Research paper

Growth enhancement of *Picea abies* trees under long-term, low-dose N addition is due to morphological more than to physiological changes

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Human activities have drastically increased nitrogen (N) inputs into natural and near-natural terrestrial ecosystems such that critical loads are now being exceeded in many regions of the world. This implies that these ecosystems are shifting from natural N limitation to eutrophication or even N saturation. This process is expected to modify the growth of forests and thus, along with management, to affect their carbon (C) sequestration. However, knowledge of the physiological mechanisms underlying tree response to N inputs, especially in the long term, is still lacking. In this study, we used tree-ring patterns and a dual stable isotope approach (δ13C and δ18O) to investigate tree growth responses and the underlying physiological reactions in a long-term, low-dose N addition experiment (+23 kg N ha⁻¹ a⁻¹). This experiment has been conducted for 14 years in a mountain *Picea abies* (L.) Karst. forest in Alptal, Switzerland, using a paired-catchment design. Tree stem C sequestration increased by ~22%, with an N use efficiency (NUE) of ca. 8 kg additional C in tree stems per kg of N added. Neither earlywood nor latewood δ13C values changed significantly compared with the control, indicating that the intrinsic water use efficiency (WUE) (A/gs) did not change due to N addition. Further, the isotopic signal of δ18O in early- and latewood showed no significant response to the treatment, indicating that neither stomatal conductance nor leaf-level photosynthesis changed significantly. Foliar analyses showed that needle N concentration significantly increased in the fourth to seventh treatment year, accompanied by increased dry mass and area per needle, and by increased tree height growth. Later, N concentration and height growth returned to nearly background values, while dry mass and area per needle remained high. Our results support the hypothesis that enhanced stem growth caused by N addition is mainly due to an increased leaf area index (LAI). Higher LAI implies that more photosynthetically active radiation is absorbed and therefore canopy-level photosynthesis is increased. We conclude that models assuming that N deposition increases tree growth through higher leaf-level photosynthesis may be mechanistically inaccurate, at least in forest canopies that are not (yet) completely closed.

**Keywords**: basal area increment, carbon isotope, leaf area dynamics, long-term nitrogen addition, nitrogen deposition, Norway spruce, oxygen isotope, tree rings.

Introduction

Nitrogen (N) is the quantitatively most important mineral nutrient for the growth of all organisms. Large amounts are required as an essential component of proteins and nucleic acids. In many pristine terrestrial ecosystems, net primary production is limited by N (LeBauer and Treseder 2008, Vitousek and Howarth 1991). Since the rise of industrialization and particularly the invention of the Habor–Bosch process, intensive livestock farming and massive fossil fuel combustion have dramatically increased the input of reactive N (NOₓ, NH₃) into terrestrial systems through atmospheric deposition (Galloway et al. 2004).
Today, globally, anthropogenic N sources are more than double the natural biological N fixation (Galloway et al. 1995). Deposition rates are predicted to remain high in industrialized countries and to increase in the developing world (Galloway et al. 2004). In particular, European forests receive up to 60 kg N ha$^{-1}$ a$^{-1}$ (MacDonald et al. 2002). As a result, forest ecosystems are shifting from being naturally N-limited to N-eutrophic or even to N-saturated (Aber et al. 1989, Dise and Wright 1995, Fenn et al. 1998). In trees, enhanced N availability can cause increased tissue N concentrations and thus higher leaf-level photosynthetic rates, leading to growth stimulation (Magill et al. 2004). However, if other factors such as light, water or other nutrients become limiting, the effectiveness of N is restricted (Aber et al. 1995, Magill et al. 1997, Bauer et al. 2004, 2010). Ongoing high-N inputs can further cause tree decline or even tree death (Schulze 1989, Aber et al. 1998, Magill et al. 2004, Hyvonen et al. 2008).

Most of our knowledge about the effects of increased N input is based on a large number of fertilizer experiments (e.g., Kenk and Fischer 1988, Miller and Miller 1988, Saarsalmi and Mäkönen 2001), while to date there are only a few experiments that investigate the effect of increased chronic atmospheric N deposition (Boxman et al. 1998, Schleppi et al. 1999b, Magill et al. 2000, Högb erg et al. 2006, Hyvonen et al. 2008, Pregitzer et al. 2008). Since fertilizer experiments are usually conducted in the context of commercial forestry, these high-dose studies focus on the effects on tree growth and productivity. The results of the long-term N-addition experiment conducted by Högb erg et al. (2006) showed that tree growth response to N addition is non-linear, making projections of the impact of low-dose, chronic N deposition problematic if they are based on studies using high addition rates.

