Effects of CO_2 Concentration on the Nutrition of Willows (*Salix phylicifolia*) Grown at Different Nutrient Levels in Organic-Rich Soil

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Willows (*Salix phylicifolia*) were grown for four months in organic rich soil at four nutrient levels (fertilization with a micronutrient-macronutrient mixture of 0, 100, 500 and 1000 kg ha^{-1} per month) and four CO_2 concentrations (300, 500, 700 and 1000 ppm). Nitrogen and phosphorus concentrations of the willows were reduced at CO_2 enhancement, the decrease being larger in the leaves and roots than in the stems. Nitrogen content of the willows plus extractable nitrate-N in the soil coincided well with the doses of nitrogen supplied, but the corresponding sums of phosphorus in the plants and soil were smaller. The total nitrogen content of willows grown in unfertilized soil was nearly two times higher than the sum of the extractable nitrate-N in soil and N content of the cuttings at the beginning of the experiment. The contents of nitrogen and phosphorus of the unfertilized willows were independent of CO_2 concentration, suggesting that CO_2 concentration did not affect through increased mineralization the availability of those nutrients to the willows.

Keywords CO_2 enrichment, nutrient status, nutrition, mineralization, *Salix*
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1 Introduction

As shown in numerous earlier experiments, CO_2 enhancement increases the photosynthetic rate and biomass production of plants (see review by Eamus and Jarvis 1989). There are many factors, however, that can limit or even hinder this production increase (Sionit and Patterson 1984, Smolander and Lappi 1984, Tolley and Strain 1985, Silvola and Ahlholm 1992). Furthermore, the increase in growth has often been found to be smaller than could be expected from short-term photosynthesis measurements (Tissue and Oechel 1987).

One important factor modulating the effect of CO_2 enhancement is nutrient status (Goudriaan and de Ruiter 1983, Tissue and Oechel 1987, Eamus and Jarvis 1989). Although the effect of a nutrient shortage in reducing the response to CO_2 has been demonstrated in many cases (e.g. Pettersson et al. 1993), an increase in the growth has been found in *Quercus alba* under conditions of severe nutrient deficiency at a doubled CO_2 concentration (Norby et al. 1986a,b). CO_2 enhancement has been shown to reduce the N concentration of plants, which means that less nitrogen is used to achieve a given amount of biomass at an elevated CO_2 concentration (Wong 1979, Overdieck et al. 1988, Curtis et al. 1989, Johnson and Lincoln 1991, Coleman and Bazzaz 1992). The reason for this reduced nitrogen concentration has been thought to lie in the lower nitrogen requirements of the plants (Hocking and Meyer 1985), in the poorer availability of nitrogen in the soil (Vessey et al. 1990) or in the accelerated ageing of plants caused by an enhanced CO_2 concentration (Coleman et al. 1993). The increase in C/N ratio at elevated CO_2 concentration is also partly caused by the accumulation of non-structural carbohydrates in plants (cf. Pettersson et al. 1993).

The increase in photosynthesis under conditions of elevated CO_2 has been found to increase carbon allocation to roots (Norby et al. 1986a, Zak et al. 1993), even if the real reason to increase in the root/shoot ratio may be the poorer availability of nutrients in soil at elevated CO_2 concentration (Pettersson et al. 1993). There is some evidence that the allocation of new carbon (root exudation and litter) to the soil increases the mineralization of older organic matter in it (Cheng and Coleman 1990), although some reports do not confirm this (Reid and Goss 1982). It has been proposed that a shortage of nutrients probably hampers increase in production to a great extent in natural ecosystems at elevated CO_2 concentrations (Kramer 1981). If increased mineralization as a result of CO_2 enhancement does occur, however, a long-term production increase could be possible in organic-rich soils.

The willows studied here were grown for four months in organic-rich soil in a greenhouse at different rates of nutrient supply and at four CO_2 concentrations. The aim was to test the effect of growth conditions on the uptake of nutrients (nitrogen and phosphorus). The extractable nitrate-N and P in soil were also measured in order to estimate the possible effect of CO_2 concentration in releasing nutrients from organic matter. The growth rhythm, biomass production and its partitioning of the same plant material have been described in a companion paper by Silvola and Ahlholm (1993).

