5 Mechanisms of N deposition effects: Review

Underlying mechanisms of nitrogen deposition effects on wood growth; shining light upon and beyond photosynthesis

Abstract

Forests have sequestered atmospheric CO$_2$ into biomass over the past decades, providing an important terrestrial C sink. The increase in aboveground woody biomass of forests is partially driven by N deposition, but underlying mechanisms for this effect remain uncertain. Ecosystem models commonly realize the positive effect of N on wood production through stimulation of photosynthesis rates. Other potential mechanisms explaining N induced growth, but less frequently (or adequately) represented by global ecosystem models, include reduced C investment in fine root production and mycorrhizal associations, and the utilization of non-structural C. We review the observational evidence on these potentially growth enhancing N effects, drawing upon fertilization experiments, meta-analysis and monitoring studies. Contrary to representations in ecosystem models, we find limited evidence for N effects on photosynthesis in mature trees and conclude that these are unlikely to fully explain observed wood growth due to N. Reduced C allocation to mycorrhizae are most promising in explaining observed direct differences in biomass production with N, although this mechanisms remains largely unquantified. Fine root production responses to N are commonly neutral or positive, therefore do not represent a plausible explaining mechanism, although uncertainties around measurement techniques remain. Non-structural C are considered likely to play an important role for N effects on wood production, however rather short–term.

We recommend stronger focus on belowground C allocation at ecosystem scale, thus considering a whole-plant C allocation approach during N fertilization experiments and monitoring efforts to unravel N effects on wood production. Uncertainties around C investments in mycorrhizae, and also FR and NSC dynamics, in response to N and other environmental drivers need to be addressed. We further recommend developing standards for BG methodologies and potential approaches for improving representations of these processes in ecosystem models.

5.1 Introduction

The enormous increase in N deposition since the industrial revolution has enriched ecosystems with N [Galloway et al., 2003, 2008], inducing a net C sink in terrestrial vegetation due to stimulated growth. The terrestrial biosphere currently acts as net sink of atmospheric C, with recent estimates

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N deposition is estimated to be responsible for 0.2–0.6 Pg C yr$^{-1}$ of this net C sink (see Chapter 3 and 4 and Zaehle et al. [2010b]). At regional and local scales, N deposition induces C sequestration at rates of 15–40 kg C kg N$^{-1}$ (Butterbach-Bahl et al., 2011, de Vries et al., 2009), mainly derived from boreal and temperate forest monitoring and experiments. Wood growth was found to increase along positive N deposition gradients in boreal and temperate climates [Magnani et al., 2007, Sutton et al., 2008, Thomas et al., 2010] and forests have responded with increased above ground (AG) wood growth in N fertilization experiments, also in the tropics [LeBauer and Treseder, 2008, Liu and Greaver, 2009, Pregitzer et al., 2008].

The underlying mechanisms of an N inducing C response are however not yet fully understood, and the quantification of N deposition effects on C sequestration in global ecosystem models is therefore hampered, which is essential for reliable future predictions. Process-based mechanisms for a wood growth-enhancing N effect have been summarized by Janssens and Luyssaert [2009] to include higher rates of photosynthesis, at the leaf and/or canopy scale, and shifts in C allocation from belowground (BG) investments to AG structures. We adopt and extend the proposed mechanisms by Janssens and Luyssaert [2009] and derive 5 potential mechanisms explaining N induced aboveground wood production (WP$_{AG}$), which we review here (mechanism A-E, Figure 5.1).

![Figure 5.1: Potential mechanisms (A-E) of stimulated aboveground wood production (WP$_{AG}$) in response to higher N availability; (A) GPP enhancement, (B) Ra reduction, (C) reduced C investment in fine roots, (D) reduced C investment in mycorrhizae, and (E) utilization of non-structural C (NSC). Note that GPP in the left column is split into autotrophic respiration (Ra) and net primary production (NPP) in the middle column, and NPP components are magnified and separated in the right column.](image)

N increases the amount of gross primary productivity (GPP), which is the amount of C assimilated through photosynthesis (Figure 5.1, mechanism A). N induced stimulation of GPP can occur at leaf level due to increased N investment in photosynthetic machinery and/or due to additional leaf area (index) (LAI) [Gough et al., 2004, Haxeltine and Prentice, 1996]. The positive connection between foliar N and photosynthesis due to the inevitable necessity of N for photosynthesis has long been recognized across functional groups and species [Field and Mooney, 1986, Kergoat et al., 2008, Peltoniemi et al., 2012, Reich et al., 1997, 1995]. Such relationships are
partly determined by functional constraints across species, but a positive relationship between N and photosynthesis has also been found within species [Kattge et al., 2009]. Assuming that higher C uptake translates directly into enhanced growth, any increase in GPP would result in proportional rise in NPP.

Changes in autotrophic respiration (R_{\text{a}}) could cause N induced wood production, if N reduces R_{\text{a}}/GPP and in turn leads to increased Carbon Use Efficiency (CUE = NPP/GPP) (Figure 5.1, mechanism B). The partition of GPP is commonly assumed to be constant at c. 0.5 CUE [DeLucia et al., 2007, Waring et al., 1998], although differences across biomes and species have emerged [DeLucia et al., 2007, Ryan et al., 1997, Tang et al., 2014]. For completeness, we will therefore review current understanding of N effects on R_{\text{a}} and whether N could explain differing CUE.

