

# Forest carbon sequestration in response to nitrogen addition: Is the foliar nutrient status an indicator?

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*Thibault Costaz (ESA/ISARA)*

*MSc Thesis in Environmental Sciences*

28/11/2016



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## **List of Abbreviations and Acronyms**

AGWB:	Aboveground Woody Biomass
ALP:	Aboveground Litter Production
Avg:	Average
BGWB:	Belowground Woody Biomass
C:	Carbon
Ca:	Calcium
C/Ca:	Carbon to Calcium ratio
CH <sub>4</sub> :	Methane
C/N	Carbon to nitrogen ratio
CO <sub>2</sub> :	Carbon dioxide
C/P:	Carbon to Phosphorus ratio
CV:	Coefficient of Variations
DRIS:	Diagnosis and Recommendation Integrated System
FAO:	Food and Agriculture Organization of the United Nations
FFCC:	Forest Foliar Co-ordinating Centre
GHGs:	Green House Gases
GPP:	Gross Primary Production
K:	Potassium
LRR:	Log Response Ratio
Mg:	Magnesium
Mn:	Manganese
n:	Number of individual
N:	Nitrogen
NBP:	Net Biome Production
N/Ca:	Nitrogen to calcium ratio
NEP:	Net Ecosystem Production
N/K:	Nitrogen to potassium ratio
N/Mg:	Nitrogen to magnesium ratio
N <sub>2</sub> O:	Nitrous oxide
N <sub>plot<sub>sp</sub></sub> :	Foliar nitrogen concentration at the specie level
N <sub>P<sub>plot<sub>sp</sub></sub></sub> :	Foliar nitrogen to phosphorus ratio at the specie level
N/P:	Nitrogen to phosphorus ratio
NPP:	Net Primary Production
N <sub>ref</sub> :	Selected nitrogen reference value for the specie
N <sub>P<sub>ref</sub></sub> :	Selected nitrogen to phosphorus ratio reference value for the specie
N/Zn:	Nitrogen to zinc ratio
P:	Phosphorus
P <sub>plot<sub>sp</sub></sub> :	Foliar phosphorus concentration at the specie level
P <sub>ref</sub> :	Selected phosphorus reference value for the specie
R <sup>2</sup> :	Coefficient of determination
RLP:	Root litter Production
S:	Sulphur
ScN <sub>pl</sub> :	Scaled foliar nitrogen concentration at the plot level
ScN <sub>sp</sub> :	Scaled foliar nitrogen concentration at the specie level
ScNP <sub>pl</sub> :	Scaled foliar nitrogen to phosphorus ratio at the plot level
ScNP <sub>sp</sub> :	Scaled foliar nitrogen to phosphorus ratio at the specie level
ScP <sub>pl</sub> :	Scaled foliar phosphorus concentration at the plot level
ScP <sub>sp</sub> :	Scaled foliar phosphorus concentration at the specie level

## **Summary**

Past and future trends of anthropogenic carbon dioxide and nitrous oxide emissions have been altering global carbon and nitrogen cycles. These effects are larger in forests, where terrestrial carbon sequestration mainly occurs through biomass production. This production (and thus carbon sequestration) is increased by nitrogen deposition. As most forest ecosystems are nitrogen and phosphorus limited, it is the forests nutrients availability that is likely to regulate their carbon response to nitrogen. The nutritional status of forests is indicated by foliar nutrient concentrations since leaves are the metabolic centre of photosynthesis process. Forest carbon responses to changes in nitrogen availability can reliably be assessed by fertilization experiments. However, such experiments only represent a single point in time and space.

In this work, many forest fertilization experiments were reviewed to assess the impact of the foliar nitrogen and phosphorus contents on the carbon responses to nitrogen addition in boreal, temperate and tropical forests. Natural differences in trees foliar nitrogen and phosphorus concentrations were scaled to a common level by using foliar critical values for nitrogen or phosphorus deficiency or by deriving typical foliar nutrient concentrations. Research findings from the literature were reviewed to gather information on foliar critical levels for nitrogen or phosphorus deficiency. Foliar nitrogen concentrations were taken from the TRY global plant-trait database, to derive typical foliar nitrogen concentration in tree species. Regression analyses were used to assess variations in the carbon response responses using the foliar nitrogen and phosphorus concentrations and ratio as explanatory variables. This relationship between the carbon responses to nitrogen addition and the foliar nutrient concentration was assessed by both linear and polynomial simple regressions analysis. Additionally, a multiple regression analysis including the foliar nitrogen and phosphorus content was used to assess the impact of nutrients interrelationship on the variations in carbon response to nitrogen addition.

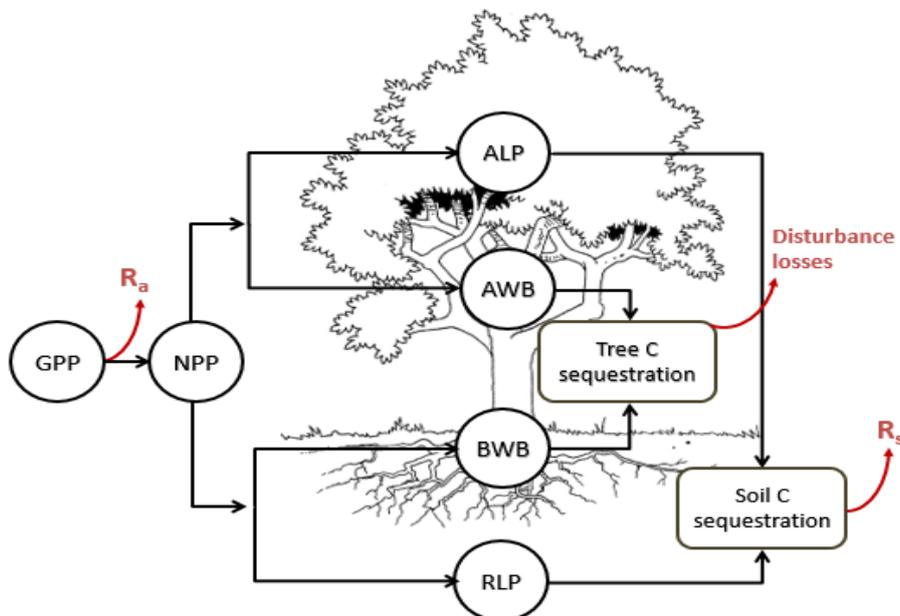
Significant correlations were found between the growth responses and all of the three explanatory variables: nitrogen addition, the foliar phosphorus concentration and the foliar nitrogen to phosphorus ratio. The carbon response to nitrogen addition negatively decreased with an increasing foliar nitrogen concentration. The foliar nitrogen concentration secondary polynomial term was only significant in the multiple regression analysis. The relationship between the growth response to the three variables was nonlinear. The multiple regression analysis highlighted the importance of nutrient interrelationship to explain variations in the carbon response to nitrogen addition. It also demonstrated the importance of the nitrogen application rate in explaining the magnitude of the growth response to nitrogen fertilization. Despite a significant correlation between the carbon response to nitrogen addition and the foliar nitrogen and phosphorus content, the models fit remained low. This suggests that other environmental parameters should be included in future models to increase reliability in predicting the carbon response of forests to changes in nitrogen availability. Although these results should be interpreted with care, they provide an important first step in future impact assessments of anthropogenic nitrogen deposition on forest carbon sequestration capacities.

## 1. Introduction

### 1.1. Background

Atmospheric concentration of greenhouse gasses (GHGs) such as carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O), have increased since the pre-industrial era due to anthropogenic emissions (Ciais et al., 2013; IPCC, 2014). Among them, CO<sub>2</sub> takes the largest part in the increased radiative forcing (Myhre et al., 2013). CO<sub>2</sub> concentrations are likely to increase in the future. Projecting long term trends in CO<sub>2</sub> emissions and sequestration is required to implement effective policies on reducing anthropogenic C emissions at an international level (Pan et al., 2011).

Forests are the main carbon (C) sink within terrestrial ecosystems (Pan et al., 2011). C sequestration by forest ecosystems is the result of gross production minus the autotrophic and heterotrophic respiration, and disturbance losses (Figure 1). It occurs mainly at two levels: (i) in the trees, where most of the primary production occurs and where the C is stored on a long-term basis in woody pools (above-ground woody production (AGWP) and below-ground-woody production (BGWP)) (e.g. branches, stem and coarse roots), and (ii) in the soil, where the organic matter produced is decomposed and stored as humus forms (de Vries et al., 2014). (Figure 1)



**Figure 1: Diagram illustrating the relationship between the different plant biomass groups.**

GPP = Gross Primary Production; NPP = Net Primary Production (NPP); Above Ground Woody Biomass (AGWB); Below Ground Woody Biomass (BGWB); Above-ground Litter Production (ALP); Root Litter Production (RLP); Autotrophic respiration (Ra) and Heterotrophic respiration (Rs). Adapted from (de Vries et al., 2014).

The C sequestration by forest is affected by several environmental drivers such as climate, N deposition, disturbance and management (de Vries et al., 2014; Fernández-Martínez et al., 2014; Kato & Tang, 2008; Valentini et al., 2000). Projection of future forest C sequestration trends requires a better understanding of the impact of these drivers on forest ecosystems (de Vries et al., 2014).

Nitrogen (N) deposition has been suggested as one of the major drivers of terrestrial C sequestration (Schimel et al., 2001). In 2010, human activity emitted about 58 Tg of N, and emissions are likely to increase in the future (Ciais et al., 2013). Most of these N emissions are deposited on land and affect the functioning of forest ecosystems at different levels such as reduced biodiversity or soil acidification (Bobbink et al., 2010). N deposition also has impact on C sequestration by forest ecosystems at both the soil and the tree level (Churkina et al., 2009; de Vries et al., 2014; Lu et al., 2011; Thomas et al., 2009; Zaehle et al., 2011).

The response of forest, in terms of C sequestration, to enhanced N deposition depends on interactions between possible N limitation with other drivers (de Vries et al., 2014). Nutrient availability was found to be a key constraints of forest net primary production (Cleveland et al., 2013; Fernández-Martínez et al., 2014; Fisher et al., 2012; Fleischer et al., 2013; Lebauer & Treseder, 2008). N (N) and phosphorus (P) are considered to be the most limiting nutrients in terrestrial ecosystems (Elser et al., 2007; Harpole et al., 2011). Other macro-nutrients such as potassium (K), calcium (Ca) and magnesium (Mg) are also expected to constrain plant productivity (Crowley et al., 2012; Fernández-Martínez et al., 2014; Moilanen et al., 2010; Reich et al., 2004). Nutrients can have synergetic effects as the availability of one nutrient modifies the plant use capacity of another. For example, sufficient P supply will increase N uptake and vice versa (Bracken et al., 2014; Saito et al., 2008).

Tree C sequestration depends on of the C/N allocation within different tree pools (e.g. stem, leaves, roots). The C/N allocation is influenced by growth limiting factors intensity, such as water availability, temperature or base cations availability. These limiting factors are mainly represented through climate differences, thus, tree C sequestration responses to N deposition vary among boreal, temperate and tropical forests (de Vries et al., 2014).

Fertilization experiments are a reliable way to assess and quantify plant growth response to nutrient availability (Pardo et al., 2011). Forest fertilization experiments have been extensively used to assess the effect of N availability on plant productivity. However, fertilization experiments represent only a single point in time and space. Generalization of fertilization trials conclusions on forest C sequestration results across a large body of studies can be done through a meta-analysis. Meta-analysis provides standardized quantitative methodologies to summarize findings of independent experiments. Effect sizes, a quantitative summary of each independent experiments, are calculated and then combined together to draw general conclusions. Experiments can be divided into subgroups to investigate whether the results differ across specific characteristics (e.g. boreal, temperate and tropical forests) (Hedges et al., 1999).

Foliar chemical analyses have been widely used in order to evaluate potential nutrition deficiency. Leaves are a metabolically active pool of forest, sensitive to changes in the available nutrient stock (Reich et al., 1997). So, Foliar nutrient content should provide an indicator for the response of forest C sequestration to changes in N availability. Procedures to assess forest nutritional status using foliar analysis are based on the principle that a given plant will respond to fertilization by a particular nutrient when the foliar concentration of this nutrient is below an established critical level (Jokela, 2004).

Criteria to distinguish sufficiency from insufficiency ranges for foliar nutrient concentrations can be based on positive relationships between foliar nutrient concentration and growth and/or outer appearance of plants (i.e. the occurrence of visual symptoms of deficiency). Variation in foliar stoichiometry is constrained within specific ranges by plants' stoichiometric flexibility, representing the ability of a given species to adapt its own elemental balance to support environmental nutrient fluctuation (Sistla & Schimel, 2012).

Studies demonstrated the importance and wide spread occurrence of nutrient co-limitation in terrestrial ecosystems (Elser et al., 2007; Harpole et al., 2011). In this context it has been debated whether single foliar nutrient concentrations or their ratios can better indicate plant nutrient availability (Güsewell, 2004). A study focusing on fertilization experiments in wetlands from Koerselman & Meuleman, (1996), defined critical N/P ratios for plants indicating N or/and P limiting conditions. The study concluded that a foliar N/P ratio below 14 shows N limitation while an N/P ratio above 16 shows P limitation. However, co-limitation by N and P is more complex to determine than the narrow range of foliar N/P ratio between 14 and 16 as values below 14 are frequently found (Güsewell et al., 2003). Similarly, such ranges can be expected for forests ecosystems. Thus, sufficiency and balances of nutrients in plant nutrition are two essential features characterizing the type of nutrient limitation (Liebig's law of the minimum as cited in Han et al. 2013). A given plant might have a balanced internal N/P ratio but the concentration of both nutrients might be below the critical levels for nutrient deficiency. Another example could be that N is in relative excess compared to P (unbalanced N/P ratio) while both internal nutrient concentrations are above their critical levels for nutrient deficiency. Assessing variations in forest C response to nutrient fertilization via foliar

nutrient status of trees require determining specific foliar nutrient ranges that characterised trees' specific nutritional states.

Besides soil nutrient availability, nutrients concentration in foliage is influenced by several other factors. Nutrient concentration in foliage varies from one species of tree to another as each of them has particular growth requirements reflecting specific strategies and adaptation (Reich et al., 2004). It is also strongly related with climatic variables (mean annual temperature and precipitation, potential evapotranspiration, irradiance and growing season length), latitude, longitude and altitude (Moilanen et al., 2010; Ordoñez et al., 2009; Reich et al., 2004). The stand's age, is another variable that influences foliar nutrient concentrations (Bonneau, 1995).

The reliability of foliar nutrient status as an indicator for tree nutrition deficiency increases with the similarities between the stand to diagnose and the stand used to establish critical levels for nutrient deficiency. In this thesis, factors influencing the foliar nutrients concentrations (as mentioned above) are taken into account by creating homogenous categories of tree stands based on their age, species and site climates. Flückiger & Braun (2003) deduced recommendations of N/P ratios normal ranges for Norway spruce between 7 and 12 and beech between 10 and 17, but these values hold mainly for temperate climate. Information on such ranges for major tree species within their climate regions is essential to assess forest C response to fertilization using foliar nutrient concentrations.

This thesis uses a meta-analysis on forest N fertilization experiments to assess the C response of trees to N addition as a function of the plant foliar status, distinguishing boreal, temperate and tropical forests.

## 1.2. Aim of the Thesis

This thesis assesses the impact of atmospheric N deposition on forest C sequestration by projecting tree growth responses to N addition in relation to site N and P limitation. The projection uses the foliar N and P content. More specifically the variations in the C to N response of tree woody biomass pools (AGWP) (Figure 1) are explained by the variation in foliar N, P concentrations and foliar N/P ratio. This should provide a first step towards predicting the impact of N deposition on forest C sequestration at the tree level. To reach this objective, this thesis addresses the following research question.

**Research Question:** Can the foliar nutrient (N and P) concentrations and ratio explain variations in the C responses of tree stands to N addition in tropical, temperate and boreal forests?

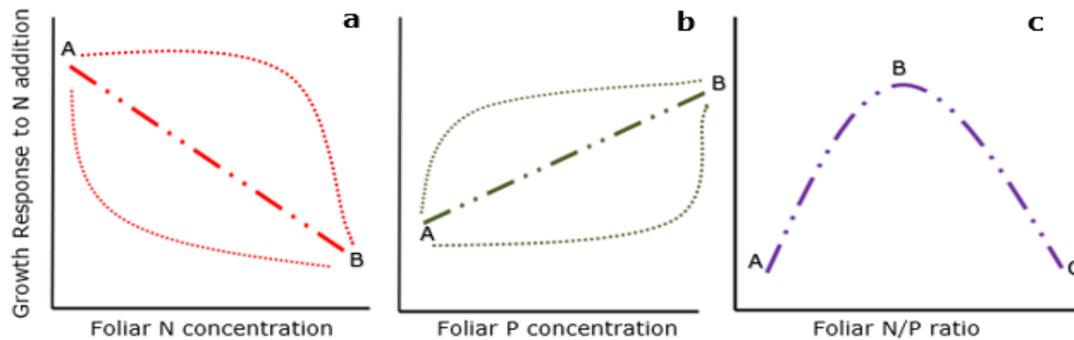
The C response to N addition is expected to decrease, as the foliar N concentration will increase. This indicates a shift from an N limited state to an N saturated state. Evidence shows that P availability constrains plants N uptake (Bracken et al., 2014). When the foliar P concentration increases, plant biomass production should increase with it. Thus, a positive relationship should appear between the growth response to N addition and the foliar P concentration. The N/P ratio indicates balances between nutrients concentrations. This should result in a positive trend between the C responses to N addition and the foliar N/P ratio until a certain plateau is reached, indicating harmonious nutrition between N and P, then once a second asymptote is passed a negative trend should appear, as nutrition unbalances are increasing (Figure 2). To test these hypotheses, this thesis uses results from a meta-analysis on forest fertilization experiments realized within the ongoing PhD of Lena Schulte-Uebbing, measuring the growth responses to N addition.