2 Material and Methods

2.1 Material

Salix phylicifolia L. is common in Finland, growing mostly in habitats with moist soil. The shoots used here were taken from natural shrubs growing on the edge of an arable field at Iiksenvaara, near Joensuu, in eastern Finland.

The shoots were collected in winter and kept moist in a cold room (+5 °C). Cuttings 25 cm long and about 1 cm thick were prepared from them and kept in water for two days before establishing the experiments. At the beginning of May they were planted individually in pots of height 20 cm and diameter 15 cm containing a sand-peat mixture in a ratio of 3:7 by volume. The earth in the pots was limed with dolomite, 5 kg m^{-3}, and fertilized with a macronutrient-micronutrient mixture (Superex 9, Kekkilä; Table 1) at four nutrient levels corresponding to the following amounts of fertilizer calculated on the basis of the surface area of the pot (kg ha^{-1}) and its volume (kg m^{-3}, in parenthesis): 0 (0), 100 (0.052), 500 (0.26), 1000 (0.52). These amounts of fertilizer were mixed as powder into the earth of the pots at first, and later on 1/4 of each amount was added weekly when watering the plants, so that the basic dose was given once a month. The zero fertilization level was used to represent the conditions under which the only source of nutrients for the plants was the mineralization of organic matter and weathering from the mineral soil. No dynamic nutrient supply was employed (cf. Ingestad 1982), but on the basis of previous growth experiments with willows, 1000 kg fertilizer ha^{-1} was thought to maintain abundant nutrient conditions (free nutrient access) throughout the whole growing season. The pots were placed in plastic baths, from which the plants received their water and fertilizer through the pores in the pots.

Table 1. Nutrient concentrations in the fertilizer used in the growth experiment (Superex 9, Kekkilä).

Nutrient concentration %				
N	P	K	Mg	S
19.4	5.3	20.0	0.2	0.3

Nutrient concentration mg kg^{-1}						
Fe	Mn	B	Zn	Cu	Mo	Co
1800	970	270	230	140	20	10

The willows were grown under natural light in four plastic chambers situated in a greenhouse, the temperature being on average 3–6 °C higher than out of doors. The CO_2 concentrations of the chambers were kept at levels of 300, 500, 700 and 1000 ppm. Four cuttings were grown in each combination of CO_2 and nutrient conditions, except that only two samples were grown at CO_2 concentrations of 500 and 1000 ppm. Thus the material comprised a total of 48 individual willows. The growth conditions and the regulation of CO_2 concentration are described in more detail by Silvola and Ahlholm (1993).

2.2 Measurements

The roots, cuttings, stems and leaves of the willows were harvested, dried and weighed separately at the beginning of September for the biomass calculations. The roots were carefully separated from the peat by hand. The biomass increment in the cuttings was determined by subtracting the initial dry weight from the weights of the cuttings harvested.

Total nitrogen in the willows and soil was analyzed by the standard Kjeldahl method (e.g. Allen 1974). NO_3-N in the soil was extracted with potassium chloride and analyzed with the Automatic Chemical Analysis System (AKEA, Instrumentarium Ltd, Helsinki), in which nitrate is reduced to nitrite, converted to a reddish-purple azo dye and analyzed by a colorimetric procedure.

Total phosphorus in the willows and soil was measured by ashing the samples and dissolving the ash in HCl. The phosphates in the soil were extracted with ammonium acetate. The analyzing of P took place spectrophotometrically (phosphomolybdate).

3 Results

3.1 Nitrogen and Phosphorus Concentrations in the Plants and Soil

The fertilization treatments greatly affected the nutrient content of the willows (Fig. 1). Average nitrogen concentrations in the leaves increased from about 1.5 to about 3 % and those in the roots from 0.8 to about 2 % as the fertilization rate increased from 0 to 1000 kg ha^{-1}. The N concentration in the stems and cuttings was lower, from about 0.5 to about 1.2 %. The phosphorus concentration in the leaves and roots increased from 0.7–1.5 to 3.5–5 mg g^{-1} and that in the stems and cuttings from 0.4–0.8 to 1–2 mg g^{-1}. The increase in CO_2 concentration mostly led to a decrease in N and P concentrations in all parts of the willows, although the effect was not allways statistically significant (Fig. 1B).

