How do plants respond biochemically to fire? Biosynthesis of photosynthetic pigments and secondary metabolites in response to this disturbance

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Abstract

Fire-prone ecosystems and their vegetation have evolved in response to this disturbance. Stress produced by fires promotes the biosynthesis of secondary metabolites which could alter subsequent fire regimes. The aim of this work was to evaluate the variation in the biosynthesis of chlorophylls, carotenoids, phenolic compounds and tannins in response to fire. Leaves of six native species were selected and three experimental burns (EB) were conducted. Spectrophotometric methods were used to quantify the metabolites. As results, a temporary variation on the biosynthesis of chlorophylls and secondary metabolites in response to fire was found. Chlorophylls and carotenoid contents decreased within a short temporal scale, but their concentrations returned to pre-event conditions one-year after disturbance. Concentrations of phenolic compounds and tannins in burnt plants increased up to two years after EB. The fast-metabolic response evidenced the plant tolerance to fire. These metabolites could be used as bioindicators of vegetation resilience after fire.

1. Introduction

Fire has always been an important ecological role in shaping the components of landscapes. In addition, it also plays a key role in the conservation and management of biodiversity in regions which have coevolved with this disturbance (e.g. the Mediterranean and the Chaco Region). In semiarid landscapes, seasonality and vegetation characteristics promote the fire occurrence (Bravo *et al.* 2001; Bravo 2010). However, within the global context of climatic and land use changes, fire regimes have been altered, becoming in increasing severity and extension events (Bravo 2010).

Plants have been biochemically prepared to respond to environmental changes through the biosynthesis of secondary metabolites. Indeed, physical stress produced by fire promotes the biosynthesis of these compounds which provide resistance to vegetation and predisposes it to new fire events which could be even more severe. Recent studies have highlighted the effect of foliar organic chemistry on flammability, focusing on volatile organic compounds (VOCs) such as terpenoids, which are characterized by reducing ignition temperatures in both foliage and litter (Ormeño *et al.* 2009; Page *et al.* 2012; Bowman*et al.* 2014; Della Rocca *et al.* 2017). Furthermore, these compounds have an important role on plant defense to herbivores and pathogens (Page *et al.* 2012; Della Rocca *et al.* 2017; Diaz-Guerra *et al.* 2018).

The role of chlorophylls, carotenoids, phenolic compounds and tannins in response to fire have still been poorly investigated. Chlorophyll is one of the main indicators of photosynthetic capacity and physiological status of plants (Cambrón-Sandoval *et al.* 2011; Callejas *et al.* 2013; Jaramillo-Salazar *et al.* 2018). Indeed, quantification of photosynthetic pigments contributes to determine the plant behavior during its development cycle under stress conditions. (Goel *et al.* 2004; Huot *et al.* 2007; Cruz *et al.* 2009). The function of carotenoids beyond being an accessory pigment in photosynthesis is the protection against photosensitization due to chlorophylls (Nisar *et al.* 2015). In addition, these compounds are considered important elements of information storage in response to environmental changes (Allred & Snyder 2008; Esteban *et al.*2015). Furthermore, foliar persistence is an important trait on plant flammability (Santacruz-García *et al.* 2019) which influences total chlorophyll and carotenoids concentrations due to the foliar absence of deciduous species during the dry and winter season (Takashima*et al.* 2004).

Phenolic compounds have an important role in plant defense against herbivore (Ganthaler *et al.* 2017; Diaz-Guerra *et al.*2018).These compounds are considered as stress bioindicators due to its high sensitivity to changes in environmental conditions (Lagadic*et al.* 1997). Indeed, Cannac *et al.* (2009) reported an increase in the synthesis of phenolic compounds in response to prescribed burnings in *Pinus laricio.* In addition to the above mentioned functions, tannins reduce the digestibility of plant tissues through the formation of indigestible complexes with dietary protein in herbivores (García 2015). Phenolic metabolites are recognized as strong antioxidant agents which protect cells against free radical damage (Lin*et al.* 2018). Hence, the renewed interest in native plants as a source of many biologically active compounds and natural antioxidants in phytomedicine (Asif 2015).

