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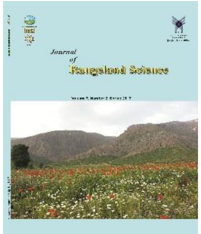

Research and Full Length Article:

Investigating Effects of a Prescribed Spring Fire on Symbiosis between Mycorrhiza Fungi and Range Plant Species

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Abstract. Fire is one of the incorporate vegetation management practices for grasslands and semi-arid rangelands. It may affect symbiotic relationship between range plants and mycorrhiza. Accordingly, this research was aimed to investigate the effects of a spring prescribed fire on the symbiotic relationships between mycorrhiza and 6 plant species. The study was conducted in a semi-arid steppe rangeland called Dehbar, Torghabeh in Mashhad, Iran. A prescribed fire was applied on 20th April 2015. Soil and plant samples were taken one month later. Colonisation and spore density were measured on the root of 6 different species. Spring fires significantly increased fungi spores in the rhizosphere of all plant species with the highest and lowest frequencies (42 and 24 per gram dry soil) obtained for *Pimpinella tragium* and *Artemisia aucheri*, respectively. Fire effects on colonization varied from high to no effect ranges. The increases in the mycorrhiza propagules after a prescribed burning during the growth season might be due to a sudden increase of nutrients from plant ashes. Although the studied plant species were different in terms of morphology (canopy and root type), phenology and life form (geophyte, perennial grasses and shrubs), the spring fire increased the colonization rate for plant species that had just started vegetative plant growth (*Stipa barbata*, *Artemisia aucheri* and *Pimpinella tragium*) but it had no effects regarding the plant species (*Poa bulbosa*, *Agropyron trichophorum* and *Astragalus gossypinus*) that were fully grown at the time of burning and/or had terminated seasonal growth period at the time of soil sampling. Therefore, in terms of plant-mycorrhizal symbiosis, a prescribed spring fire might increase the competitive advantage of perennial late season species as compared to annual early season species which are mostly ephemeral or invasive plants.

Key words: Rangelands, Ecological restoration, Plant interactions, Soil biology

Introduction

Fire is considered as one of the plant composition management practices for improving meadows and semi-arid rangelands and may affect various aspects of growth and development of plant communities such as flowering, seed dispersal, germination and seedling establishment, plant mortality and biomass (Ehsani *et al*., 2013). Fire temporally reduces the vegetation cover and the amount of available forage; however, in long term, it can have positive or negative effects on soil and vegetation properties (Faryabi *et al*., 2012). Fire enhances the diversity of vegetation and gives an entirely new life to some plants (Mesdaghi, 2003). It may change soil structure, nutrient and water availability for plants (Azul *et al*., 2010). Fire effects on soil are dependent on soil type, vegetation type, fire intensity, and the time intervals between fires (Harrison, 2005). Successive fires in the pine (Ponderosa) forests of the south-western US negatively affected belowground biomass and nutrient cycling processes in a long-time assessment (Stendell*et al.,* 1999*).* Fire may also change the dynamic relationships in the root-soil media where roots, micro-organisms and biotic factors interact with each other. It can affect mycorrhiza-fungi relationship (Leone and Lovreglio, 2003). Burning and subsequent forage harvesting significantly affected the richness and abundance of soil-fungi (Augustine *et al*., 2014); however, in some areas, the host plant colonization by the fungus was not affected by the fire (McMullan-Fisher *et al*., 2011). Fire heat can potentially destroy survival of fungi in the upper soil layers (Hart *et al*., 2005). A few of the fungus can reproduce after a fire, but others are usually destroyed by fire. (Bruns *et al*., 2002).The fire effects are also dependent on the symbiotic plant species; for example, burning significantly reduced the rate of Vesicular Arbuscular Mycorrhizae (VAM) colonization with *Festuca trichophylla* but it enhanced that of *Nardus stricta* (Baskin and Baskin, 1989). Low symbiotic relation with mycorrhiza may reduce plant capability to revive from injuries (Fowler *et al*., 2004). Arbuscular Mycorrhizal Fungal (AMF) communities can also affect the availability of nutrients in the soil. Plants that were inoculated with the mycorrhiza had a higher level of organic and inorganic matter in their tissues. They also showed a better performance in crop production and a higher resistance to stresses (Friese and Allen, 1991). Root fungal colonization plays a critical role in the establishment of seedlings, and having access to the underground resources, water and nutrients (Teste *et al*., 2009).