As mentioned by Janssens and Luyssaert [2009], shifts in C allocation from BG to WP_{\text{AG}} may involve shifts away from fine root (FR) and coarse roots (mechanism C, Figure 5.1) or C may be diverted from mycorrhizae (mechanism D, Figure 5.1). C allocation shifts from roots to shoots have reported to be a key response to rising N availability in Europe and North America [Erisman and Vries, 2000, Magill et al., 2004], and such shifts are well known from herbaceous plants [Krause et al., 2012]. Optimal allocation theory predicts that increased nutrient availability lessons required investments in root expansion and exploration [Bloom et al., 1985]. As another important BG component, mycorrhizae are mutualistic fungi that strongly control plant-soil nutrient and C exchange by providing nutrients (e.g. N and P) to the plant in exchange for C, so that up to 80% of plant N uptake (and 75% of P uptake) may occur via mycorrhizae [Read, 1991, Van Der Heijden et al., 2008]. N availability could reduce the reliance of trees on mycorrhizae for nutrient uptake, reducing the C costs of obtaining nutrients and consequently allowing greater C investments in WP_{\text{AG}}. This has recently been proposed to be the responsible mechanism for observed increases in wood production due to N in mature trees[Talhelm et al., 2011].

A fifth mechanism concerns the utilization of non-structural C (NSC) with higher N availability, which could induce WP_{\text{AG}} by activating stored NSC (mechanism E, Figure 5.1) [Fatichi et al., 2014, Körner, 2003], which aids plants to cope with unfavourable conditions such as droughts [van der Molen et al., 2011] and reflect the plants balance between C assimilation (source) and C demand for growth and maintenance (sink) [Chapin et al., 1990, Körner, 2003]. N exerts fundamental controls on growth due to stoichiometric constraints of expanding tissue. If N requirements for growth (or other nutrients for that matter) are not met, assimilated C may enter storage pools and added N at a later stage may induce growth due to the utilization of such storage C [Fatichi et al., 2014, Körner, 2013]. The growth limitation hypothesis, entails that growth is limited by environmental conditions and nutrients, thus not only indirectly via limitations on C assimilation [Körner, 2003].

Other NPP components include root exudation, reproduction, volatile organic C, active N/P uptake, or the production of other complex molecules [Drake et al., 2012, Vicca et al., 2012]. Most relevant for C–N interactions could be root exudation of C, estimated to be 1–5% of GPP [Grayston et al., 1997], but considerably affecting rhizospheric dynamics [Finzi et al., 2015, Phillips et al., 2011]. Due to data paucity on N effects on these NPP components and the rather small C allocation fractions assigned to them, we regard their potential for explaining N induced WP_{\text{AG}} as low and do not discuss them here further.

Many terrestrial ecosystem and landsurface models include N cycle dynamics and C–N interactions, albeit focusing on N effects on C assimilation and to a lesser extent on C allocation.
and BG dynamics [De Kauwe et al., 2013, Zaehle et al., 2014]. Most C–N ecosystem models incorporate a linear connection between foliar N and the photosynthesis parameter, $V_{c_{max}}$, controlling GPP, which levels off at a certain maximum [Smith et al., 2014, Wang et al., 2010, Zaehle et al., 2010b]. C allocation schemes vary among models, while some have replaced fixed schemes with functional dependencies, e.g. including a negative N control on root allocation (see overview in De Kauwe et al. [2013]). Separation of FR and coarse root pools is not always realized, and on top of this, coarse roots may be classified as wood, compromising a clear AG–BG separation [Smithwick et al., 2014]. Most models also consider the dependency of respiration rates on N content of the tissue, causing higher rather than lower respiration with increasing N concentrations [Zaehle et al., 2014]. Explicit representations of mycorrhizae and NSC dynamics are commonly neglected in global ecosystem models [Fatichi et al., 2014, Hobbie, 2006].

In contrast to the N-photosynthesis focus in terrestrial ecosystem models, it has increasingly emerged that other factors than GPP are likely exerting strong controls on forest biomass production (BP). In a global forest dataset (n=49) [Luysseart et al., 2007, Vicca et al., 2012] fertile forests allocated a greater portion of GPP to biomass ($58 \pm 3\%$) than low-nutrient forests ($42 \pm 2\%$), a difference of 16% in biomass production efficiency (BPE = BP/GPP) [Vicca et al., 2012]. Also Litton et al. [2007] found a higher fraction of GPP invested in wood in fertile forest compared to low-fertility forests. GPP alone is thus not the sole predictor of BP and consequently Mercado et al. [2011] was able to explain only $c. 30\%$ of observed stem growth with modeled GPP estimates in the Amazon Basin. The observed shifts in BPE with N availability may only be explained by changes in NPP allocation or the overall separation of GPP into NPP and $R_a$ (mechanisms B-E), suggesting that internal processes, independent of GPP (mechanism A), are likely further controlling N effects on BP.

Based on this apparent mismatch between the modeled and observed C–N effects, we summarize and evaluate observational evidence on the relevance of each above-mentioned mechanism in explaining a positive N effect on $W_{AG}$ in mature forest ecosystems (mechanism A-E, Figure 5.1). We draw upon monitoring studies, fertilization experiments and meta-analyses hope to constrain the likely acting mechanisms of plant growth enhancing effect of N deposition and inform future model development. The proposed mechanisms are discussed in sequence; A: N effects on GPP in section 5.2.1, B: N effects on $R_a$ in section 5.2.2, C: N effects on FR allocation in section 5.2.3, D: N effects on mycorrhizae in section 5.2.4, and finally E: N effects on NSC in section 5.2.5). Subsequently we discuss implications of observational evidence for future measurement and modeling efforts in section 5.3.

