# Experimental study in microcosms of the effect of fire on the characteristic vegetation of Mediterranean temporary ponds

Étude expérimentale en microcosmes de l'effet du feu sur la végétation caractéristique des mares temporaires méditerranéennes

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> Received: 20 September, 2021; First decision: 4 October, 2021; Revised: 25 November, 2021; Final decision: 26 November, 2021.

## Abstract

Fire is an important disturbance factor of ecosystems. Its impact on temporary ponds remains poorly documented. In Morocco, temporary ponds are numerous and mostly found in forested and agricultural environment. Forest fire has been increasingly frequent over the last years eventually spreading to the temporary pools. In addition, some farmers light fire to get rid of the biomass of crop residues. No assessment of the impact of fire on the plant biodiversity of temporary ponds was made. We tested the impact of fire on soil samples in an experiment in controlled conditions. Soil samples were taken from five ponds in the Middle Atlas divided into four series of 25 samples each. The first series served as a control while those of the 2nd. 3rd and 4th series were subjected respectively to a blowtorch flame sent for 30, 120 and 600 seconds. We measured the temperature of the soil surface for each treatment after fire using an infrared thermometer. We put all soil samples in germination

**Keywords:** temporary ponds characteristic species, species richness, seed density, fire intensity, natural restoration, seed bank.

conditions by watering them daily. During the second year, we manually disturbed the soil samples and then germinated them by watering them daily. Unlike low-intensity fires (fires of agricultural land), high-intensity fires (forest fires) significantly reduced the total density of seeds, those of annuals and aquatic annuals, as well as the species richness of the same groups. The effect of fire also varied according to the species and the duration of exposure. Natural restoration of the vegetation of the temporary ponds even after an intense fire is still possible but probably slow. Improved environmental conditions after fire and the presence in the soil of a long-lived seed bank are relevant indicators of restoration success.

### Résumé

Le feu est un agent important de perturbation des écosystèmes. Son impact sur les mares temporaires reste très peu documenté. Au Maroc,

**Mots-clés:** espèces caractéristiques des mares temporaires, richesse en espèces, densité de semences, intensité de feu, restauration naturelle, banque de semences.

les mares temporaires sont nombreuses et majoritairement concentrées soit dans un environnement forestier soit agricole. Le feu de forêt y est devenu fréquent ces dernières années. En plus, certains agriculteurs pratiquent le feu pour se débarrasser de la biomasse des restes des cultures. Dans ce pays, aucune évaluation de l'impact du feu sur la biodiversité des mares temporaires n'a été faite. Nous avons testé dans une expérimentation en conditions contrôlées, l'impact du feu sur des échantillons de sol prélevés à partir de 5 mares du Moyen Atlas et répartis en 4 lots de 25 échantillons chacun. Le premier a servi de témoin tandis que les 2<sup>e</sup>, 3<sup>e</sup> et 4<sup>e</sup> lots ont été soumis respectivement à une flamme de feu envoyée d'un chalumeau pendant 30, 120 et 600 secondes. Nous avons mesuré la température de la surface du sol des échantillons de chaque traitement après feu par un thermomètre infrarouge. Les échantillons ont été mis en germination et arrosés chaque jour. Durant la deuxième année, nous avons perturbé manuellement les mêmes échantillons de sol puis les avons mis en germination en les arrosant quotidiennement. Contrairement aux feux de faible intensité (feux de terrains agricoles), les feux de forte intensité (feux de forêt) ont diminué significativement la densité totale des semences, celles des annuelles et des aquatiques annuelles ainsi que la richesse en espèces des mêmes groupes. L'effet du feu a varié aussi en fonction des espèces et la durée d'exposition. La restauration de la végétation des mares temporaires après un feu de forte intensité est possible mais probablement lente. L'amélioration des conditions environnementales après feu et la présence dans le sol d'une banque de semences longévives sont des indicateurs pertinents du succès de la restauration.

## Introduction

Fire has often been considered as a discrete event in time that disturbs the structure of the ecosystem (community and population), limits biomass, through complete or partial destruction of plants (Grime 1979; Pickett & Withe 1985). The majority of fires that affect natural ecosystems (i.e. forests, savannas, etc.) are triggered by nature (lightning, high temperatures during episodes of intense drought) or by man himself through his activities (Archibald et al. 2012). The proportion of fires caused by natural factors is only 2% while the share caused by man is 98% (Jhariya & Raj 2014). Man-made fires are either criminal in origin or due to recklessness or neglect (Neyisci 1985; Mol & Kucukosmanoglu 1997; Mustafa 2009).

The extent of the damage caused by a fire varies according to its intensity (Pereira *et al.* 2011;

Heydari et al. 2012), its size, its occurrence frequency (Wright & Heinselman 1973) and its seasonality (Doamba et al. 2020). There are notable and varied effects depending on the composition and diversity of the vegetation (Bucini & Lambin 2002; Schroeder & Perera 2002; Capitanio & Carcaillet 2008; Aref et al. 2011; Pourreza et al. 2014b). Fire can affect natural ecosystems by altering plants (Buhk et al. 2007) and by modifying the structure of ecosystems (Freeman & Kobziar 2011) and soil properties (Certini 2005; Aref et al. 2011; Scharenbroch et al. 2012; Pourreza et al. 2014a). Post-fire changes can be assessed through the use of indicators reflecting both soil and vegetation characteristics (Ryan 2002; Pourreza et al. 2014b). Among the indicators of species diversity, species richness, alongside others such as equity, can be used to analyze trait diversity (Magurran 1988).

