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Changes in Root Starch Contents of Mature Beech (*Fagus sylvatica* L.) Along an Ozone and Nitrogen Gradient in Switzerland

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Summary

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Ozone and nitrogen deposition as anthropogenic pollution factors have been shown to affect carbon partitioning in plants. Both pollution factors have increased in recent decades, frequently exceeding the critical levels and loads for forests. Therefore, root starch contents of *Fagus sylvatica* L. were determined along an ozone and nitrogen gradient in Switzerland at 20 forest sites of mature trees. The starch contents were analyzed enzymatically. We found a negative correlation for the root starch contents with ozone and nitrogen, with an antagonistic interaction term, indicating that one pollution factor might alleviate the effect of the other.

Introduction

Ozone and nitrogen are two of the most important anthropogenic pollution factors for natural ecosystems such as forests. In recent years, ambient ozone doses often exceeded the critical level of 10 ppm*h above AOT40 daylight (accumulated exposure over a threshold of 40 ppb) (FUHRER & al. 1997). Ozone doses at these levels can impair carbon partitioning and thus lead to a decrease of root carbohydrate contents (COOLEY & MANNING 1987). Similarly, enhanced nitrogen loads are also known to affect C-allocation (WALLENDA & al. 1996). During the last decades,

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nitrogen deposition has increased exceeding the critical N load for forests of 10 - 20 kg N ha^{-1} yr^{-1} in about 90 % of Swiss forests by now (RIHM 1996).

Several belowground parameters, e.g. carbohydrate reserves in roots, seem to be appropriate indicators for environmental changes. They are sensitive to stress and should be affected prior to the occurrence of visible aboveground symptoms (VOGT & al. 1993). Particularly, LUX & al. 1997 have shown that ambient ozone doses of 12.3 and 19.6 ppm*h above AOT40 daylight lead to a reduction in root starch content of beech and spruce trees compared to filtered air. Similar effects were found by WALLENDA & al. 1996 for spruce trees grown under enhanced N supply.

Both experiments were conducted on young trees being exposed to either enhanced ozone doses or nitrogen loads, but not a combination thereof. Since each factor plays an important role in the C-allocation of trees, it seems essential to explore carbohydrate fluxes and carbohydrate storage as a function of both atmospheric nitrogen input and ambient ozone levels. So far, only very few studies have taken this into account. GRULKE & al. 2001 examined the impact of both pollution factors on mature *Pinus ponderosa* trees in California, showing that root biomass and root starch content were reduced at more polluted sites in comparison to cleaner ones. To our knowledge, no such studies have been conducted on mature deciduous trees in temperate regions so far. *Fagus sylvatica* L. is one of the main tree species in Switzerland and young trees are known to be sensitive to ozone (BRAUN & FLÜCKIGER 1995) and nitrogen (FLÜCKIGER & BRAUN 1999). Recent work also suggests an impact of nitrogen and ozone on the growth of mature beech (BRAUN & al. 1999). Therefore, we studied the effect of both pollution factors on the root starch content of mature beech trees in Switzerland.

Material and Methods

We selected 20 forest sites of mature beech with presumably acidic soil conditions below 1100 m asl along an ozone and nitrogen gradient in Switzerland.

Using the nitrogen deposition model of RIHM & KURZ 2001, calculated N-loads varied between sites from 29 to 71 kg ha^{-1} yr^{-1} (Fig. 1). In 1999, modeled ozone doses (ACHERMANN & RIHM 1997) ranged from 7 to 36 ppm*h above AOT40 daylight (accumulated ozone dose above the threshold of 40 ppb) (Fig. 1). In January and February 2000 we sampled roots and adhering rhizospheric soil in two different soil depths (upper soil layer: 0 - 15 cm and lower soil layer: 35 - 60 cm) of six to seven trees per site.

The adhering soil was carefully removed from the roots and dried at 40 °C. For the pH-analysis 15 ml of a $CaCl_2$-solution (0.01 M) were added to 6.0 g of the dried soil. Samples were shaken at regular intervals and the pH of the solution was measured after at least one hour, using a double junction glass electrode (713 pH Meter, Metrohm, Herisau, Switzerland).