5.2 Main literature review

5.2.1 N effects on GPP

Below we summarize observational evidence to test hypothesis that N availability has a positive effect on $W_{AG}$ via increases in GPP (mechanism A, Figure 5.1). We expect that to be case only if certain conditions are met: (1) Leaf-level photosynthesis is limited by N (stage 1 of N saturation Aber et al. [1998, 1989]) and the forest does not suffer from any other limitations acting on photosynthesis, e.g. supply of light, water, other essential nutrients, or high concentrations of ozone or heavy metals, and (2) The canopy is not closed so that investments in additional leaf area are beneficial. Thus under N limited photosynthesis and an open canopy, the combined optimal investment of C and N in leaf area and leaf-level photosynthesis is thought to elevate GPP.

In both cases, growth itself is limited by C, so that it is beneficial for the plant to invest N
in leaf area and/or leaf-level photosynthesis in order to assimilate more C. GPP increases with N availability until photosynthesis is not limited by N anymore, and leaf area expands until canopy closure, or until growth is not limited by C any longer. The positive N effect on GPP is thus non-linear along an axis of time and/or total N addition, comparable to the non-linear response of foliar N or other indicator variables according to the N saturation hypothesis [Aber et al., 1998, 1989].

**N effects on foliar N**

Foliar N is commonly measured and monitored due to its relatively low cost, and the vast majority reports an increase in foliar N after N fertilization or across gradients of N deposition (Table 5.2.1), but see also [Boggs et al., 2007, Liu et al., 2013, Magill et al., 1997]). The translation of N availability into foliage N is well documented but its functionality is not, as N may not exclusively be invested in photosynthetic machinery. The foliar N photosynthesis relationship may in fact be de-coupled at the high end of foliar N. Presumably due to other factors becoming limiting to photosynthesis, additional N fails to translate into increased rates of photosynthesis but rather serves storage purposes [Bauer et al., 2004, Kattge et al., 2009]. Unfortunately, few studies are able to assess the functionality of foliar N during controlled fertilization experiments in mature forests, thus its exact role in controlling GPP and subsequent growth in mature forests remains uncertain.

**N effects on GPP from fertilization experiments**

Direct studies of N effects on photosynthesis in mature trees, involving direct measurements at the leaf-level in experimental control design are rare, but have recently been conducted at a number of sites [Krause et al., 2012, Talhelm et al., 2011, Wortman et al., 2012]. We combined those with earlier research [Bauer et al., 2004, Brix and Ebell, 1969, Elvir et al., 2006, Schaberg et al., 1997, Teskey et al., 1994], and have thus summarized results from 8 independent studies assessing N effects on photosynthesis in mature forests (Table 5.2.1). At two sites, multiple species were assessed for leaf-level photosynthesis responses to N (n=3 in Elvir et al. [2006] and Wortman et al. [2012]), leaving us with 12 semi-independent observations in mature tree stands.

Significant positive effect of N fertilization on leaf-level photosynthesis were made in 3 cases (Table 5.2.1, Teskey et al. [1994], Elvir et al. [2006] and Wortman et al. [2012]). In Teskey et al. [1994], positive photosynthesis effects occurred in combination with foliar N increases, but the response was too small to explain increases in LAI and biomass, and could also not be detected at a later stage [Murthy et al., 1996]. In Elvir et al. [2006], leaf-level photosynthesis (and foliar N) responded positively in Sugar Maple stands, accompanied by increased wood growth [Elvir et al., 2003]. Sugar Maple was the only species (out of 3) with a positive response in photosynthesis to N fertilization in the experiment (see also Elvir et al., 2010]). In Wortman et al. [2012], high-light photosynthetic efficiency increased by 11% in an Oak/Chestnut/Birch stand following N treatment, however leaf N and potentially accompanying growth effects were not assessed.

In the vast majority of cases (7/12) no evidence of a photosynthetic response to N fertilization in mature trees could be found (Table 5.2.1). Brix and Ebell [1969] found no response in leaf-level photosynthesis after 3-years, while wood production and LAI responded positively. No evidence for leaf-level photosynthesis increase was found after 8-year treatment of Red spruce in NE United States [Schaberg et al., 1997]. In this case, effects on wood production were not assessed and the lack of photosynthetic response, despite elevated foliar N, was attributed to lower foliar Ca and Mg levels [Schaberg et al., 1997]. At the American beech site and the Red spruce site, no responses in leaf-level photosynthesis after a 13-year N treatment were detected [Elvir et al., 2006], although wood production increased at the American beech site [Elvir et al., 2003]. The lack of
photosynthetic response was again inferred to be due to limitations of other essential nutrients [Elvir et al., 2006].

Talhelm et al. [2011] extensively measured leaf-level photosynthesis after N fertilization at the Great Lakes, where previously increases in wood growth have been reported however not accompanied by changes in leaf biomass and leaf area index [Pregitzer et al., 2008]. No stimulation of leaf-level photosynthesis by fertilization could be identified by direct leaf gas exchange measurements and isotope analysis, although levels of foliar N have risen significantly. The lack of response could not be ascribed to indications or evidence of other nutrient limitations [Talhelm et al., 2011], nor could any other explanation be detected with certainty (see section 5.2.4 for further discussion). Krause et al. [2012] similarly measured no change in photosynthesis following isotope signals after a tree stem C sequestration increase of 22%. Positive changes in leaf area (and dry mass) of the not completely closed canopy were hypothesized to increase stem biomass, although convincing quantification of this effect could not be made. Changes in photosynthetic efficiency after a 1-year high N treatment in Norway Spruce remained also absent, albeit wood production was not assessed [Wortman et al., 2012].

We found two studies reporting negative N effects on photosynthetic capacity. Photosynthetic efficiency was decreased in a beech stand, albeit not significantly and without an assessment of foliar N, wood growth and LAI [Wortman et al., 2012]. In the second case, photosynthetic capacity decreased after high N treatment, with increased foliar N and without an assessment of LAI [Bauer et al., 2004]. This site has however also experienced growth reduction and higher mortality, thus experiencing detrimental effects of N saturation [Magill et al., 2004]. This observation is thus not directly relevant in the context of explaining N induced wood growth here, which we expect to occur in earlier stages of responses to accumulating N availability [Aber et al., 1998, 1989].