The response of plant diversity to fire is highly dependent on the fire's severity (Wright & Heinselman 1973). Effects of fire on plant species diversity and composition depend on site conditions and fire intensity. Serious fires could reduce the diversity of plant species and lead to the modification of the composition of plant communities (Lecomte et al. 2005) by destroying plants and their seeds, which represent genetic sources ensuring the conservation of plant biodiversity. Conversely, low intensity transient fires could improve nutrient availability (Certini 2005; Knoepp et al. 2009) and increase plant species richness and diversity (Hutchinson et al. 2005; Marozas et al. 2007). Likewise, low intensity fires could disrupt the competitive relationships between species and promote the expression of weakly competitive species, more particularly rare species (Pourreza et al. 2014b), by offering more ecological niches which are favorable to them.

The impact of fire on terrestrial ecosystems such as forests, savannas, grasslands, etc., has been widely studied (Keeley 1986; Baeza *et al.* 2002; Alday *et al.* 2009; Elliott & Vose 2005; Adel *et al.* 2013; Doamba *et al.* 2020), but its effects on wetlands, in particular temporary ponds, remain very poorly documented. These original environments by their hydrological functioning, characterized by the alternation of flooding and drying phases, are real hotspots for biodiversity (Rhazi *et al.* 2012; El Madihi 2020). Their heritage value has often been highlighted because of the large number of species they contain but above all because of the rarity of several of them (Grillas *et al.* 2004; Rhazi *et al.* 2005; El Madihi 2020). The characteristic vegetation of these environments is largely dominated by annual species (Deil 2005), more particularly aquatic annuals, given their strong adaptation to variations in the conditions of their environment (Grillas *et al.* 2004; Rhazi *et al.* 2005; El Madihi 2020).

The work carried out regarding the effect of high intensity fires on forest ecosystems has highlighted the extent of the damage caused to both species richness and plant diversity. Fires of low or intermediate intensity do not seem to negatively affect species richness and diversity, and even in some cases fires of these intensities can stimulate the germinations of seeds of some scrubland species, such as the genus *Cistus* (Roy & Sonié 1992; De Luis *et al.* 2005; Clemente *et al.* 2007; Santana *et al.* 2014).

In Morocco, temporary pools are very numerous and mainly concentrated either in a forest or agricultural environment. Forest fires have become frequent there in recent years (IRES 2009). In addition, some farmers practice fires to get rid of biomass from crop residues. In both situations, fire spreads and affects the neighboring temporary pools, which can also happen in other Mediterranean countries (Grillas *et al.* 2004).

In Morocco, as in several other countries, no assessment of the impact of fire on the plant biodiversity of temporary pools has been made. The aim was to test the impact of the intensity of the fire on the vegetation characteristic of temporary pools, mainly dominated by annuals, mainly aquatic annuals, given the remarkable adaptation strategies they adopt.

The objectives of this work were:

- 1- To study the effect of fire intensity on seed density and total specific richness as well as the effect on annual species including aquatic annuals
- 2- To evaluate the success of the natural restoration of the characteristic vegetation of temporary ponds after fire.

We carried out an experiment under controlled conditions to study the effect of fire on the characteristic vegetation of ponds. We also took into account the effect of grazing by simulating its effect.

# Methods

## Study area

The study targeted temporary ponds scattered within different sectors of the Middle Atlas, particularly Ifrane, Azrou and Timahdite (Figure 1).

The Middle Atlas, oriented North-East, South-West constitutes the central element of the Moroccan mountainous ensemble; it is

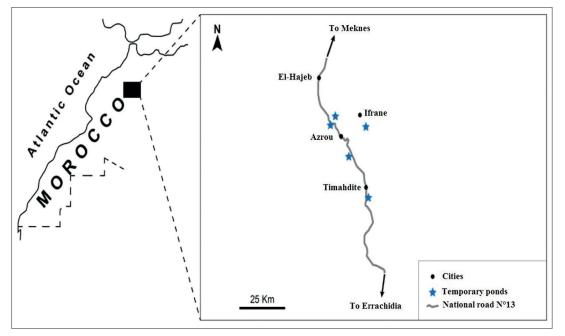


Figure 1 – Location map of sampled temporary ponds

limited to the north by the Taza corridor which separates it from the Rif chain, to the south of the High Atlas and the upper Moulouya, to the east by the valley of the middle Moulouya and to the west of the plateau central (Touabay *et al.* 2002). This massif is characterized by an important faunistic and floristic diversity; it is habitat to a very high forest potential and a set of interesting permanent and temporary wetlands, which, according to Braun Blanquet (1936) represent real floral gems due to the number of plant species they shelter and the rarity of several of them.