In times of climate change, the additional question arises whether enhanced atmospheric N deposition can help to mitigate climate change by increased C sequestration (Reay et al. 2008, De Vries et al. 2009). Nitrogen-induced growth enhancement can be measured by the N use efficiency (NUE), which is defined as the amount of biomass C sequestered per unit of N deposition. To determine this effect size, several approaches have been used in the past: (i) correlations between long-term forest growth data and N deposition (Solberg et al. 2009), (ii) tracing the fate of N with $^{15}$N-labelling experiments, combined with the C/N ratio of the related compartment (Nadelhoffer et al. 1999, Krause et al. 2012), (iii) model-based simulation of the C response to N deposition (Sutton et al. 2008, Wamelink et al. 2009) and (iv) the results of long-term, low-dose N-addition experiments (Högb erg et al. 2006, Hyvonen et al. 2008, Pregitzer et al. 2008). Considering the results of all these approaches, NUE is within the range of 7–725 for total biomass (Nadelhoffer et al. 1999, Magnani et al. 2007, Sutton et al. 2008, Wamelink et al. 2009, Thomas et al. 2010), with the most common range of 15–40 for aboveground biomass (Hyvonen et al. 2008, De Vries et al. 2009). However, in some experimental studies, trees reacted with declined growth or even died due to high N loads (Magill et al. 1997, 2004; Hyvonen et al. 2008), thus resulting in a negative NUE.

However, the physiological mechanisms explaining how N deposition triggers tree growth are still under debate. Although the strong positive correlation between foliar N concentrations and photosynthetic rates is well known (Field and Mooney 1986, Evans 1989), some results of chronic N deposition studies showed no increase in leaf-level photosynthesis despite a significant increase in foliar N concentration (Schaberg et al. 1997, Elvir et al. 2006, Talhelm et al. 2011). Some authors suggest that other nutrients such as magnesium (Mg) or calcium (Ca) limited photosynthesis (Schaberg et al. 1997, Bauer et al. 2004, Elvir et al. 2006). However, some other processes have also been observed to affect leaf physiology and morphology: increases in foliar respiration (Schaberg et al. 1997), changes in leaf size (Schaberg et al. 1997, Bauer et al. 2004, Talhelm et al. 2011) and decreases in photosynthetic NUE (Schaberg et al. 1997, Bauer et al. 2004). Therefore, a better understanding of tree physiology may be a key to better predicting future changes in forest functioning. Direct tree physiological measurements over long periods are almost impossible due to logistical and financial constraints and to the difficulty of scaling up leaf- or branch-level measurements to the ecosystem. Stable isotopes in tree rings from long-term experiments, which record physiological processes at the time the ring was formed, are a valuable tool for obtaining integrated physiological responses to environmental changes including N deposition (McCarrol and Loader 2004, Barbour 2007, Savard 2010).

The aim of the present study was to investigate the impact of long-term (14 years), low-dose N addition on (i) tree physiology and morphology and (ii) the carbon (C) sequestration potential of a temperate forest ecosystem at Alptal, Switzerland. Tree physiological responses to chronic N addition were assessed by combining the analyses of the stable isotopes of carbon ($\delta^{13}$C) and oxygen ($\delta^{18}$O) in early- and latewood cellulose. Morphological changes of the canopy were tracked by foliar morphological and chemical analyses. Changes in the C sequestration potential were investigated by measures of early- and latewood basal area increment (BAI). We compared our tree-ring-based NUE estimates with those previously obtained from $^{15}$N fate at the same site.