Vegetation strategies for tolerance to environmental disturbances should be studied as a challenge within the current scenario of climate and land use change. The traditional ecological approach considers that fire tolerance is exclusively related to post-disturbance vegetation regeneration strategies, which include the resprouting ability from bud banks and/ or germination through seed banks (Clarke *et al.*2013). However, this work proposed the evaluation of plant tolerance to fire from a biochemical approach which had not been previously explored by any study in the fire ecology area. The link between the burnt biomass during a fire event and the biochemical response of plant could be considered as a plant tolerance to fire indicator. Considering that fire tolerance is directly related to the fire severity (Bran *et al.* 2007), and fire severity is related to the intensity and duration of the event and it indicates the degree to which vegetation has been affected by this disturbance; the burnt biomass is a measure of fire severity (Montorio *et al.* 2007).

The Chaco Region forests have been strongly affected by anthropic disturbances as wildfires, livestock, logging and mechanical treatments to silvopastoral systems (Bravo *et al.* 2014; Ledesma *et al.* 2018). Forest management strategies include tools such as mechanical treatments and prescribed burning to shrub clearing and to improve pastures for livestock (Talamo & Caziani 2003; Kunst *et al.* 2012; Grau *et al.* 2015; Santacruz-Garcia *et al.* 2019). However, the response of native vegetation to this disturbance possibly reveals an adaptation and tolerance to fire (Bowman *et al.* 2014; Bravo *et al.* 2014). The knowledge of the effect of foliar chemical composition on vegetation response to fire contributes to understand the patterns of postfire recovery of native vegetation in burnt forests. In addition, it could provide tools to wildland fire management strategies (Cannac *et al.* 2009; Ormeño *et al.*2009; Jaureguiberry 2012; Della Rocca *et al.* 2015, 2017).

The objectives of this work were a) to evaluate the effect of experimental burns in the concentrations of photosynthetic pigments as chlorophylls and carotenoids, phenolic compounds and tannins in leaves of six woody species, b) to determine the temporal dynamics of the biosynthesis of chlorophylls and secondary metabolites in response to fire and c) to establish the relationship between the plant tolerance to fire (according to the burnt biomass during experimental burns) with the biochemical response of plants. We evaluated the following hypothesis: a) Fire promotes a variation in the concentration of plant metabolites (Cannac *et al.* 2007, 2009; Ormeño *et al.* 2009; Della Rocca*et al.* 2017). The total chlorophyll and carotenoid contents decrease (Carter & Knapp 2001; Jaramillo-Salazar *et al.* 2018), whereas, phenolic compounds and tannins increase after fire events (Cannac *et al.* 2007), b) The stability and post fire recovery is dependent on biochemical responses as antioxidants biosynthesis that promote the plant tolerance and its resilience to fire regime (Bowman*et al.* 2014; Enright *et al.* 2014), c) Vegetation affected by fire increases the biosynthesis of natural bioactive compounds with potential applications in health and agroforestry sectors (Karjalainen*et al.* 2009; Edwin-Wosu *et al.* 2017) and d) The biochemical response of plants to fire is related to the amount of burnt biomass (Hoffmann & Moreira 2002; Keeley *et al.* 2011).

This study represents the first effort to evaluate a set of metabolites involved in plant responses to fire since a strict biochemical approach. The most studies evaluate response to fires without considering ecophysiological behavior. The knowledge of plant biochemical adaptation strategies to fire could contribute to understand the mechanism of tolerance and resilience of plants in natural environments. A temporary variation on the concentration of chlorophylls and secondary metabolites in response to fire could reveal the role of these compounds as bioindicators of plant tolerance to fire.

2. Material and methods

2.1 Experimental Site

The study area was located in Argentina Western Chaco Region. The sampling sites was located in the Experimental station "Francisco Cantos", Santiago del Estero, Argentina (28°03'S, 64°15'E). The climate is seasonal semiarid, the mean temperature was 20.6 °C and the mean annual precipitation was 731.2 mm during the studied years (2016-2018) (INTA climatic record, 2000-2018 annual series). Soils are regosols (Lorenz 1995).

Typical vegetation includes a mosaic of forest, grasslands, shrublands and savannas. Native forest is distributed in three strata; the dominant tree species of the upper stratum are *Aspidosperma quebracho-blanco* Schltdl. and *Schinopsis lorentzii* (Griseb.) Engl., reaching over 20 m height. The medium stratum includes*Sarcomphalus mistol* (Griseb.) Hauenschild, reaching from 7 to 12 m height. In the understory, thorny species as *Atamisquea emarginata* Miers ex Hook. & Arn, *Celtis ehrenbergiana*(Klotzsch) Liebm (tala), and *Schinus johnstonii* F.A. Barkley are common.