## **Experimental design**

We identified and selected five temporary ponds in the Middle Atlas for sampling (Figure 1), taking into account criteria related to their accessibility. These ponds are located on rocky ground given the geological nature of the Middle Atlas region. Samples of intact soil were taken from these ponds. A total of four series of 25 samples each were taken using a metal square (10cm wide and 5cm deep) driven into the soil with a hammer.

The first series served as a control while those of the 2nd, 3rd and 4th series were subjected to a blowtorch flame of approximately 15cm of length (Figure 2), applied during 30 seconds, 120 seconds and 600 seconds respectively. We measured the temperature of the soil surface for each treatment after fire using an infrared thermometer. We put all the soil samples in germination (from November 2017 until 30 June 2018) and we watered them daily. During the second year, we slightly disturbed the soil samples simulating the activities of herbivores attracted by water and pond vegetation, which often leads to the resurfacing of seeds buried in the soil. Afterwards, we put the soil samples in germination by watering them daily. During the two years, every 15 days, the plants germinated from the seeds were removed as soon as they could be identified in order to avoid competition between individual plants.

We assigned the names of the listed species using the catalog of the vascular flora of Morocco (volumes 1 and 2) (Fennane & Ibn Tattou 2005). We considered *Isoetes velata*. L as a facultative annual because the germinations came from the spores and not from the bulbs (Grillas *et al.* 2004).

## Data analysis

We tested the means between fire treatments, of seed density and species richness (total, annuals and aquatic annuals), as well as for each species by using a variance analysis (ANOVA). We analyzed the means comparisons between pairs of treatments using the Tukey-Kramer test.

We tested the means between the two states (after fire and after soil disturbance) of seed density and species richness of the same ecological groups by ANOVA for treatments: control, 30s and 120s. For the 600s treament, we used the Kruskal-Wallis test.

We tested the relationship between seed density and species richness for each ecological group by nonparametric correlation using Spearman's rho.

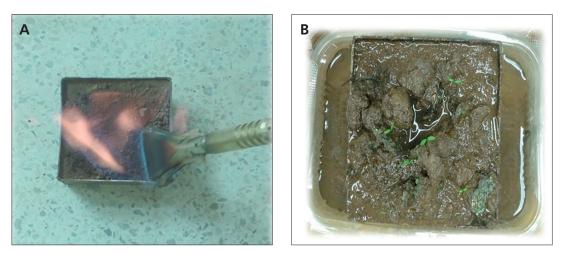


Figure 2 – Soil sample subjected to a blowtorch flame (A); new germinations (B).

# Results

The measured temperatures after each treatment were:  $19^{\circ}C \pm 4^{\circ}C$  for the control treatment,  $37^{\circ}C \pm 3^{\circ}C$  for the 30s treatment,  $84^{\circ}C \pm 5^{\circ}C$  for the 120s treatment and 130°C  $\pm 10^{\circ}C$  for the 600s treatment.

During the first year, after fire treatment, we identified 12 species in total (with 10 species for the control treatment, 11 species for the 30s treatment, 9 species for the 120s treatment and 0 species for the 600s treatment) against 8 species during the second year, after manual soil disturbance, (with 5 species for the control treatment, 7 species for the 30s treatment, 6 species for the 120s treatment and finally 3 species for the 600s treatment).

## First year after fire treatments

## Seed density

The total density of germinated seeds (F = 22.37, DF = 2, P < 0.0001), the density of annuals (F = 22.40, DF = 2, P < 0.0001), particularly, those of aquatic annuals (F = 21.10, DF = 2, P < 0.0001), decreased significantly with increasing fire intensity. They were significantly higher in the control and 30s treatments, but significantly lower for the 120s treatment. We did not observe germination for the 600s treatment (Figure 3).

The impact of fire intensity on seed density also varied depending on the species (Table 1).

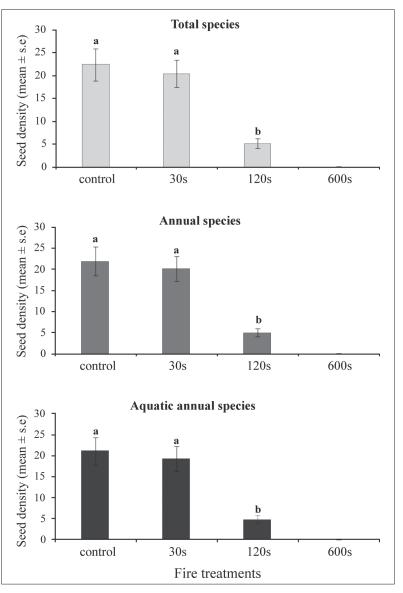


Figure 3 – Results of the variance analysis on the comparison, between fire treatments, of the seed density of different species groups. Different letters above columns indicate significantly different means (Tukey-Kramer test, P < 0.05).

 Table 1 – Results of variance analysis, on the comparison between fire treatments, of the germinated seed density for each species.