Material and methods

Study site and low-dose N addition

The experimental site of this study is located in the Alptal valley, on the northern edge of the Alps in central Switzerland (47°02′N, 8°43′E, 1200 m a.s.l.). The climate is cold and wet
with a mean annual temperature of 6 °C and a mean annual precipitation of 2300 mm (30% as snow), reaching a maximum in June (270 mm) and a minimum in October (135 mm). The vegetation period lasts from June to September. Atmospheric inorganic N deposition is moderate with 12 kg N ha⁻¹ a⁻¹ of bulk deposition and 17 kg N ha⁻¹ a⁻¹ in throughfall, equally divided between NO₃⁻ and NH₄⁺ (Schleppi et al. 1999a). The soil C/N ratio of 19.4 (Providoli et al. 2006) indicates that N availability is moderate. Parent rock material is flysch, and soils are clay-rich gleysols with an average of 48% clay, 46% silt and 6% sand, implying a low permeability and a water table close to the surface throughout the year (Hagedorn et al. 1999). Two different soil types are determined by the distinct microtopography: (i) on mounds, umbric gleysols with mor (raw humus), Ah and oxidized or partly oxidized Bg or Br horizons; (ii) in depressions, where the water table frequently reaches the surface, mollic gleysols with an anmoor (muck humus) topsoil, a thin Lf horizon and an almost permanently reduced Bg or Br horizon (Hagedorn et al. 2001). On average, soils are snow-covered from mid-November to April. The slope is ~20% with a west aspect. The naturally regenerating mature Norway spruce (Picea abies (L.) Karst.) stand (15% Abies alba Mill.), with trees up to 260 years old, has a relatively low single-sided leaf area index (LAI) of 3.8 (Schleppi et al. 1999b). The average dominant tree height is 30 m. According to humus type and light conditions, a well-developed ground vegetation forms patches of different botanical associations (Schleppi et al. 1999b).

A low-dose N-addition experiment was conducted since April 1995 on two small catchments (each ~1500 m²) within the spruce forest. One catchment was subjected to an increased average deposition of ~23 kg N ha⁻¹ a⁻¹ as NH₄NO₃—(Table 1) added to rainwater and sprinkled 1.5 m above ground, i.e., under the tree canopy but above the ground vegetation (Schleppi et al. 1998). A second catchment was acting as a control, receiving only rainwater from the sprinkler system. The treatment was applied automatically during precipitation events (~200 times per year) to mimic natural atmospheric N deposition as realistically as possible. The water used for irrigation was replaced by the occasional application of a concentrated NH₄NO₃ solution on the snow, using a backpack sprayer.

**Assessment of tree growth and isotopic analyses**

Within each of the two plots, half of the dominant trees were felled in 2010 (n = 15) to obtain stem disks for tree-ring analyses. Stem disks were taken at 10 m height because large compression wood sections were formed at breast height due to slow but consistent downslope movement of the terrain. Usage of the first 10 m, the most valuable part of the stem, for timber sale further restricted our choice. The mean tree age of the felled trees at 10 m height was 142 years (±4 SE) on the control and 143 years (±3 SE) on the treated plot.

Early- and latewood width were measured from the bark to the pith on each disk along six radii at 60° angles from each other. Ring widths were measured using the WinDENDRO tree-ring analysis system (Regent Instruments Inc., Québec, Canada) attached to a digital scanner (Epson Expression 10000 XL, Seiko Epson, Nagano, Japan). Disk sections were scanned at 1500 dpi and measured to 0.01 mm accuracy. Each measured section was visually adjusted for accurate ring boundary detection and then independently verified. The six ring-width chronologies obtained per disk were averaged to obtain one chronology per tree. The averaged ring width of each dated tree was plotted, cross-dated visually and then statistically by (i) the percent agreement in the signs of the first differences of two time series (the so-called *Gleichläufigkeit*) and (ii) the Student’s *t*-test, which determines the degree of correlation between the curves. No false or missing rings were observed. Ring-width series were cross-dated using COFECHA (Holmes 1983), and standard methods were used to build an averaged series for each plot (Fritts 1976). The averaged early- and latewood width from the six measured radii was converted to early- and latewood BAI. To examine the mean growth trend of the dominant trees at each plot, BAI for each year was averaged over all individuals per plot.