Direct assessments of photosynthesis have been made in combination with a positive wood growth response in 6/12 cases (bold face in Table 5.2.1). Wood growth could be linked to a positive photosynthesis response in two cases [Elvir et al., 2006, Teskey et al., 1994], while no photosynthesis response was detected in the other four cases [Brix and Ebell, 1969, Elvir et al., 2006, Krause et al., 2012, Talhelm et al., 2011]. Out of the two cases with increased wood growth and photosynthesis, for Teskey et al. [1994] the rise in photosynthesis was too small to fully explain increase in wood growth. Therefore, we found only one case where a positive growth response could be linked to a rise in photosynthesis, which was at the Sugar Maple stand and at a site where two other forest types, Red spruce and American beech, showed no response [Elvir et al., 2003, 2006].

In three cases, increased LAI and wood production occurred in response to N fertilization [Brix and Ebell, 1969, Krause et al., 2012, Teskey et al., 1994]. Leaf-level photosynthesis responses were small or absent [Brix and Ebell, 1969, Teskey et al., 1994], so that additional N was potentially directly invested in leaf area. In one case, LAI was relatively low (LAI = 3.8) and although measurements were not precise enough to detect changes in LAI with N, the authors judged that other indications were present that an increase in LAI provided the best possible explanation for the observed positive wood production [Krause et al., 2012].
Table 5.1: N fertilization experiments assessing leaf-level photosynthesis in mature trees; main reference, location, species, climate, age in years at experiment start (planting year), duration N treatment in years, followed by direction of response to N treatment (⇔=neutral, ↑=positive, ↓=negative, X=not assessed) of foliar N concentration (FN), leaf-level photosynthesis (LP), above-ground wood production (WP_{AG}), leaf area index (LAI), cases in bold face combine photosynthesis and wood growth assessment.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Species</th>
<th>Climate</th>
<th>Age #yrs</th>
<th>FN</th>
<th>LP</th>
<th>WP_{AG}</th>
<th>LAI</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teskey et al. [1994]</td>
<td>Florida, US</td>
<td>Slash pine</td>
<td>ST</td>
<td>23 (1963)</td>
<td>2</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>Small positive photosynthetic response not sufficient to explain wood growth, LAI more likely</td>
</tr>
<tr>
<td>Wortman et al. [2012]</td>
<td>Switzerland</td>
<td>Oak, chestnut, birch</td>
<td>TE</td>
<td>NA 1 X+</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Positive photosynthesis response, but WP, LAI and foliar N not assessed</td>
</tr>
<tr>
<td>Brix and Ebell [1969]</td>
<td>-</td>
<td>Douglas fir</td>
<td>-</td>
<td>20 (1944)</td>
<td>3</td>
<td>X</td>
<td>⇔</td>
<td>⇔</td>
<td>No photosynthesis and LAI response, but not sufficient explanation for WP increase</td>
</tr>
<tr>
<td>Schaberg et al. [1997]</td>
<td>Vermont, US</td>
<td>Red spruce</td>
<td>TE</td>
<td>NA 8</td>
<td>↑</td>
<td>⇔</td>
<td>X</td>
<td>X</td>
<td>No photosynthesis response, but foliar N increased, attributed to other limiting nutrient</td>
</tr>
<tr>
<td>Elvir et al. [2006]</td>
<td>Maine, US</td>
<td>American beech</td>
<td>TE</td>
<td>NA 13</td>
<td>↑</td>
<td>⇔</td>
<td>X</td>
<td>X</td>
<td>No photosynthesis response although foliar N and WP increased, attributed to other limiting nutrient</td>
</tr>
<tr>
<td>Elvir et al. [2006]</td>
<td>Maine, US</td>
<td>red spruce</td>
<td>TE</td>
<td>NA 13</td>
<td>↑</td>
<td>⇔</td>
<td>⇔</td>
<td>X</td>
<td>No photosynthesis and WP response, although foliar N increased</td>
</tr>
<tr>
<td>Talhelm et al. [2011]</td>
<td>Michigan, US</td>
<td>Sugar maple</td>
<td>TE</td>
<td>94 (1900)</td>
<td>16</td>
<td>⇔</td>
<td>⇔</td>
<td>⇔</td>
<td>No photosynthesis and LAI response although foliar N and WP increased, nutrient deficiencies not detected</td>
</tr>
<tr>
<td>Krause et al. [2012]</td>
<td>Switzerland</td>
<td>Norway spruce</td>
<td>TE</td>
<td>&lt;260 (1750)</td>
<td>14</td>
<td>⇔</td>
<td>⇔</td>
<td>⇔</td>
<td>No photosynthesis response although foliar N increased, WP stimulated by LAI increase in open canopy</td>
</tr>
<tr>
<td>Wortman et al. [2012]</td>
<td>Switzerland</td>
<td>Norway spruce</td>
<td>TE</td>
<td>NA 1 X+</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>No photosynthesis response, but WP, LAI and foliar N not assessed</td>
</tr>
<tr>
<td>Wortman et al. [2012]</td>
<td>Switzerland</td>
<td>Beech</td>
<td>TE</td>
<td>NA 1 X+</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>Negative photosynthesis response, but WP, LAI and foliar N not assessed</td>
</tr>
<tr>
<td>Bauer et al. [2004]</td>
<td>Harvard Forest, US</td>
<td>Red pine</td>
<td>TE</td>
<td>74 (1926)</td>
<td>12</td>
<td>⇔</td>
<td>⇔</td>
<td>⇔</td>
<td>Negative photosynthesis response however also associated with negative WP</td>
</tr>
</tbody>
</table>