 \*\*\* P < 0.0001; \*\* P < 0.001; \* P < 0.01; ns: not significant; -: absence of results; Fr. occ: Frequency of occurrence; A: Annual; P: Perenial.</td>

| Fire Treatments : Means (SE)     |      |     |    |     |               |               |               |      |         |
|----------------------------------|------|-----|----|-----|---------------|---------------|---------------|------|---------|
| Espèces                          | Туре | F   | DF | Р   | Control       | 30s           | 120s          | 600s | Fr. Occ |
| Callitriche brutia Petagna       | A    | 8.6 |    |     |               | 7.1 (2.2) a   | 1.1 (0.3) b   | 0.0  | 57      |
| Juncus bufonius L.               | А    | 2.9 | 2  |     |               |               | 0.6 (0.3) b   | 0.0  | 22      |
| Callitriche truncata Guss.       | А    | 1.3 |    | ns  | 0.9 (0.5) a   | 1.0 (0.6) a   | 0.5 (0.2) a   | 0.0  | 16      |
| <i>lsoetes velata</i> A. Braun   | А    | 1.1 | 2  |     |               | 0.3 (0.2) a   |               | 0.0  | 13      |
| Ranunculus peltatus Schrank      | А    | 3.3 | 2  | *   | 3.3 (1.3) ab  | 6.8 (2.9) a   | 1.6 (0.8) b   | 0.0  | 26      |
| Ranunculus batrachioides Pomel   | А    | 8.8 | 2  | *** | 2.8 (0.6) a   | 2.9 (0.8) a   | 0.5 (0.2) b   | 0.0  | 35      |
| Polygonum aviculare L.           | А    | 8.7 | 2  | *** | 0.8 (0.2) a   | 0.9 (0.2) a   | 0.2 (0.1) b   | 0.0  | 27      |
| Lythrum thymifolia L.            | А    | 4.4 | 2  | * * | 0.5 (0.2) a   | 0.3 (0.1) ab  | 0.1 (0.04) b  | 0.0  | 18      |
| Eleocharis palustris (L.) R. Br. | Р    | 0.9 | 2  | ns  | 0.08 (0.05) a | 0.08 (0.07) a | 0.03 (0.08) a | 0.0  | 12      |
| Elatine macropoda Guss.          | А    | 1.1 | 2  | ns  | 0.4 (0.2) a   | 0.3 (0.1) a   | 0.3 (0.2) a   | 0.0  | 13      |
| Glyceria fluitans (L.) R. Br.    | А    | -   | -  | -   | -             | 0.04 (0.02)   | -             | -    | 1       |
| Chara sp.                        | А    | -   | -  | -   | -             | -             | 0.08 (0.08)   | -    | 1       |

It was significant on species Callitriche brutia Petagna., Juncus bufonius L., Ranunculus peltatus Schrank., Ranunculus batrachioides Pomel., Polygonum aviculare L. and Lythrum thymifolia L., but has no effect on other species, Callitriche truncata Guss., Isoetes velata A. Braun., Eleocharis palustris (L.) R. Br. and Elatine macropoda Guss.

### Species richness

Total species richness (F = 58.003, DF = 2, P < 0.0001), the richness of annuals (F = 55.08, DF = 2, P < 0.0001), notably those of aquatic annuals (F = 53.32, DF = 2, P < 0.0001), decreased significantly with increasing fire intensity. We found it significantly higher in the control and in the 30s treatments, which did not differ from each other, but significantly lower for the 120s treatment. No species appeared in the 600s treatment (Figure 4).

# Second year after manual soil disturbance

#### Seed density

The total density of germinated seeds (F = 3.13, DF = 3, P = 0.0292), the density of annual species (F = 3.11, DF = 3, P = 0.0297), and more specifically, that in annual aquatic species (F = 2.97, DF = 3, P = 0.0356), differed significantly between the fire treatments. We found them significantly higher for the 30s fire treatment than for the 600s treatment. The other treatments show intermediate values (Figure 5).

## Species richness

The total species richness (F = 2.27, DF = 3, P = 0.0845), annual species richness (F = 2.13, DF = 3, P = 0.1008), and aquatic annual species richness (F = 2.16, DF = 3, P = 0.097) did not vary significantly between fire treatments (Figure 6).

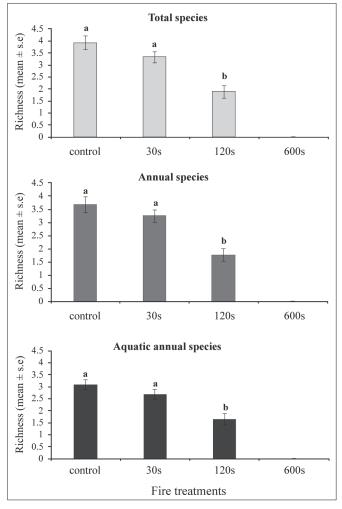


Figure 4 – Results of the variance analysis on the comparison, between fire treatments, of the species richness of different ecological groups. Different letters signify that the difference between the means is significant (Tukey-Kramer test, P < 0.05).

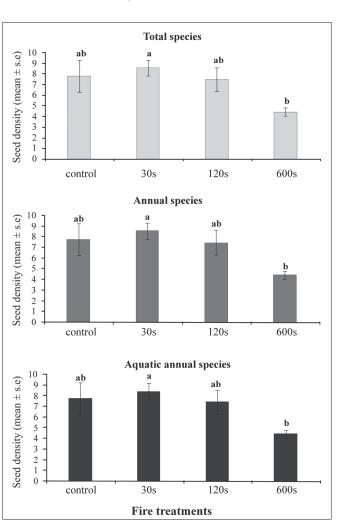


Figure 5 – Results of the variance analysis on the comparison, between fire treatments, of the seed density of different species groups after slight manual soil disturbance. Different letters above columns indicate significantly different means (Tukey-Kramer test, P < 0.05).