Yearly height growth was determined from yearly images of the tree tops by photogrammetric methods and by direct measurement of internode lengths of the felled trees (control: *n* = 13; N-addition plot: *n* = 8). A 22-year period (1986–2008) was selected for tree-ring isotopic analyses. It included 9 years before treatment and 13 years during N addition. To account for the circumferential variability of the isotopic signal from tree rings (Leavitt 2010), two radial segments (~7 × 7 mm) per disk at an angle of 180° to

**Table 1.** Average over 14 years of the monthly amount of NH₄NO₃ sprayed on the treated plot during precipitation events using sprinklers.

<table>
<thead>
<tr>
<th>Month</th>
<th>NH₄NO₃-N (kg ha⁻¹ month⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.7</td>
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<tr>
<td>2</td>
<td>0.6</td>
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<tr>
<td>3</td>
<td>2.7</td>
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<td>2.0</td>
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<tr>
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</tr>
<tr>
<td>11</td>
<td>1.1</td>
</tr>
<tr>
<td>12</td>
<td>0.8</td>
</tr>
<tr>
<td>Total</td>
<td>22.8</td>
</tr>
</tbody>
</table>
each other were cut from each disk using a band saw. To avoid the interspersion of the isotopic signal caused by sawing and to ensure that tree-ring boundaries were clearly distinguished, the surface of each side of the bars was prepared using a core microtome (Gärtnner and Nievergelt 2010). The absolutely dated tree rings from the two bars per tree were then separated into early- and latewood with a scalpel under a stereo microscope (Leica Wild M3B, Wetzlar, Germany; ×40 magnification) to build a composite sample. The thin chips were then coarsely milled with a coffee grinder prior to the purification of the α-cellulose (Rinne et al. 2005, Boettger et al. 2007). To ensure homogeneity and to obtain a fine and uniform particle size of the α-cellulose, samples were treated in an ultrasonic homogenizer and finally freeze-dried to remove any residual water prior to mass spectrometry (Laumer et al. 2009). For the δ¹³C and δ¹⁸O analyses, samples of dry α-cellulose (ca. 1 and 1.2 mg, respectively) were weighed into individual tin foil capsules and sealed. Samples were either combusted in an element analyzer (EuroVector EA3000, HEKAtech GmbH, Wegberg, Germany) for δ¹³C or pyrolyzed in a high-temperature conversion element analyzer (also HEKAtech). The resulting gases were analyzed on an isotope ratio mass spectrometer (IRMS, Delta V Advantage, Thermo Fisher Scientific, Waltham, MA, USA). All δ¹³C and δ¹⁸O values were expressed relative to their respective standards, PeeDee belemnite and Vienna Standard Mean Ocean Water:

\[
\delta^{13}C \text{ or } \delta^{18}O = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right)
\]

where \( R \) is the ratio of \(^{13}\)C to \(^{12}\)C atoms or \(^{18}\)O to \(^{16}\)O atoms of the sample and standard. Measurement precision was better than 0.3‰ for both δ¹³C and δ¹⁸O.

**Stable isotope theory**

Stable isotopes of carbon and oxygen are valuable tools for understanding past tree physiological processes, including responses to chronic N addition. Farquhar et al. (1989) linked the C isotope ratio (δ¹³C) of C₃ plants to their mean growing season water-use efficiency (WUE). This link from δ¹³C plant tissues to WUE can be made because the isotopic discrimination of plants is linearly connected to the c/cᵢ ratio, where \( c \) is the partial pressure of CO₂ in the leaf intercellular spaces and cᵢ is that of the ambient air (Farquhar et al. 1982). However, interpreting C stable isotope ratios for WUE is still ambiguous because changes in WUE can be a result of changes in photosynthetic capacity (A), stomatal conductance \( (gₛ) \) or both. Therefore, additional information is needed to define which of these two factors is the most important. Under specific conditions the oxygen isotope ratio in tree-ring cellulose can help solve this problem (Scheidegger et al. 2000, Grams et al. 2007).

**Foliar analyses**

To determine foliar properties, needle samples from the seventh whorl from five tree tops per plot were collected yearly (Schleppi et al. 1999b). In the laboratory, all needle-bearing twigs were clipped and the most recent five needle age classes were separated. After drying to constant weight (65 °C), 120 needles (in three batches) of each age class per tree were arranged without overlap on a glass plate and then scanned using a scanner with backside illumination (Snapscan 1236s, AGFA, no longer commercially available). The projected needle area was then analyzed from the digital images using our software PIXSTAT v1.1 (http://www.wsl.ch/dienstleistungen/produkte/software/pixstat). After having them ground, needles were analyzed with a C and N analyzer.