Fire has been an ecological event of the Chaco Region from late 19th century (Torella & Adámoli 2005; Kunst *et al.* 2014). Since the last century, the Chaco vegetation has experienced changes in the land use and anthropogenic disturbances which altered its fire regime. The fire season in Chaco region coincides with the long dry and cold season which extends from May to October. Along this period, the moisture content and phenological state of vegetation vary and can alter the plant flammability and the vegetation response to fire (Santacruz-García*et al.,* 2019).

The experiment was conducted on a typical forest of this region characterized by wildfires and mechanical treatments to control shrub encroachment and to improve the pasture growth for livestock (Talamo & Caziani 2003; Kunst *et al.* 2012; Grau *et al.* 2015; Santacruz-García *et al.,* in press). Six woody species were selected according their representativeness in the three forest strata within the study area (Araujo *et al.* 2008). Botanical family, growth habit and foliar persistence were considered in the selection of the species, as that these traits were used in the analysis and the subsequent discussion. The species selected were: *A. quebracho-blanco* (Apocynaceae, tree, evergreen); *S. lorentzii*(Anacardiaceae, tree, deciduous); *S. mistol* (Rhamnaceae, tree, deciduous); *A. emarginata* (Capparaceae, shrub, evergreen), *C. ehrenbergiana* (Celtidaceae, shrub, deciduous) and *S. johnstonii* (Anacardiaceae, shrub, evergreen). Names of species follow the nomenclature system devised by the *Instituto de Botánica Darwinion* , Universidad Nacional de La Plata (Buenos Aires, Argentina).

2.2 Characterization of Experimental Burns

To evaluate the chemical response of vegetation to fire, three experimental burns (EB) were carried out in three successive years (2016-2018). In the sampling site, 85 individual plots of 2 m x 2 m were randomly established. Thirty plots were burnt each year, with five replications (individual plants) for each species. Individuals of*A. emarginata* were not burnt in 2018 because their absence in the plot destined to burn during this year. An individual plant of each species, DBH < 15, located in the center of each plot was tagged. A low fine fuel load was used, 4000 Kg DM ha^{-1}, corresponding to the aboveground biomass in native grass in the Chaco region (Kunst *et al.* 2012; Bravo*et al.* 2014; Ledesma *et al.* 2018). In plots where the fine fuel load did not reach the expected amount, dry grass biomass previously weighed, was homogeneously distributed.

EB were conducted at the end of the fire season each year (September-October), in coincidence with the flammability peak observed in the Chaco region (Ledesma *et al.* 2018; Santacruz-García*et al.* 2019). Meteorological conditions during the EB dates were monitored every 30 minutes (air temperature -°C-, air relative humidity -%-, wind speed –km h^{-1}- and wind direction) for the maintenance of acceptable thresholds of fire behavior. A drip-torches was used to lit fire setting a head fire. Burnt biomass (BB; %) was estimated visually by two operators and then averaged to reduce the experimental error.

2.3 Evaluation of Chlorophyll and Carotenoid contents

The chlorophyll and carotenoid contents were determined in plants burnt during the 2016 as a preliminary approach to evaluate the initial response of vegetation to fire. Samples of five burnt individuals of each species were collected six and twelve months after the EB 2016. As control, five individual unburnt plants of each species were randomly collected in the same sampling site, in both dates (6 and 12 months after the EB date). During the trial time period, species went through different phenological states: at six months after EB (April 2017, autumn season in Southern hemisphere), deciduous species shed their foliage but evergreen species could maintain their leaves and continue growing; at twelve months after EB (October 2017, springtime season in Southern hemisphere), species restarted their active growth or actively growing.

Representative leaf samples of each individual plant (0.6 g of dried material) were extracted with 50 mL of acetone using a blender (Pro Scientific, Oxford, USA) and filtered under vacuum. This procedure was repeated twice, and these three extracts were combined, and then, transferred to diethyl ether by adding small portions of the acetone extract and large amounts of water in a separatory funnel. Ethereal extract was separated and taken to a 10 mL final volume. Extracts were prepared in duplicate. Determinations were performed using a UV/vis spectrophotometer (7315 Spectrophotomether, Jenway, Staffordshire, England) and absorbances were measured at 430, 642 and 660 nm. Comar and Zscheile equations were used to calculate chorophylls *a, b* and total contents (Goodwin 1976). Total carotenoid contents were calculated using the carotenoid absorption coefficients (Goodwin 1976).