Foliar nutrients concentration indicating a specific nutritional status will vary from one tree species to another as each tree species has different nutrient requirements and growth strategies (Reich et al., 2004). To allow comparison of the foliar nutrient concentration extracted in the meta-analysis over different studies and tree species, these concentrations need to be standardized over a reference that scales natural differences in foliar nutrient concentration to a same level.

One way to scale these natural differences is to use the foliar nutrients critical levels for deficiency.

**Sub-research question 1:** What are the ranges in foliar N and P concentration and N/P ratio standing for either sufficient/balanced or insufficient/unbalanced tree nutritional status?

The use of foliar nutrient concentrations to assess nutrients limitation requires the identification of critical levels representing reference values corresponding to tree (in)sufficient and (un)balanced nutritional status (Mellert & Göttlein, 2012). Criteria to distinguish this nutritional status via nutrients concentrations in foliage is usually based on the positive relationships between a given nutrient concentration and tree growth response to the same nutrient addition or on the outer appearance of visual symptoms linked to severe deficiency.



**Figure 2: Diagram showing expected C response to N addition against the foliar N (a); P (b) and N/P ratio (c).** Letter (A, B and C) on the graph indicates the overall direction of the relationship.

Another way to scale the foliar nutrient concentration of different tree species to a same level is to use the foliar nutrient concentration the most often found in those species as reference point.

**Sub-research question 2:** What are the typical foliar N and P concentrations and N/P ratio of forest tree species?

This second sub-research question aims at attributing at each tree population a typical foliar nutrient concentration value. A 'typical' foliar nutrient concentration is a representative concentration of a given nutrient in foliage such as an average or any measures of central population values that characterise a large sample of a particular tree species.

Once the foliar nutrient concentration between tree species are comparable, the relationship between the foliar nutrient level and the C response to N addition of experimental plots present in the meta-analysis can be assessed.

**Sub-research question 3:** Is the foliar N and P status correlated to the C to N responses of the stands?

This third sub-question tested the hypothesis previously stated by assessing eventual trends between the foliar N and P status of experimental plots (independent variables) and the variations in the corresponding C response to N addition (dependent variable).

In order to reach answer, the main research question this thesis is organized into two main phases, the first phase correspond to the assessment of reference values for foliar nutrient concentration to account for natural interspecific differences (sub-question one and two). The second phase corresponds to the assessment of the correlation between the foliar nutrient concentration and the C response to nutrient addition (sub-question three).

The second chapter presents the methodology used to assess the relationship between the C responses to N addition of independent forest fertilization experiments and the scaled or non-scaled foliar N and P contents. The resulting relationships are described in the third chapter. Additionally, special attention is paid to the differences among boreal, temperate and tropical forests. The fourth chapter aims at discussing the limitations of this thesis in relation to the methodology and the gathered data. Finally, general conclusions on the nature and reliability of the foliar N and P contents in predicting tree C responses to N addition are drawn in the fifth chapter.

## **2. Research Methodology**

This chapter presents the methodology used in order to answer the different research questions raised in the previous chapter. The first section detailed the meta-analysis on forest fertilization experiments framework. The second section is related to the scaling procedures used to standardize the natural differences in foliar nutrient concentrations. The third section explains the statistical analysis performed to assess the relationship between the C response to N fertilization and the nutrient concentration in foliage.

### **2.1 Assessing Carbon Responses to Nitrogen Addition: A Meta-analysis**

This thesis is built on a meta-analysis established as part of the Ph.D. thesis of Lena Schulte-Uebbing. This meta-analysis focuses on experimental studies assessing tree woody biomass increment responses to N addition. This thesis uses the data collected under this meta-analysis framework. This section aims at describing the meta-analysis procedure used to collect and analyse the data.

#### **2.1.1 Data Collection**

A search string composed of specific key words such as forest, fertilization, woody biomass was used in Scopus to create an initial pool of studies possibly matching the requirement for this meta-analysis Scopus returned 2115 studies as result.

From these initial 2115 studies compiled via Scopus, a set of criteria were applied in order to further select studies for the analysis. This set of criteria is presented as follow:

- (i) Experiments are performed under field conditions or in open top chambers;
- (ii) The experimental design includes a control treatment (or any treatment allowing quantifying the effect of the fertilizer of interest);
- (iii) The trees are older than ten years (consider as mature tree). Because of the specificity of fruit tree cultures, orchards were excluded from the analysis;
- (iv) The measured response variables were measured at least one year after the fertilization treatments;
- (v) Experiments using organic fertilizer were excluded as organic fertilizer affects the soil C content and thus it is difficult to isolate the effect of fertilization;
- (vi) The response variables are measured on trees that represent at least 90% or more of the considered ecosystems (Lebauer & Treseder, 2008).

When the studies reported results for more than one level of nutrient addition (e.g. different rates of nutrient addition or/and different plot locations), those were considered as independent observation (Curtis & Wang, 1998; Lebauer & Treseder, 2008). In case of studies presenting data from several years, the latest reported measurement was extracted.

Additional background information such as geographic and flora characteristics and soil parameters are included. The climate region in which each observation was derived using google earth in combination of the Food and Agriculture Organization of the United Nations (FAO) thermal zones (Figure 3).

For data availability reasons, only measurements representing the AGWP were extracted. If studies reported proxies for increase in woody biomass, such as diameter at breast height, tree height or basal area, allometric equations were used to calculate the related woody biomass increment. Studies providing only end data on relative biomass response were excluded from the meta-analysis as it does not allow the calculation of absolute biomass change.

This thesis focuses on C sequestration at the tree level; therefore, only studies with aboveground woody biomass (AGWP) response parameter grouped were selected in this case. (Figure 1).

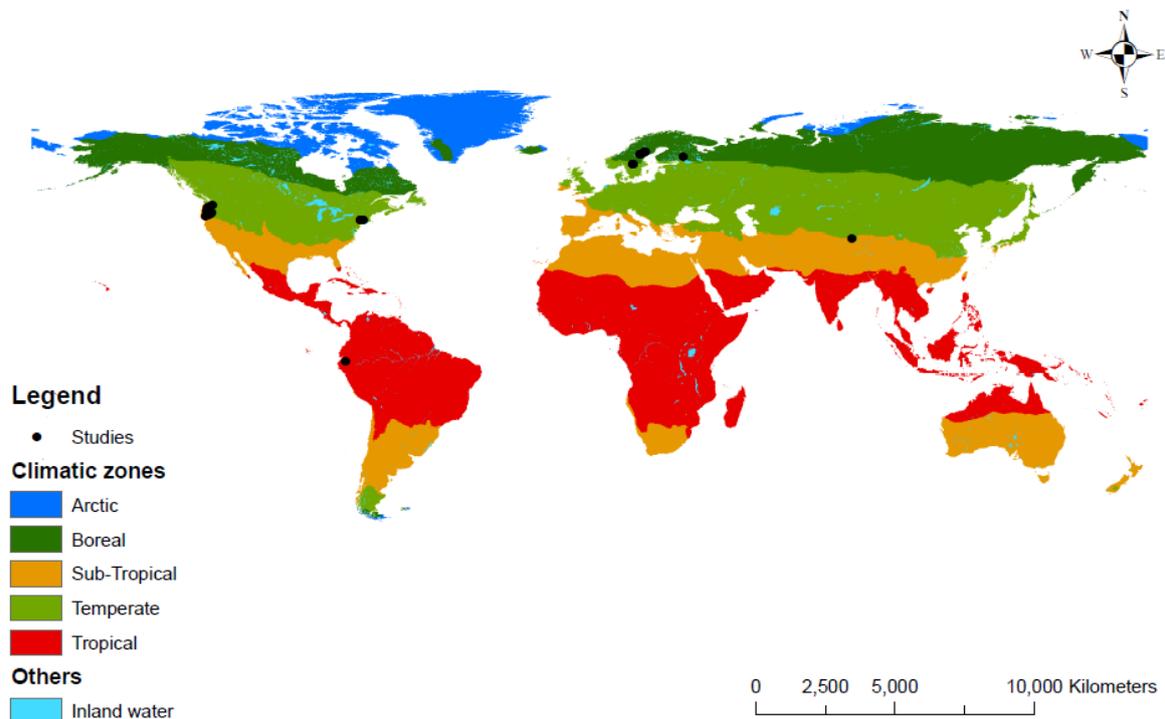
Additionally, only studies reporting foliar macronutrients concentration (N, P, K, Ca, Mg and Mn) were selected for this thesis framework. For the purpose of the analysis, only the foliar nutrients contents from unfertilized control plots were extracted.

If more than one measurement representing different times of the experiment is available per study, the earliest sampling measurement was selected.

If foliar nutrient concentrations were measured for several species in the same experimental plot, data from all available species were included individually.

When data were presented in the form of figures, they were digitized using Plot digitizer (published by the Free Software Foundation).

Finally, seven studies representing 38 independent observations were included in this thesis framework. Their distributions are mostly in temperate region of the northern hemisphere (Figure 3).



**Figure 3: Global distribution of experiments included in the meta-analysis (based on the FAO world thermal climate zones)**

### 2.1.2 Analysis

Based on the data extracted from the selected studies, a dataset of woody biomass response to N addition was compiled. The explanatory variables are the foliar N, P concentration and N/P ratio, the response variable is the C response per kg of N added. The response variable extracted from the selected studies represents the effect size of the experiments and needs to be expressed on a common scale to allow comparison. The relative sensitivity was calculated in order to extract a response variable comparable between studies (Rustad et al., 2001).

The relative sensitivity (RS) is calculated as follows:

$$RS = \frac{\left( \frac{\bar{X}_E - \bar{X}_C}{\bar{X}_C} \right) * 100}{eN} \quad \text{EQ 1}$$

Where: RS is given in % C response per Kg N added;  $\bar{X}_E$  and  $\bar{X}_C$  are the mean from the experimental and control group respectively in Kg C. ha<sup>-1</sup>. yr<sup>-1</sup> and  $eN$  is the average N addition over the period of measurement in Kg N.

With the standard error as:

$$SE_{RS} = \frac{\sqrt{\left(\left(\frac{SE_E}{\bar{X}_E}\right)^2 + \left(\frac{SE_C}{\bar{X}_C}\right)^2\right) * RR}}{eN} \quad \text{EQ 2}$$

And

$$RR = \frac{\bar{X}_E}{\bar{X}_C} \quad \text{EQ 3}$$

Where: SE\_RS is the Standard error of the Relative sensitivity given in % C response per Kg N added;  $\bar{X}_E$ ,  $\bar{X}_C$ ,  $SE_E$  and  $SE_C$  are the mean and the standard error from the experimental and control group respectively in Kg C. ha<sup>-1</sup>. yr<sup>-1</sup>; RR is the Response Ratio and  $eN$  is the average N addition over the period of measurement in Kg N.

## 2.2 Assessing the Foliar Nutrient Status of Forest Plots

To allow comparison of the foliar nutrient concentration extracted in the meta-analysis over different studies and trees' species, these concentrations needed to be standardized over a reference that scale natural differences in foliar nutrient concentration to a same level (2.1). This section presents the two approaches used to scale the foliar N and P concentration and ratio of tree species to a same level (sub-research questions one and two).

### 2.2.1 Assessing Ranges in Foliar Nutrient Status for (in)-Sufficient and (un)-Balanced Nutrition

The first approach is to scale the foliar N and P concentration and ratio of tree species according to an indicative threshold value for single nutrient deficiency.

The use of foliar nutrient concentration to assess the nutritional status of trees requires the identification of critical levels representing reference values corresponding to tree (in)sufficient nutritional status (Mellert & Göttlein, 2012). This section intends to describe the methodology chosen to answer the first research question:

**Sub-research question 1:** What are the ranges in foliar N and P concentration and N/P ratio standing for either sufficient/balanced or insufficient/unbalanced tree nutritional status?

A literature review on trees' critical foliar nutrient concentration indicating the threshold between deficient and adequate nutrient supply has been conducted. The objective of this review was to collect current knowledge on foliar nutrient thresholds for trees to use as references to diagnose trees single nutrient deficiency.

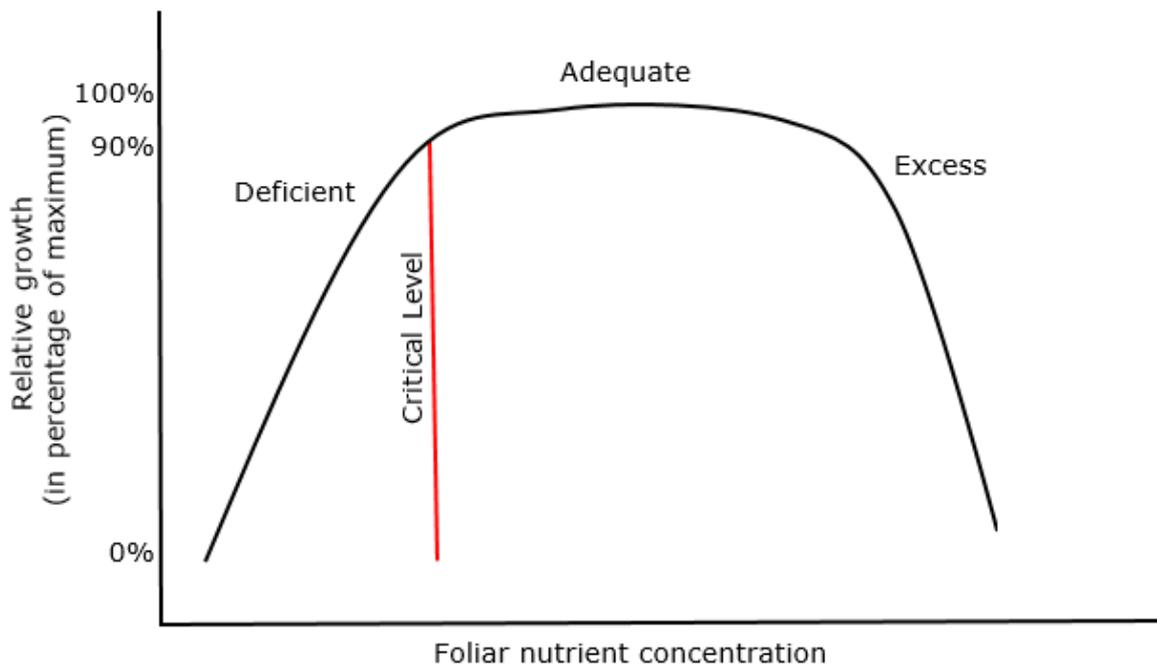
The critical level (CL) approach is the most widely used methodology when using foliar analysis to assess the nutritional status of a forest (Gregoire & Fisher, 2004). It compares current nutrient concentration in foliage to reference values or ranges defining the nutritional status of the plant.

The reliability of this method lies in the similarities between stand used in establishing the critical level and the stands to diagnose (Benton, 1993). As foliar nutrient concentration vary in function of climate, age and tree species (Reich et al., 2004), these factors are taken into account to increase consistency between the stands present in the meta-analysis and established critical values for nutrient deficiency found in the literature.

Conventionally, the "critical level" is defined as the foliar nutrient concentration at which the stand reach a given percentage relative to the maximum growth (Figure 4) (Reuter 1997; Bonneau 1995; Ulrich and Hills, 1967) as cited in Gregoire & Fisher 2004). However, criteria used in establishing critical levels for nutrient deficiency are often based on visual leaf symptoms, reaction to fertilisers or other physiological parameters (Mellert & Göttlein, 2012; Reuter, 1997).

Regarding the tree nutritional status, there is no actual convention on specific terminology and often different terminology are employed to describe the same nutritional status (Mellert & Göttlein, 2012; Reuter, 1997). This complicates the comparison of several critical values with each other.

