Fluorescence procedures to assess the photosynthetic resilience in Scots pines after a surface fire

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Abstract. Forest fire represents one of the most serious abiotic stress factors that influence the function and productivity of ecosystems globally. Siberian pine forests are often exposed to forest fires but they are not always harmful to them. We have analyzed the effects of repeated heat stress on the photosynthetic apparatus of Scotch pines before and after surface fire exposure that occurred in the growing season in 2014. The survey area was the forest steppe zone of Krasnoyarsk region (South Siberia). First, we investigated the changes in the temperature-dependent responses of photosynthesis in the needles by means of fast and delayed chlorophyll fluorescence. Our results are indicative of some acclimation of Scotch pines after fire to the repeated high temperature stress. Also, this paper discusses the possibility of using chlorophyll fluorescence parameters for evaluating the presence of physiological changes after the fire affects. The time after fire effect and growth season were found to be important to assess the repair of photosynthesis and pigment content.

1. Introduction

Siberian forests play a major role in the global climate system, they are crucial for terrestrial biodiversity, and they are a supply of major natural resources. Forest fires happen frequently in Siberia. From 4.5 thousand to 27 thousand forest fires annually occur in this part of the world [1]. Sixty percent of forest fires occur in pine forests [2]. In this area, fires have always been a factor of evolutionary significance, since these forests are characterized by a high fire frequency.

Fires, as a stress factor, may induce metabolic changes in trees. Heat stress due to fire effects is exerting strong effects on plant physiology and the resultant forest dynamics. During forest fires, the temperature gradient in the crown layer is very steep and heat events can have a major impact on photosynthetic features of plants. Plant resilience at individual and community levels can be assessed through ecological consequences of fires on plant communities. Trees are long-living organisms, and forest ecosystems need to adapt to changing environmental conditions in particular to high temperatures at fires. Different procedures such as chlorophyll fluorescence records are applied for exploring the processes involved in plant’s ability to survive and recover after an extreme stress [3]. Most studies investigating the photosynthetic temperature responses of plants have focused on responses of chlorophyll fluorescence and the underlying biochemical mechanisms. In fact, physiological and metabolic processes change at temperatures higher than the optimum [4].

Currently, the main challenge is not only to define a parameter that may be used to assess the heat sensitivity of photosynthesis, but also for how long trees can conserve stress memory (features and durations of the metabolic changes in plants) after the fire influence. We need to understand not only how trees respond to stress events but also whether they will be able to recover. This is essential to understand the ability of pine forests to maintain their viability in spite of environmental variations.

This paper aims to find out if Pine trees have resilience to repeated heat stress after low-intensity fires and what method can be used to assess high temperature effects.

2. Materials and methods

2.1. Plant materials and experimental treatments

The studied area was located in a forest-steppe zone of Krasnoyarsk region in the territory of “Pogorelskiy bor” station of Sukachev Forest Institute, Siberian Branch of the Russian Academy of Sciences (56°22′07 N 92°57′17 E). The sample plot was the one with a surface fire of 2014. For comparison with the burned site, we sampled plants from an unburned, 20- year -old P. sylvestris stand. The unburned site was similar in size, age, plant community composition and ecological conditions to the P. sylvestris stand at the burned site. The sample territories were chosen in 2015 year, the measurements were taken in June, July, and September. Studies were performed in a grass pine forest. Ten model pine trees were selected for each plot. Intensity of fires was estimated on the basis of burn marks on tree stems. A fire injury of tree trunks was marked after the fire. The average height of a ring quench layer (a charred bark surface) was <0.6 m and we therefore considered this to be a low intensity fire [5]. The basic data on the forests in the test areas, and the after fire effect, are presented in Table 1

Table 1. Forestry taxation properties of the **experimental areas** under investigation

Test area, no.	Forest composition	Average				Fire period	Growth class
		Diameter, cm	Height, m	Height to crown, m	Fire intensity		
SP 1	10 P	11,7	7,8	2,9	low intensity	May 2014	I
SP 2	10 P	12	7,9	3	control	-	I

