Analysis of laser-induced fluorescence in wood plants under nitrogen soil pollution

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Investigations aimed to reveal peculiarities of laser-induced fluorescence (LIF) of the Siberian stone pine (*Pinus sibirica Du Tour*) and the warty birch (*Betula pendula Roth.*) have been carried out for different concentrations of easily hydrolyzable nitrogen in soil. Along with remote LIF-signal analysis of experimental transplants and reference plants at λ = 685 and 740 nm, needles and leaves pigmentation as well as nitrogen concentration in soil have been measured. An optimal nitrogen level as well as wood plants response to nitrogen surplus in soil have been ascertained.

Introduction

Monitoring of the Earth's vegetation cover is the issue of importance for predicting the sustained development of our civilization. Due to globality of the problem, remote monitoring techniques are advanced today. A significant progress in laser shortwave radiation sources stimulates the use of LIF techniques for remote monitoring of vegetation. These techniques are based on observation and analysis of fluorescent properties of the pigments belonging to the plant photosynthetic system and having a wide-band fluorescence in the visible and UV ranges, as well as on chlorophyll fluorescence in two narrow bands at λ = 685 and 740 nm.

The aim of this work was to study the correlation between fluorescent properties of wood plant chlorophyll and the nitrogen soil pollution. The study was based on results of the laboratory experiments,[1,2] which showed a transformation of pigment bands to occur in reflectance and fluorescence spectra of plants under variations of mineral nutrition. It can be assumed that some change in root nutrition should result in deformation of reflectance and fluorescence spectra in the ranges determined by the chlorophyll state and density. In support of this assumption, field experiments were carried out to study the LIF in 685 and 740-nm bands, as well as reflection properties of birch leaves and Siberian stone pine needles in 500–700 nm bands under the influence of easily hydrolyzable nitrogen added to soil.

Experiment

In the experiments, from five- to seven-year plants of the Siberian stone pine (*Pinus sibirica Du Tour*) and the warty birch (*Betula pendula Roth.*) were used. The choice was determined by a high sensitivity of foliaged trees and a resistance of conifers to different soil pollutants.[3,4] The experiment was carried out from the second decade of July to the second decade of August. In the beginning of July the samples were transplanted from the "Kedr" experimental seadling nursery of the Kaltai forestry into ~ 4-dm^3 cone-type polyethylene vessels, which were 70 m distant from the lidar. For this period, nitrogen in the form of ammonium nitrate, which can be either a fertilizer or a pollutant depending on the concentration, was applied four times at 5-day intervals.

Experimental plants were divided into three groups. Soil of the first (reference) group remained unfertilized; an optimal (pre-determined) dose of the fertilizer was added to soil of the second group while an extra dose – to soil of the third one. The nitrogen concentration in soil was determined in the beginning of the experiment before fertilizing and at the end after fourfold nutrition by the Shkonde–Koroleva method.[5] Samples of two-year Siberian stone pine needles and birch leaves were taken from all the groups before and after each fertilization. Chlorophyll concentration in needles and leaves was measured with an UV-1601PC spectrophotometer; the LIF of birch and Siberian stone pine transplants was studied with the use of a lidar.[6]

Discussion

Figure 1 presents data on nitrogen concentration in soil at the end of the experiment. As is seen, the total nitrogen content is species-specific and depends on the fertilizer amount.

In comparison with control measurements before nutrition, the concentration of easily hydrolyzable

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nitrogen decreases for birch and increases for Siberian stone pine in case of optimal doses of the fertilizer.

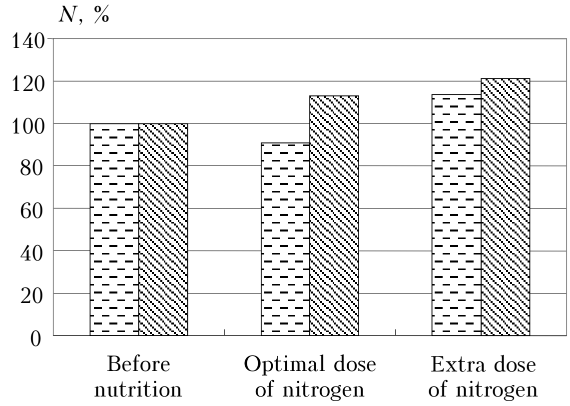

Fig. 1. Relative abundance of the easily hydrolyzable nitrogen in soil at the end of experiment: ▤ corresponds to birch and ▧ to Siberian stone pine.

This can be explained by more active uptake of soil nitrogen by birch. It should be noted, that extra doses of the fertilizer result in an increased concentration of easily hydrolyzable nitrogen in soil both for birch and Siberian stone pine.