In this thesis, the foliar nutrient concentration is divided into three ranges: Deficient, Adequate and Excessive. The deficient range corresponds to the foliar nutrient concentration that is associated with visual symptoms of deficiencies and foliar nutrient concentration values that are above the critical threshold for visual deficiency symptoms but where growth is still limited by nutrient availability. The adequate range represents the foliar values where only marginal changes in the tree physiological state in terms of growth or biomass production can be associated with an increase in nutrient concentration in foliage. Finally, within the excessive range, growth will start to decrease and toxicity symptoms will appear in different parts of the plant (Figure 4)



**Figure 4: Schematic representation of the meaning of the terms used to classify the nutrient status of trees**

(Bonneau, 1995; den Burg, 1990; Reuter, 1997)

The literature review focused on foliar N and P concentrations representing the threshold between the deficiency state and adequate state for mature trees (above 10 years old). Indeed, the nutrient requirement for a tree decreases as the tree grows older (Bonneau, 1995). The primary sources, where the original foliar nutrient concentration thresholds were derived, were preferentially included in the literature review. When the original data could not be accessed, secondary sources were used with reference to the cited papers where the values originate.

Background information on the criteria on which the foliar nutrient thresholds are based on, such as growth rate or visual symptoms and the methodology used to derive the cited thresholds level, such as the age or the position of the collected leaves on the tree, the sampling period or specificity about the experiment (e.g. irrigated plot), etc. were additionally collected.

Climate parameters have a major influence on foliar nutrient concentration (Ordoñez et al., 2009; Reich et al., 2004). Climatic regions (Boreal, Temperate, Sub-Tropical and Tropical) were included in the review as categorical information in order to increase the reliability of the CL methodology. The species were clustered by climatic region accordingly and the foliar thresholds concentration selected for scaling between species differences were made climatic region wise.

If the literature review cannot provide nutrient thresholds for a given macro-nutrient deficiency for a species in the meta-analysis, it is assumed that, for a given species the foliar N (or P) concentration is comparable to other species within the same functional type and climate cluster. There is evidence that these clusters of species might have similar foliar nutrient concentrations, when assessed at the global scale (Reich et al., 1997; Woodward et al., 2004; Wright et al., 2004). For these species, the foliar nutrient thresholds were extrapolated using the following decisional tree:

- (i) Same species in the same climate;
- (ii) Same genus in the same climate;
- (iii) Same family in the same climate;
- (iv) Same functional type in the same climate;
- (v) Same species across all climate;
- (vi) Same genus across all climate;
- (vii) Same family across all climate;
- (viii) Same functional type across all climate.

When more than one foliar nutrient threshold was found for a given specie in the meta-analysis, the average of these values was taken as a representative threshold for this specie.

Regarding the N/P ratio, critical levels were often derived by dividing the lower limit (Critical level for deficiency) of one nutrient by the higher limit of the second nutrient (Critical level for excess) and vice versa (Flückiger & Braun, 2003; Mellert & Göttelein, 2012). Despite the wide used of this methodology, the resulting adequate ranges are very large and does not match with physiological state of (un)balanced nutrition (Flückiger & Braun, 2003).

Stefan et al. (1997) with the Forest Foliar Co-ordinating Centre (FFCC) published a report on the evaluation of the foliar nutrient contents for 16 European countries. From this database, three ranges of nutrient ratios were derived using the critical levels for foliar nutrient concentration fixed by the third Forest Foliar Expert Panel Meeting. These European levels does not correspond to any physiological status of the tree but allows comparison among countries (Croise et al., 1999; Stefan et al., 1997). Stefan et al. (1997) reports critical levels for only the four following genera: Spruce, Pine, Beech and Oak for temperate climate. Critical levels for N/P ratio were grouped by leaf type of these four main genera (Needle-leaved and Broadleaf) and used across all climate regions.

### 2.2.2 Assessing Typical Ranges of Foliar Nutrient Concentrations.

The second approach exposed in this thesis is to scale the foliar nutrient concentration and ratio of tree species according typical level of foliar nutrient concentration.

**Sub-research question 2:** What are the typical foliar N and P concentrations and N/P ratio of forest tree species?

Data on foliar nutrient concentrations and ratios from the worldwide plant trait database TRY<sup>1</sup> are used to extrapolate critical levels for deficiency. TRY assembles many datasets from studies conducted for different research purposes allowing access to a large collection of data on plant traits. DIVERSITAS/IGBP and the Max Planck Institute direct the TRY project for Biogeochemistry. The TRY network project was chosen to derived typical foliar N concentration for tree species.

The following traits were requested to the TRY project:

- (i) Leaf N content per leaf dry mass;
- (ii) Leaf P content per leaf dry mass;
- (iii) Leaf potassium (K) content per leaf dry mass;
- (iv) Leaf sulphur (S) content per leaf dry mass;
- (v) Leaf calcium (Ca) content per leaf dry mass;
- (vi) Leaf magnesium (Mg) content per leaf dry mass;
- (vii) Leaf C/N ratio;
- (viii) Leaf C/P ratio;

<sup>1</sup> [www.try-db.org/TryWeb/Home.php](http://www.try-db.org/TryWeb/Home.php)

- (ix) Leaf N/P ratio.

Beside this collection of traits requested for this thesis analysis, another publicly available dataset was used, on the categorical traits for species present in the TRY database. This dataset contains information such as on genus, family, plant phylogeny, plant growth forms, leaf type or leaf phenology.

The dataset provided on the requested traits, is not restricted to tree growth forms and need further data processing to extract relevant information to derived typical foliar N concentration.

#### 2.2.2.1 *Data processing*

The database compiled by the TRY project is an aggregation of several datasets provided by several authors. For reasons of knowledge property, some of the dataset containing the requested traits required involvement as co-author in eventual publication. These datasets were excluded, by convenience in the process of data ownership.

The remaining data were combined in a single dataset. The records of foliar N concentration for plant species present in this dataset were further selected based on the following criteria:

- (i) Only tree growth forms;
- (ii) The measurements were performed on mature trees (older than ten years old);
- (iii) Observation with missing coordinates were excluded;
- (iv) Only tree species with a leaf type and leaf phenology corresponding to broadleaf/needleleaf and deciduous/evergreen respectively.

Additional information was collected on the genus, family, leaf type and leaf phenology from the categorical traits for species in TRY dataset.

Functional types were derived using the combination of leaf type (Broadleaved or Needle-leaved) and leaf phenology (Deciduous or Evergreen).

Information on the climatic regions corresponding to each observation was added using their coordinates. The climatic regions are based on the FAO thermal zones overlay reduced to four categories (tropical, sub-tropical, temperate and boreal) (Figure 5). ArcGIS software was used to extract this information.

The final dataset used in the analysis, contains 773 observations of foliar N concentration for 154 trees species (Table A1).

The locations of these observations are mainly for temperate and sub-tropical climates with a majority of points in the norther hemisphere (Figure 5).

The foliar P concentration and N/P ratio were not retained for this analysis as their number of records were too low ( $n < 50$ ).

#### 2.2.2.2 *Data analysis*

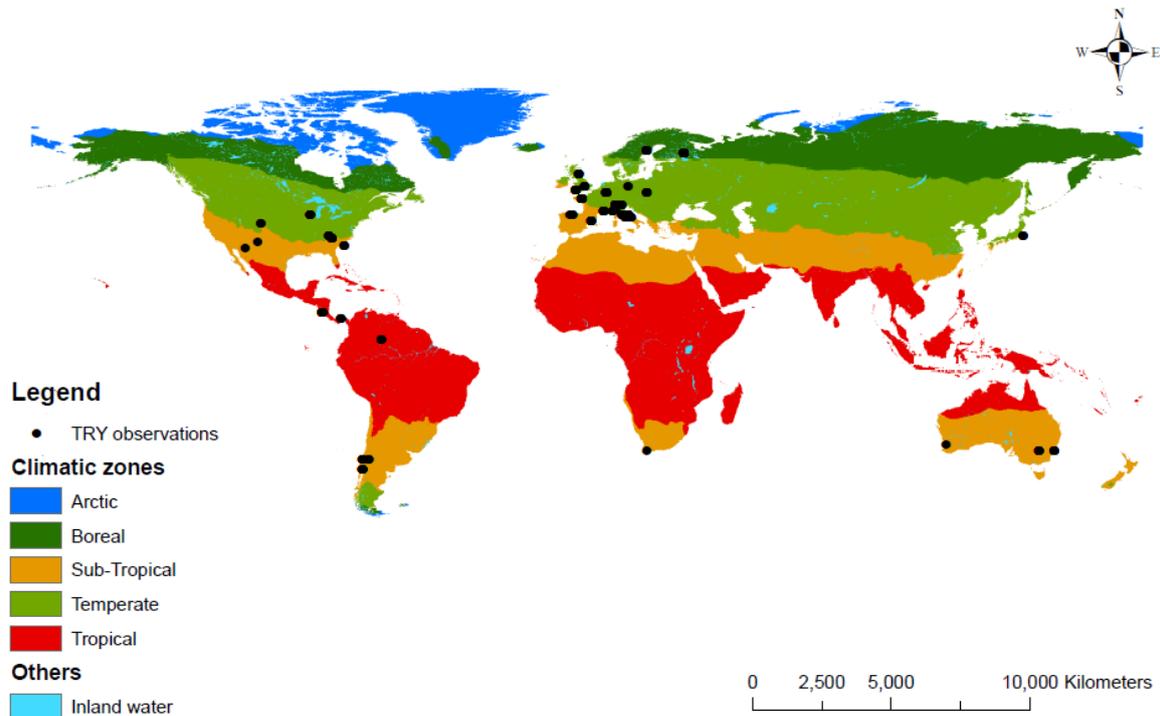
Based on the data extracted in Section 2.2.2.1, typical values for foliar N concentration were derived from the population central value. As explained in section 2.2.1, trees species were sorted by climate zone to increase similitude with the trees present in the meta-analysis. Moreover, the minimum number of observations per tree species in a given climate zone is set at 25 ( $n = 25$ ) in order to reach a certain statistical relevance in the derived typical foliar N concentration.

The typical foliar N concentration for tree species present in the meta-analysis was calculated from the average of foliar N concentrations from observations in the TRY database. If there are not enough records of foliar N concentration for a species, the same methodology as 2.2.1 is applied:

- (i) Same species in the same climate;
- (ii) Same genus in the same climate;
- (iii) Same family in the same climate;

- (iv) Same functional type in the same climate;
- (v) Same species across all climate;
- (vi) Same genus across all climate;
- (vii) Same family across all climate;
- (viii) Same functional type across all climate.

The averages and the coefficient of variations were given for all available levels at which typical foliar N concentration are calculated.



**Figure 5: Location of the different observations for foliar N concentration for mature trees present in the TRY database**

### 2.3 Assessing the Relationship between the Carbon Responses of Trees to Nitrogen Addition

The relationship between the aboveground woody biomass production in response to N fertilization and the foliar nutrient concentration and ratio was analysed using a regression analysis.

**Sub-research question 3:** Is the foliar nutrient status correlated to the C to N responses of the stands?

In order to answer this question, the foliar N and P concentrations have to be scaled to a comparable level based on either, the foliar N and P critical levels for deficiency or on the foliar N and P typical concentrations. The scaled foliar N, P and N/P were derived by dividing the foliar N, P or N/P of the tree species presents in the experimental plots by the foliar N, P or N/P reference concentrations (critical levels or typical concentrations) respectively as in the following example for N.

$$ScN_{sp} = \frac{N_{plot_{sp}}}{N_{ref_{sp}}} \text{ EQ 4}$$

Where:  $N_{plot_{sp}}$  is the foliar N concentration of the experimental plot for a given species and  $N_{ref_{sp}}$  is the reference foliar N concentration selected for this species. Similar ratios were calculated for foliar P concentration and N/P ratio.

If a given experimental plot is composed of several tree species, a weighted average of each  $ScN_{sp}$  ratio ( $ScP_{sp}$  and  $ScNP_{sp}$ ) was calculated according to the different species proportion of this plot.

Following the principle of Occam's Razor<sup>2</sup> states that "for a set of equally good explanations for a given phenomenon, the correct explanation is the simplest explanation" (Crawley, 2007), simple linear regressions were conducted to analyse the relationship of the response variable (RS) and for each of the explanatory variables.

The linear model used is of the form:

$$y_i = \alpha + \beta x_i + \varepsilon_i \text{ EQ 5}$$

Where:  $\alpha$  is the intercept;  $\beta x_i$  is the slope and  $\varepsilon_i$  is the random error (Dalgaard, 2008). In this analysis the  $y_i$  is the relative sensitivity (dependent variable) and  $x_i$  are the foliar N or P concentrations or the N/P ratios (independent variables).

The homogeneity of the variance and normality of the error of the models were graphically assessed in order to check the models assumptions (Crawley, 2007).

The Log response ratio was used as dependent variable, in case the model assumptions were not met using the relative sensitivity. The log response ratio is the most widely used effect size in ecological meta-analysis (Hedges et al., 1999). It has the advantage to linearize the metrics having for effect to normalize the distribution of the sample distribution (Hedges et al., 1999).

Log Response Ratio (LRR):

$$LRR = \log\left(\frac{\bar{X}_E}{\bar{X}_C}\right) \text{ EQ 6}$$

The LRR does not include the effect of the N application rates. To compensate the data were subdivided into two groups of N application rates (Low and High). The low N application rates group was defined as the tree stands fertilized at a rate below the average of N application rates for this meta-analysis. Respectively the high N application rates group are the tree fertilized at a rate above this average.

To test non-linear relationships between the C response to N addition and the foliar N and P contents, polynomial regression models were used. The models tested the best combination of first, second and third order for each explanatory variables (e.g. Foliar N<sup>2</sup> or Foliar N<sup>3</sup>). The best and simplest model was kept based on significant improvement of each model compared to the previous via Analysis of Variances of the models.

Besides simple regression analysis, a multiple regression analysis was carried out to assess the explanatory power of the combination of foliar N and P concentrations and ratio on the C response to N addition variations. As previously mentioned, in order to meet the models assumptions, the LRR was used as dependent variable while the non-transformed foliar N, P and N/P were used as independent variables. The N application rate was assessed as a fourth explanatory variable to account for its effect on tree growth response.

A maximum model of the form (see below) including the four explanatory variables, their quadratic terms ( $x^2$ ) and all their interactions was used at first. Stepwise model simplifications were applied in order to remove non-significant terms and interactions (Crawley, 2007).

$$y = \alpha + \beta_1 x + \beta_2 x^2 + \dots + \beta_k x^k + \varepsilon \text{ EQ 7}$$

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<sup>2</sup> William of Occam was a 14th-century English philosopher.

### **3. Results**

This chapter presents the overall results of the C response to N addition present in the studies included in the meta-analysis. It also displays the foliar nutrient thresholds and typical concentration found via the literature review and the TRY database as well as the resulting selected reference values for the species present in the database. Finally, it presents the results of the regression analysis.

#### **3.1 Carbon Responses to Nitrogen Addition: A Meta-analysis**

This section presents the resulting C response to N addition (relative sensitivity) calculated from the meta-analysis on forest fertilization experiments.

It is expected that in an N limited forest, the addition of N fertilizer will increase the soil N availability and the plant N concentration that should stimulate photosynthesis and thus results in an increase in biomass production. In these forests, a positive C response to N addition is expected. Inversely, in an N saturated forest, N fertilization will increase soil acidity, decrease mycorrhizae production limiting resources access, and reduce biomass production. For these forests, a negative C response to N addition is likely to happen.

Figure 6 shows that a majority of the plots did not displayed a significant positive nor negative C response to N addition (error bars overlap with 0). The possible reasons for these insignificant responses to N additions experiments are numerous (e.g. nutrient deficiency other than N, soil characteristics, etc.).

An important information that rises out of this dataset is the spread of observations across the three forest types considered in this thesis (tropical, temperate and boreal). Temperate forests are in majority while only a few plots represent boreal and tropical forests. The tree types are mainly coniferous trees (needle-leaved) with 30 observations, among which *Pseudotsuga menziesii* prevails with 16 observations out of 30 (Figure 6).

The average by climate is in line with the long held assumption that tropical forests are generally less N limited than boreal forests (Reich et al., 2004). Similarly, coniferous forests have been found to show higher responses to N addition than broadleaved forests. This can be explained by physiological adaptation of coniferous trees to low nutrient environment and a lower N uptake capacity, leading to a stronger growth response sensitivity to N fertilization (Fleischer et al., 2013; Liu & Greaver, 2009).