## Comparison of seed density and species richness between the two states (post-fire state and after manual soil disturbance state)

## Seed density

The total density of germinated seeds, annual species and aquatic annual species were significantly higher after fire than after soil disturbance for the control and 30s treatments. Conversely, for the 600s treatment, they were significantly higher after soil disturbance than after fire. We found no significant difference between the two states for the 120s treatment (Table 2).

## Species richness

The total species richness, annual species richness and aquatic annual species richness were significantly lower after manual soil disturbance than after fire for the control and 30s treatments, whereas they were significantly higher after manual soil disturbance than after fire for the 600s treatment. We found no significant difference for the 120s treatment between the two conditions (Table 3).

# Correlation between seed density and species richness

We found seed density and species richness positively correlated with each other for all ecological groups (Figure 7).

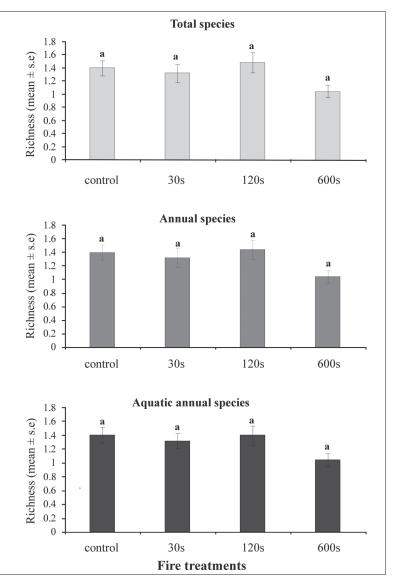


Figure 6 – Results of the variance analysis on the comparison, between fire treatments, of the species richness of different ecological groups after slight manual soil disturbance. Different letters above columns indicate significantly different means (Tukey-Kramer test, P < 0.05).

# Discussion

The results of this experiment carried out in microcosms revealed various effects of the intensity of fire disturbance on seed density and species richness during the first year of the experiment, and during the second year after a slight manual disturbance of the soil, simulating the impact of herbivores.

During the first year, the high intensities of fire disturbance (120s and 600s) negatively affected the seed density and the species richness, of the main ecological groups characteristic of temporary pools, given their strong correlation. Conversely, the low to moderate disturbance intensities (30s) led to results identical to those of the control treatment by promoting the density of seeds and the species richness of these ecological groups. These results confirm the relevance of the theoretical model of Grime (1979) "Humped back model" which establishes a relation between the intensity of the disturbance and the density or the species richness and that the latter would be important for the moderate intensities but low for extreme disturbance intensities.

The negative impact of fire on the soil seed bank of various ecosystems, through a reduction in the number of viable seeds, has been highlighted by many authors (Whelan *et al.* 2002; Wills & Read 2007; Lipoma *et al.* 2018) and has also been proven on the egg bank and the species richness of the characteristic fauna of Mediterranean temporary pools (Montcusí *et al.* 2019). Three non-mutually exclusive hypotheses could explain the reduction in seed density and subsequently species richness.

| Table 2 – Results of the variance analysis on the comparison between the 1 <sup>st</sup> year (state after fire) and the 2 <sup>nd</sup> year (state after manual |
|---|
| soil disturbance), of the total seed density, seed density of annual species and seed density of aquatic annual species.  |

