred											x	x			
oxred_Ndeptotox					x					x					x
oxred_Ndeptotred							x					x			
oxred_Ndepwetox					x					x			x		

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
oxred_Ndepwetred								x						x				
Pdep	x		x			x			x			x	x		x			x
wc2.1_30s_bio_1	x		x	x		x			x			x			x	x		x
wc2.1_30s_bio_2				x						x	x							
wc2.1_30s_bio_3	x	x		x			x	x										
wc2.1_30s_bio_4	x						x					x	x					
wc2.1_30s_bio_5						x						x	x		x			
wc2.1_30s_bio_6						x	x					x	x	x				x
wc2.1_30s_bio_7	x						x					x	x	x				x
wc2.1_30s_bio_8			x	x	x				x	x	x				x	x	x	x
wc2.1_30s_bio_9	x	x	x	x	x	x	x	x	x	x	x	x			x	x	x	x
wc2.1_30s_bio_10													x					
wc2.1_30s_bio_11						x	x						x					x
wc2.1_30s_bio_12				x	x					x	x			x			x	x
wc2.1_30s_bio_13				x		x					x	x			x		x	x
wc2.1_30s_bio_14		x		x		x				x	x			x		x	x	

wc2.1_30s_bio_15	x	x			x		x						x	x				
wc2.1_30s_bio_16											x	x					x	x
wc2.1_30s_bio_17		x		x	x	x		x		x	x					x	x	x
wc2.1_30s_bio_18			x		x			x							x	x		
wc2.1_30s_bio_19			x		x						x				x		x	
et0_yr		x	x	x	x	x		x	x	x	x	x		x	x	x	x	
ai_et0	x	x		x	x				x	x	x				x		x	
PETseasonality	x	x	x	x	x	x	x		x	x	x			x		x	x	x

Appendix V: Global distribution and drivers of forest biome foliar N:P ratios

Table S5.1 See table S4.6

Table S5.2 See table S4.7

Table S5.3 See table S4.8a and S4.8b

Table S5.4

	vars	Trop	TempN	TempB	Bor
1	var28		x	x	
2	var29		x		x
3	var30				
4	var31	x			x
5	var41	x	x	x	
6	var42				
7	BD				
8	BS	x	x	x	
9	CACO3		x		
10	CEC	x			
11	CLAY				
12	ECE				
13	ESP	x			
14	EXAL				
15	EXCA		x	x	
16	EXH	x			
17	EXK	x	x	x	x
18	EXMG				
19	EXNA				
20	GRAV				
21	GYP				
22	OC				
23	PBR				
24	PHCA	x	x	x	

25	PHH2O	x	x	x	
26	PHK		x	x	
27	PNZ				
28	SAND				
29	SILT				
30	TC				
31	TK	x			
32	TN				
33	TP				
34	TS				
35	VMC1				
36	VMC2				
37	VMC3				x
38	modelslat	x			
39	inorganic_Ndepdry	x			x
40	inorganic_Ndeptot	x			x
41	inorganic_Ndepwet				
42	organic_Ndepdry				
43	organic_Ndeptot				
44	organic_Ndepwet				
45	oxred_Ndepdryox				x
46	oxred_Ndepdryred				
47	oxred_Ndeptotox				x
48	oxred_Ndeptotred				
49	oxred_Ndepwetox				
50	oxred_Ndepwetred				
51	Pdep		x		x
52	wc2.1_30s_bio_1				
53	wc2.1_30s_bio_2			x	
54	wc2.1_30s_bio_3				
55	wc2.1_30s_bio_4				
56	wc2.1_30s_bio_5				
57	wc2.1_30s_bio_6	x			x
58	wc2.1_30s_bio_7	x			
59	wc2.1_30s_bio_8		x	x	x

60	wc2.1_30s_bio_9		x	x	x
61	wc2.1_30s_bio_10				x
62	wc2.1_30s_bio_11				x
63	wc2.1_30s_bio_12			x	
64	wc2.1_30s_bio_13		x		x
65	wc2.1_30s_bio_14			x	
66	wc2.1_30s_bio_15				
67	wc2.1_30s_bio_16				x
68	wc2.1_30s_bio_17			x	x
69	wc2.1_30s_bio_18		x	x	
70	wc2.1_30s_bio_19	x			
71	et0_yr	x	x	x	x
72	ai_et0		x	x	
73	PETseasonality	x	x	x	