#### **3.2. Foliar Nutrient Status of Forest Plots**

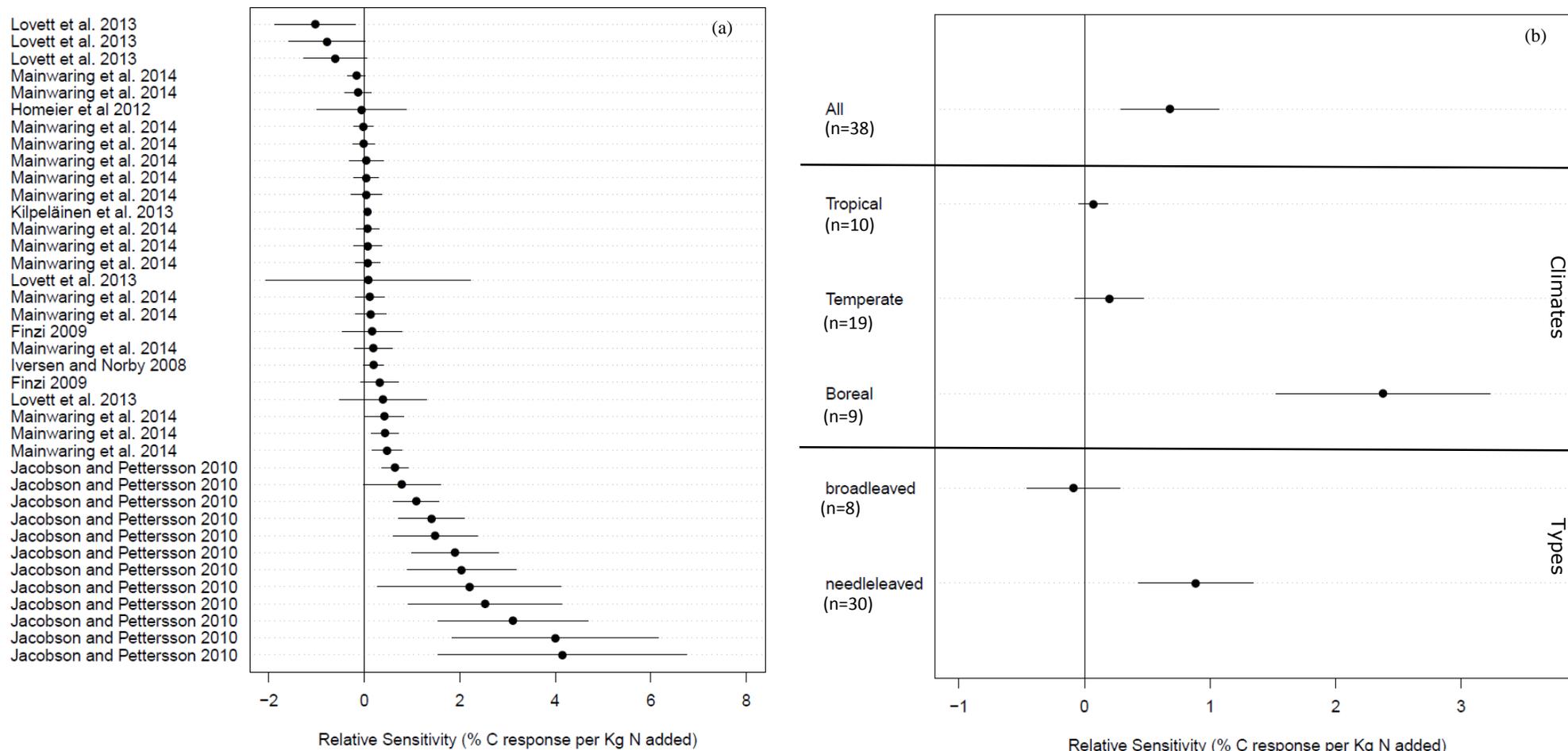
This section presents the results of the two approaches taken to scale the natural differences in foliar N concentration of tree species.

The trees species present in the database sorted by climate zones with their corresponding genus, family and functional types, are displayed in Table 1. This classification is the basis for the references values selection.

Section 3.2.1 gives the results of the literature review on foliar N and P concentrations critical levels for deficiency whereas section 3.2.2 presents the derivation of typical foliar N concentration from the TRY database. Section 3.2.3 gives the selected reference values for N, P concentrations and N/P ratio.

##### **3.2.1 Ranges of Foliar Nutrient Concentrations or Ratios for (in)-Sufficient and (un)-Balanced Nutrition**

The literature review resulted in 18 studies representing, foliar N critical levels for deficiency of 86 different tree species in 24 families for a total of 112 observations. When sorted by climate zones (boreal, temperate, sub-tropical and tropical), temperate regions are the most represented with 68 records, then sub-tropical regions with 26 observations, 15 and 3 observations for tropical and boreal regions.



**Figure 6: C response to N addition of the independent experimental plots included in the database (a) and average by climates zones or tree types (b).**

The error bars represent two times the standard error of the mean. On the right the average C response to N addition for all study (All) or grouped by climate regions (Tropical, Temperate and Boreal) or by tree types (broadleaved and needle-leaved). The n indicates the number of independent experimental plots in each groups. The error bars are two times the standard error of the experimental plot (A) or of the mean (B).

**Table 1: Tree species present in the meta-analysis sorted by climate zones**

Climate	Functional type	Family	Genus	Species
Boreal	needle-leaved evergreen	Pinaceae	Picea	<i>Picea abies</i>
			Pinus	<i>Pinus sylvestris</i>
Temperate	broadleaved deciduous	Aceraceae	Acer	<i>Acer rubrum</i>
				<i>Acer saccharum</i>
		Betulaceae	Betula	<i>Betula alleghaniensis</i>
				<i>Betula papyrifera</i>
		Fagaceae	Fagus	<i>Fagus grandifolia</i>
			Quercus	<i>Quercus rubra</i>
		Hamamelidaceae	Liquidambar	<i>Liquidambar styraciflua</i>
		Juglandaceae	Carya	<i>Carya ovata</i>
		Oleaceae	Fraxinus	<i>Fraxinus americana</i>
		Rosaceae	Prunus	<i>Prunus serotina</i>
		Salicaceae	Populus	<i>Populus grandidentata</i>
Boreal	needle-leaved evergreen	Pinaceae	Pinus	<i>Pinus sylvestris</i>
			Pseudotsuga	<i>Pseudotsuga menziesii</i>
			Tsuga	<i>Tsuga canadensis</i>
				<i>Tsuga heterophylla</i>
Sub-Tropical	needle-leaved evergreen	Pinaceae	Pseudotsuga	<i>Pseudotsuga menziesii</i>
Tropical	broadleaved evergreen	Euphorbiaceae	Alchornea	<i>Alchornea lojaensis</i>
			Hieronyma	<i>Hieronyma fendleri</i>
		Melastomataceae	Graffenrieda	<i>Graffenrieda emarginata</i>
		Myrtaceae	Myrcia	<i>Myrcia sp</i>

The average and coefficient of variations were calculated in case of, for a same tree species within the same climate region, several studies gave a foliar N critical value. In this situation, the average was retained as reference value for the tree species present in the database. Concerning the critical levels for the foliar P, the literature review resulted in 17 studies including 86 species in 23 families for a total of 112 observations. When sorted by climate regions, 67, 27, 15 and 3 observations were included for temperate, sub-tropical, tropical and boreal regions respectively (Table 3).

The criteria used to determine these critical levels for N and P deficiency were classified into six categories:

- (i) **90% maximum growth:** Foliar nutrient concentration corresponding at 90% of potential maximum growth
- (ii) **Database:** Derived from databases previously established such as the Forest Foliar Coordinating Centre (FFCC).
- (iii) **Visual symptoms:** From visual symptoms indicating deficiency such as chlorosis on leaves for N shortage.

- (iv) **Mix:** A mixed of several criteria (e.g. visual symptoms and 90% growth response)
- (v) **Fertilizer trial:** Growth response (tree height or increments) in response to fertilizer application in field trials.
- (vi) **Unknown:** Not specified in the study

Critical levels found in the literature review show, for some values, large variations within the same species in the same climate. *Pinus pinaster* in sub-tropical regions have a critical level for N deficiency between 6 mg. g<sup>-1</sup> (Reuter, 1997) and 12 mg. g<sup>-1</sup> (Álvarez-Álvarez et al., 2011) or *Pseudotsuga menziesii* in temperate climate with a foliar N critical value for deficiency between 12 mg. g<sup>-1</sup> (den Driessche, 1979) and 17 mg. g<sup>-1</sup> (Mohren et al., 1986) (Table 3). This might be explained by the criteria used to establish these critical levels (Table 2) and by the site specificities (e.g. microclimate, soil characteristics)

**Table 2: List of criteria used to determine foliar critical levels for N and P deficiency.**

Criteria	Reference
<b>90% maximum growth</b>	
	Croise (1999)
	Lamb (1977)
	Morhen et al (1986)
	Quesnel et al (2006)
	Richards and Bevege (1969)
	Wang and Klinka (1997)
<b>Database</b>	
	Álvarez-Álvarez et al (2011)
	Mellert and Göttlein (2012)
	Tausz et al (2004)
	den Driessche (1979)
<b>Visual symptoms</b>	
	Drechsel and Zech (1990)
<b>Mix</b>	
	Kopinga and van den Burg (1995)
	Reuter (1997)
<b>Fertilizer trials</b>	
	Wells and Crutchfield (1969)
<b>Unknown</b>	
	Díaz-Maroto et al (2009)
	Garisson et al (2000)
	Jokela (2004)
	Moilanen et al (2015)

### 3.2.2 Typical Ranges of Foliar Nutrient Concentrations and Ratios

The typical values for foliar N concentration derived from the TRY database with 474 observations were divided into climate regions sub-groups. Table 4 displays the average (Avg), coefficient of variation (CV) and number of observation (n) for the tree species, genus, family and functional types sorted by climate regions that had more than 25 records in the TRY database. An important point to notice in the structure of the resulting typical values is the low numbers of species representing the needle-leaved evergreen in both boreal and temperate regions with *Pinus sylvestris* and *Picea abis* accounting for 73.8% and 64.3% of the total observations respectively. Similarly, *Quercus petraea* represents 60.7% of the total observations for broadleaved deciduous in sub-tropical regions (Table 4). In contrary, broadleaved evergreen trees in sub-tropical and tropical regions have very few observations (59 and 27 respectively) and are composed of a multitude of different tree species (data not shown).

**Table 3: Critical foliar N and P concentrations (in mg. g<sup>-1</sup>) for single nutrient deficiency for various tree species obtained from the literature review. Observations are grouped by climatic region.**

Avg N (P) represent the average critical levels and CV N (P) are the coefficient of variations for N and P respectively.

Species	Reference	Critical Foliar N concentration	Avg N	CV N	Critical Foliar P concentration	Avg P	CV P
<b><i>Boreal</i></b>							
Picea glauca	Quesnel et al (2006)	10.6	11.3	0.09	1.6	2.1	0.31
	Wang and Klinka (1997)	12.0			2.5		
Pinus sylvestris	Moilanen et al (2015)	12.0			1.3		
<b><i>Temperate</i></b>							
Acer campestre	Kopinga and van den Burg (1995)	18.0			1.3		
Acer negundo	Kopinga and van den Burg (1995)	17.0			1.3		
Acer platanoides	Kopinga and van den Burg (1995)	23.0			1.6		
Acer pseudoplatanus	Kopinga and van den Burg (1995)	23.0			1.6		
Acer saccharinum	Kopinga and van den Burg (1995)	19.0			1.4		
Alnus glutinosa	Kopinga and van den Burg (1995)	26.0			1.6		
Alnus incana	Kopinga and van den Burg (1995)	25.0			1.6		
Betula pendula	Kopinga and van den Burg (1995)	23.0			1.6		
Carpinus betulus	Kopinga and van den Burg (1995)	20.0			1.3		
Gleditsia triacanthos	Kopinga and van den Burg (1995)	20.0			1.5		
Robinia pseudoacacia	Kopinga and van den Burg (1995)	26.0			1.4		
Fagus sylvatica	Croise (1999)	20.0			-		
	Kopinga and van den Burg (1995)	21.0	19.9	0.06	1.4	1.3	0.11
	Mellert and Göttlein (2012)	18.7			1.2		
Quercus palustris	Kopinga and van den Burg (1995)	21.0			1.3		
Quercus petraea	Croise (1999)	20.0	19.9	0.01	1.1	1.3	0.17
	Mellert and Göttlein (2012)	19.8			1.4		
Quercus robur	Croise (1999)	20.0			1.1		
	Kopinga and van den Burg (1995)	21.0	20.3	0.03	1.4	1.3	0.13
	Mellert and Göttlein (2012)	19.8			1.4		

**Table 3 (continued)**

<b>Species</b>	<b>Reference</b>	<b>Critical Foliar N concentration</b>	<b>Avg N</b>	<b>CV N</b>	<b>Critical Foliar P concentration</b>	<b>Avg P</b>	<b>CV P</b>
Liquidambar styraciflua	Kopinga and van den Burg (1995)	18.0			1.3		
Aesculus hippocastanum	Kopinga and van den Burg (1995)	19.0			1.3		
Liriodendron tulipifera	Kopinga and van den Burg (1995)	26.0			1.8		
Fraxinus excelsior	Kopinga and van den Burg (1995)	23.0			1.6		
Platanus x acerifolia	Kopinga and van den Burg (1995)	20.0			1.5		
Crataegus laevigata	Kopinga and van den Burg (1995)	19.0			1.4		
Crataegus monogyna	Kopinga and van den Burg (1995)	19.0			1.4		
Sorbus aria	Kopinga and van den Burg (1995)	19.0			1.3		
Sorbus aucuparia	Kopinga and van den Burg (1995)	19.0			1.3		
Populus alba	Kopinga and van den Burg (1995)	19.0			1.6		
Populus deltoides	Kopinga and van den Burg (1995)	22.0			3.0		
Populus nigra	Kopinga and van den Burg (1995)	25.0			1.6		
Populus tremula	Kopinga and van den Burg (1995)	19.0			1.4		
Populus x canescens	Kopinga and van den Burg (1995)	18.0			1.6		
Populus x euramericana	Kopinga and van den Burg (1995)	26.0			1.6		
Salix alba	Kopinga and van den Burg (1995)	22.0			1.4		
Salix triandra	Kopinga and van den Burg (1995)	23.0			1.7		
Salix viminalis	Kopinga and van den Burg (1995)	23.0			1.7		
Tilia americana	Kopinga and van den Burg (1995)	27.0			1.8		
Tilia cordata	Kopinga and van den Burg (1995)	21.0			1.5		
Tilia platyphyllos	Kopinga and van den Burg (1995)	22.0			1.6		
Tilia x euchlora	Kopinga and van den Burg (1995)	22.0			1.5		
Tilia xvulgaris	Kopinga and van den Burg (1995)	25.0			1.6		
Ulmus carpinifolia	Kopinga and van den Burg (1995)	23.0			1.6		
Ulmus glabra	Kopinga and van den Burg (1995)	23.0			1.6		
Ulmus x hollandica	Kopinga and van den Burg (1995)	23.0			1.6		
Larix decidua	Croise (1999)	18.0			1.4		

**Table 3 (continued)**

<b>Species</b>	<b>Reference</b>	<b>Critical Foliar N concentration</b>	<b>Avg N</b>	<b>CV N</b>	<b>Critical Foliar P concentration</b>	<b>Avg P</b>	<b>CV P</b>
Abies alba	Croise (1999)	13.0			1.2		
Abies grandis	Garisson et al (2000)	11.5			1.5		
Picea abies	Croise (1999)	13.0	13.5	0.05	1.3	1.4	0.10
	Mellert and Göttlein (2012)	14.0			1.5		
Picea engelmannii	den Driessche (1979)	15.0			1.8		
Picea glauca	den Driessche (1979)	15.0			1.8		
Picea sitchensis	den Driessche (1979)	15.0			1.8		
Pinus contorta	den Driessche (1979)	13.0	12.5	0.06	1.5	1.4	0.16
	Garisson et al (2000)	12.0			1.2		
Pinus laricio	Croise (1999)	10.0			0.9		
Pinus nigra	Reuter and Robinson (1997)	15.0			1.3		
Pinus pinaster	Croise (1999)	9.0			0.9		
Pinus sylvestris	Croise (1999)	15.0	14.5	0.05	1.5	1.4	0.10
	Mellert and Göttlein (2012)	14.0			1.3		
Pseudotsuga menziesii	Croise (1999)	15.0	14.5	0.12	1.4	1.4	0.11
	den Driessche (1979)	12.0			1.6		
	Garisson et al (2000)	14.0			1.2		
	Morhen et al (1986)	17.0			1.5		
	Reuter (1997)	14.5			1.5		
Tsuga heterophylla	den Driessche (1979)	12.0			2.6		
<b>Sub-Tropical</b>							
Quercus petraea	Díaz-Maroto et al (2009)	15.0			1.0		
Quercus robur	Díaz-Maroto et al (2009)	15.0			1.0		
Acacia dealbata	Reuter (1997)	24.0			1.0		
Ceratopetalum apetalum	Reuter (1997)	6.0			0.4		
Eucalyptus delegatensis	Reuter (1997)	14.0			1.1		
Eucalyptus diversicolor	Reuter (1997)	11.0			0.6		