Extractable N and P concentrations in the soil were very low at the fertilization rates of 0 and

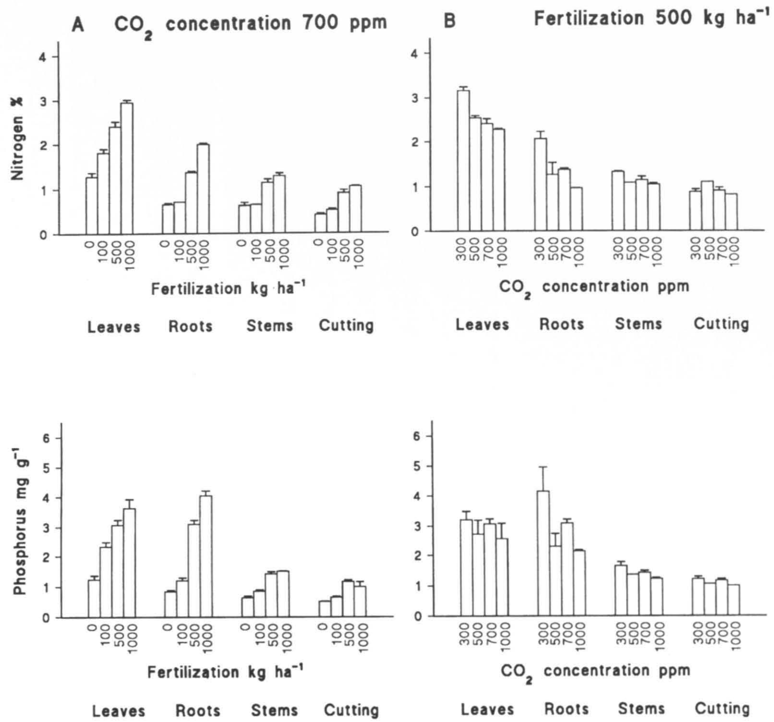

Fig. 1. Examples of the effects of fertilization (A) and CO_2 concentration (B) on nitrogen and phosphorus concentrations (mean ± S.E.) in different components of *Salix phylicifolia*.

100 kg ha^{-1} (NO_3-N mostly < 0.2 mg dm^{-3}, P < 3 mg dm^{-3}, Fig. 2), but the effect of the nutrient supply could be seen at higher fertilization levels, the maximal NO_3-N concentration being approx. 170 mg dm^{-3} and that of phosphorus approx. 40 mg dm^{-3} at the rate of 1000 kg ha^{-1}. The effect of CO_2 concentration on extractable nutrient concentrations in the soil could not be seen at the 0 and 100 kg ha^{-1}, but at higher fertilization rates the elevated CO_2 concentration led to lower nutrient concentrations in the soil. Soil nutrient concentrations were considerably higher at 300 ppm CO_2 in particular than at the higher CO_2 concentrations. Extractable nutrient concentrations in the soil were many times lower than the total nutrient concentrations, which were 429–754 mg dm^{-3} in the case of nitrogen and 80–171 mg dm^{-3} in the case of phosphorus (data not shown).

3.2 Nitrogen and Phosphorus Contents in the Willows and Soil

The nutrient uptake of the willow specimens was calculated by multiplying the dry weights (the biomass increment of the cuttings) of the various components of the willows by the corresponding nutrient concentrations. These amounts and the amounts of nutrients given during the growing season and the amounts of extractable P and NO_3-N available in the soil at the end of the growth experiments are presented in Fig. 3. The willows growing in unfertilized soil contained