Roots were washed with cold water and treated with ultrasonic waves to clean them carefully. Medium-sized root parts (diameter: 1.0 - 2.5 mm) were selected for starch analysis. After rinsing them twice with demineralized water they were padded dry with tissue-paper, shock-frozen in liquid nitrogen and finally freeze-dried (Pirani, Edwards High Vacuum, Crawley, England). Dried samples were ball-milled (Retsch MM2, Retsch GmbH & Co KG, Haan, Germany) and stored at –20°C in plastic bags over silica gel until further analysis.

The starch content of the freeze-dried roots was analyzed enzymatically as described by BOEHRINGER-MANNHEIM 1989. Forty mg of the powdered plant material were washed three times with 1 ml ethanol (80% v/v) to remove mono- and oligosaccharides. The remaining pellet was dried again (Speed Vac Plus SC 110 A, Savant Instruments Inc., Holbrook, NY) and digested for 30 min at 60°C using 1ml of dimethylsulfoxide/HCl (8 M) in a 4 : 1 (v/v) ratio. After neutralization of the digest, starch was hydrolyzed with amyloglucosidase from *Aspergillus niger* (Boehringer, Mannheim, Germany). Free glucose was assayed using hexokinase/glucose-6-phosphate-dehydrogenase as described by BOEHRINGER-MANNHEIM 1989.

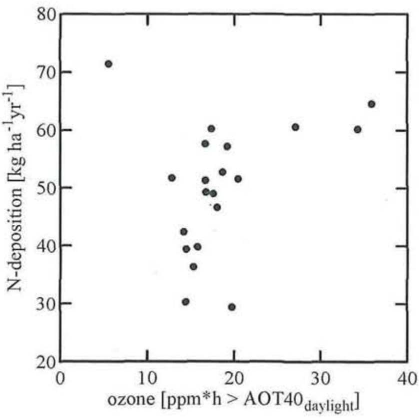

Fig. 1. Ozone concentration and nitrogen deposition for the study sites. Each dot represents one study site (n = 20).

For statistical analysis SYSTAT 9 (SYSTAT Inc., Evanston, IL, USA) and S-PLUS 2000 (MathSoft Inc., Cambridge, MA, USA) were used. The effect of N-deposition and ozone-dose on the root starch content was quantified using a linear mixed effects model with the site as a grouping variable. Differences in soil pH and starch content of the roots between soil layers were determined by a t-test for dependent samples. The residuals of the tests were checked for normal distribution.

Results

The rhizospheric soil was acidic for the selected sites with even more acidic pH values in the lower: 3.92 ± 0.06 (mean ± SE) compared to the upper soil layer: 4.13 ± 0.05 (mean ± SE). Differences were significant at $p < 0.001$, df = 119 (t-test for dependent samples). Starch values of individual root samples ranged from 3 to 68 mg g^{-1} d. wt. irrespective of the soil layer (Fig. 2).

Ozone and nitrogen loads were negatively correlated with the root starch contents of both soil layers (Table 1). Both pollution variables lead to a significant reduction of the root starch content showing a much stronger correlation with the ozone dose in comparison to the nitrogen load. The interaction term indicated that the effect was strongest for low and medium pollution situations (defined as: below 25 ppm*h > AOT 40 daylight and below 60 kg N ha^{-1} yr^{-1}), whereas for sites with high ozone and nitrogen loads the effect got weaker. Root starch content was not affected by soil pH in either soil layer.

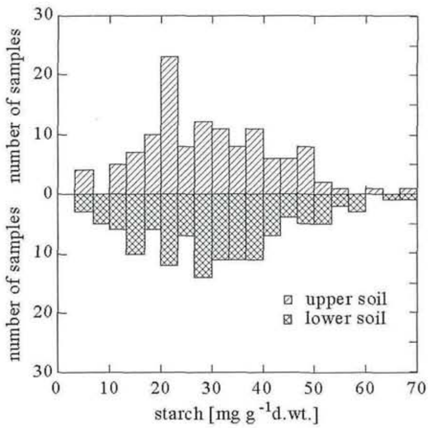

Fig. 2. Root starch contents of the two soil layers (upper soil layer: 0-15 cm, lower soil layer: 35-60 cm).