2.4 Total Phenolic Compounds and Tannin Assessments

In order to analyze the effect of temporal variations on total contents of phenolic compounds and tannins, leaf samples of five individual plants of each species, burnt during each EB (2016, 2017 and 2018) were collected in November 2018 for this experiment. In November 2018, the closest date to EB tested, the leaves of all studied species were fully expanded for sampling. As control, samples of five individual plants of each species were collected in the same month in an unburnt plot of the same study area. The results correspond to different individual plants collected simultaneously in November 2018, to ensure that all plants were exposed to the same environmental conditions before analysis. Every plant was burnt once, and no one was treated twice.

Representative samples of each individual plant (0.1 g of dried material) was extracted with 10 mL of acetone/water (70/30, v/v) in an ultrasonic bath (HH-S Water Baths, Bioamerican Science, Buenos Aires, Argentina) periodically during 60 min, at room temperature. Extracts were centrifugated at room temperature for 10 min at 10000 rpm. The supernatant completed to 10 mL with the acetone/water mixture (70/30, v/v), was used to the phenolic compounds and tannin contents assessments according to García (2015).

Folin-Ciocalteu method was used to assess the total contents of phenolic compounds. For calibration purposes, gallic acid (GA) was taken as a reference chemical standard. GA solutions (0.05, 0.06, 0.10 and 0.15 mg mL^{-1}) were prepared in distilled water. An aliquot of 0.15 mL of each extract was transferred to a 5 mL volumetric flask containing distilled water (1.05 mL) and Folin-Ciocalteu reagent (0.65 mL). The volume was completed with 3.15 mL of anhydrous sodium carbonate (20% w/v). The mixture was stirred and heated at 50 °C for 10 min in the same conditions as the blank without plant extract. The absorbance of the samples was measured at 725 nm in a UV/Vis spectrophotometer. Results were calculated and expressed as GA equivalents 100 mg^{-1} of sample (García 2015).

For extractable condensed tannins (ECT) determinations, the butanol/ HCl assay was used. In a volumetric flask, 3 mL of butanol/ hydrochloric acid (95/5, v/v) was added to 0.5 mL of each extract. Each mixture was stirred and heated in a water bath at 95 °C for 60 min and then, it was cooled in ice bath up to reaching room temperature. Afterwards, sample absorbance was measured at 550 nm. ECT were calculated using the tannin absorption coefficients (Goodwin 1976) and results were expressed as g of cyanidin-3-glucoside 100 g^{-1} dry weight (García 2015).

2.5 Statistical analysis

For assessments of chlorophyll *a,* and total chlorophyll contents, data were analyzed through a generalized linear model (GLMs), using the species, the fire data (6 or 12 months after EB), and the treatment (Burnt or Unburnt) as fixed effects. A generalized linear mixed model (GLMM) was used to assess chlorophylls *b* and carotenoid contents, using Normal distribution, and the species, the fire data (6 or 12 months after EB), and the treatment (Burnt or Unburnt), as fixed effects. For assessments of total phenolic compounds and tannin contents a generalized linear mixed model (GLMM) was also performed, using Normal distribution and the species and EB year as fixed effects. For the evaluation of plant tolerance to fire, a principal component analysis (PCA) was performed, using BB and the total contents of the biochemical compounds evaluated in this work. The first axis of the PCA was used to obtain a biochemical response value. The correlation between BB and the biochemical response was performed through a Pearson's correlation coefficient. The statistical software used was Infostat/2017 (InfoStat Group, Universidad Nacional de Córdoba, Argentina) with an $\alpha = 0.05$.