Three branches from the lower parts of crowns were cut from each tree using lopping shears and they were exposed to a variety of influences: 70% relative humidity; 14-h light at 26 +- 2 °C/10-h darkness at 22+- 2°C (light intensity of 150 μmol/(m2 s)). The exposition lasted for 5 days under laboratory conditions. We used the method of artificial stress influence. The branches were heated in thermostat at temperatures of 45°C. The time of heating was 10 minutes and then the indexes of delayed and fast fluorescence were measured. The functionality of photosynthetic apparatus was investigated for four days after the temperature treatment. The plants were in a jar with water in order to avoid water stress. Exposure to 26°C was used as a control.

2.2. Fluorescent measurements

2.2.1. Delayed fluorescence. We investigated the parameter of delayed fluorescence (DF) that demonstrates photosynthetic pine needle activity for the evaluation of pine needles resistance to high temperature stress, from trees within burned vs. unburned plots. We indicated DF on a fluorimeter “Foton – 10” that was produced at the Department of Ecology and Natural Resource Management at Siberian Federal University [6]. A high sensitivity of photosynthesis to environmental factors, and the sensitivity of DF to changes in different photosynthetic processes have made DF a useful tool for testing plant reactions under stress conditions [7].

2.2.2. Prompt fluorescence. In addition to the parameter of delayed fluorescence (DF), we used Fv/Fm as a criterion for evaluating heat resilience of the needles of Scotch pine, as it is a rapid and non-destructive index to detect stress damage. The parameter Fv/Fm is often used to assess the maximal photochemical quantum yield and the efficiency of the PSII reaction centers (YII) in a dark adapted state that provides valuable information about the function of PSII (W. R. Chen,2012). Changes in PSII efficiency were measured using Junior-PAM modulated fluorometer (Walz, Germany). The ratio

Fv/Fm was used as an estimate of maximal quantum yield of photochemistry [8]. Minimum and maximum fluorescence yields (F0, Fm) were measured after 10 minute dark-adaption. Data were presented as the mean ± standard error for each case (n = 10).

2.3. Pigment analysis

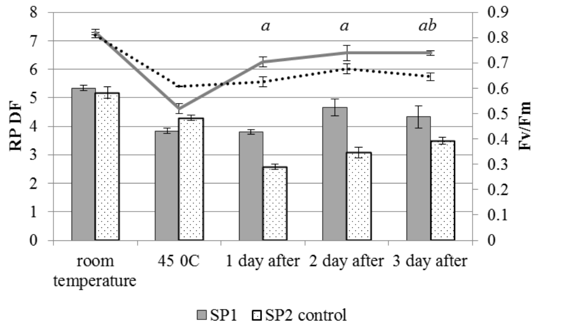
Photosynthetic pigments were extracted from fresh leaf samples in 85% acetone. Absorbance of the extracts was measured using Analytic Jena 2000 spectrophotometer (Germany). Photosynthetic pigment content was calculated in mg/g dry weight to allow comparison among species, using the three equations suggested by Lichtenhaler (1987) [9].

2.4. Statistical analysis

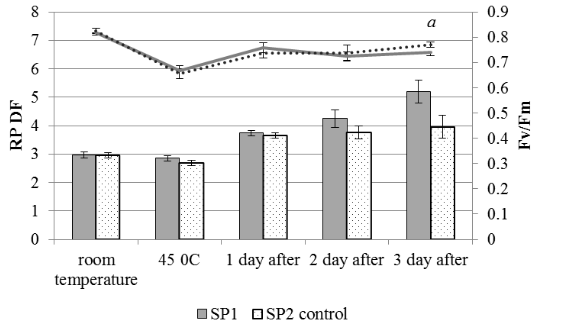
Statistical analysis was carried out using STATISTICA software (v. 11.5). The data about delayed fluorescence are the mean of ten replicates, and chlorophyll content is the mean of three replicates.