**Statistical analyses**

Time series of BAI, internode length and isotope ratios were subjected to statistical intervention analysis after calculating the difference between N treatment and control. With this approach, the difference in growth level between the two plots is eliminated, resulting in a single time series where the identification of a treatment effect is possible by time series analyses. The resulting data were then used to fit an autoregressive (ARIMA 1,0,0) model. For BAI and tree-ring δ¹³C, we used a linear increase as an explanatory intervention model to test for a treatment effect. For tree height growth, the convex part of a sine-shaped function, and for tree-ring δ¹⁸O, a step function were used. The autocorrelation function, the partial autocorrelation function and the Ljung-Box Q-test were checked for all ARIMA residuals to make sure that no significant deviations occurred.

For needle properties (N concentration, dry mass, area and mass per area), we again calculated the difference between the mean values of the N addition and the control plot. A non-linear sigmoidal Boltzmann function was fitted to the resulting data. Because the choice of an intervention model is always somewhat arbitrary, trends were further analyzed using the rank-based non-parametric Mann–Kendall statistical test (Mann 1945, Kendall 1975). However, testing yearly height growth was not possible because the requirement for monotonic changes was not fulfilled.

ARIMA intervention analyses were conducted using the SPSS 17.0 statistics package (SPSS, Chicago, IL, USA), while the Mann–Kendall test was carried out using the free software package R (R Development Core Team 2011). Curve fitting was done using Origin 6.1 (OriginLab Corporation, Northampton, MA, USA).

**Nitrogen-use efficiency of the trees**

We determined the NUE of the added N (NUEₘₐₓ) by calculating the additional C sequestered during the 14 years of N treatment,
divided by the cumulated N addition (312 kg N ha⁻¹). The NUE of the ambient N deposition (NUEₐmb) was calculated by dividing the annual total amount of C sequestered in stem wood by the atmospheric throughfall N deposition (17 kg ha⁻¹ year⁻¹).

Annual tree volume increment was calculated considering individual BAI and tree height from all trees >6 m in height and assuming that stems are cone shaped. Measurements from the felled trees were then combined to allometric relations between diameter and height measured on all the trees to obtain growth values for the trees that were not felled. The amount of C sequestered was then determined using a wood density for earlywood of 300 kg m⁻³ and for latewood of 700 kg m⁻³. The C content of wood was measured to be 50%. To obtain values for the total aboveground biomass, C sequestered into the tree crowns was also calculated. For this, we assumed that yearly litterfall is equal to yearly needle and branch production. The measured yearly litterfall was 0.23 kg m⁻² on the control plot and 0.30 kg m⁻² on the N-treated plot, with a C concentration of 50% (the mean of 14 years). Details of litterfall sampling can be found in Schleppi et al. (1999b).

**Results**

**Tree growth**

Before the experimental N addition, tree height growth on both N-treated and control plots showed very similar values with a mean annual growth of 8.6 cm in height, except in the year 1990 (Figure 1). Trees on the N-treated plot responded already in the first year of the N addition (April 1995). Increased height growth on the N-treated plot was then observed for 8 years with a peak after 5 years. In the ninth year, height growth rates had returned to the background level. The convex sine-shaped ARIMA model fitted to the yearly height growth data showed a significant growth change (Table 2). When we compare the mean yearly height growth of the period before N addition (1989–94) with the mean of the four years with the highest differential growth rates (2000–2003), N addition significantly increased height growth for those 4 years by almost 4.5 cm per year, or 64%.

Analyses of BAI showed that, prior to the treatment, trees on the control plot had, in general, higher growth rates (mean annual BAI of 936 mm² a⁻¹) than trees on the N-treated plot (625 mm² a⁻¹). The Mann–Kendall test showed that there was no trend for the annual BAI before N addition (1960–94; τ = −0.173; P = 0.147). Trees from both plots showed the same inter-annual growth patterns (Figure 2). After the treatment started in 1995, growth rates on the N-addition plot increased for the entire ring and earlywood BAI, while growth rates on the control plot remained unchanged. The ARIMA (1,0,0) intervention analyses showed that latewood BAI did not significantly change but earlywood BAI responded to N addition with a highly significant linear increase. Adding early- and latewood, annual BAI changed significantly, following also a linear trend (Figure 2). Statistics for the ARIMA models can be found in Table 2.