* wood growth assessment made by Elvir et al. [2003], # wood and LAI assessment made by Pregitzer et al. [2008] + reported elemental ratios but not absolute concentrations in Wortman et al. [2012], $ assessed in Magill et al. [2004]
CHAPTER 5. MECHANISMS OF N DEPOSITION EFFECTS: REVIEW

N effects on GPP along natural gradients

Observational studies along N availability gradients provide indirect evidence of a functional relevance of N availability for photosynthesis. Mitchell and Hinckley [1993] found a higher photosynthetic rate in Douglas fir associated with higher foliar and soil N concentrations when comparing 2 trees. Also, N deposition was positively correlated with photosynthesis at the canopy scale for FLUXNET forest sites [Fleischer et al., 2013], however only for evergreen needleleaf forests below a level of 8 kg N ha$^{-1}$ yr$^{-1}$. Co-correlated climatic variables however prevented drawing an unambiguous connection between N deposition and canopy photosynthesis. Clear links to foliar N and LAI could not be made with the available data, nor was wood production assessed at flux tower sites, hampering confinement of this relationship to underlying causes [Fleischer et al., 2013]. Nonetheless, the apparent lack of any N deposition effect above 8 kg N ha$^{-1}$ yr$^{-1}$, which concerned most forests included in the study, indicated that N availability was not affecting canopy photosynthesis above this level of N deposition [Fleischer et al., 2013]. An analysis of a global forest dataset [Luysseart et al., 2007] revealed that N deposition was positively related to LAI but not to GPP, however the range of LAI values over which this assessment was made were not reported [Fernández-Martínez et al., 2014].

5.2.2 N effects on autotrophic respiration

$R_a$ is commonly positively related to tissue N concentrations [Burton et al., 2002, Chapin III, 1980, Reich et al., 2007] as higher tissue N content leads to increased maintenance costs. Foliage $R_a$ commonly varies with foliar N and photosynthesis rates [Atkin et al., 2015, Kattge et al., 2009, Reich et al., 2007], and also a large meta-analysis of $>$2500 measurements found that respiration scales linearly with N across and within species and plant organs [Reich et al., 2008]. The scaling of $R_a$ with GPP and N is however not completely straightforward: At high ends of leaf N, GPP leveled off earlier than $R_a$, reducing CUE [Campbell et al., 2007, Kattge et al., 2009]. This is thought to be due to higher respiration costs associated with dealing of excess energy and preventing cell damage [Amthor, 2000]. In another study, growing season maintenance respiration scaled with N content in 1-yr old needles, but foliage $R_a$ did not relate to photosynthesis [Vose and Ryan, 2002]. Also Bond-Lamberty et al. [2004] found no relationship between belowground $R_a$ and AG, BG or total NPP. More recently, the leaf respiration dependence on N was found to differ between PFTs, and a control of temperature that a given species prevails in was identified [Atkin et al., 2015].

The $R_a$/GPP ratio also found to increase with increasing stand age, thus rather working towards lower BPE with age, since both, $R_a$ and GPP, decline with age but GPP at a faster rate [Tang et al., 2014, Turnbull et al., 2005]. Although interactions of age and N availability, and other controlling factors, are not completely resolved, observations of N reducing $R_a$ are generally rare, and are not conveying a direct link to date. For example, reduced belowground $R_a$ occurred after N fertilization despite a threefold increase in AG biomass [Olsson et al., 2005], and in another case N addition reduced rhizosphere respiration, which accounted for the main part of reductions in soil respiration [Sun et al., 2014]. However, in both cases belowground $R_a$ was derived from total soil C efflux measurements, where confounding effects on lessened root or mycorrhizae production are challenging such assessments of N effects on BG $R_a$ (see section 5.2.3 and 5.2.4).

5.2.3 N effects on fine root allocation

Belowground plant biomass is mainly constituted in the coarse root pool, reported to contain the analogue of 10–90% of AG woody biomass, mostly ranging from 20–30% [Burton and Pregitzer,
FR biomass constitutes only c. 1–3% of total biomass Gower et al. [1992], Xiao et al. [2003], Yuan and Chen [2010], Yuste et al. [2005]. Reports of C investment in these two pools are highly variable, but in any case substantial, as 22 to 63% of total NPP was invested in FR and coarse roots across 12 sites [Waring et al., 1998]. Despite the small biomass C pool, FR involved in absorptive functioning undergo rapid turnover, and FR production was estimated to account for c. 22% of global NPP [McCormack et al., 2015]. Other estimates of NPP fractions allocated to FR production are 32% in boreal ecosystems [Yuan and Chen, 2010], 27 ± 11% in tropical ecosystems [Malhi et al., 2011], and 10–36% at stand scale [Xiao et al., 2003, Yuste et al., 2005]. We focus on FR responses to N here (mechanism C, Figure 5.1), as we expect physiological responses to (changing) environmental conditions to occur in FR [Smithwick et al., 2014]. Furthermore, FR responses to N availability are frequently assessed, while direct assessments of coarse root responses to N are rare.

**N effects on fine root biomass**

FR biomass responses to N fertilization are highly variable, but most frequently are reported as reduced or neutral [Axelsson and Axelsson, 1986, Gower and Vitousek, 1989, Haynes and Gower, 1995, Vogt et al., 1987]. A recent meta-analysis derived a significant albeit weak negative relationship between FR biomass and soil fertility in boreal forests [Yuan and Chen, 2010]. Neutral FR biomass responses to N addition were reported at the long-term N experiments at Harvard and Aber forest [Emmett et al., 1995, Magill et al., 2000, 2004], although positive AG productivity responses occurred.