3. Results

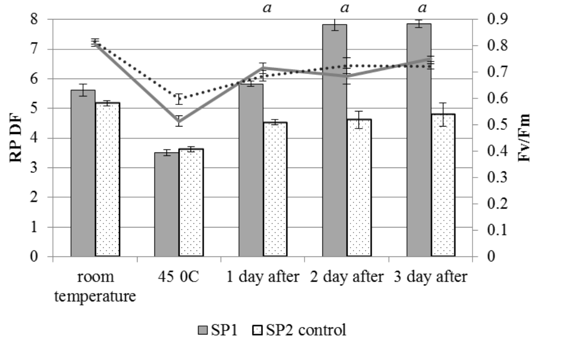
The delayed fluorescence (at room temperature) as well as the ratio Fv\Fm was not significantly different for the pairs of burned and unburned plot in June, July and September prior to the artificial stress (figure a, b, c). The figure shows the dynamics of photosynthetic rates after heating of branches under laboratory conditions due to 45°C. In this way, we determined the general heat resistance, when cells could switch to defensive and protective reactions and resist against damage. We detected variation in photosynthetic pine needle activities between burned plot and control plot after repeated artificial heat stress. The parameter of delayed fluorescence in pine needles was reduced after artificial heat treatment at 45 ° C for control plot and burned sample plots. The fast chlorophyll fluorescence was measured simultaneously with the delayed chlorophyll fluorescence. The lines in figures reflect quantum efficiencies of pine needles after an artificial stress (YII).



a)



b)



c)

Figure The average of relative delayed fluorescence (RP DF) and PSII quantum yield (Fv/Fm) for the needles that were selected in A) June, B) July, C) September (SP1 **-** plots after fire effects; SP 2– control plots).
The lines reflect Fv/Fm
a - Statistically significant differences ($P<0.05$) for (RPDF)
b - Statistically significant differences ($P<0.05$) for Fv/Fm)

Figure (a) shows the simulated effects of high temperature (45 °C) on chlorophyll fluorescence of pine needles that was investigated in June. The relative indicator of delayed fluorescence measured immediately after the high temperature stress was reduced in plants from all plots. Even greater decrease was observed in photosynthetic activity for the needles from plots the next day after heating. This reaction was possible due to the fact that the plants were in a phase of shoot growth, and the flow of plastic substances (metabolites) was directed to the support of growth. It may be due to the cells containing a smaller amount of the thermal protector.

The Chl fluorescence ratio Fv/Fm showed similar behavior related to the rate of delayed fluorescence. A temperature of 45 °C was already at the threshold of damaging levels, it resulted in the decrease of Fv/Fm due to the influence of 45 °C in relation to the initial level. The maximal quantum yield of dark adapted needles (Fv/Fm) was around 0.5 – 0.7 in Scotch pines after heating in the thermostate. The original level of photosynthesis was restored within 3 days under laboratory conditions. A higher level of delayed and prompt fluorescence was noted for conifer trees from plot exposed to stressful temperatures after fire in 2014. Altogether, burned plot were characterized by a higher level of the relative measure of delayed fluorescence on the first, second and third day after an artificial stress at a high temperature.

Figure (b) shows the simulated effects of high temperature (45 °C) on chlorophyll fluorescence of pine needles that was investigated in July. The parameter of delayed fluorescence markedly decreased for all plots during July at room conditions compared to the previous and subsequent periods of this study. For coniferous trees grown at natural conditions, the inhibition of photosynthesis in air occurs at leaf temperatures of about 30 °C. Thus, higher air temperatures in July may influence the decrease in photosynthetic activity.

The maximal quantum yield of dark adapted needles (Fv/Fm) did not change, and was consistently high (around 0.7–0.8), suggesting a high efficiency of primary photochemistry, but it decreased under severe thermal stress at 45°C. Ten minute heating at the temperature of 45 °C did not lead to a significant decrease of RI DF while Fv/Fm decreased by about 12 % in relation to the initial levels for the needles of pine trees from all plots. However, the measured rate of RI DF after heat treatment became higher than the initial rate due to the exposition under laboratory conditions. Generally, ten minute heating at the temperature of 45°C for twigs resulted in greater suppression of photosynthetic activity for the trees growing in a control plot, but we did not observe significant differences in quantum efficiencies.