The mean annual BAI during N addition (1995–2008) showed an increased growth of ~24% compared with the mean value before N addition (1960–94). Considering the differing density of early- and latewood, tree stems sequestered ~22% more C per year due to the added N. Earlywood C sequestration (+28%) reacted much more than latewood (+15%). Relating the amount of C sequestered in stem biomass on the control plot to the atmospheric N entering the ecosystem, NUEₐmb was 95 kg C/kg N for the period 1980–94. For the same period, i.e., prior to the start of the experimental N addition, stems on the (later) N-treated plot sequestered 70 kg C/kg N. Including C sequestered annually into the canopy by assuming that litterfall equals foliage and branch production, the total aboveground tree biomass accumulated 155 kg C/kg N. Counting only the experimental addition of 23 kg N per year and its effect on stem wood production, NUEadd was 8 kg C/kg N. The C/N ratio of plant material was determined for stem wood as 612, for bark and branches as 111 and for needles as 41 (control) or 40 (N addition).

**Tree-ring isotopes**

The δ¹³C values of tree-ring cellulose showed a decreasing trend over time for early- and latewood on both plots in the same magnitude as the Suess effect (Keeling 1979) (Figure 3). Analyzed δ¹⁸O values in tree-ring cellulose showed almost identical values for both the control and the N-treated plot (Figure 4). The ARIMA intervention analysis as well as Mann–Kendall’s trend test showed no significant change in the

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*Figure 1. Least square means of the yearly height growth for trees from the control (n = 4) and N-added plots (n = 5). The transient increase in the N-added plot is significant (see Table 2).*

*Figure 2. Annual tree volume increment was calculated considering individual BAI and tree height from all trees >6 m in height and assuming that stems are cone shaped. Measurements from the felled trees were then combined to allometric relations between diameter and height measured on all the trees to obtain growth values for the trees that were not felled. The amount of C sequestered was then determined using a wood density for earlywood of 300 kg m⁻³ and for latewood of 700 kg m⁻³. The C content of wood was measured to be 50%. To obtain values for the total aboveground biomass, C sequestered into the tree crowns was also calculated. For this, we assumed that yearly litterfall is equal to yearly needle and branch production. The measured yearly litterfall was 0.23 kg m⁻² on the control plot and 0.30 kg m⁻² on the N-treated plot, with a C concentration of 50% (the mean of 14 years). Details of litterfall sampling can be found in Schleppi et al. (1999b).*
isotopic signal of δ¹³C or δ¹⁸O values in tree-ring cellulose owing to the 14 years of N addition (Table 2). Hence, N addition had no significant effect on intrinsic WUEi, photosynthetic rate and stomatal conductance.

Needle properties

Needle N concentration increased significantly (P = 0.0059) 4 years after the start of the N addition, reaching a peak from 1999 to 2001 followed by a decline (Figure 5). After 4–5 years, the needle N concentration returned nearly to pre-treatment values. Needle area significantly increased (P < 0.001) in the fourth year after N addition, staying at a high level during the rest of the N addition period compared with the control. Needle dry mass also significantly increased after the fourth year of N addition (P < 0.001) and then consistently remained higher than the control. No significant difference was found for the needle mass per area (Figure 5).

Discussion

Tree physiological changes due to N addition

Using the Farquhar et al. (1989) model to interpret our δ¹³C data (Figure 3), we found that the A/gs ratio remained constant and thus WUEi of the N-treated trees did not change. Several authors have proposed that stomatal conductance might change in parallel with leaf photosynthetic rates to maintain a constant CO₂ partial pressure in the leaf intercellular spaces (c) (Wong et al. 1979, Sheriff et al. 1986, Saurer et al. 1997, Balster et al. 2009). The interpretation of our δ¹⁸O values (Figure 4) with the conceptual model of Scheidegger et al. (2000) showed that leaf stomatal conductance did not change due to N addition, nor did the photosynthetic rates. Important for the use of this conceptual model is the requirement that the δ¹⁸O values of the source water and of the atmospheric water vapour are similar for the investigated trees. In our study, the two experimental plots are on the same gentle slope very close to each other. They are thus subjected to the same micro-meteorological conditions. The irrigation water used as a vector for the N spreading was collected on site and also applied (without N addition) to the control. We can thus assume that no bias occurred and that differences in δ¹⁸O and thus in gs would be caused by the N addition alone.