**Table 3 (continued)**

<b>Species</b>	<b>Reference</b>	<b>Critical Foliar N concentration</b>	<b>Avg N</b>	<b>CV N</b>	<b>Critical Foliar P concentration</b>	<b>Avg P</b>	
Eucalyptus dunnii	Reuter (1997)	18.0			1.1		
Eucalyptus fastigata	Reuter (1997)	20.0			1.5		
Eucalyptus globulus	Reuter (1997)	12.0			1.1		
Eucalyptus grandis	Reuter (1997)	16.0			1.0		
Lophostemon confertus	Reuter (1997)	11.0			0.7		
Araucaria cunninghami	Richards and Bevege (1969)	13.5			1.1		
Araucaria heterophylla	Reuter (1997)	14.0			1.6		
Casuarina glauca	Reuter (1997)	19.8			1.0		
Pinus canariensis	Reuter (1997)	11.9			1.0		
Pinus caribaea	Reuter (1997)	12.9			1.7		
Pinus contorta	Reuter (1997)	15.5			1.5		
Pinus elliotii	Jokela (2002)	10.0			0.9		
Pinus halepensis	Reuter (1997)	9.5			1.0		
	Tausz et al (2004)	10.0	9.8	0.04	1.0	1.0	0.00
Pinus pinaster	Álvarez-Álvarez et al (2011)	12.0			1.2		
	Reuter (1997)	6.0	9.0	0.33	1.0	1.0	0.15
	Tausz et al (2004)	9.0			0.9		
Pinus radiata	Reuter (1997)	12.0			1.4		
	Tausz et al (2004)	12.0	12.0	0.00	1.2	1.3	0.11
Pinus taeda	Jokela (2002)	12.0			1.2		
	Wells and Crutchfield (1969)	-			0.9	1.0	0.24
<b><i>Tropical</i></b>							
Terminalia ivorensis	Drechsel and Zech (1990)	15.2			1.1		
Cassia siamea	Drechsel and Zech (1990)	-			1.8		
Brosimum alicastrum	Drechsel and Zech (1990)	22.7			0.8		
Triplochiton scleroxylon	Drechsel and Zech (1990)	36.3			2.6		
Gmelina arborea	Drechsel and Zech (1990)	18.1			1.1		
	Reuter (1997)	18.6	18.4	0.02	1.1	1.1	0.00

**Table 3 (continued)**

<b>Species</b>	<b>Reference</b>	<b>Critical Foliar N concentration</b>	<b>Avg N</b>	<b>CV N</b>	<b>Critical Foliar P concentration</b>	<b>Avg P</b>	<b>CV P</b>
Tectona grandis	Drechsel and Zech (1990)	14.4	15.5	0.10	1.2	1.3	0.11
	Reuter (1997)	16.5			1.4		
Anacardium occidentale	Drechsel and Zech (1990)	15.3			1.9		
Hevea brasiliensis	Reuter (1997)	33.1			2.0		
Acacia mearnsii	Drechsel and Zech (1990)	31.7			1.4		
Azadirachta indica	Drechsel and Zech (1990)	-			1.4		
Cordeauxia edulis	Drechsel and Zech (1990)	12.0			1.1		
Eucalyptus camaldulensis	Drechsel and Zech (1990)	12.2	13.4	0.13	1.2	1.5	0.28
	Reuter (1997)	14.6			1.8		
Eucalyptus deglupta	Lamb (1977)	21.0			-		
Nauclea diderrichii	Drechsel and Zech (1990)	14.6			-		

The magnitude of the increase in CV is however, different between needle-leaved and broadleaved tree with a delta CV below 0.05 or above 0.10 respectively. This is explained by the lower number of species for needle-leaved evergreen trees present in the TRY dataset than for broadleaved trees. It is also in line with observations made by other studies that needle-leaved trees generally have more comparable foliar nutrient concentrations than broadleaved trees (Mellert & Göttlein, 2012).

### 3.2.3 Reference Values for Tree Species present in the database.

Reference values to scale the natural differences in foliar N and P concentration were selected based on the results presented in the previous sections (Sections 3.2.1 and 3.2.2). As not all of the tree species within their climate regions were found in neither the literature review nor the TRY database, the selection of reference values followed the decisional tree explained in Sections 2.2.1 and 2.2.2. The following list describes the coding systems used to notify at which level of integration the selected reference value corresponds:

- (i) **A:** Same species in the same climate regions;
- (ii) **B:** Same genus in the same climate regions;
- (iii) **C:** Same family in the same climate regions;
- (iv) **D:** Same functional type in the same climate regions;
- (v) **E:** Same species across all climate;
- (vi) **F:** Same genus across all climate;
- (vii) **G:** Same family across all climate;
- (viii) **H:** Same functional type across all climate; and
- (ix) **I:** Same tree type (broadleaves/needle-leaved) across all climates.

Consequently, the average of all the species grouped under the same level of integration was calculated and served as reference values for the tree species present in the database (Table 5).

The level of integrations for the reference values is rather heterogeneous with only six species within their climate region that were found in the literature review on critical levels for N and P deficiency and only one for the foliar N typical concentrations. The N critical level for deficiency is relatively high (33.1 mg. g<sup>-1</sup>) for *Alchornea lojaensis* and *Hieronyma fendleri* (Euphorbiaceae), two tropical broadleaved evergreen species in comparison of the two other tropical broadleaved evergreen species *Graffenrieda emarginata* and *Myrcia sp* (18.1 and 14.9 mg. g<sup>-1</sup> respectively). Tropical broadleaved evergreen trees show the high variations between species in critical values for N deficiency compared to boreal and temperate needle-leaved evergreen and broadleaved deciduous trees (Table 5).

The N/P reference values are from a single source Stefan et al. (1997), and represents only temperate climate. As no broadleaved evergreen trees were included in Stefan et al. (1997), the critical N/P ratio levels for broadleaved evergreen trees is the same as the one from broadleaved deciduous trees (Beech and Oak).

**Table 4: Typical value of foliar N concentration (in mg. g<sup>-1</sup>) for tree species classified by genus, family and functional types within climate zones derived from the TRY database.**

Only the species, genus, family and functional type with more than 25 observations are displayed. Avg is the average foliar N concentration (in mg. g<sup>-1</sup>); CV the coefficient of variation associated to the average and N is the number of observations.

Climate	Functional type	Family	Genus	Species	Avg	CV	N	
Boreal	needle-leaved evergreen				8.93	0.18	84	
		Pinaceae			8.93	0.18	84	
			Pinus		8.67	0.16	62	
				Pinus sylvestris	8.67	0.16	62	
Temperate	broadleaved deciduous				24.81	0.25	124	
		Fagaceae			23.64	0.14	56	
			Fagus		24.31	0.08	41	
			Fagus sylvatica	24.31	0.08	41		
	needle-leaved evergreen					11.62	0.26	84
		Pinaceae				11.62	0.26	84
		Picea			12.12	0.21	64	
			Picea abies	12.54	0.21	54		
Sub-Tropical	broadleaved deciduous				22.01	0.35	346	
		Fagaceae			19.32	0.21	307	
			Quercus		19.12	0.21	282	
				Quercus petraea	19.10	0.21	210	
			Quercus robur	20.20	0.17	56		
		Rosaceae			39.73	0.13	38	
			Prunus		40.32	0.09	37	
				Prunus persica	40.32	0.09	37	
	broadleaved evergreen				11.49	0.27	59	
Tropical	broadleaved evergreen				18.25	0.29	27	

**Table 5: Reference values for foliar N and P concentration (in mg. g<sup>-1</sup>) and ratio for trees species present in the database for meta-analysis, sorted by climate.**

Values are calculated average based on the values from Table 3 (critical) and Table 4 (typical). A: Same species and climate regions; B: Same genus and climate regions; C: Same family and climate regions; D: Same functional type climate regions; E: Same species across all climate; F: Same genus across all climate; G: Same family across all climate; H: Same functional type across all climate; I: Same leave type across all climate.

Species	N Critical	N critical Ref	P Critical	P Ref	N/P	N/P Ref	N Typical	N typical Ref
<b>Boreal</b>								
<i>Picea abies</i>	11.30	<b>B</b>	2.05	<b>B</b>	17.0	<b>H</b>	8.93	<b>C</b>
<i>Pinus sylvestris</i>	12.00	<b>A</b>	1.30	<b>A</b>	17.0	<b>H</b>	8.67	<b>A</b>
<b>Temperate</b>								
<i>Acer rubrum</i>	20.00	<b>B</b>	1.44	<b>B</b>	25.0	<b>H</b>	24.81	<b>D</b>
<i>Acer saccharum</i>	20.00	<b>B</b>	1.44	<b>B</b>	25.0	<b>H</b>	24.81	<b>D</b>
<i>Betula alleghaniensis</i>	23.00	<b>B</b>	1.60	<b>B</b>	25.0	<b>H</b>	24.81	<b>D</b>
<i>Betula papyrifera</i>	23.00	<b>B</b>	1.60	<b>B</b>	25.0	<b>H</b>	24.81	<b>D</b>
<i>Fagus grandifolia</i>	19.90	<b>B</b>	1.30	<b>B</b>	25.0	<b>H</b>	24.31	<b>B</b>
<i>Quercus rubra</i>	20.12	<b>B</b>	1.28	<b>B</b>	25.0	<b>H</b>	23.64	<b>C</b>
<i>Liquidambar styraciflua</i>	18.00	<b>A</b>	1.30	<b>A</b>	25.0	<b>H</b>	24.81	<b>D</b>
<i>Carya ovata</i>	21.12	<b>D</b>	1.51	<b>D</b>	25.0	<b>H</b>	24.81	<b>D</b>
<i>Fraxinus americana</i>	23.00	<b>B</b>	1.60	<b>B</b>	25.0	<b>H</b>	24.81	<b>D</b>
<i>Prunus serotina</i>	19.00	<b>B</b>	1.35	<b>C</b>	25.0	<b>H</b>	24.81	<b>D</b>
<i>Populus grandidentata</i>	21.50	<b>B</b>	1.80	<b>B</b>	25.0	<b>H</b>	24.81	<b>D</b>
<i>Pinus sylvestris</i>	14.50	<b>A</b>	1.40	<b>A</b>	17.0	<b>H</b>	11.62	<b>C</b>
<i>Pseudotsuga menziesii</i>	14.50	<b>A</b>	1.44	<b>A</b>	17.0	<b>H</b>	11.62	<b>C</b>
<i>Tsuga canadensis</i>	12.00	<b>B</b>	2.60	<b>B</b>	17.0	<b>H</b>	11.62	<b>C</b>
<i>Tsuga heterophylla</i>	12.00	<b>A</b>	2.60	<b>A</b>	17.0	<b>H</b>	11.62	<b>C</b>
<b>Sub-tropical</b>								
<i>Pseudotsuga menziesii</i>	10.47	<b>C</b>	1.14	<b>C</b>	17.0	<b>H</b>	10.28	<b>G</b>
<b>Tropical</b>								
<i>Graffenrieda emarginata</i>	18.13	<b>D</b>	1.54	<b>D</b>	25.0	<b>I</b>	18.25	<b>D</b>
<i>Alchornea lojaensis</i>	33.10	<b>C</b>	2.00	<b>C</b>	25.0	<b>I</b>	18.25	<b>D</b>
<i>Hieronyma fendleri</i>	33.10	<b>C</b>	2.00	<b>C</b>	25.0	<b>I</b>	18.25	<b>D</b>
<i>Myrcia sp</i>	14.92	<b>C</b>	1.50	<b>C</b>	25.0	<b>I</b>	18.25	<b>D</b>

### 3.3 Impacts of the Foliar Nutrient Status on the Carbon Responses to Nitrogen Addition

The following sections present the results of this assessment. The first section presents the evaluation of relationships between the  $ScN_{pl}$  or  $ScP_{pl}$  or  $ScNP_{pl}$  (Scaled foliar N, P and N/P ratio respectively) and the relative sensitivity while the second section displays the regression analysis using the unscaled foliar N and P concentrations and ratios (independent variables) against the log response ratio (dependent variable) for needle-leaved trees only. A third section provides the results of the multiple regression analysis assessing interactions effects of combined foliar nutrient concentrations and ratio on the C response to N addition.

#### 3.3.1 Relationship between the Scaled Foliar Nitrogen and Phosphorus Status and the Carbon Response to Nitrogen Additions

The relationship between the relative sensitivity and the  $ScN_{pl}$  (both typical and critical),  $ScP_{pl}$  and  $ScNP_{pl}$  variables are visually assessed as the assumptions of homogeneity and normality of the residue for the linear regression were not met for these models (Figure 7).

There were no apparent trends coming out of these graphics. The tree C response to N addition (relative sensitivity) seemed to increase as the  $ScN_{pl}$  typical values increases (Figure 7a). This goes against previous expectations of a decreasing tree growth response to N addition with an increasing foliar N concentration (Figure 2). This was mainly due to the strong positive C response to N addition of boreal forests. Inversely, the tree relative sensitivity to N addition slightly seemed to decrease with an increasing  $ScN_{pl}$  critical value (Figure 7b). Boreal forests showed the same strong positive C response to N addition as in the previous graphic, despite a  $ScN_{pl}$  above one indicating an N sufficient status. The foliar N status of tropical forest did not predict the C response to N addition of tropical forest as they remain close to 0% C response per Kg N added all along the x axis ( $ScN_{pl}$  critical values). Only temperate forests appeared to have a sparse decreasing relative sensitivity to N additions as their foliar N concentration increases shifting from an N deficient ( $ScN_{pl} < 1$ ) to an N sufficient status ( $ScN_{pl} > 1$ ). The tree C response to N addition seemed hardly to increase with an increasing  $ScP_{pl}$ . This is especially due to sub-tropical forests that displayed an increase in tree growth response to N addition with a foliar P concentration shifting from a P deficient ( $ScP_{pl} < 1$ ) to a P sufficient status ( $ScP_{pl} > 1$ ) (Figure 7c). The relative sensitivity to N addition showed a steep increase that pick before decreasing as the  $ScNP_{pl}$  increases. However, none of the  $ScNP_{pl}$  values did not display unbalances of N over P foliar concentrations ( $ScNP_{pl} < 1$ ) (Figure 7d).

Both of the  $ScN_{pl}$  (typical and critical), displayed two different patterns of points indicating a strong effect of the scaling procedure on the outcomes. In order to remove this strong effect, the relationship between the relative sensitivity and the foliar N and P content of the tree was assessed using the non-scaled foliar N, P and N/P concentrations. Only the needle-leaved trees were selected for the following analysis as the scaling procedure was accounting for natural differences between tree species. The model assumptions were not met and the relationship between the relative sensitivity and the foliar N or P concentration or N/P ratio was assessed visually (Figure 8).

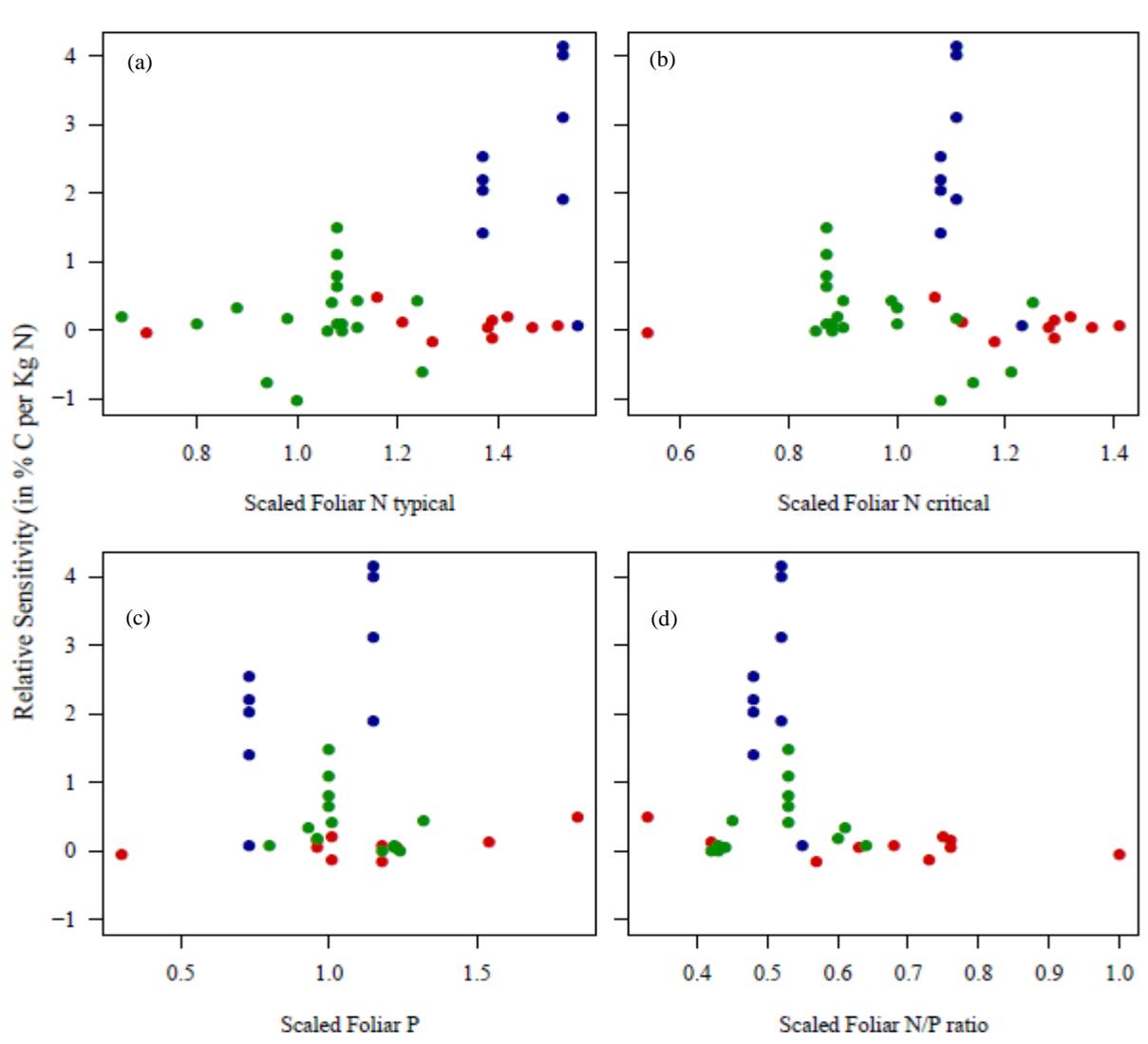
In this case, the C response to N addition decreases as the foliar N concentration increases, however the relationship between these two variables did not seem linear but decreases with an asymptote. Boreal forests clearly showed a higher relative sensitivity to N addition at the exception of one point than the temperate or sub-tropical forests. This is in line with the findings of section 3.1 (Figure 6), where boreal forests add a significant positive C response to N addition.

