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Short communication

Evaluation of fire severity via analysis of photosynthetic pigments: Oak, eucalyptus and cork oak leaves in a Mediterranean forest

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A B S T R A C T

Few studies to date have examined the effect of the high temperatures attained during wildfire events on the pigments present in forest foliage. Here, we seek to analyse the main photosynthetic pigments in the leaves of the oak, cork oak and eucalyptus following a wildfire. We also subject leaves of these last two species to a range of contact temperatures (100–500 °C) in the laboratory using a muffle furnace. The samples were left in the muffle for two hours at 100, 150, 200, 250, 300, 350, 400 and 500 °C, in line with other soil study models (Úbeda et al., 2009; Düdaite et al., 2013). At temperatures above 250 °C, chromatography fails to detect any pigments. A minimal increase in temperature degrades chlorophyll, the process being more rapid in eucalyptus than in cork oak, while it increases pheophytin concentrations.

1. Introduction

Fire severity describes the response of ecosystems to fire and can be used to describe the effects of fire on soils, the water system, the flora and fauna, the atmosphere and human society (Bento-Gonçalves et al., 2012). The term fire severity was born out of the need to provide a description of how fire intensity affected ecosystems, particularly following wildfires where direct information on fire intensity was absent and effects are often quite variable within and between different ecosystems (Keeley, 2009).

The main problem faced by the literature is that of identifying an unequivocal means of determining fire severity. One of the most frequently cited is that developed by DeBano et al. (1998) which categorises a burnt area as presenting low, moderate and high fire severity depending on the percentage area of the total burnt forest that has been exposed to a high intensity fire episode. Yet, it has been well documented that in a single wildfire different parts of the forest burn at different severities and it is important that they be identified to understand the specific effects of the fire episode and associated environmental processes (Úbeda et al., 2006).

In controlled or prescribed fires a thermocouple can be used (Mataix-Solera et al., 2002) to measure the temperatures reached in the soil, but in wildfires the methods have to be indirect. For example, the following methods have been used to determine the degree of severity of a wildfire: the colour of the ashes (Úbeda et al., 2009), the number and diameter of remaining branches (Moreno and Oechel, 1989), Near Infrared Spectroscopy (Guerrero et al., 2007), and changes in soil colour (Lentile et al., 2006). The general conclusion is, however, that it is important to know how fire severity affects different sections of the overall burnt area so as to know whether it is necessary or not to initiate post-fire management (Francos et al., 2016).

By means of remote sensing, it has been shown that the reflectivity of a burnt area increases as a result of the loss of leaf chlorophyll and/or increase in the proportion of bare soil (De Santis and Vaughan, 2009). However, few studies to date have examined the effects of temperature on chlorophyll, the pigment that captures the light energy needed for photosynthesis, and, hence, on this process of photosynthesis (Chen et al., 2012). There are, in fact, two basic classes of plant pigments: chlorophylls and carotenoids. According to the definition furnished by Rowan (1989), chlorophylls are greenish pigments that contain a porphyrin ring. This is a stable ring-shaped molecule around which electrons are free to migrate. Because the electrons move freely, the ring has the potential to gain or lose electrons easily, and hence the potential to provide energized electrons to other molecules. This is the

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fundamental process by which chlorophyll captures the energy from sunlight. Carotenoids, on the other hand, are usually red, orange, or yellow pigments, and include the familiar compound carotene, which gives carrots their colour. These compounds are composed of two small six-carbon rings connected by a chain of carbon atoms. As a result, they do not dissolve in water, and have to be attached to membranes within the cell. Moreover, carotenoids cannot transfer sunlight energy directly to the photosynthetic pathway, but have to pass their absorbed energy to chlorophyll.

The objective of this study, therefore, is to propose a new indirect method for determining the severity of a wildfire or a prescribed fire in different areas of the same burnt forest by analysing the photosynthetic pigments of leaves. In conducting this research, we have worked with three types of predominant vegetation in an area in the north of the Iberian Peninsula affected by a wildfire in 2016. Additionally, we have used an autochthonous species and an introduced species in a subsequent laboratory test.

2. Study area

Blanes occupies the south-eastern section of the Catalan Coastal Ridge, marking the limit between the Gavarres Massif and the Montseny. It is considered the first town on the Costa Brava in Catalonia and lies within the *comarca* of La Selva. Blanes has a Mediterranean climate, with an annual precipitation of around 630 mm characterised by irregular, torrential rain events, and a mean annual temperature of 15 °C. In terms of its geomorphology, Blanes can be divided into two areas: the southern half of the municipality is characterised by a gently sloping plain, while the northern half has a more pronounced relief, reaching a maximum height of 309 m a.s.l. It is in this second area of predominantly late-Hercynian granites that samples were taken for this study. The soils are mainly deep leptosols with some rocky outcrops. The area is dominated by forests of oak (*Quercus ilex*) and cork oak (*Quercus suber*) with white pine (*Pinus halpensis*), and eucalyptus *(Eucalyptus globulus)* introduced by man.