3. Results

3.1 Evaluation of Chlorophyll and Carotenoid contents

A wide range of total chlorophyll contents among the different species and treatments were observed, varying from 256.7 ± 81.3 (burnt plants, six months after EB, *A. emarginata)* to 1601.9 ± 225.2 (control plants, twelve months after EB, *C. ehrenbergiana)* µg g^{-1} dry weight (Table 1). A decrease in total chlorophyll synthesis was observed at the two sampling dates evaluated; nevertheless, there were significant differences only in the first sampling date (6 months since EB 2016). In the second sampling date (12 months after EB 2016), results have not indicated significant differences in the total chlorophyll contents (Fig. 1A, Table 1). Chlorophyll *a* contents have shown a significant decrease in the first sampling date; whereas, in the second sampling date there was no significant differences respect to control (Fig. 1B, Table 1). Neither of the dates of measurement have shown significant differences in the chlorophyll *b* contents of the samples treated (Fig. 1C, Table 1). Considering the foliar persistence, total chlorophyll contents of deciduous species were significantly higher than those of evergreen species. Total chlorophyll contents of deciduous species have shown a significant decrease in the second sampling date respect to the first sampling date. Total chlorophyll contents of evergreen species have not shown significant differences in neither of the dates of measurement (Fig. 1D).

The mean concentration of total carotenoids varied from 74.4 ± 35.3 (burnt plants, six months after EB, *A. emarginata)* to 774.5 ± 136.8 (control plants, twelve months after EB, *C. ehrenbergiana)*µg g^{-1} dry weight. The total carotenoid contents have decreased in burnt plants respect to control in each sampling date, but differences among treatments were not significant. Nevertheless, total carotenoid contents were significantly higher in the second sampling date than in the first one, in both treatments (Fig 2A, Table 1). Deciduous species have shown significantly higher total carotenoid contents than evergreen species among control plants, in burnt plants these differences were not significant (Fig. 2B, Table 1).

3.2 Total Phenolic Compounds and Tannin Assessments

The mean concentration of total phenolic compounds (TPC) in the leaves varied from 6.2 ± 1.2 (EB 2017, *A. emarginata*) to 480.9 ± 178.8 (EB 2017, *S. mistol*) mg of gallic acid 100 $mg^{-1}$of sample (Table 2). A significantly increase in the concentration of TPC was observed in burnt plants respect to control. The increasing of TPC was observed for two months after EB in new leaves fully expanded in resprouts post-fire, to twenty-six months after EB in leaves fully expanded. These contents have not returned to normal values

26 months later (Fig. 3A). All studied species have shown significant differences in TPC concentration. The species *S. lorentzii* and *S. johnstonii* have shown the highest concentrations of this metabolic group whereas *A. emarginata* showed the lowest concentration (Fig. 3B, Table 2). There was no observed a tendency related to foliar persistence.

The mean concentration of extractable condensed tannins (ECT) varied from 0.02 ± 0.01 (EB 2016 and EB 2017, *C. ehrenbergiana*) to 2.36 ± 0.89 (EB 2016, *S. mistol*) g of cyanidin-3-glucoside 100 g^{-1} dry weight. ECT concentration in burnt plants varied significantly from control plants (Table 2). The results have shown a non-significant decrease in ECT contents, two months after EB as compared with control plants. Fourteen months after EB, ECT concentrations have not shown a significant difference respect to control, whereas, at twenty-six months after EB, ECT contents have shown a significant increase respect to control plants (Fig. 3C, Table 2). *S. mistol* have shown the highest ECT concentrations whereas *A. quebracho-blanco* and *C. ehrenbergiana* showed the lowest concentration of this secondary metabolite (Fig. 3D, Table 2). The results have not shown a tendency related to foliar persistence and CV showed a high intra and interspecific variation in TPC and ECT contents (Fig 3, Table 2).

3.3 Plant tolerance to fire: Association between burnt biomass and the plant biochemical response to fire

The first to axes of the PCA performed to evaluate the plant biochemical response to fire using BB and total contents of the post-fire biosynthesized metabolites explained 71% of the data. The first PCA axis (PC1) explained 43% of the variation in the data and was positively associated with BB and with all the biochemical compounds evaluated except with ECT and TPC. The second PCA (PC2) explained 28% of the variation and separated the bioactive compounds biosynthesized post-fire from BB (Figure 4A). The Pearson's correlation coefficient between BB and the first component of the plant biochemical response showed a significant association between them (Pearson's $P = 0.40$,P -value= 0.0014; Fig. 4B).