Fluorescence properties of plants, i.e., the ratio of the fluorescence signal at λ = 685 nm to the signal at λ = 740 nm are shown in Figs. 2 and 3. This ratio for birch plants of the reference group is about 3 (see Fig. 2). A weak time dependence of signal intensity for this group reflects a seasonal variability. The signal increase is characteristic of the second and third groups in 7–14 days after the first ammonium nitrate addition, as well as the asymptotic approach of signals in all three groups to about 3 to the end of the experiment.

Substantially different dependence is revealed for fluorescence properties of Siberian stone pine needles (Fig. 3). The reference group is characterized by a high LIF stability, while a contrast maximum is

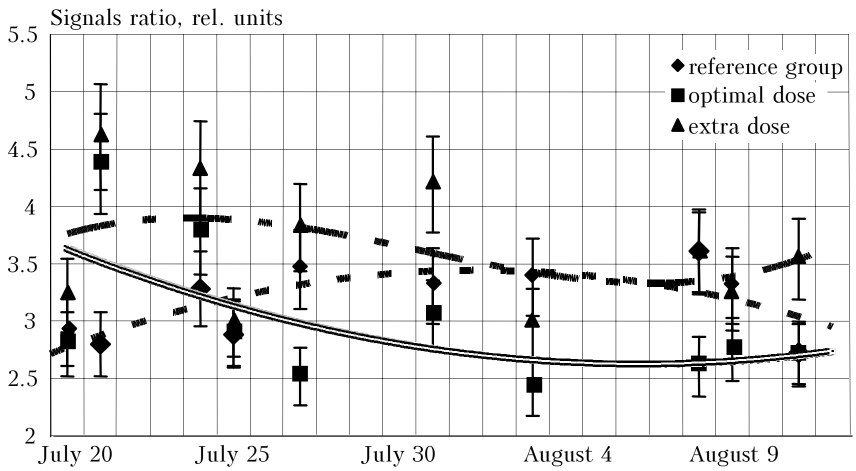

Fig. 2. Ratio of fluorescence signals at 685 and 740-nm wavelengths for birch transplants at different concentrations of easily hydrolyzable nitrogen added to soil.

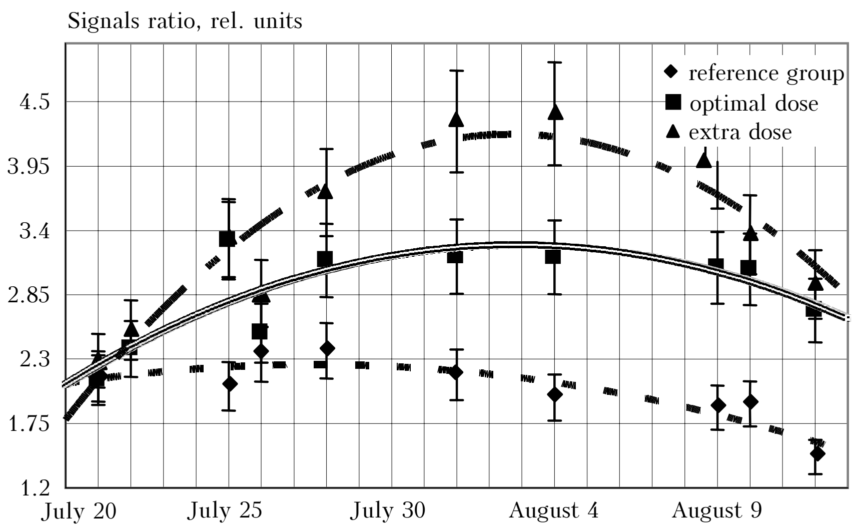

Fig. 3. Ratio of fluorescence signals at 685 and 740-nm wavelengths for Siberian stone pine needles at different concentrations of easily hydrolyzable nitrogen added to soil.