3. Methods

We conducted two studies: one in an area affected by the wildfire, the other in a laboratory simulation.

In July 2016, a wildfire broke out in Blanes (NE Spain), affecting 27 ha and forcing the evacuation of some 400 people. Forty land and thirteen aerial firefighters worked to extinguish the fire that broke out at 12:51 p.m. on 24 July 2016 and which was brought under control at 10:15 a.m. on 25 July. The fire affected a mixed forest of oak, cork oak and eucalyptus. We collected leaves from each species: some that were unburned and some that had been affected by the fire. Among the burned leaves we identified three groups based on the leaf colour as an indication of the degree of burning: low, medium or high. The fire was fierce enough to burn the forest shrub land but it did not reach the crown. This meant that the condition of the leaves in the canopy of the forest was attributable to the hot air convection caused by the wildfire and the smoke produced during combustion.

The second study was conducted by sampling unburned eucalyptus and cork oak leaves. It is not possible to collect leaves at specific temperatures during a fire in order to identify their pigments; therefore, a laboratory experiment represents a suitable alternative for obtaining an approximate idea of the impact of temperature on leaf pigments. Unburnt leaves were freeze-dried to remove all moisture, thus simulating the effect of heat, and then passed through the muffle furnace. The samples were left in the muffle for two hours at 100, 150, 200, 250, 300, 350, 400 and 500 °C, in line with other soil study models (Úbeda et al., 2009; Düdaite et al., 2013).

The pigments were extracted from c. 1 g sediment dry weight in 4 ml of 90% acetone with a probe sonicator (Sonopuls GM70 Delft, The Netherlands) (50 W, 2 min). The extract was centrifuged (4 min at 3000 rpm, 4 °C) and filtered through a Whatman Anodisc 25 (0.1 μm) and analysed by ultrahigh-performance liquid chromatography (UHPLC). The pigment was analysed using a modification of the method described by Buchaca and Catalan (2007). The UHPLC system (Acquity, Waters, Milford, MA, U.S.A.) was equipped with an Acquity UPLC HSS C18 SB column (dimensions: 100 × 2.1 mm, particle size: 1.8 μm) and photodiode array (λ 300–800 nm) and fluorescence (λ excitation 440 nm, emission 660 nm) detectors. The detector was set at 440 and 660 nm for carotenoid and phorbin peak integration, respectively. After sample injection (4.5 μl), pigments were eluted by linear gradient from 100% solvent B (51:36:13 methanol:acetonitrile: MilliQ water, v/v/v 0.3 M ammonium acetate) to 75% B and 25% A (70:30 ethyl acetate:acetonitrile, v/v) for 3 min, followed by 0.45 min of isocratic hold at 75% B and 2 min of linear gradient to 99.9% solvent A. Initial conditions (100% B) were linearly recovered in 0.65 min. The flow rate was 0.7 ml min^{-1}. Pigments were identified by verifying retention times and absorption spectra to a library based on commercial standard mixtures (DHI, PPS-MiX-1) and extracts from pure cultures of algae and bacteria. Integration and data storage were performed with Empower 2 Chromatography Manager software. The concentration of pigments in the leaves was calculated using Buchaca's formula (1) (2006):

$$C = A_p \cdot (1/V_{inj}) \cdot (V_{est}/P_{dw}) \cdot F \cdot (1/6 \cdot E) \quad (1)$$

where C is the concentration of pigment in the peak area, Vinj is the injected volume, Vest is the volume of the extract, Pdw is the dry weight of the sediment extracted, F is the flow flowing through the mobile phase and E is the extinction coefficient, considering that the absorbance/voltage device relationship is 1/1 and the passage of light detection system is 1 cm. Concentrations are expressed in μg/g.

4. Results

The results for the samples collected after the wildfire of July 2016 (Table 1) show that the total concentration of pigments in the control leaves for each of the species differs markedly: eucalyptus has a total concentration of 530 μg/g, oak of 409 μg/g and cork of 33 μg/g.

Cork oak leaves presented the lowest concentration of all pigments, but most notably of chlorophylls.

Chlorophyll *a*, the main pigment involved in photosynthesis, presented a similar relative percentage in the control leaves of all three species. However, post-fire, the percentage of chlorophyll in cork oak fell from 59 to 7%, whereas in the other species this percentage value remained largely constant.

β carotene is the only pigment detected in all burnt samples, generally the concentration falling as the degree of burning increased. However, in the leaves of the cork oak, the opposite was true, although the absolute concentration was low.

Pheophytin *b* is the main pigment presented by the leaves after burning. In the controls, it was either not present or only present at very low concentrations, while in the burnt leaves of the eucalyptus and cork oak it presented concentrations of around 26 and 78 μg/g, respectively.

Both major classes of pigments were detected in the tests. In the case of the chlorophylls, the highest concentrations were recorded for chlorophyll *a* followed by chlorophyll *b*, pheophytin *a* and pheophytin *b*. In the case of the carotenoids, we detected β