4. Discussion

4.1 Evaluation of Chlorophyll and Carotenoid contents

The decrease in total chlorophyll and chlorophyll *a* contents observed six months after EB could be attributed to physiological stress induced by fire (Carter & Knapp 2001). Therefore, the quantification of photosynthetic pigments contributes to the assessment of plant response to environmental variables (Huot *et al.* 2007; Cruz *et al.*2009). EB could affect functional status of the woody species studied, at least in a short temporal distance from disturbance (six months), in which significant differences respect to control plants were observed. Nevertheless, twelve months after EB there were non-significant differences in chlorophyll *a* contents respect to control, suggesting a remarkable recovery of plant physiological stability in this native species.

The EB have not produced significant effects in chlorophyll *b*contents, this result could be related to the subsidiary nature of this pigment in photosynthesis. Chlorophyll *a* is the principal pigment in photosynthesis process, whereas chlorophyll *b* is considered as collector antenna that absorbs light in a different wavelength range to pass on to chlorophyll *a* (Cambrón *et al.* 2011; Jaramillo-Salazar *et al.* 2018). The maintenance of chlorophyll*b* contents even at short time after EB, could suggest a greater stability of the studied species to keep the photosynthetic activity, instead of the decrease in chlorophyll *a* contents caused by the fire effect (Cambrón *et al.* 2011; Jaramillo-Salazar *et al.*2018).

The higher total chlorophyll content in deciduous than evergreen species observed in control and burnt plants, revealing a homogeneous response at community level. The evergreen species have lower photosynthetic capacity than deciduous species in relation to higher dry mass content and lower specific leaf area (Takashima *et al.* 2004; Santacruz-García *et al.,* 2019) However, the lack of significant differences in chlorophyll contents observed in our work, could be attributed to a high resilience of the Chaco region species regardless of foliar persistence, life form and phylogeny (Takashima *et al.*2004).

The carotenoid contents followed a similar decreasing tendency that chlorophyll *b* contents, with non-significant differences among treatments in each sampling date. However, these secondary metabolites increased significantly in the second sampling date after the growth recess period. These results reaffirm the role of carotenoids as antioxidants in response to environmental stress conditions beyond the physical stress produced by wildfire (Kunst & Bravo 2003; Manrique Reol 2003; Esteban *et al.* 2015). Postfire environments are characterized by high radiation level and evapotranspiration of plants and soil; therefore, high concentrations of carotenoids in leaves could enhance the recovery not only of plant biomass, but also, could improve the main metabolic processes related to net primary productivity (Stylinski *et al.* 2002; Saitoh *et al.* 2012; Esteban*et al.* 2015; Huang *et al.* 2019).

The higher total carotenoid contents in deciduous than those in evergreen species in both treatments could be related to the higher proportion of sclerified tissues in the last-mentioned species. The sclerenchyma is a tissue without alive protoplasm therefore, with absence of chlorophylls and carotenoids (Arambarri *et al.* 2011). This result is reaffirmed by the absence of significant differences in total carotenoid contents among deciduous and evergreen species in burnt plants, as perennial species probably produced new leaves with low sclerenchyma proportion after EB. These findings suggest a great potential of the studied species to recover their photochemical efficiency, which could be related to the resilience of native vegetation to this disturbance (Allred & Snyder 2008; Esteban *et al.* 2015). Anatomical studies contribute to improve the knowledge about plant-environment relationship (Arambarri *et al.* 2011), biochemical processes and to comprise the differential responses of distinct species growing in a given environment (Manrique Reol 2003).

4.2 Total Phenolic Compounds and Tannin Assessments

The significant increase in phenolic compounds concentration observed in burnt plants is a consequence of the thermal stress produced by fire. Alonso *et al.* (2002) mentioned variations in phenolic compounds in *Pinus pinaster* forests after first months of thermal stress in bole and crown. Cannac *et al.* (2009) considered these compounds as good stress bioindicators due to their high sensitivity to changing environmental conditions. In our work, the increased concentration of phenolic compounds could be attributed both, to the EB as to changes in postfire environmental conditions (high radiation level, higher daily and seasonal thermal amplitude and soil evaporation) observable even 26 months after EB. The changes in the UV-radiation produce an increase in the biosynthesis of phenolic compounds (Alothman *et al.* 2009) as a product of the stimulation of the plant antioxidant defenses to resist pathogens (Diaz-Guerra *et al.* 2018). Nevertheless, Bowman*et al* . (2014) also mentioned the difficulty to assign this type of vegetation responses to fire as an adaptation to promote plant flammability since it could also represent a mechanism of tolerance of species to disturbance.