From high-dose fertilization experiments in temperate forests, it is well known that the first response is an increase in foliar N content followed by an increase in foliar biomass and a return to nearly background foliar concentrations (Aber et al. 1986).
Increased foliar N concentration might be accompanied by an increased pool of proteins linked to electron transport and carboxylation that enhances photosynthetic rate ($A$). The increased C assimilation per unit leaf area has been suggested to provide the assimilates required to increase foliar biomass (Gough et al. 2004). Later, fertilizer N initially incorporated into photosynthetic proteins may be re-allocated to developing foliage, which in turn decreases the pool of photosynthetically active proteins per unit leaf area, thus bringing $A$ down to background values. Other fertilization experiments also found an increase in production per unit of foliage biomass (Brix 1983) or in $A/g_s$ (Brooks and Coulombe 2009) lasting as long as 3–4 years, followed by an increased LAI. This typical sequence of changes observed in fertilizer experiments is quite similar to our findings from a long-term, low-dose N-addition experiment.
ectomycorrhizal fungi are an important C sink (Hobbie 2006) known for herbaceous plants. Furthermore, it is recognized that tree growth rates to shifts in C allocation, this effect is well although so far no study exists that relates N-induced higher aboveground growth might be caused by shifts in C allocation. Braun et al. (2010) hypothesized that N deposition might induce foliar phosphorus (P) limitation. Needle analyses at Alptal prior to the N addition indicated scarce supply with P and Mg (Schleppi et al. 1998) possibly precluding an increase of A. In addition to increased foliar N concentration, Talhelm et al. (2011) found no base cation deficiencies, and therefore hypothesized that the increase in aboveground growth might be caused by shifts in C allocation. Although so far no study exists that relates N-induced higher tree growth rates to shifts in C allocation, this effect is well known for herbaceous plants. Furthermore, it is recognized that ectomycorrhizal fungi are an important C sink (Hobbie 2006) and chronic N deposition can lead to a shift of C allocation from tree tissue to ectomycorrhizal fungi (Lilleskov et al. 2002, Van Diepen et al. 2007, 2011, Högberg et al. 2010).

Our results from the dual-isotope approach combined with analyses of foliar properties support the view that enhanced stem growth caused by N addition is primarily due to increased stand LAI rather than to physiological changes. Unfortunately, our data on stand-level LAI (Schleppi et al. 1999b) are not precise enough to show changes due to the N addition, but increased needle area and needle dry mass are good indicators that LAI must have increased indeed. With a higher stand LAI, the amount of absorbed photosynthetically active radiation increases, and therefore canopy-level photosynthesis can also increase (Balster and Marshall 2000). A significant increase in stand LAI is only possible in forest stands where the canopy is not fully closed (Alptal: LAI = 3.8). If the canopy is already closed and light interception is near its maximum, trees may respond with higher leaf-level photosynthetic rates (Janssens and Luyssaert 2009). Hence, this may explain why additional N can have different impacts on leaf-level photosynthesis depending on the considered study.

Carbon sequestration due to N addition

At the Alptal site, two different approaches were used to investigate how elevated N deposition alters C sequestration in forests. Here, we directly measured tree-ring width, whereas in Krause et al. (2012) we tracked the fate of N deposition using a $^{15}$N tracer. For the comparison of the two approaches, we calculated NUE using $^{15}$N recovery results for the tree compartments from our earlier study and combined them with the corresponding C/N ratio (method of Nadelhoffer et al. 1999). On the N-treated plot, 14 years after the tracer application, Krause et al. (2012) recovered $\sim 1\%$ of the applied $^{15}$N tracer in stem wood, resulting in an NUE$_{add}$ of $\sim 5$ kg C/kg N. Nine years after the addition of the tracer to the control plot, 0.7% was recovered in stem wood, resulting in an NUE$_{add}$ of $\sim 3.5$ (note that, in spite of the tiny amount of tracer added, the calculated NUE is for this small addition, thus NUE$_{add}$ and not for ambient deposition).