| Ecological groups               | Treatments | State                | Means (±SE)  | ANOVA          |    |        |  |
|---------------------------------|------------|----------------------|--------------|----------------|----|--------|--|
|                                 |            |                      |              | F              | DF | Р      |  |
|                                 | Control -  | 1 <sup>st</sup> year | 22.32 (2.67) | 14.86          | 1  | 0.0003 |  |
|                                 |            | 2 <sup>nd</sup> year | 7.76 (2.67)  |                |    |        |  |
|                                 | 30s -      | 1 <sup>st</sup> year | 20.28 (2.17) | 14.47          | 1  | 0.0004 |  |
|                                 |            | 2 <sup>nd</sup> year | 8.56 (2.17)  |                |    |        |  |
| Total seed density              | 120s -     | 1 <sup>st</sup> year | 5.08 (1.05)  | 2.59           | 1  | 0.1141 |  |
|                                 |            | 2 <sup>nd</sup> year | 7.48 (1.05)  |                |    |        |  |
|                                 |            |                      |              | χ2             | DF | Р      |  |
|                                 | 600s -     | 1 <sup>st</sup> year | 0.00 (0.00)  | 135.06         | 1  | 0.0001 |  |
|                                 |            | 2 <sup>nd</sup> year | 4.40 (0.26)  |                |    |        |  |
| Seed density of annuals         | Control    | 1 <sup>st</sup> year | 21.80 (2.63) | 14.24          | 1  | 0.0004 |  |
|                                 |            | 2 <sup>nd</sup> year | 7.76 (2.63)  |                |    |        |  |
|                                 | 30s -      | 1 <sup>st</sup> year | 20.00 (2.15) | 14.11          | 1  | 0.0005 |  |
|                                 |            | 2 <sup>nd</sup> year | 8.56 (2.15)  |                |    |        |  |
|                                 | 120s -     | 1 <sup>st</sup> year | 4.88 (1.02)  | 3.10           | 1  | 0.0842 |  |
|                                 |            | 2 <sup>nd</sup> year | 7.44 (1.02)  |                |    |        |  |
|                                 |            |                      |              | X <sup>2</sup> | DF | Р      |  |
|                                 | 600s -     | 1 <sup>st</sup> year | 0.00 (0.00)  | 135.06         | 1  | 0.0001 |  |
|                                 |            | 2 <sup>nd</sup> year | 4.40 (0.26)  |                |    |        |  |
| Seed density of aquatic annuals | Control    | 1 <sup>st</sup> year | 21.04 (2.57) | 13.26          | 1  | 0.0007 |  |
|                                 |            | 2 <sup>nd</sup> year | 7.76 (2.57)  |                |    |        |  |
|                                 | 30s -      | 1 <sup>st</sup> year | 19.12 (2.15) | 12.37          | 1  | 0.0010 |  |
|                                 |            | 2 <sup>nd</sup> year | 8.40 (2.15)  |                |    |        |  |
|                                 | 120s -     | 1 <sup>st</sup> year | 4.76 (1.03)  | 3.25           | 1  | 0.0775 |  |
|                                 |            | 2 <sup>nd</sup> year | 7.40 (1.03)  |                |    |        |  |
|                                 |            |                      |              | Χ2             | DF | Р      |  |
|                                 | 600s -     | 1 <sup>st</sup> year | 0.00 (0.00)  | 135.06         | 1  | 0.0001 |  |
|                                 |            | 2 <sup>nd</sup> year | 4.40 (0.26)  |                |    |        |  |

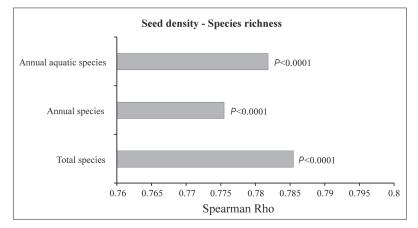


Figure 7 – Spearman's nonparametric correlation between seed density and species richness for different ecological groups.

The first hypothesis targets the direct effect of the fire through its flame, of which the length remains variable depending on the nature of the fuel and its biomass (Cox & Austin 1990), which may destroy the seeds located in the superficial horizons (Cain & Shelton 1998; Crowley et al. 1999; Bebawi et al. 2000; Odion & Davis 2000; Humphrey & Schupp 2001; Bebawi & Campbell 2002; Korb et al. 2004; Vermeire & Rinella 2009). Destruction of seeds could involve on the damage of their different tissues or at least vital parts, such as the embryo and/or reserve tissues (Silvertown 1999). This observation could apply in situations of high disturbance by fire (120s) and (600s). The second hypothesis concerns the indirect effect of fire through the temperatures generated by the size of the flames (Ryan & Noste 1985) and the duration of exposure of seeds to fire (Baskin & Baskin 2014). The high intensities of fire disturbance could generate high temperatures, which either inhibit seed germination (Lloret 1998) or become lethal for the seeds by losing their viability (Lamont & Barker 1988; Bradstock et al. 1994; Lloret 1998). In addition to the duration of exposure to fire, the impact of lethal temperatures for seed germination and viability