Boreal needle-leaved forests had a critical level for N deficiency around 12mg. g<sup>-1</sup> (Table 5), and positively responded to N addition while indicating a sufficient N nutrition. Temperate forests had a critical level for N deficiency between 12 and 14.5 mg. g<sup>-1</sup> (Table 5) and seemed to have a decreasing relative sensitivity to N addition with an increasing foliar N concentration. Sub-tropical forests had a critical level for N deficiency of 10.47 mg. g<sup>-1</sup> (Table 5) and appeared to have a growth response that decrease slightly as the foliar N concentration increases. In overall, the asymptote is quickly reached

and does not allow drawing any relationship between the relative sensitivity and the foliar N (Figure 8a).

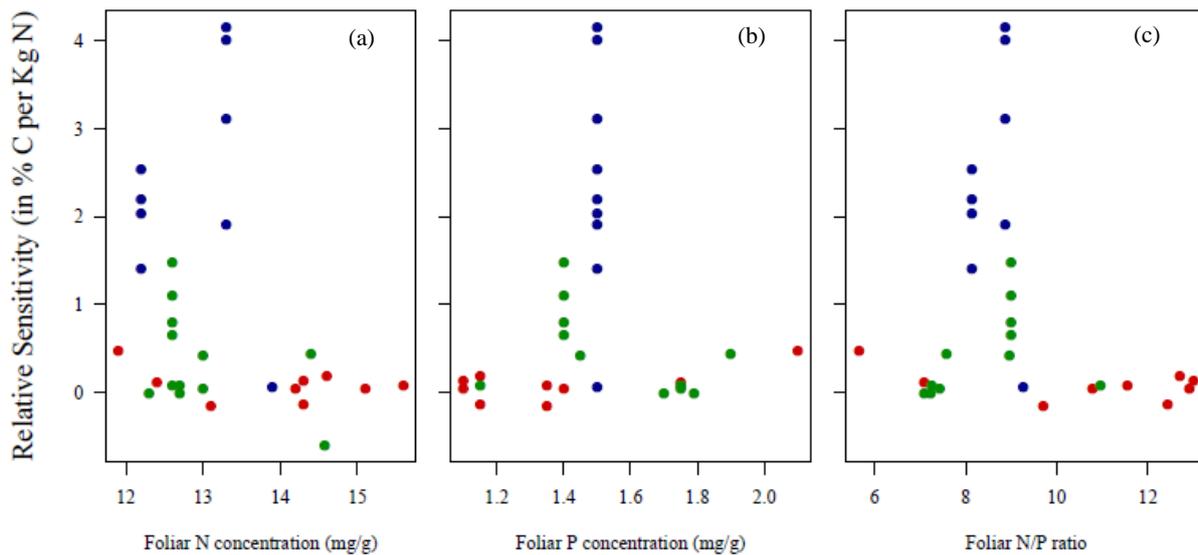
Variations in the relative sensitivity to N addition did not seem to be explained by the foliar P concentration. Boreal forests showed a wide variation range of C response to N addition for the same foliar P concentration; thus, no appearing relationship between the two variables could be found. Sub-tropical forests showed a sparse increase in relative sensitivity to N addition as the foliar P concentration increases. Temperate forests displayed variations that are more heterogeneous than the sub-tropical forests. Their C response to N addition seemed to decrease as the foliar P concentration increases. This goes against the previous hypothesis (Figure 2) of an increasing growth response to N addition as the foliar P concentration increases (Figure 8b).

The tree C response to N addition displayed a strong peak for an N/P ratio of 8.5. Boreal forests showed a wide variation range of C response to N addition for similar foliar N/P ratios values. Sub-tropical forest presented a slight decreasing C response to N addition as the foliar N/P ratio increases. Temperate forests had the closest expected pattern (Figure 2) with a relative sensitivity increases to N addition that first increase to a peak value and then decreases with an increasing foliar N/P ratio (Figure 8c).



**Figure 7: Relative sensitivity (in % C per Kg N) against the scaled foliar N typical (a) and critical (b), the scaled foliar P (c) and the scaled foliar N/P ratio (d).**

Colours represent the three climatic regions (Blue: Boreal; Green: Temperate and Red: Tropical and Sub-tropical).



**Figure 8: Relative sensitivity (in % C per Kg N) of needle-leaved trees against the foliar N (a) and P (b) concentration (in mg. g<sup>-1</sup>) and N/P ratio (c).**

Colours represent the three climatic regions (Blue: Boreal; Green: Temperate and Red: Sub-tropical).

### 3.3.2 Regression Analysis for Needle-leaved Trees

This section presents the results of the regression analysis between LRR (dependent variable) and the non-scaled foliar N or P concentration or N/P ratio (independent variables) (Figure 9).

The C response to N addition appeared to have a significant decreasing trend with an increasing foliar N concentration. However, the regression model did not fit properly the data, as the coefficient of determination ( $R^2$ ) remain very low (0.10). Boreal forests mainly stand out of the confidence intervals and their C responses to N addition seemed to increase as the foliar N concentration increases (Figure 9a).

The C response to N addition displayed an increasing trend with an increasing foliar P concentration. However, the relationship was not found significant ( $p$ .value > 0.05) and the coefficient of determination ( $R^2 = 0.05$ ) also indicated a poor fit of the model to the data (Figure 9b). Once the boreal forests were removed, the relationship between the log response ratio and the foliar P concentration remained statistically insignificant although a weak increasing trend appeared (data not shown).

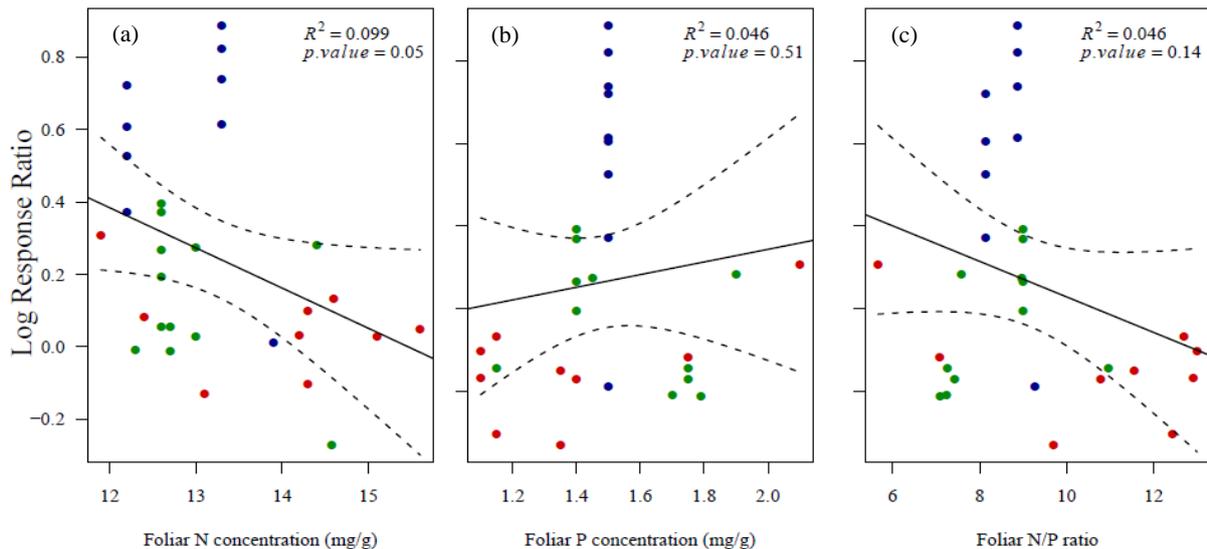
As expected, no significant linear trend was found between the log response ratio and the foliar N/P ratio. Only, sub-tropical forests showed a C response that slightly decreases with increasing foliar N/P ratio.

LRR, unlike the relative sensitivity, does not take into account the N fertilization rates. To correct this, the data was grouped into two categories, low N addition rates and high N addition rates based on the overall N application rate average (see Section 2.3).

Figure 10 presents the results of these linear regressions analysis between the log response ratio for needle-leaved tree the foliar N or P concentration (in mg·g<sup>-1</sup>) or N/P ratio.

For the low N application rate group, all of the three explanatory variables (foliar N and P concentration and N/P ratio) significantly explained variations in the log response ratio.

The trend line between the log response ratio linearly decreases as the foliar N concentration increases. The coefficient of determination ( $R^2 = 0.20$ ) point out a rather poor model fit, but better than in Figure 9a. Whilst boreal forests showed the highest LRR, sub-tropical forests C response to N addition did not seem to increase nor decrease as the foliar N increases (Figure 10a and S1).



**Figure 9: Linear regression between the Log response ratio of needle-leaved trees and the foliar N (a) and P (b) concentrations (in  $\text{mg} \cdot \text{g}^{-1}$ ) and N/P ratio (c).**

The  $R^2$  is the coefficient of determination for the regression models. The solid line represents the trend and the two dotted lines the 95% confidence interval. The equations define the intercept and coefficients for the model trend lines. Colours represent the three climatic regions (Blue: Boreal; Green: Temperate and Red: Sub-tropical).

The trend line between the LRR and the foliar P concentration increases with an  $R^2$  of 0.20. Boreal forests displayed a singlewide variation in LRR for an exact same foliar P concentration and sub-tropical forests C response to N addition did not appear to either increase or decrease as the foliar P concentration increases (Figure 10b and S1).

The C response to N addition showed a linear decreasing relationship as the foliar N/P ratio increases. The fit of the model indicates a poor fit of the model to the data ( $R^2 = 0.25$ ). Boreal forests displayed a high C response to N addition associated to a low N/P while sub-tropical forests did not seem to have significant positive C response to N fertilization as the foliar N/P ratio increases, although the majority of them have a relatively high foliar N/P ratio (Figure 10c and S1).

For the high N application rate group, none of the explanatory variables (foliar N and P concentration and N/P ratio) significantly explained variations in the log response ratio (Figure 10)

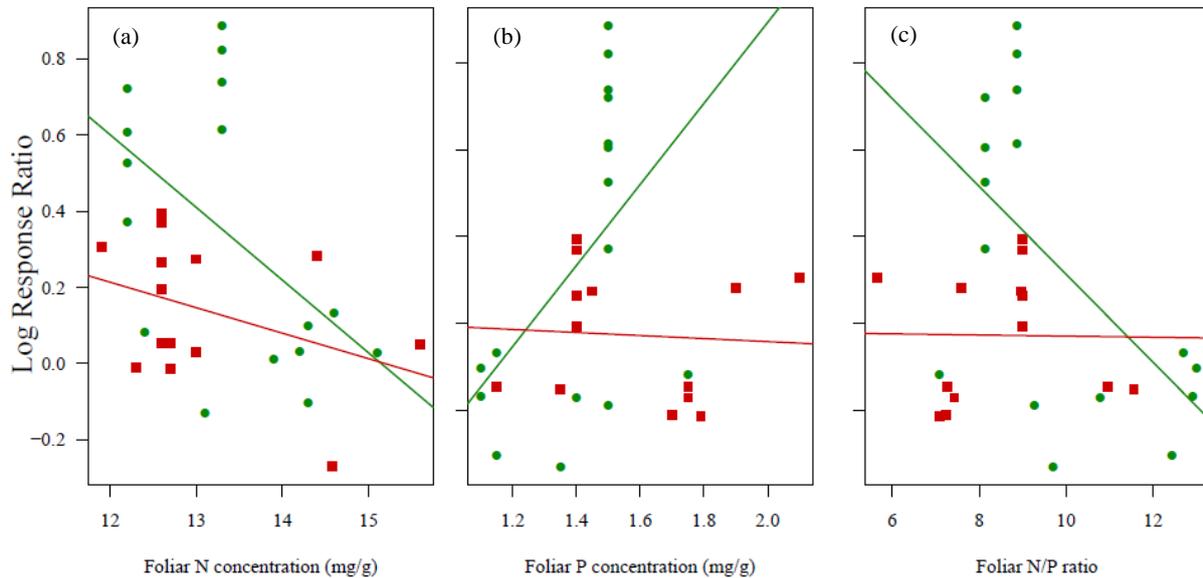
Although the log response ratio seemed to linearly decreases as the foliar N increases the data points, the model fit is very poor ( $R^2 = 0.07$ ) (Figure 10a and S1). The log response ratio appeared to stay almost constant with an increasing foliar P as well as for an increasing foliar N/P ratio (Figure 10b and 10c).

This indicates that the variations in the growth response to N addition might be better explained by a combination of several foliar nutrient concentrations than by single nutrient concentration. The differences in the relationship between the log response ratio and the foliar N and P concentration and N/P ratio when separated in low and high N addition rate groups shows that the foliar N and P concentration as well as N/P ratio significantly explain the variations in C response to N addition. However, a shift towards other explanatory variables occurred when the N addition rates increases.

As mentioned in the Section 3.3.1, the relationship between the C response to N addition appeared to be non-linear. To test this non-linear relationship, polynomial regression models were used (Section 2.3) for needle-leaved trees (Figure 11).

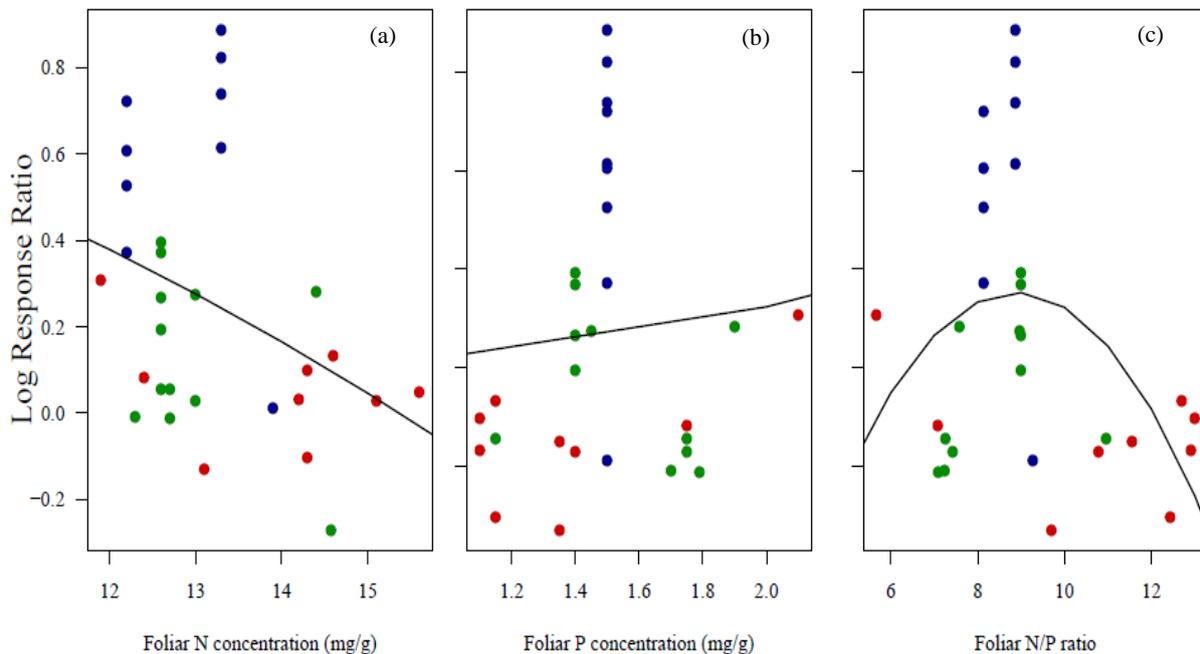
The growth response to N addition significantly decreases with an increasing foliar N concentration. For this model the quadratic term for foliar N concentration (foliar N concentration<sup>2</sup>) is displayed (Figure 11a), however, this non-linear regression did not significantly improve the model fit (data not shown). Variations of the log response ratio was significantly explained by the square foliar P concentration indicating an asymptotic curve reaching a plateau as the foliar P concentration

increases (Figure 11b). Both the foliar N/P ratio and its quadratic terms significantly improve the model fit to variations in the growth response to N addition. The trend line starts with an increase before reaching a short plateau and then decreases with an increasing foliar N/P ratio indicating first a more and more harmonious nutrition (C response to N addition increases) and shift back to an unbalanced nutrition (C response to N addition decreases) (Figure 11c).