Thus, the results for NUE from both approaches agree well, considering all possible error sources. Due to the fact that, after 14 years, the soil stores almost 60% of the applied $^{15}$N tracer from which trees are taking up a certain amount each year (Krause et al. 2012), the time since the tracer addition plays an important role in the results of the $^{15}$N approach.

In the study by Krause et al. (2012) on the same site, we recovered ca. 11% of the $^{15}$N tracer in the total aboveground tree biomass on the N-treated plot and ca. 9% on the control plot, resulting in an NUE$_{add}$ of 15 and 10, respectively. Unfortunately, we were not able to calculate the NUE$_{add}$ for the total aboveground biomass using the tree-ring approach because we have no accurate data on how needle and branch production changed due to N addition. In their review, De Vries et al. (2009) suggested a most likely range of aboveground NUE in forests of 15–40. They considered the studies on NUE in aboveground biomass based on four approaches. Our results with NUE values of 15 and 10 for aboveground biomass, respectively, lie at the bottom end of the range defined by De Vries et al. (2009).

The comparison of NUE data from different experimental studies is challenging because not only the duration of the experiments varies but also the form of the added N. Another widely neglected issue is the fact that edaphic properties (Morford et al. 2011) and the related legacy of historical N-deposition rates can have a considerable influence on N availability. Recently, Högberg (2012) proposed the use of the soil-internal N supply (based on N mineralization rates) relative to the actual N deposition as a criterion to capture the differing N history in N-addition experiments. However, measuring the N mineralization rates is difficult, quite laborious and time-consuming, and the methodology is still under debate (Hart and Firestone 1989, Hatch et al. 2000, Durán et al. 2012). An alternative criterion for comparing the differing N status of ecosystems could be the soil C/N ratio. The forest floor C/N ratio has been shown to reflect gross mineralization rates (Hart et al. 1994, Gundersen et al. 1998), and is therefore considered to be an easily obtainable indicator of the N status of the ecosystem. At Alptal a soil C/N ratio of 19.4 indicates moderate N availability.

The relatively small response to N addition of C sequestration in trees at Alptal demonstrates that N deposition plays a
minor role in mitigating climate change by removing CO₂ from the atmosphere. Carbon sequestration due to N addition might even be offset by several other factors. The C pool of forest soils may be altered by N deposition, but evidence is strongly contradictory: some studies show that N deposition may decrease soil C, others demonstrate no change and still others suggest that C pools may increase (Reay et al. 2008). In addition, the net impact of N addition on sequestered CO₂ from the atmosphere may be counter-acted in terms of their global warming potential (GWP) by increased N₂O emissions (Papatheodorou et al. 2001, Lu et al. 2010) and decreased CH₄ uptake (Castro et al. 1995, Sitaula et al. 1995, Steudler et al. 1989, Gunderson et al. 2012). Furthermore, other interactions may occur in the long term; for example, increased growth rates may be coupled with reduced tree longevity (Bigler and Vegel 2009, Bugmann and Bigler 2011, Rötheli et al. 2012), thus leading to earlier C release and offsetting the growth-related enhanced CO₂ uptake caused by N addition.

Conclusions
We have provided evidence that long-term, low-dose N addition is of minor importance for sequestering atmospheric C into tree biomass and for mitigating climate change. We further recommend that, when accounting for the influence of N deposition on the GWP of forests, not only the counter-acting processes and factors reviewed above (soil C pool, N₂O, CH₄ or reduced tree longevity) need to be considered, but also the time scale over which the additional C sequestered in biomass is removed from the active C cycle.

Further, our results support the view that enhanced stem growth caused by N addition is due to an increased stand LAI more than to physiological changes, at least in forest canopies that are not yet completely closed. Higher LAI implies that more photosynthetically active radiation is absorbed and therefore canopy-level photosynthesis is increased. Our data thus show that models that extrapolate the key role of N for photosynthesis (Evans 1989) to the effects of N deposition on forest productivity (Aber et al. 1997, Gu et al. 2010, De Vries and Posch 2011) may not always be mechanistically accurate.

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Conflict of interest
None declared.

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References


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