Figure (c) shows the simulated effects of high temperature (45 °C) on chlorophyll fluorescence of pine needles investigated in September. The inhibition of photosynthesis was observed after a short exposure (10 min) to high temperature (45 °C) for the needles from all our plots. The needles from sample plots restored their original levels of photosynthetic activity within a day. After additional stress treatment, a visible positive effect of fire that occurred in 2014 was observed. It was expressed in a higher level of delayed fluorescence compared to its initial rate due to the exposition under laboratory conditions. Obviously, even this sublethal treatment could provoke a certain increase in the heat tolerance of trees which endured the stress due to the fire that happened one year ago.

The parameter of delayed fluorescence for the needles from a sample plot where surface fire occurred in 2014 showed higher photosynthetic activities compared to control needles collected during early and late summer after heating. The measurement of PSII photochemistry in the dark-adapted state (Fv/Fm) was assessed for each sample plot but there were no significant differences ($P < 0.05$) between the pre- and post-heat event measurements in the needles that were selected in July and September for the first experimental area with the plot where the fire occurred in 2014.

Pigment content is one of the indicators of plant responses to different stress factors of the environment and one of informative parameters describing photosynthesis activity and physiological plant state. Measurements of pigment content provide direct information about photosynthesis potential. The average numbers for each sample plot are presented in table 2. The amount of chlorophyll (a+b) decreased in July and September for the plot where the fire occurred in 2014. According to Girs (1964) [4], the chlorophyll content in needles was higher than in unburned trees at the beginning of the growing season (June) the next year after the fire. This was in agreement with our experimental data and the chlorophyll content amounted to 2.26 and 2.06 mg/g for SP1 (burned plot) and SP2 (control plot) respectively. Thus, the fire of low-intensity did not have a significant effect on the leaf pigments.

Table 2. The average of Chlorophyll and Carotenoids content of needles, mg/g of dry mass

	Chlorophyll a+*b*		Car	
	SP1	SP2	SP1	SP2
June	2.26±0.12	2.06±0.40	0.29±0.02	0.28±0.03
July	2.07±0.15	2.24±0.11	0.24±0.01	0.25±0.004
September	2.11±0.30	2.19±0.30	0.26±0.01	0.25±0.03

Chlorophyll fluorescence measurements are a popular method for evaluating the impact of stress factor on photosynthesis [3, 10]. In this study, we tested the applicability of the measurement of prompt and delayed chlorophyll fluorescence to detect the response of Scotch Pines after a low-intensity surface fire to repeat heat stress factors. The results confirmed that the investigated fluorescence signal parameters were sensitive to the heat stress in different degrees.

The maximal quantum yield of photochemistry (Fv/Fm) in needles that were selected in July and September was unchanged between control and burned plots where the surface fire occurred in 2014. In particular, after an artificial stress, the values of Fv/Fm were the same between control and burned trees. So, in this study, we did not demonstrate a possibility of applying this parameter to evaluating the duration of saving the metabolic changes in trees.

4. Conclusion

We demonstrated that all the burned trees from a sample plot with a surface fire of 2014 were characterized by a higher level of photosynthetic activity after repeated heat stress. This can lead to the conclusion that some physiological processes in plants are modified by ex-stress events. These changes can have a positive effect during repeated actions of stress factors. Furthermore, the fluorescence method can be used to diagnose pine needle thermic resilience and assess high-temperature effects. In particular, we recommend applying a relative index of delayed fluorescence as an index to assess the duration of conservation of the metabolic changes of photosynthetic apparatus for several years after a fire. The ratio Fv/Fm that shows the photochemical efficiency of PSII can be only used to describe responses in forest stands after the immediate effect of stress factors.

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