The non-significant differences in tannin contents at two-months after EB could mean that the biosynthesis of these compounds have not responded directly to fire effect. Tannin biosynthesis is considered among the most important plant defense mechanisms due to their capacity to reduce the digestibility of plant tissues (García 2015). The increasing tannin concentration two years after EB could be related to the presence of herbivores and pathogens in the post-fire environmental conditions. This indirect response of vegetation to fire could be an "exaptation" (plant characters with specific functions that incidentally increases flammability) (Bowman *et al.* 2014).

Species selected to this study showed significant differences in phenolic compounds and tannin contents which represent an interesting variability of these biochemical traits at community level. These results indicated that a homogeneous response of plants to fire cannot be expected. Further, the high coefficients of variation on the concentration of secondary metabolites suggest a high intrapopulation variability that plays an important role in adaptation to biotic and abiotic environmental conditions (Valares 2011; Galindo-Segura 2018). The higher total phenolic compounds have been observed in both Anacardiaceae species, *S. lorentzii* and *S. johnstonii* . In addition to the above species, *S. mistol* (Rhamnaceae), have shown the highest tannin contents. Both botanical families are characterized by their VOCs and these compounds are related to plant flammability (Ormeño *et al.* 2009; Bowman *et al.* 2014; Guedes *et al.* 2016; Hernández *et al.* 2016; Della Rocca *et al.* 2017; Bonatto-Schimitberger *et al.* 2018).

3.3 Plant tolerance to fire: Association between burnt biomass and the plant biochemical response to fire

The impact of fire on vegetation depends mainly on three factors, the plant storage reserves to regrowth post-disturbance, the characteristics of the fire event and the growth form of the species (Bravo *et al.* 2003). The greater availability of plant reserves allows a greater investment in defense and regeneration structures to guarantee their establishment post-disturbance (Clarke *et al.* 2013). Moreover, as the main defense mechanism, plants synthesize bioactive compounds whose function is to grant them protection against environmental disturbances (Balasundram *et al.* 2006; Martins *et al.* 2011).

Due to the loss of aerial biomass during the disturbance, plants active the production of resproutings as a survival strategy to recover their vegetative structure (Clarke *et al.* 2013). This disturbance response is conditioned by the availability of biochemical reserves in buds and meristems. Therefore, the re-sprouting ability is closely related to the plant biochemical composition, as these compounds allows the production of new sproutings (Clarke *et al.* 2013; Pérez-Harguindeguy *et al.* 2013). Our results suggest that in low-intensity events (as the QE performed in this work), the correlation between the plant biochemical response to fire and the amount of burnt biomass during EB, could be considered as an indicator of plant tolerance to fire. Thus, this study allows us to suggest that its association contributes to the post-disturbance vegetation establishment.

Our results showed that EB caused a decreasing of chlorophylls and carotenoid contents within a short temporal scale, but their concentrations returned to pre-event conditions one year after disturbance. These results seem to indicate that the photosynthetic process and recovery of biomass may be insured under natural conditions of our study area. Antioxidant compounds as phenolic and tannins showed an increase even 26 months after EB indicating that their protective effect on metabolic processes is prolonged. These findings confirm our hypothesis suggesting that a fire event produces changes in the biosynthesis of metabolic compounds, but their fast recovery seem to indicate a high "resilience" of native vegetation but also at metabolic level. Indeed, these compounds could be bioindicators of plant tolerance to fire, due to the strong correlation between the burnt biomass during EB and the plant biochemical response to fire. The high variation coefficients observed in our results may be attributed to the intraspecific variation of the native vegetation. This intrapopulation variability is recognized as the resilience motor of the species and their environment (Valares 2011; Galindo-Segura 2018). The responses observed after EB could not be related exclusively to fire but to a combination of drought, high UV-radiations and pathogens adaptations typical of post-fire environments, which has been described for other authors as "exaptations" (Bowman *et al.* 2014). More studies about quantifications at physiological level are desirable to assess their direct effect on biomass production. An unconsidered aspect is the beneficial effects of tannin contents in native vegetation digestibility which represents an interesting future research line for restoration activities in areas affected by wildfires.

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