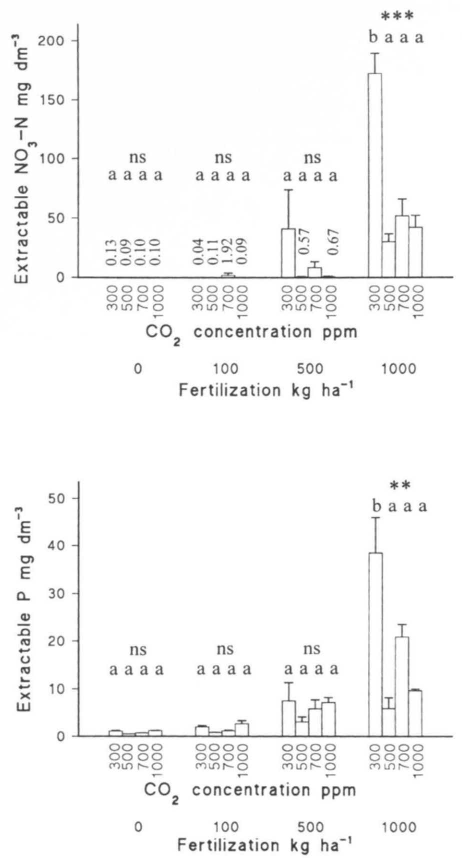

Fig. 2. Concentrations of extractable nitrate-N and phosphorus (mean ± S.E.) in the soil in which willows had been grown for four months at various fertilization rates and CO_2 concentrations.

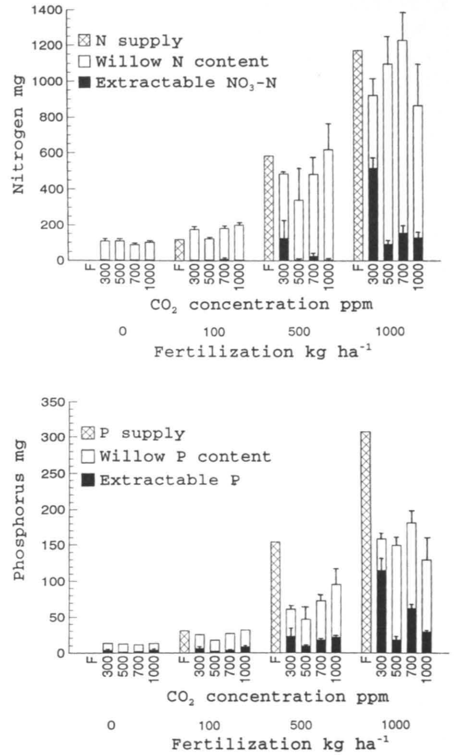

Fig. 3. Nitrogen and phosphorus budgets of willows (*Salix phylicifolia*) grown for four months at various CO_2 and nutrient levels. The groups of columns show the nutrient supply (F) to the willow specimens and the sum of extractable nutrients in the soil at the end of the experiment plus the nutrient content of the willows (mean ± S.E.). The nutrient content was obtained by multiplying the biomass production of the different components of the willows by the corresponding nutrient concentrations.

about 110 mg nitrogen, and there was less than 0.5 mg nitrate-N in the pot soil. At the fertilization rate of 100 kg ha^{-1} the average nitrogen content of the willows was about 200 mg, which is slightly less than the N content of those in the unfertilized soil plus the nitrogen given in the fertilizer. At the fertilization rates of 500 and 1000 kg ha^{-1}, the sums of the N content of the willows plus the nitrate-N available in the soil were on average slightly smaller than the amounts of N given in the fertilizer. 10–40 % of the nitrogen given was still left in the soil as NO_3-N at 1000 kg ha^{-1}, and there was also a small amount of unused NO_3-N in the soil of the 500 kg ha^{-1} experiment. At higher nutrient levels CO_2 enhancement increased nitrogen accumulation in the willows, but at lower nutrient levels no clear effects of CO_2 could be seen in either the nitrogen content of the willows or the nitrate-N in the soil.

The amounts of extractable phosphorus in the soil were higher than those of nitrogen at low fertilization rates (Fig. 3). At 100 kg ha^{-1} the phosphorus contents of the plants approximately corresponded to the amounts of phosphorus given, but at higher fertilization rates the phosphorus content of the willows was markedly lower than the phosphorus supplied to the soil. The effect of CO_2 concentration on phosphorus uptake was not very clear, even though CO_2 enhancement tended to increase the phosphorus content of the willows at higher nutrient levels and to reduce the extractable phosphorus in the soil.