Similarly, BG biomass, combining FR and coarse roots, was not related to N deposition in an analysis of a global forest dataset, while AG biomass and LAI were positively related to N deposition (see also section 5.2.1; Fernández-Martínez et al. [2014]). Most available studies thus detect an increase in shoot:root biomass ratio with N due to AG stimulation, and reduced or neutral responses in BG biomass. Positive response of FR biomass to N fertilization on the other hand have mostly been linked to low initial N conditions [Davis et al., 2004, Raich et al., 1994, Stober et al., 2000].

**N effects on fine root production**

Due to considerable FR turnover rates, responses of FR production to N are expected to be most relevant for evaluating underlying mechanisms for N induced WP and AG. FR production responses to N are however strongly debated; so was FR production hypothesized to increase with N availability due to higher turnover and mortality rates, in turn leading to frequently observed FR biomass reductions [Nadelhoffer, 2000]. Reports of reduced FR production with fertilization in concert with increased AG production (i.e. litterfall) were however also made by Haynes and Gower [1995] and others. Reconciling all reports of FR responses remains challenging as applied methodologies differ, their assumptions are hard to test and different methods often yielded contrasting results [Haynes and Gower, 1995, Malhi et al., 2011, McCormack et al., 2015, Nadelhoffer, 2000, Smithwick et al., 2014].

Recently, a meta-analysis of FR production in response to N addition, as well as an assessment along a natural nutrient gradient, has supported the positive response of FR production to N availability [Yuan and Chen, 2012]. The authors estimated a 27% increase in FR production globally, which is slightly lower than the estimated 30% AG response of NPP [LeBauer and Treseder, 2008]. An earlier meta-analysis of N fertilization experiments [Liu and Greaver, 2010] showed an overall neutral response in FR litter production, which was only positive in temperate
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Some further indirect evidence was found in a meta-analysis by Janssens et al. [2010], where reduced soil C respiration in response to N was linked to reduced microbial biomass, which could however not be linked to reduced FR production. It was therefore hypothesized that reduced C allocated to mycorrhizae must underline the reduced supply of C to soil microbes [Janssens et al., 2010]. The dominant type of mycorrhizae present is likely further influencing the tree’s responses to N, e.g., tree species associated to arbuscular mycorrhizae responded with positive growth to N deposition and all tree species experiencing reduced survival due to N were associated to ectomycorrhizae [Thomas et al., 2010].

There is thus considerable evidence linking mycorrhizal dynamics to N availability, commonly suggesting reduced mycorrhizae abundance or colonization with N, however clearly not exclusively and no changes and increased abundance are also observed [Mohan et al., 2014]. Furthermore, elevated CO$_2$ commonly increases mycorrhizae abundance, and many other interactions with environmental drivers such as warming or P fertilization, as well as the load of N availability,
further control ecosystem responses [Mohan et al., 2014, Treseder, 2004]. Also, as mentioned above, differing species-level responses of tree–mycorrhizal associations, and resulting shifts in mycorrhizae communities are often observed, which likely alter overall C demand [Cox et al., 2010, Egerton-Warburton and Allen, 2000, Mohan et al., 2014]. Only few studies have actually addressed mycorrhizae production and C investment response to N availability [Mohan et al., 2014]. Therefore ecosystem scale mycorrhizae C allocation in response to N availability remain poorly understood and drawing conclusions thereon from observations made to date is challenging if not impossible.

5.2.5 N effects on non-structural C dynamics

Non-structural carbon (NSC) reserves may be substantial, holding c. 2 times the amount of canopy C in evergreen needleleaf and up to 4 times in deciduous trees [Hoch et al., 2003]. In temperate forest trees NSC have been estimated to contain 8% of living biomass [Richardson et al., 2013], thus are large enough to support noticeable growth. NSC reserves are highly dynamic and mean concentrations are generally maintained to be no less than 55% of their maximum [Hoch et al., 2003]. Importantly, NSC reserves may be accessible to plants up to a decade after that C was assimilated [Richardson et al., 2013].

Dynamics of NSC pools could explain, or at least contribute to, N induced WPAG if C from NSC were utilized for such growth stimulation. Under conditions were N is limiting growth, assimilated C could accumulate as NSC, which is then utilized for wood production when N becomes available again. Direct observations of N effects on NSC are infrequent to date, but N fertilization was found to reduce the amount of glucose and fructose in leaves and stems [Lavola and Julkunen-Titto, 1994] and C investment in NSC decreases with higher N availability [Peñuelas and Estiarte, 1998], which both supports NSC mediation of N controls on growth. More recently, Doughty et al. [2015] found a significant temporal decoupling of photosynthesis and plant C expenditure in the Amazon, implying an underlying control of NSC. Others have also found that NSC provides a conclusive explanation for observed time lags between C assimilation and growth [Gough et al., 2009, Richardson et al., 2013], and NSC dynamics have been linked to other environmental conditions limiting growth [Fajardo et al., 2012, Körner, 2003]. N availability clearly has the potential to exert similar controls on NSC dynamics, as a major limiting nutrient for growth and functioning. However, direct linkages between N induced wood production and the utilization of storage C have not been made to our knowledge (see also Fatichi et al. [2014]).