| Ecological groups               | Treatments | State                | Means (±SE) | ANOVA          |    |          |  |
|---------------------------------|------------|----------------------|-------------|----------------|----|----------|--|
|                                 |            |                      |             | F              | DF | Р        |  |
|                                 | Control    | 1 <sup>st</sup> year | 3.92 (0.22) | 63.67          | 1  | < 0.0001 |  |
|                                 |            | 2 <sup>nd</sup> year | 1.40 (0.22) |                |    |          |  |
|                                 | 30s -      | 1 <sup>st</sup> year | 3.32 (0.19) | FD 47          | 1  | < 0.0001 |  |
|                                 |            | 2 <sup>nd</sup> year | 1.32 (0.19) | 53.47          |    |          |  |
| Total species richness          | 120s -     | 1 <sup>st</sup> year | 1.88 (0.21) | 1 74           | 1  | 0.1922   |  |
|                                 |            | 2 <sup>nd</sup> year | 1.48 (0.21) | 1.74           |    |          |  |
|                                 |            |                      |             | Х2             | DF | Р        |  |
|                                 | C00.       | 1 <sup>st</sup> year | 0.00 (0.00) | 130.83         | 1  | < 0.0001 |  |
|                                 | 600s -     | 2 <sup>nd</sup> year | 1.04 (0.06) |                |    |          |  |
| Annual species richness         | Control    | 1 <sup>st</sup> year | 3.68 (0.22) | 50.76          | 1  | < 0.0001 |  |
|                                 |            | 2 <sup>nd</sup> year | 1.40 (0.22) |                |    |          |  |
|                                 | 30s -      | 1 <sup>st</sup> year | 3.24 (0.19) | 48.08          | 1  | < 0.0001 |  |
|                                 |            | 2 <sup>nd</sup> year | 1.32 (0.19) |                |    |          |  |
|                                 | 120s -     | 1 <sup>st</sup> year | 1.76 (0.19) | 1.37           | 1  | 0.2469   |  |
|                                 |            | 2 <sup>nd</sup> year | 1.44 (0.19) | 1.57           |    | 0.2469   |  |
|                                 |            |                      |             | X <sup>2</sup> | DF | Р        |  |
|                                 | 600s -     | 1 <sup>st</sup> year | 0.00 (0.00) | 130.83         | 1  | < 0.0001 |  |
|                                 |            | 2 <sup>nd</sup> year | 1.04 (0.06) | 130.65         |    |          |  |
| Aquatic annual species richness | Control    | 1 <sup>st</sup> year | 3.08 (0.16) | 50.04          | 1  | < 0.0001 |  |
|                                 | Control    | 2 <sup>nd</sup> year | 1.40 (0.16) | 50.04          |    |          |  |
|                                 | 30s -      | 1 <sup>st</sup> year | 2.68 (0.16) | 33.75          | 1  | < 0.0001 |  |
|                                 | 202        | 2 <sup>nd</sup> year | 1.32 (0.16) |                |    |          |  |
|                                 | 120s -     | 1 <sup>st</sup> year | 1.64 (0.19) | 0.75           | 1  | 0.3891   |  |
|                                 | 1205       | 2 <sup>nd</sup> year | 1.40 (0.19) | 0.75           | ļ  | 0.5091   |  |
|                                 |            |                      |             | X <sup>2</sup> | DF | Р        |  |
|                                 | 600s -     | 1 <sup>st</sup> year | 0.00 (0.00) | 130.83         | 1  | < 0.0001 |  |
|                                 |            |                      | 1.04 (0.06) | 10.00          |    |          |  |

Table 3 – Results of the variance analysis on the comparison between the 1<sup>st</sup> year (state after fire) and the 2<sup>nd</sup> year (state after manual soil disturbance), of the total species richness, annual species richness and aquatic annual species richness.

also depends on species diversity (Lloret 1998; Baskin & Baskin 2014). In the same perspective, Dessent et al. (2019) showed that soil temperatures exceeding a potential threshold of 70 and 80°C affect the number of germinating seeds and could possibly lead to a change in the distribution of species. Such a view agrees perfectly with our results. In the treatment (120s), the temperature generated by the flame was  $84^{\circ}C \pm 5^{\circ}C$  and could have an inhibitory effect leading to a decrease in seed density and species richness. In the treatment (600s), the temperature generated by the flame of  $130^{\circ}C \pm 10^{\circ}C$  could have a lethal character, thus explaining the absence of germination and expression of species. The last hypothesis advanced by Standish et al. (2007) implicates the inhibitory effect on the germination of seeds of certain species by smoke from fires and could partly explain the significant reduction in the size of seed stocks in the two treatments (120s) and (600s).

No negative impact was highlighted by the low intensity of disturbance by fire (30s) (Esposito *et al.* 2006) which had rather favored the density of seeds and the total species richness, those of annuals including aquatic annuals. According to Certini (2005) and Knoepp *et al.* (2009), low intensity fires, improve nutrient availability and contribute to increase in diversity and the specific richness (Hutchinson *et al.* 2005).

On the one hand, low intensity fires can improve species diversity and species richness by providing more favorable ecological niches and avoid the competitive exclusion of priority species for conservation such as rare species (Pourreza *et al.* 2014b) like, *Ranunculus batrachioides* Pomel, *Elatine macropoda* Guss, etc. In temporary pools, a similar effect of low-intensity fire was highlighted on the *anostraca* eggs of the fauna characterizing these habitats (Wells *et al.* 1997). The temperature generated by the fire that lasted (30s) was  $37^{\circ}C \pm 3^{\circ}C$  and had no negative impact on either seed density or species richness. Nevertheless, according to Moore & Wein (1977) a low intensity fire can destroy a small proportion of superficial seeds, but many of them located in the superficial organic horizon can escape and germinate.

The effect of fire also varies according to the species and the duration of exposure, as has also been demonstrated by Judd, (1993), Tierney & Wardle (2005) and Torres et al. (2006). In our experiment it happeared preponderant on some species but not on others. Indeed, the position of the seeds, whether they are outside or inside the fruit (Moya et al. 2008), in the central stone of the fruit or in parietal position (Mercer et al. 1994), the thickness of the fruit wall and that of the seeds (Bradstock et al. 1994), the size of seeds and fruits (Bradstock et al. 1994; Judd 1994), their water content (Judd 1994), are all key elements, proposed to explain this interesting result.

During the second year, manual disturbance of the soil lead to the resurfacing of seeds, which resulted in an increase in the density of germinated seeds and species richness (Rhazi et al. 2005; Sahib 2010). The distribution of seed stocks along a vertical gradient of soil depth is varied (Rhazi 2001; Grillas et al. 2004). The most important seed density and diversity are found in the shallow horizons of the soil (Rhazi 2001; Rhazi et al. 2004; Fenner & Thompson 2005) then decrease significantly towards the deep horizons. Thus, fire effect is strongly associated to soil depth and more soil surface is close, the effects of fire are important (Baskin & Baskin 2001; Cochrane 2003; Tesfaye et al. 2004).