Table 1. Regression parameters for root starch contents of the upper and lower soil layer. Ozone data are given as ppm*h above AOT40 daylight and nitrogen data (N-depo) as kg ha^{-1} yr^{-1}. For each soil layer: number of observations: 124; number of groups: 20 sites.

	Coefficient	SE	p-value
UPPER SOIL LAYER:			
(Intercept)	124.154	31.487	< 0.001
ozone 99	- 6.044	1.987	0.0078
N-depo	- 1.411	0.494	0.0115
N-depo × ozone 99	0.093	0.031	0.0094
Lower soil layer:			
(Intercept)	155.701	34.304	< 0.001
ozone 99	- 8.076	2.165	0.0018
N-depo	- 1.791	0.539	0.0043
N-depo × ozone 99	0.120	0.034	0.0029

Discussion

Root starch content of the investigated mature beech trees was a negative function of both ozone dose and nitrogen load (Table 1). However, ozone had a much stronger effect than nitrogen. The combination of the two pollution factors was also significantly correlated with root starch content. The positive value of the interaction term (Table 1) indicated that the effect of ozone and nitrogen was decreased at sites with simultaneously high N loads and ozone doses. These findings are consistent with the results of other studies with deciduous tree seedlings. For example PÄÄKKÖNEN & HOLOPAINEN 1995 found that sufficient nitrogen supply can confer young *Betula pendula* with greater resistance to ozone. In another study, *Populus tremuloides* seedlings were grown with an excess or even a toxic supply of nitrogen. They also tended to be less responsive to ozone in terms of growth rates (PELL & al. 1995). Despite these findings and the notion of one factor alleviating the effect of the other, the root starch contents for the high pollution groups in our experiment were still below the levels of sites with low nitrogen deposition and low ozone concentrations. Similar to our results, GRULKE & al. 2001 found a reduced root starch content in mature *Pinus ponderosa* trees at sites with high ozone and high N deposition compared to cleaner sites. In contrast, the bole starch content of *Pinus ponderosa* was significantly increased at the most polluted sites (GRULKE & al. 1998).

However, the mechanism behind the observed carbon partitioning due to enhanced ozone concentrations or nitrogen loads is not yet fully understood. As an effect of ozone, BORTIER & al. 2000 suggested carbon retention in the leaves for repair of damaged foliage and a decreased phloem loading. For nitrogen, a decreased root starch content might be due to a reduced carbon translocation from the shoot together with a higher need for carbon skeletons for amino acid synthesis under increased inorganic nitrogen supply (WALLENDA & al. 1996). Why one factor might alleviate the other under high pollution conditions remains open.

A reduced root starch content might coincide with other effects for the trees. Elevated ozone doses might result in a higher carbon retention in the shoot for ozone detoxification and lower carbon allocation to the roots leading to alterations in whole tree biomass after several years (TAKEMOTO & al. 2001). As a consequence, trees might become more susceptible to stresses like drought, windthrow, and root diseases (TAKEMOTO & al. 2001). Similar findings are stated for an enhanced nitrogen deposition. Besides a lowered carbon allocation to the roots (WALLENDA & al. 1996) the fine root production is decreased (NADELHOFFER 2000) and shoot/root ratio increased (FLÜCKIGER & BRAUN 1998) with a higher nitrogen availability. COOLEY & MANNING 1987 provided evidence that changes in carbohydrate partitioning may even affect the way plants interact with other organisms, particularly symbionts, but also pathogens. This has been shown for ozone (ANDERSEN & RYGIEWICZ 1995) and also for an enhanced nitrogen supply (WALLENDA & al. 1996).

The change in carbon allocation resulting in a reduced root starch content in mature beech trees may lead to various additional effects. Enhanced ambient ozone concentrations and nitrogen supply as they can be observed in Switzerland might play an important role in tree health of *Fagus sylvatica* L.

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