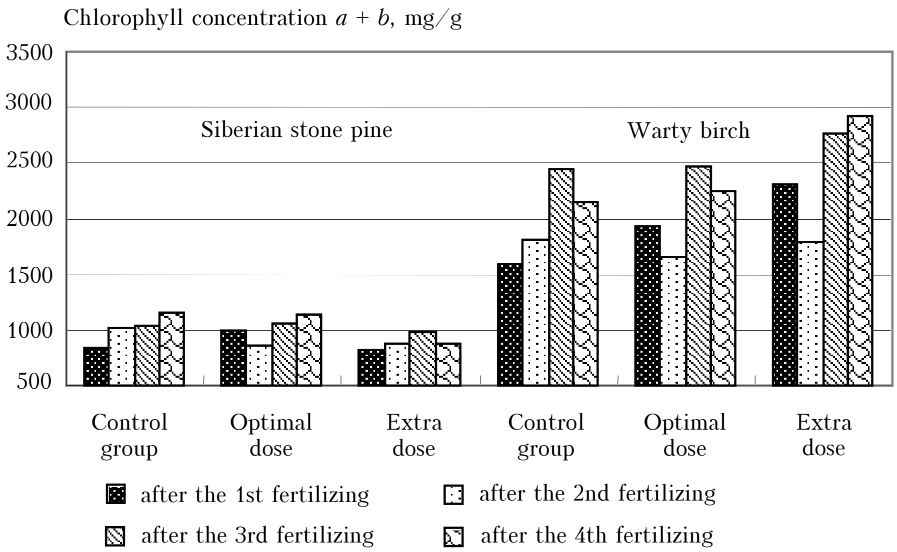

Fig. 4. Chlorophyll concentrations *a* and *b* in Siberian stone pine needles and warty birch leaves after adding different concentrations of ammonium nitrate to soil.

observed in groups with optimal and extra nitrogen concentration in soil. This fact is an evidence of the resistance of conifers to variations of easily hydrolizable nitrogen concentration in soil. This is also proves the hypothesis that extra doses of the soil fertilizer can inhibit the activity of enzymes responsible for ammonia and nitrate nitrogen uptake by plants.[7]

Consider photometric measurements of chlorophyll concentrations. The results on *a* and *b* chlorophyll concentrations are shown in Fig. 4. Note, that an absolute pigmentation of Siberian stone pine needles was 2.5–3 times as low as of birch leaves at all stages of the experiment.

Low chlorophyll concentration is a common feature of conifers as compared to foliaged trees, which manifests itself in fluorescence signal intensities.

In Siberian stone pine needles, the chlorophyll concentration at optimal fertilizer doses sharply increases after the first fertilization and is virtually the same as in the reference group after subsequent fertilizing. Such a pigmentation stability is connected with peculiarities of the nitric metabolism in conifers. Extra nitrogenous nutrition decreases photosynthetic pigmentation in Siberian stone pine needles. According to the above-mentioned, it can be concluded that extra doses of ammonium nitrate inhibit nitrogen uptake by Siberian stone pine roots.

The nitrogen nutrition affects warty birch pigmentation quite different. Optimal doses of the fertilizer do not initiate significant changes in photosynthetic pigmentation, but already next day manifest themselves in LIF characteristics. First, the ratio of fluorescence signal intensities $f = F(685)/F(740)$ increases significantly at optimal and high concentrations of nitrogenous matters in soil, which is the evidence of beginning deviation from the functional standard and plant abiosis (see Fig. 2). The fluorescence signal weakens with fertilizer reinforcing, which is connected with adaptation processes in response to stress conditions. Evidently, there occurs some regulation in primary photosynthates aiming at a decreased buildup of photosynthetic products in conditions when they cannot be taken up completely by plants. This manifests itself in reduction of chlorophyll concentrations in the second and third experimental groups. A further fertilization results in accumulation of nitrogenous matters in soil, decrease of fluorescence intensity f, and visual damages, e.g. degradation of leaves of the bottom storey.

In parallel with LIF recording and standard biochemical analyzing of pigment concentrations, measurements of spectral characteristics in living leaves have been carried out. Observation of the chlorophyll absorption band edge both in absorption and reflectance spectra of living leaves is of interest for remote monitoring of the vegetation cover with laser sensing techniques. Since the edge spectral width is not large (~ 40 nm), the absorption–scattering technique can be used for remote sensing of chlorophyll in living leaves.

Conclusion

Thus, optimal concentrations of easily hydrolizable nitrogen result in sharp increase of chlorophyll concentrations in Siberian stone pine needles just after the first fertilizing. Further nitrogenous fertilization decreases chlorophyll concentrations to the level inherent to the control group of plants. Accounting for the Siberian stone pine capability to restrict nitrogen uptake by roots, it can be explained why the changes in its fluorescence parameters appear only after the second fertilization.

Under extra nitrogenous nutrition, birch leaves accumulate pigments in a higher degree as compared to the control group. Contrary to the Siberian stone pine, nitrogen uptake by the birch does not decrease, while the ratio of fluorescence intensities sharply increases already after the first fertilization.

When recording absorption and reflectance spectra using a spectrophotometer with an integrating sphere, 30–40% dips in absorption spectra were observed for some sampling leaves in the 680–740-nm range.

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