**Figure 10: Linear regression between the Log response ratio of needle-leaved trees and the foliar N (a) and P (b) concentrations (in  $\text{mg} \cdot \text{g}^{-1}$ ) and N/P ratio (c).**

The equations define the intercept and coefficients for the model trend line. Colours: low N addition rates (Green) and high N addition rates (Red).



**Figure 11: Polynomial regression between the Log response ratio of needle-leaved trees and the foliar N (a) and P (b) concentrations (in  $\text{mg} \cdot \text{g}^{-1}$ ) and N/P ratio (c).**

Colours represent the three climatic regions (Blue: Boreal; Green: Temperate and Red: Sub-tropical).

Similar non-linear trend lines were obtained using a generalized additive model allowing non-specific assumptions about the parametric form of the function (FigureS2). This confirmed the non-linearity of the relationship between the C response to N addition and the foliar N and P status as well as the form of the functions used in the polynomial regression model.

### 3.3.1 Multiple Regression Analysis for Needle-leaved Trees

A multiple analysis was run to test the combination effect of the foliar N and P concentrations and N/P ratio on the C response to N addition. The N addition rate was added to compensate the fact that the log response ratio does not take it into account. Second order parameters were also added to this multivariate regression model. Table 6).

The results of this multivariate model were highly significant in explaining variations in the C response to N addition. The model had a multiple coefficient of determination of 0.89. This result must be interpreted with care. Although this model was simplified by removing non-significant quadratic terms and interactions, the model included, at first, all four explanatory variables with all their quadratic terms and possible interactions. This does not bring much scientific meaning in explaining variations in the C response of forests to N additions. However, the results confirmed the significant interaction effect of N and P together in explaining the growth response to N additions.

The foliar N concentration describes better the variation in the log response ratio than its quadratic terms. In contrast, the foliar P concentration was significantly better at explaining variations in the C response to N addition with its quadratic terms. No change in the p.value between the foliar N/P ratio and its quadratic terms could be detected. This is in line with the findings of the previous polynomial regression (Section 3.3.2).

All the interactions including the foliar N concentration, except one, were significant. In contrast, only the interactions between the foliar P or the foliar N/P and the N addition rate were retained in this model. Surprisingly, the third and the fourth level interactions were significantly explaining the variations in the C response to N addition. This highlights the importance of synergic effect of nutrients on characterising the nutritional status of the trees.

**Table 6: Multiple regression analysis between the Log response ratio of needle-leaved trees and the foliar N and P concentrations (in mg. g<sup>-1</sup>); N/P ratio and the N addition rate (including quadratic terms).**

	<b>Coefficients</b>	<b>Std error</b>	<b>p-Value</b>
<b>(Intercept)</b>	-537.32	131.24	0.0011
<b>Leaf N</b>	154.84	23.31	>0.0001
<b>Leaf P</b>	103.71	63.22	0.12
<b>Leaf N/P</b>	-52.47	7.79	>0.0001
<b>eN</b>	86.83	16.04	>0.0001
<b>(Leaf N)^2</b>	1.62	0.47	0.004
<b>(Leaf P)^2</b>	94.46	13.93	>0.0001
<b>(Leaf N/P)^2</b>	3.65	0.59	>0.0001
<b>Leaf N * Leaf P</b>	-62.25	10.66	>0.0001
<b>Leaf N * Leaf N/P</b>	-6.41	1.22	>0.0001
<b>Leaf N * eN</b>	-11.11	2.01	>0.0001
<b>Leaf P * eN</b>	-40.10	7.21	>0.0001
<b>Leaf N/P * eN</b>	1.67	0.29	>0.0001
<b>Leaf N * Leaf P * eN</b>	3.87	0.68	>0.0001
<b>Leaf N * Leaf P * Leaf N/P * eN</b>	0.16	0.03	>0.0001

## **4. Discussion**

### **4.1 Meta-analysis**

This study assessed the relationship between the forest trees' C response to N addition and the foliar N and P contents. A meta-analysis on forest fertilisation experiments was conducted to gather information on tree growth response to N fertilisation and on the initial foliar N or P concentration of these forests.

Despite the large body of literature reviewed to gather information on both forest aboveground growth responses to N addition and foliar N or P initial concentration, only a very few amount of studies (n=7) were included in the meta-database. Within this thesis timeframe, only half of the 2115 studies returned by Scopus were entirely reviewed for selection. From these studies, 27 were retained for analysis. Among them, 7 measured aboveground woody biomass responses to N fertilisation and foliar chemical analysis.

The forest plots present in the meta-database were mainly from needle-leaved temperate forests (12 out of 19 plots). Needle-leaved sub-tropical forests (Tropical forests resulted in a combination of nine sub-tropical needle-leaved forests plots and one tropical broadleaved evergreen forest plot), spatial distribution was very close to most of the temperate needle-leaved forests plots. The study of Mainwaring et al. (2014) accounts for 16 of the 21 forest plots for both temperate and sub-tropical needle-leaved forest plots present in the database. The location of Mainwaring et al. (2014) experimental plots were classified into two different climate regions using their latitudes and longitudes coordinates but geographically fall close to an arbitrary climate boundary. These forests' plots climatic conditions might be more similar than the applied climates zones classification suggest. Continuous climatic variables such as mean annual temperature and mean annual precipitation should better differentiate the influence of climatic conditions on forest growth (Ordoñez et al., 2009; Reich et al., 2004).

Boreal forest displayed the highest relative sensitivity to N addition. However, from the nine boreal forest plots present in the database, eight of them are from the single study of Jacobson & Pettersson (2010). In this case, the experimental plots of Jacobson & Pettersson (2010) were all complemented with Ca, Mg and Boron fertilisation that might explain the strong positive C response to N addition of these plots. This highlights the importance of implementing multiple foliar nutrient contents in modelling variations in tree C responses to N addition.

Foliar analysis interpretations are delicate and significant variations in the foliar nutrient content can occur within the same tree species in a given climate and age category. Sources of variations that affect the foliar chemistry of a tree (Croise et al., 1999; Jokela, 2004) include:

- (i) Within trees of a same population;
  - The social status of the tree;
  - The genotypes related to the genetic characteristics of each individual tree;
  - The physiological status of the tree;
- (ii) Between leaves of the same tree;
  - Leaf age;
  - Leaf position in the tree (e.g. shaded vs full light exposure);
- (iii) Time of sampling;
  - Year due to different climatic factors;
  - period of the year for seasonal variation in internal nutrient content;
  - day of sampling due to variation in the metabolic activity of the tree;
- (iv) Analytical procedure;
  - Inter-laboratory reliability in the analytical procedure employed to extract the nutrient from the leaf material.

Although these variations weaken the comparability of foliar nutrient concentration between studies, they cannot be accounted for within a meta-analysis framework. In this meta-analysis, foliar nutrient concentrations were measured, for 21 of the experimental plots, prior to the start of any fertilization

trials and 23 were measured from control plots. Among the latest, 17 were taken at the end of the experiment and five sampled before the start of the experiments. The sampling methodology in terms of the period of the year and tree and/ or leaves selection and chemical analysis procedures intrinsically differed from one study to another. Although sampling period should be kept as consistent as possible, it was considered that foliar nutrient concentration would not significantly change between the start and the end of the fertilization trial within the control plot. Indeed, methodological international guidelines for foliar analysis has been developed to harmonised foliar analysis and thus provide a powerful tool to monitor forest nutritional status (Mellert & Göttelein, 2012). A good example of consistent large scale foliar survey for forest nutritional status assessment is the one realized by Stefan et al. (1997). This study provides reliable foliar nutrient thresholds for assessing forest nutritional status. However, to my actual knowledge, this type of work has only been realized for European forests.

## 4.2 Scaling the Foliar Nitrogen and Phosphorus Status

One of the main issues that have been raised by this thesis is how the natural differences in the foliar nutrient content of trees can be scaled to a common level This was done using two distinct procedures. The first consisted of using critical levels for N and P deficiency, and the second derived typical foliar N concentration.

### 4.2.1 Foliar Critical Level for Nitrogen and Phosphorus Deficiency

The literature review on critical levels for N and P deficiency conducted is lacking background information on the site and stands characteristics used to establish foliar critical levels for N and P deficiency due to difficulties in getting access to primary sources of information. Among the 18 studies for foliar critical levels of N deficiency and 17 studies for foliar critical level for P deficiency, only five studies actually establish foliar critical levels for N and P deficiency. The remaining studies all quote secondary authors. Consequently, detailed information, such as experimental settings, soil characteristics, sampling period or tree and leaves selection, and climatic parameters were not reported.

Additionally, the criteria used for establishing critical levels are relatively heterogeneous between studies. This particularity in this literature review was already encountered by den Burg, (1990) literature's compilation on foliar nutrient thresholds. Each of these criterions has its own advantages and shortcomings compared to the others:

- (i) Visual symptoms are easy to apply, as they do not require laboratory analysis. However, an absence of visual symptoms for a given stand does not always indicate an adequate nutrition. Nutrient deficiency affecting stand growth are above a given level of severity that does not produce visual deficiency symptoms (Ballard and Carter (1986) as cited in Carter 1992). Visual symptoms display by a plant can be misleading as several another factor may cause similar visual abnormalities on plants (e.g. pests) (Carter, 1992).
- (ii) The foliar nutrient concentration corresponding to 90% of potential maximum growth is able to detect nutrient deficiency affecting growth before the severity thresholds producing visual symptoms. However, this criterion requires the calculation of a reliable growth response to nutrient fertilization curve. As such curves are difficult to produce growth index are often use as a replacement. Additionally, field experiments that aim to establish foliar critical levels for nutrient deficiency and that result in a 10% growth reduction from a potential maximum must provide a field environment where no other limiting factors than the studied nutrient occurs (Reuter, 1997).
- (iii) Fertilizer trials have the advantage to base the foliar nutrient concentrations to an actual growth response to fertilizer application. It assesses short-term growth response (e.g. first-year tree height or diameter increment) to fertilizer application. However, as for the 90% potential maximum growth criterion, they must limit all other limiting factors other than the studied nutrient (Carter, 1992; Reuter, 1997).
- (iv) A Foliar database, such as the FFCC provides a consistent and reliable source of information as it results from large scale foliar analysis survey realized within a

standardized framework. Although this database classified foliar nutrient content into three classes allowing comparison of trees foliar nutrient content across species and countries, it is not related to a physiological status of the tree. Consequently, it does not distinguish nutrient deficiency affecting tree growth (Croise et al., 1999; Stefan et al., 1997). Others (e.g. Mellert & Göttlein 2012) used literature review to report foliar critical levels for nutrient deficiency and derived their own critical levels.

The different criteria and the lack of background information on the sites and stands characteristics to establish foliar critical levels for nutrient deficiency created inconsistent reference values. Indeed, critical values for N and P deficiency do not represent the same tree nutritional status of deficiency between each other. To overcome this, critical levels were selected accordingly in order to increase similitudes between each threshold. The resulting scaled foliar N and P concentration, however, appeared to not show any clear relationship with the growth response to N addition. Hence, the use of non-scaled foliar N and P concentration for needle-leaved evergreen trees.

Foliar N concentration above 25-30 mg·g<sup>-1</sup> indicates a nutritional status within a high or even toxicity range (de Vries et al., 2000). Despite the fact that these observations hold mainly for Europe several, foliar critical values for N deficiency above 25 mg·g<sup>-1</sup> appeared a few times in the literature review such as for *Populus nigra* or *Tilia Americana*. Additionally, not all species present in the meta-database were found in the literature review. To derived foliar critical levels for these species, averages were calculated at the genera, family or functional type within or across all climates regions (Section 3.2.3). This adds more inconsistencies on the foliar scaling procedure bias that were discussed in the previous point.

The foliar critical level approach is limited by several conceptual shortcomings (Luyssaert et al., 2007). Besides being affected by several environmental factors such as climate, seasons, age, tree competition, stress, and sampling procedures. The critical level approach is partly dependent on the availability of other nutrients (Pritchett, 1979 as cited in Schutz & de Villiers 1987).

To overcome this problem, other techniques using foliar analysis were developed such as the Diagnosis and Recommendation Integrated System (DRIS) (Beaufils 1971 and Walworth & Sumner 1987 as cited in Gregoire & Fisher 2004; Luyssaert et al. 2004). The DRIS system is based on the concept of nutrient balance and interrelationship of nutrients, thus provides an indication of the order, from most limiting to least limiting nutrients (Schutz & de Villiers 1987). It provides a nutritional diagnosis from plant's tissue nutrient concentration independently of the age, the sampled organs (Leaves, stem, etc.) or the time of sampling (Gregoire & Fisher, 2004).

The application of DRIS is divided into three main components (Gregoire & Fisher, 2004; Schutz & de Villiers, 1987):

- (i) Creation of a database on plant analyses with corresponding yield information. These data are collected from fertilizer experiments or surveys and should represent wide ranges of environmental conditions. The more observations, the better are the precision of the DRIS norms.
- (ii) Establishment of norms: From the created database, the observations are divided into yield categories (two or three). The obtained population must have a normal distribution, thus, it is sub-divided based on their yield. Each of the available nutrient concentrations is expressed in all the possible ways (e.g.: N, N/P, N/K, N/Ca, N/Mg, N/Zn, etc.). The more combinations and the more sensitive is the DRIS norms.
- (iii) Calculation of indices: An index is derived from an equation where the plant to diagnose nutrient concentrations are compared to the previously calculated norms.

The DRIS system shows limits in predicting biomass response to fertiliser addition when plant nutrient are within the sufficiency ranges as this system focuses on the balances aspect (Benton, 1993; Gregoire & Fisher, 2004; Luyssaert et al., 2004).

The study of Luyssaert et al. (2004) developed a nutrition profile of the tree that accounts for multiple nutrient concentrations and ratios. This technique uses large-scale foliar surveys to calculate groups

of trees with similar nutrition profile. However, it does not fully discriminate types of nutrient deficiency between the different groups.

Foliar analysis is a powerful tool to assess forest nutritional status and has been widely used through different interpretation techniques (e.g. Critical Levels, DRIS, and Nutrition Profile) however, none of these techniques fully characterise forest nutrition quality in regards to tree growth responses to change in nutrient availability. Holistic approaches on plant nutritional status related to growth responses are needed in order to improve the use of foliar chemical analysis to explain or predict variations in forest C sequestration in response to N deposition (Gregoire & Fisher, 2004; Luysaert et al., 2004; Mellert & Göttlein, 2012).

#### 4.2.2 Typical Foliar Nitrogen Concentration

Background information in the TRY database on the data collection and the sites characteristics are lacking. This limits the creation of a consistent and reliable foliar nutrient concentration database.

The spatial repartition and numbers of the observations did not cover a wide diversity of environmental factors. Tropical forests were represented by only three sites, and 27 entries for broadleaved evergreen trees and 18 for broadleaved deciduous, yet they represent 44 different tropical species in total. The foliar dataset gathered through the TRY project was not sufficient to derive representative typical foliar N concentration. The small amount of data also created a bias as the tree species that have more observations had a higher relative weight in the calculated average than other species with fewer observations.

High foliar N concentrations were encountered in this foliar dataset, highlighting the impact of the small number of observation had on deriving typical foliar N concentration. As an example, *Prunus serotina* has an average N concentration in foliage of  $40 \text{ mg}\cdot\text{g}^{-1}$ , which is rather high for an average according to den Burg, (1990). This might be due to site characteristics as the data entries came from a single site. *Prunus serotina* account for almost all the records (37 out of 38) of its family (Rosaceae) within temperate climates.

Similar to the critical level approach, typical values for N concentration in foliage do not account for nutrients interrelationship and ratios, hence, the potential lack of correlation between the scaled foliar N and P ratio and the relative sensitivity to N addition.

The foliar scaling procedures impact on the correlation between the relative sensitivity to N addition and the scaled foliar N concentration using both critical levels and typical concentration is well displayed by the Figures 7a and 7b. The correlation results in two different distribution of points. This lead to the choice of using non-scaled foliar nutrient concentration in the correlation assessment that follows Section 3.3.1.

### 4.3 Correlation between the Foliar Nitrogen and Phosphorus Status and the Carbon Responses to Nitrogen Addition

The relationship between the C responses to N addition of needle-leaved forest displayed a decreasing trend with an increasing foliar N concentration, an increasing trend with an increasing foliar P concentration and a bell shaped curve with the foliar N/P ratio. However, the regression models had low coefficients of determinations indicating that the independent variables (foliar N and P concentration and foliar N/P ratio) did not perfectly explain variations in the C response to N addition. The low  $R^2$  found in this simple regression analysis are in line with the study of Li et al. (2016) that assessed the correlation of foliar P concentration and N/P ratio with the response ratio of aboveground biomass production and found an  $R^2$  of 0.067 and 0.28 respectively for a simple linear regression analysis.