The post-soil disturbance phase revealed a difference in seed densities only between the treatments (30s) and (600s) with significantly greater values for the treatment (30s) than for the treatment (600s) probably due to the still high inter-treatment variance. While the species richness did not differ significantly between treatments following the improvement in species richness.

In addition, the comparison for each treatment, between the two years (after fire and after slight manual soil disturbance), of seed densities and species richness of ecological groups, highlighted a change in the density of germinated seeds and species richness according to the treatments. In most seed germination experiments under optimal conditions, the germination rate is very high during the first year (Rhazi 2001; Grillas *et al.* 2004). Similarly, moderate disturbances often promote germination and induce a high species richness (Rhazi *et al.* 2004; Rhazi *et al.* 2005). These results agree with those obtained for the control and (30s) treatments.

The high intensity of fire disturbance resulted in the destruction of a relatively large fraction of seeds located in the upper soil horizon in the (12s) treatment and a total destruction of the abundant seeds in the shallow horizon (Rhazi et al. 2007) in the (600s) treatment. Manual soil disturbance led to the resurfacing of the seeds, facilitating their expression. This led to an improvement in the density of germinated seeds and subsequently in species richness. Moreover, three species (Callitriche truncata Guss, Callitriche brutia Petagna and *Ranunculus batrachioides* Pomel), absent during the post-fire phase, appeared in the treatment (600s) during the post-soil disturbance phase. Burying seeds in the soil protects them from fire (Baskin & Baskin 2001; 2014) but greatly reduces their germination rate (Rhazi 2001). They germinate only after their resurfacing by herbivore soil disturbances (Grillas et al. 2004; Deil 2020). Annual species have particular life history traits, including the production of many seeds, often small (Sahib 2010; Rhazi et al. 2012), facilitating the burying of a fraction of them in the soil through the slits while keeping their viability for decades (Grillas et al. 2004; Poschlod & Rosbakh 2018; Deil 2020). The close relationship between seed stocks and vegetation in temporary pools has been widely demonstrated (Zedler 2000; Rhazi et al. 2005, 2007, 2012; Bouahim 2010; Bouahim et al. 2014; El Madihi et al. 2017; Deil 2020). Thus, improvement in species richness after manual soil disturbance was highly dependent on residual populations maintained in the soil as seed stocks (Rhazi et al. 2005; Sahib 2010; Rhazi *et al.* 2012).

The extent and severity of damage vary greatly with the fire intensity (Pereira *et al.* 2011; Heydari *et al.* 2012), with effects on seed stocks and plant diversity (Bucini & Lambin 2002; Schroeder & Perera 2002; Capitanio & Carcaillet 2008). Fire can affect natural ecosystems, such as temporary pools, by acting on plants (Buhk *et al.* 2007), changing the trajectory of plant successions (Freeman & Kobziar 2011) and modifying

physicochemical properties (Certini 2005; Scharenbroch *et al.* 2012) and biological (Pourreza *et al.* 2014a) of the soil. Fire can change plant species diversity and richness through changes in site conditions (Harrison *et al.* 2003; Aref *et al.* 2011; Pourreza *et al.* 2014b). Despite this impact, the ecological restoration of post-fire plant biodiversity remains possible.

The results of our experiment strongly suggest that even after a high intensity of fire disturbance, plant diversity could return to its initial reference state (Le Floc'h & Aronson 1995; Rhazi et al. 2005, Buisson 2011) but probably gradually through a dynamic of plant succession requiring a few years. The success of restoring plant biodiversity in temporary ponds depends on several parameters according to Zedler (2000). The improvement of the site's environmental conditions and the presence of a long-lived seed bank are among the conditions considered necessary for the restoration of plant biodiversity in wetlands (Zedler 2000) including temporary ponds (Grillas et al. 2004; Rhazi et al. 2005; Sahib 2010).

# Conclusion

The fire can have various effects depending on its intensity. Intense fires, like those in forests, have negative impacts on the size of seed stocks and on the plant biodiversity of temporary pools. On the other hand, low to moderate intensity fires, such as those practiced by farmers to remove crop biomasses, can promote plant diversity in these ecosystems, but this probably depends on the types of crops (e.g. Triticum crop vs Zea crop). In both cases, restoration sensu stricto was still possible, but with different speeds depending on the intensity of the fire. The improvement of post-fire environmental conditions and the presence of a perennial seed bank are relevant indicators of the success of post-fire restoration. However, recurring fires could constitute a danger for the conservation of plant diversity in the medium or long term, particularly in a perspective linked to climate change with still hot and dry summers, favorable to the outbreak of fires. Thus, the effects of recurrent fire on the plant biodiversity of temporary ponds should be tested in order to identify key elements for their conservation.

## Acknowledgements

We would like to thank the anonymous reviewer and the Editor (Dr E. Buisson) for their relevant remarks which contributed to the improvement of this manuscript. This work has been achieved with the financial support of the Ministry of Higher Education, Scientific Research and Innovation of Morocco (MESRSI) in the framework of the BiodivRestor program [RESPOND Project 1-2021]

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