5.3 Discussion

5.3.1 Likelihood of mechanisms

Following the presented literature review, we regard it as highly unlikely that the N–photosynthesis effect fully explains observed N effects on wood production in mature trees (mechanism A, Figure 5.1, section 5.2.1). Despite known clear relationships between N and photosynthesis, unambiguous evidence for an N enhancing wood production effect in mature trees via stimulation of photosynthesis did not emerge. Only in one study, out of six who combined photosynthesis and wood production measurements, could positive wood production be associated to a rise in leaf-level photosynthesis in mature trees (Sugar Maple in Elvir et al. [2006]). In addition, only in 3 out of 12 studies, assessing photosynthesis after N addition, could a positive response be detected. Thus for the majority of mature forest stands under investigation, photosynthesis was not limited by N,
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although positive $WP_{AG}$ still occurred. This conclusion drawn from a systematic assessment of N effects on photosynthesis from fertilization experiments in mature forest stands clearly supports the finding by Vicca et al. [2012] and others [Fleischer et al., 2013, Litton et al., 2007, Mercado et al., 2011], who have previously demonstrated that other factors beyond N effects on photosynthesis must play an important role in determining wood production.

Given current observational evidence, we consider the possibility that reductions in $R_a$ explain observed increases in wood production due to N in mature forests as highly unlikely (mechanism B, Figure 5.1, section 5.2.2). Although direct assessments are challenging and N may affect whole-tree $R_a$ indirectly by changes in C (and N) allocation, so that we cannot completely rule out any reductions in whole-tree $R_a$ with N. The literature however generally supports the positive connection between N tissue content and $R_a$, and presented no compelling reason to assume that reduced $R_a$ is of relevance for explaining increased $WP_{AG}$.

We consider the reduction of FR production or biomass due to N as unlikely in explaining the observed N effect on wood production (mechanism C, Figure 5.1, section 5.2.3). FR biomass and production responses to N should not be equated and may exhibit different responses [Nadelhoffer, 2000]. For FR biomass, observations suggest a general reduction with N, although reports vary. Since the FR biomass pool is however very small, potential reductions in FR biomass due to N are unlikely to explain decreases in $WP_{AG}$. FR production has been most frequently reported to respond neutral or to increase with N by a number of recent meta-analyses, while generally supporting the assumption of increased shoot:root ratios due to N [Bloom et al., 1985, Janssens et al., 2010, Liu and Greaver, 2010, Yuan and Chen, 2012]. Thus although shoot production was more favored, we expected responses in FR production to be the inverse of AG responses in order to explain N induced $WP_{AG}$, so that an actual diversion of C is taking place, which was however not the case.

Many lines of evidence for N induced reduction in C investments to mycorrhizae exist and we regard the diversion of C from mycorrhizae in favor of $WP_{AG}$ as a plausible explanation (mechanism D, Figure 5.1, section 5.2.4). However, quantified assessments of C investment in mycorrhizae (and in combination with N induced wood production) are lacking so that we can only join the speculation and make an informed judgment on its likelihood [Krause et al., 2012, Talhelm et al., 2011]. Observations do however indicate potentially large C investments in mycorrhizae, have demonstrated their responsiveness to environmental drivers, including N, and their control on ecosystem functioning [Cox et al., 2010, Mohan et al., 2014, Pritchard et al., 2014, Thomas et al., 2010, Treseder, 2004]. We highly recommend considering mycorrhizae as a potential explanation for observed $WP_{AG}$ due to N.

We regard the utilization of NSC with N availability as a likely contributor to induced $WP_{AG}$. Although no direct observations exist and its relevance remains speculative, studies clearly demonstrate the substantial size of the NSC pool, its relevance in determining growth and its dynamic response to environmental conditions. Thus the utilization of NSC due to rising N availability is a likely relevant mechanism controlling N induced wood production, in particular in explaining relatively short–term lag effects between C assimilation and wood production.

5.3.2 Implications for measurements

Gas exchange measurement techniques assessing photosynthesis are well established and has arguably led to the C assimilation focus in terrestrial ecosystem modeling to date. We however
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recommend to re-evaluate the common assumption that photosynthesis dominates N effects on wood production. We recommend investigating N effects on leaf-level photosynthesis in mature trees, more thoroughly, in concert with foliar N, LAI and wood production, as has been undertaken in some recent efforts [Krause et al., 2012, Talhelm et al., 2011, Wortman et al., 2012]. Leaf-level photosynthesis measurements must allow integration over time due to the non-linear nature of processes [Gough et al., 2004], as well as an integration over the whole canopy, for which flux measurements have proven to be useful [Fleischer et al., 2013].

Uncertainties associated to FR responses to N were mostly related to current measurement techniques and lack of standards [Malhi et al., 2011, McCormack et al., 2015, Nadelhoffer, 2000, Smithwick et al., 2014]. We therefore highly recommend future refinements of these. Although we judged FR responses as unlikely to explain N effects on WP, we recommend incorporating FR assessments as a standard in ecosystem monitoring. Consistent classification and measurement strategies for roots needs to be developed and adopted, including the separation of C allocated to root biomass, root production, and other components such as root exudates, as has recently been developed by McCormack et al. [2015]. FR responses can lead to significant changes in the rhizosphere and subsequently on C–N feedbacks, which requires future investigation [Finzi et al., 2015, Phillips et al., 2011].

Measurement techniques for assessing mycorrhizae lack behind other BG components, in particular assessments of actual C investments in mycorrhizae. To date, estimates of C investments in mycorrhiza are often based on budget analysis of unaccounted components (and their uncertainties) and represent therefore the largest unknown in C allocation studies [Malhi et al., 2011]. Furthermore, techniques of C allocation to and within mycorrhiza are often based on differing C budget calculations so that direct comparisons are hampered [Hobbie, 2006]. Additionally indicator types of mycorrhizae dynamics are manifold, and the suitability of abundance measures, colonisation rates, or flowering events, etc., as indicators for plant C investments remains uncertain. We strongly recommend developing a standards for C allocation to mycorrhizae. Such framework should aim for integration with FR dynamics, given that a conceptual separation of root and mycorrhizae activity may be unrealistic [Drake et al., 2012, McCormack et al., 2015].