4 Discussion

CO_2 enhancement reduced N and P concentrations in the willows, the decrease being less marked in the stems than in the leaves and roots. Similar concentration decreases in plants have been found to take place under various nutrient conditions by Wong (1979), Hocking and Meyer (1985) and Larigauderie (1988), for instance. The reason may lie in an increased amount of soluble carbohydrates in plants, in the reduced availability of nutrients in the soil or in a lower physiological demand for nutrients at enhanced CO_2 concentrations. Since the decrease has also been found to take place in the presence of substantial amounts of nutrients, one could assume that the reason must be the reduced nutrient requirement. The experiment of Vessey et al. (1990) does not support this conclusion, however, for when the plants were growing in flowing-solution culture, where nitrate was uniformly distributed and constantly re-supplied to the roots, the N concentration in the plants was independent of the CO_2 concentration. The authors thus conclude that in fact the lowered nutrient availability in the root system in porous media and not a reduced nutrient demand has been the reason for the decreased nutrient concentration in plants often found at elevated CO_2 concentrations.

The effect of CO_2 concentration on growth was here small at fertilization rates of 0 and 100 kg ha^{-1} but amounted to over a 100 % increase at higher fertilization rates (Silvola and Ahlholm 1993). The results are in accordance with those of Pettersson et al. (1993). In their experiment, at a suboptimal N supply the growth was controlled by N supply (not CO_2) but at free acces nutrient conditions the growth was depending on CO_2 concentration. These results differ from those by Norby et al. (1986a,b), who have found a remarkable growth increase in *Quercus alba* despite severe nitrogen deficiency. Since the nutrient concentrations in the plants are reduced at elevated CO_2 concentration, this will gradually lead to a decrease in soil fertility due to the lower nutrient content of the detritus. In organic-rich soil, however, there are large amounts of nutrients bound in the organic matter. Contradictory results exist regarding the effect of "new carbon" on the decomposition of "old carbon" in soil. Some experiments have indicated that the plant roots reduce the decomposition of organic matter in soil (Reid and Goss 1982, 1983, Sparling et al. 1982), but in many other experiments living roots have been seen to stimulate soil organic matter decomposition (Helal and Sauerbeck 1984, 1986, Cheng and Coleman 1990). The proportion of the biomass accounted for by the roots has been found to increase with increasing CO_2 concentration, especially under nutrient-poor conditions (Norby et al. 1986a), and CO_2 enhancement increases photosynthesis more than it does biomass increment, especially in cases of nutrient deficiency leading to an accumulation of soluble carbohydrates (Silvola and Ahlholm 1992, Pettersson et al 1993). Potential sinks for the "extra" carbon assimilated could be the root exudates and the accelerated growth and turnover of fine roots (cf. Norby et al. 1987, Zak et al. 1993). If stimulated mineralization of nutrients in the soil takes place due to elevated CO_2 (cf. Zak et al. 1993), this could compensate for the nutrient shortage in organic-rich soils due to the reduction in nutrients in the detritus.

If CO_2 enhancement accelerates mineralization in soil, an increase in microbial activity and biomass could be expected. In the experiment of Norby et al. (1986a), CO_2 enhancement did not increase the density of rhizosphere bacteria, but the total bacterial biomass in the soil had probably increased because of the increased root biomass. Also Zak et al. (1993) found an increase in the microbial biomass and nitrogen mineralization at elevated CO_2. It remained open, however, whether the question was about an acceleration of N cycling or also that of N release from "native" soil organic matter. Here the uptake of nutrients by the willows at the fertilization rates of 0 and 100 kg ha^{-1} did not depend significantly on CO_2 concentration. It thus seems that here at low nutrient levels the small increase in biomass production was compensated for by a reduced nutrient concentration, and that CO_2 enhancement did not increase the availability of nutrients for the willows.

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