The foliar N concentration did not seem to explain the C response to N addition of temperate and sub-tropical needle-leaved forests (Figure 9 and 11). These forests have been saturated by decades of N deposition that can explain the lack of growth response to N fertilisation (Fleischer et al., 2013). The study of Braun et al. (2010) found no correlation between the foliar N concentration and the N deposition and only a slight increase of the foliar N concentration with N addition. Braun explained

these results suggesting N saturated plots do not lead to more increases in the plant N tissue. This is supported by the theory of stoichiometry flexibility stating the ability of a given individual to regulate, to a certain extent, its own nutritional balances (Sistla & Schimel, 2012).

The C response to N addition of temperate forests seemed to decrease with an increasing foliar P concentration. This is in contradiction to the idea of an increasing N uptake with an increasing foliar P concentration and thus, increasing growth response to N addition. The foliar P concentration is representative of non-fertilized plots, however, the foliar P concentration tends to decrease when N is applied as a fertiliser, probably due to a reduction of P uptake by inhibition of mycorrhizae (Braun et al., 2010). These variations in the foliar P concentration could not be discriminated in the meta-analysis database. This also suggested that the foliar P concentration will not improve N uptake in an N-saturated environment.

When the data was sub-divided into two groups of high and low N addition rate, the relationship between the C responses to N addition was significantly correlated to the foliar N and P concentration and to the foliar N/P ratio for the low N application group. On the other hand, for the high N application group, variations of the log response ratio were not correlated to any of the three independent variables. This suggested that for high N rates and/ or N environments other factors might explain tree growth response to N addition. The study of Mainwaring et al. (2014), that accounts for most of the temperate and sub-tropical needle-leaved evergreen forest present in the database, found that the C responses of Douglas-fir trees to N fertilisation were mostly explained by the Ca soil availability via the soil Ca/N ratio.

As many studies suggested it, nutrient interrelationship has been found to significantly explain variations in forest C response to N addition. More and more studies show the importance of nutrient co-limitations on terrestrial ecosystems biomass productions and the importance to include them in future analysis (Elser et al., 2007; Fernández-Martínez et al., 2014; Harpole et al., 2011). The multivariate analysis in Section 3.3.3 did highlight the importance of nutrient synergies in assessing variations in forest C responses to N addition.

Soil characteristics or soil types are known to influence tree growth (Gregoire & Fisher, 2004). A study realised by Fernández-Martínez et al. (2014) on the net ecosystems production (NEP) and C use efficiency (CUE), classified nutrient-rich and nutrient-poor forests based on a combination of soil parameters and foliar nutrients concentrations and tree history. This study found strong evidence that the NEP and CUE were higher in a nutrient-rich forest than in a nutrient-poor forest. This showed that including soil group characteristics in combination of the nutritional status of the tree might lead to better results in explaining the C response of tree stand to nutrient additions.

Other environmental drivers are expected to control forest C response to N addition than the nutrients availability. In a theoretical non-nutrient limited forest the first expected factors to limit growth are light irradiance, water stress or temperature (Holdridges, 1947 as cited in Fisher et al. 2012). A second, type of factor that are expected to affect forest C responses to N additions are ozone or other toxic compounds that will affect tree health and thus reduce its biomass productions (de Vries et al., 2014). The impacts of all these environmental factors, and their interactions on forest C sequestration capacity should be further assessed in order to better predict forest growth variation to N deposition.

## **5. Conclusions**

This thesis used a meta-analysis on forest fertilisation experiments to assess the impact of foliar N and P contents on the variations in C response to N addition of forest trees. The meta-analysis resulted in 38 experimental plots mainly represented by needle-leaved evergreen trees and temperate forests. Boreal forests displayed a positive and higher C response to N addition than the temperate and tropical forests with a nearly null growth response to N addition. This supported the long-held, nutrient gradient hypothesis, stating an increase in N availability with a decreasing latitudes or altitudes.

Prior assessing the relationship between the C response to N addition and the foliar N and P contents, natural differences in foliar nutrient concentrations were scaled to a common level. This was done using two different procedures. The first procedure consisted in reviewing the literature to gather information on foliar N and P concentrations and ratio standing for insufficient or unbalanced nutritional status (sub-question 1). The second procedure consisted in deriving typical foliar N concentration of forest tree species (sub-question 2).

The literature review resulted in 112 observations representing 86 tree species. Original sources, in most of the case, could not be accessed. Foliar N and P critical levels for nutrient insufficiency varied from one study to another. This is mainly due to heterogeneity in the criteria used to established foliar critical level for N or P deficiency (e.g. visual symptoms). Foliar N/P ratios, for nutrient unbalance levels were only retained from the study of Stefan et al. (1997). Although, they do not indicate any physiological unbalances in the tree nutrition, they allow the comparison of foliar N/P ratio between countries. The study of Stefan et al. (1997) derived critical foliar N/P ratio for the following genera: Spruce, Pine, Beech and Oak. These critical levels were extended to other genera based on matching functional types with the one present in the meta-database (e.g. needle-leaved evergreen).

The global plant trait database, TRY, was used to create a large database on foliar nutrient concentration, in order to derive typical values for N concentration in foliage. After selection, only a small amount of data entries remained in the database. Therefore, the range of conditions covered by this dataset and the number of entries were too poor to derive representative typical foliar N concentration for tree species.

A common limit to both of these scaling procedures is that they did not account for nutrients interrelationship. Many different forest nutritional status diagnostics exist, such as the DRIS or nutrition profile approaches. They all have their advantages and limits, and a holistic approach to tree nutritional statuses is yet to be ascertained to predict growth responses to changes in nutrient availability.

The correlation between the C response to N addition and the foliar N and P content (sub-question 3) was assessed using the calculated relative sensitivity to N addition (in % C per Kg N added) and the scaled foliar concentration. The assumptions of variance homogeneity and normality of the residues were not met to carry a regression analysis, however, no clear relations between the variables were found using visual assessment. The foliar scaling procedures displayed a strong effect on the results as the relative sensitivity appeared to positively increase with an increasing scaled foliar N concentration by typical values and decreases with an increasing scaled foliar N concentration by critical levels.

To overcome foliar scaling effect and to meet regression analysis assumptions, the log response ratio for only needle-leaved evergreen trees was used. The C response to N addition for needle-leaved evergreen trees (hereafter simply referred as C response to N addition) showed significant correlation with the foliar N and P concentrations and the foliar N/P ratio. The C responses to N addition were decreasing linearly with an increasing foliar N concentration, indicating a shift from a deficient to a sufficient N status. The relation between the C response to N addition and the foliar P concentration had a significant model fit by its quadratic term (foliar P concentration<sup>2</sup>) implying a nonlinear response that suggested the presence of a plateau at higher foliar P concentration. The C response to N addition showed a significant correlation with the foliar N/P ratio and its quadratic terms, displaying at first a growth increase with an increasing N/P ratio till a plateau was reached, indicating

a harmonious nutrition between N and P. A decrease in growth response was then seen as the N/P ratio continues to increase, displaying a shift from a harmonious to an unbalanced nutrition.

The univariate regression analysis with the data being split into high and low N application rates showed a significant correlation between the C response to N addition for the low N application rate group and a non-significant correlation for the high N application rate group. Other parameters such as soil Ca availability might better explain the growth response to N addition as the N application rate increases.

The multivariate analysis further confirmed the nonlinear relationship between the C response and the foliar N and P concentration and N/P ratio as all quadratic terms were significant. The N application rate had a major influence on explaining the magnitude of the C response to N fertilisation. The importance of the N and P interrelationships impact on the growth response to N addition was also confirmed by a significant correlation between the C response to N addition and the third level interaction, including the foliar N and P concentration and the N application rate.

In overall, this thesis showed that tree C responses to N addition was significantly correlated to the foliar N and P contents. The nature of this relationship appeared to be non-linear and better characterised by multiple variables than a single variable with an important interaction effect of foliar N and P concentration in explaining variations in the C response to N additions. Other environmental parameters, influencing forest biomass production such as ozone toxicity, soil characteristics, both micro and macro-nutrients as well as their interactions need to be further assessed in order to improve models reliability in predicting variations in the C response of forest to change in N availability.

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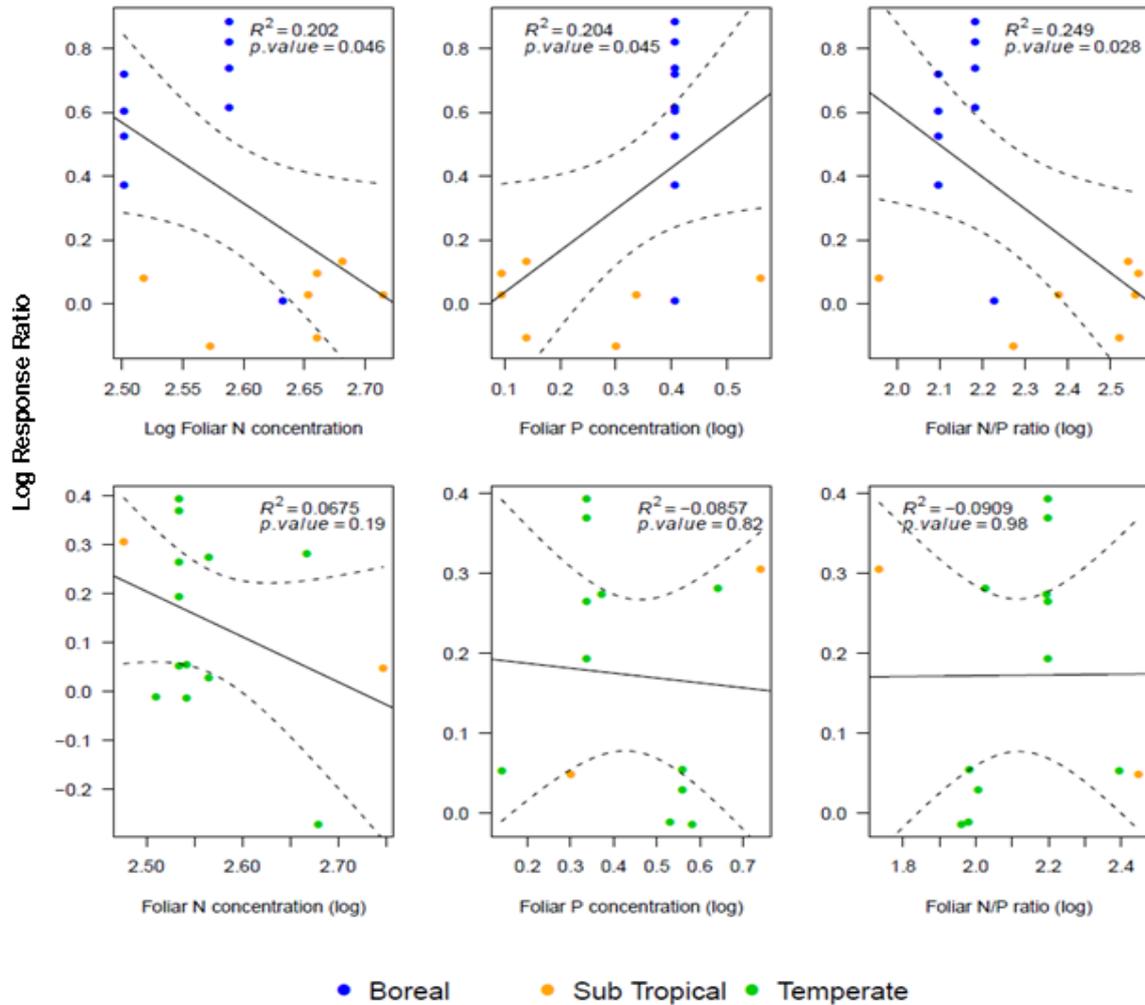
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## Appendices

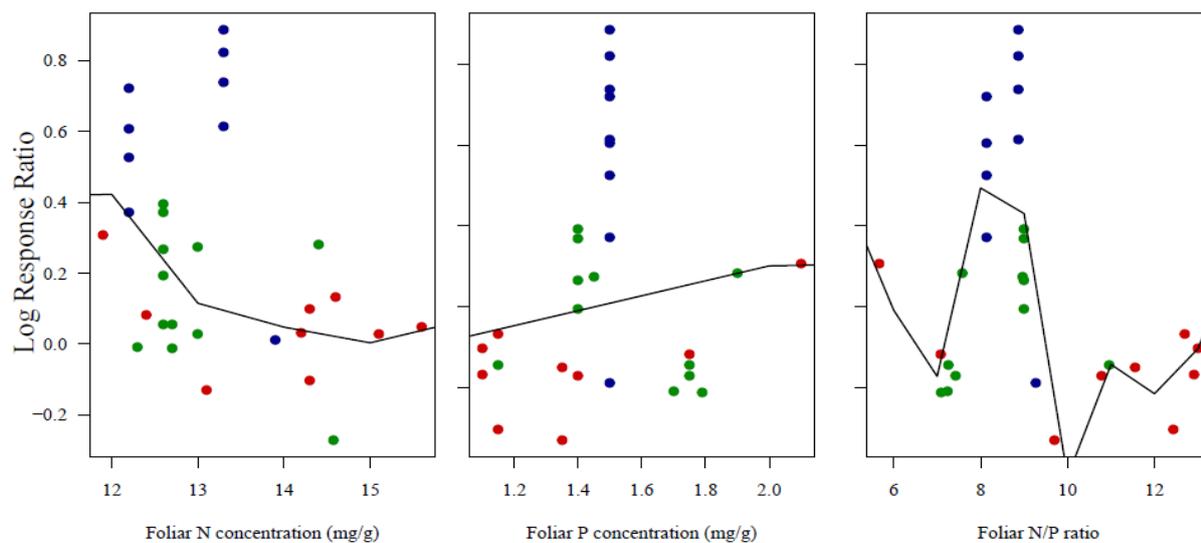
**Table A1: Presentation of the foliar N concentration database created from the TRY project dataset**

<b>Variable</b>	<b>Description</b>	<b>Type</b>	<b>Unit</b>	<b>Factors / Descriptive statistics</b>	<b>summary</b>
<b>AccSpeciesName</b>	Name of tree species	Nominal	None	154 species: <ul style="list-style-type: none"> <li>• Quercus petraea: (n=216)</li> <li>• Picea abies: (n=76)</li> <li>• Fagus sylvatica: (n=64)</li> <li>• Pinus sylvestris: (n=64)</li> <li>• Quercus robur: (n=58)</li> </ul>	
<b>Genus</b>	Genus of tree species	Nominal	None	101 genera <ul style="list-style-type: none"> <li>• Quercus: (n=297)</li> <li>• Pinus: (n=87)</li> <li>• Picea: (n=86)</li> <li>• Fagus: (n=64)</li> </ul>	
<b>Family</b>	Family of tree species	Nominal	None	51 families <ul style="list-style-type: none"> <li>• Fagaceae: (n=364)</li> <li>• Pinaceae: (n=178)</li> <li>• Rosaceae: (n=53)</li> <li>• Fabaceae: (n=18)</li> </ul>	
<b>ObservationID</b>	Unique identifier for each observation	Nominal	None	N=773	
<b>LeafN</b>	Foliar N concentration	Numeric	mg. g <sup>-1</sup>	$\bar{x}$ =17.926 $\mu$ =18.76 $\sigma$ =68.83	
<b>Funct</b>	Functional type of tree species	Nominal	None	4 functional types: <ul style="list-style-type: none"> <li>• BD (n=488)</li> <li>• BE (n=105)</li> <li>• ND (n=1)</li> </ul>	

				• NE (n=179)
<b>ClimZ</b>	Climatic regions	Nominal	None	4 climatic regions Tropical: (n=45) Sub-Tropical: (n=417) Temperate: (n=227) Boreal: (n=84)
<b>nhdep</b>	Ammonia deposition	Numeric	Kg.ha <sup>-1</sup> .yr <sup>-1</sup>	$\tilde{x}$ =7.17 $\mu$ =6.59 $\sigma$ =14.29
<b>nodep</b>	N oxide deposition	Numeric	Kg.ha <sup>-1</sup> .yr <sup>-1</sup>	$\tilde{x}$ =5.76 $\mu$ =5.39 $\sigma$ =5.03



**Figure S1: Linear regression between the logarithm of the foliar nutrients concentrations and the log response ratio of needle-leaved trees.** The upper graphs represent plots with low N application rate and the lower graphs are the plots with high N application rate. R2 is the coefficient of determination and p.value for  $\alpha = 5\%$ . The two dotted lines represent the 95% confidence intervals around the regression line.



**Figure S2: Generalized additive regression between the Log response ratio of needle-leaved trees and the foliar N concentrations and N/P ratio.** Colours represent the three climatic regions (Blue: Boreal; Green: Temperate and Red: Sub-tropical)