Respiration measurements at organ level are well established, although not without pitfalls, but whole-plant assessments of Ra are challenging so that BP is often used as a proxy for NPP or tissue-level measurements of Ra are up-scaled with given assumptions. Sufficient observational evidence suggests that the former approach is not reasonable [Vicca et al., 2012]. BG assessments of Ra are challenging due to difficulties in separating Ra from heterotrophic components, and the fact that published estimates of root respiration vary in their definition thereof [Bond-Lamberty et al., 2004]. We recommend to attempt to standardize definitions and measurement techniques thereof, and to further aim for novel approaches addressing soil and rhizosphere C dynamics (see above).

NSC measurements have been mainly conducted in association to temperature limitations [Körner, 2003] and there has been no study relating NSC dynamics with N supply and wood production to date, as Fatichi et al. [2014] pointed out. We recommend studying NSC dynamics after N fertilization in mature trees to resolve magnitude and time components of such effects, ideally in concert with estimations of the above-mentioned processes in a whole-plant C allocation scheme.
5.3.3 Implications for modeling

The focus on photosynthesis in implemented C–N interactions in terrestrial ecosystem models, is likely underestimating the influence of C allocation and BG processes on observed wood production. We recommend developing and exploring modeling approaches that modify the strong control of GPP on NPP, and the subsequent determination of biomass and wood production, currently implemented in global scale ecosystem models. This literature review and other work has convincingly shown that internal C allocation dynamics, BG dynamics and in particular N effects thereon can alter wood production substantially, which need to be adopted and explored by ecosystem models. Broadly speaking, C allocation schemes need to reflect functional relationships between environmental conditions, resource acquisition and C investments. Model evaluations with elevated CO\textsubscript{2} experimental data (FACE) showed that models with functional relationships of C allocation among roots, wood and foliage performed best, while fixed allocation coefficients did not prove suitable in predicting the elevated CO\textsubscript{2} response [De Kauwe et al., 2014].

Current representation of root processes in ecosystem models hinders correct quantification of biosphere processes [McCormack et al., 2015, Smithwick et al., 2014]. Ecosystem models currently lack dynamic FR responses to altered soil conditions [Smithwick et al., 2013] which are necessary to predict ecosystem responses to N. A two-pool FR approach, splitting into an absorptive and transport pool (equivalent to leaves and twigs), has already successfully been adopted [Gaudinski et al., 2010, Parton et al., 2010] and allowed for more accurate representations of root processes, reducing uncertainty in root production and turnover. Including such dynamics would also allow modeling reduced wood growth with high N due to root stress [Smithwick et al., 2013]. Such adverse N effects are likely to be increasingly important as vast areas receive high doses of N deposition for decades now [Lamarque et al., 2013].

Global ecosystem models do not consider mycorrhizal associations yet, despite their recognized importance in forest C cycling and functioning for decades [Read, 1991]. The substantial uncertainty surrounding mycorrhizae controls on plant (and soil) C cycling, and the resulting C–N interactions, as well as the relatively limited knowledge on the diverse ecology and distribution of e.g. >1000 different species of ectomycorrhizal fungi, might explain this mismatch. Nonetheless, several modeling approaches have been developed recently to include mycorrhizae in ecosystem models, e.g. the framework by Phillips et al. [2013], or the optimal C allocation scheme by Brzostek et al. [2014] based on the FUN model [Fisher et al., 2010]. Model approaches should aim for an approach that integrates FR and mycorrhizae dynamics due to their near inseparable nature [McCormack et al., 2015]. Some small-scale models have developed dynamic NCS pool approaches more than two decades ago [Cropper and Gholz, 1993], and have shown to improve in particular the representation of time lag effects between C assimilation and growth when considering a two-pool NSC approach [Gough et al., 2009, Richardson et al., 2013]. A more elaborate approach of C sink-source modeling has been proposed by Fatschi et al. [2014], which would allow among others for the inclusion of NSC, as well as mycorrhizae, albeit many unquantified processes and parameters likely remain to date.

5.4 Conclusion

Given the observational evidence, it is highly unlikely that the current mechanisms of C–N interactions incorporated into global ecosystem models are truly representative of the underlying ecological processes. The strong focus on N controls of photosynthesis in ecosystem models is unlikely to fully explain observed N induced wood growth. Reduced C investments in mycorrhizae
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with rising N availability, is likely to further control that response. FR production was less likely to explain N induced wood growth, given observational evidence to date. However, large uncertainties around the quantification of both BG components remain. We recommend placing a stronger BG focus during observational efforts and experimental studies, and attempting whole-plant C allocation budgets. Allocation shifts to WPAC away from mycorrhizae, could not explain long-sustained increases in wood production with N, but rather would result in a transition to higher BPE with N. Similarly, NSC has the potential to provide C for N induced wood growth, but likely limited to short-term lag effects. The literature review has shown that the focus on N controls on GPP should be complemented with N controls on BG C investments to mycorrhizae and FR, as well as internal plant C allocation to NSC pools.

It is clear that internal plant C allocation dynamics can only shift the efficiency of BP from GPP, thus BPE. It is therefore constrained by GPP and any further long-term increase in BP must be sustained by increases in GPP. Furthermore, increases in BP due to N may only partly explain the observed terrestrial C sink, and it needs to be further elucidated to what degree the additional C allocated to wood has counteracted C that would have otherwise been lost from ecosystems. This literature review has highlighted the potential of C allocation dynamics in determining N effects on BP, and similar assessment of N effects on soil C dynamics, and C turnover dynamics, are required for completing the assessment of N effects on the terrestrial C sink.

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