Periodic fire can increase soil organic matter and pH (Anderson and Menges, 1997) which may be useful for seed germination and survival (Gibson, 2010). On the other hand, fire may decrease symbiotic mycorrhizal fungi in the upper layers of the soil and also led to the death of host trees (Dahlberg, 2002; Perry *et al*., 1989; Hoeksema *et al*., 2010). Destruction of host plants by fire has some devastating effects on mycorrhizal networks (Oswald *et al*., 1989; Treseder *et al*., 2004; Bergner *et al*., 2004); however, the reduction in symbiotic relationship after fire is more destructive for plants than mycorrhiza (Fowler *et al*., 2004). In the United States, fire is an important tool for the development and dynamics of grasslands (Bi, 2006; Gargand Manchanda, 2009). Fire management can affect the activity of AMF. Fire can be effective in plant communities and soil. For example, in grasslands, spring fire increases soil moisture and soil moisture changes during the growth period (Millerand Jastrow, 2000). Frequent fires may reduce the amount of available nitrogen, increase carbon to nitrogen ratio and can affect the growth of micro-organisms and plants (Harley and Smith, 1983; Axelrod, 1985). All these factors can affect the mycorrhizal fungi and hence, change the structure of both fungal and plant community; however, Fire has a variety of effects on fungal communities (Christensen, 1997).

Due to the use of fire as a method for rangeland restoration (Valentine, 1989; Jankju, 2009) and frequent wildfires in some semi-arid rangelands, understanding the effects of fire on the components of rangeland ecosystems is important and necessary. Most of previous studies usually report the inadvertent effects of fire on the component of natural ecosystems (Baghestani *et al*., 2004; Ghasemi, 2014); however, they had measured effects of natural/wild fires that had been occurred outside of the growing season i.e. during the summer drought. But studies on the effects of prescribed on rangelands have rarely considered the growth season (shariatmadari, 2011). Especially, the effect of fire on plant-mycorrhiza relationships has never been considered. Therefore, this research was designed to study the effects of a prescribed fire at the beginning of a growth season (spring) on the symbiotic relationship between mycorrhiza and plant species in a semi-arid rangeland in north-eastern Iran.

Materials and Methods

Study area

Dehbar rangeland is located in Torghabeh, Khorasan Razavi, Northeast of Iran (Fig. 1) at 36°08'50" to 36°20'39" Northern latitude and 59°09'18" to 59°26'25" Eastern longitude with the average elevation from see level given as 1793 m. Mean annual precipitation for 63 years (1951- 2014) is 301.1 mm, which mainly occurs during winter and early (April-May) spring in the forms of snow and rain. The mean annual temperature is 9.1°C. The climate of region is semi-arid cold based on Dumbarton method. Total canopy cover of control site was 71% but it was reduced to 28 and 30 % in the spring and autumn burning sites, respectively.

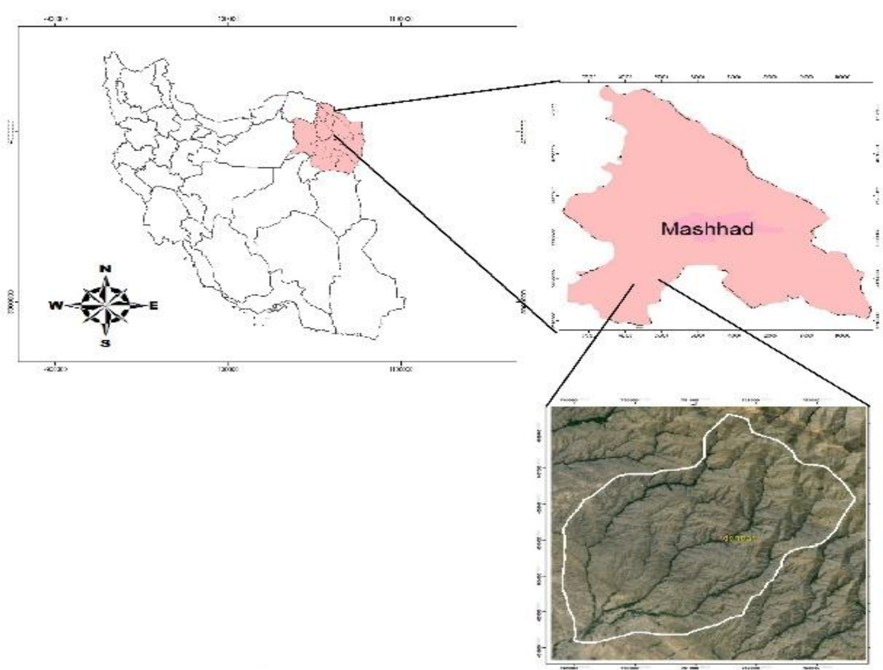

Fig. 1. Study area map

Data collection

Four macro plots with a similar size (10×15m) were chosen in a northern slope aspect with the slope rate given as 40%. Each macro-plot was divided into two similar size plots, one as a control and the second one for the prescribed burning. Fire breaks were created around each plot to prevent fire spreading on the neighbouring areas. Plant growth season in Dehbar starts at 10-20thMarch. Thus, we applied the prescribed burning on 20th April; soil and plant samples were taken one month later on 20th May 2015. Since the humidity percent of soil and fuel (aboveground plant biomass) was high at the time of burning, we used natural gas for an even and complete burning. Fuel gas was not a major source of fuel, but it was used to extend fire in the areas with low plant cover only in spring time burning.

Plant Species

Since the effects of fire and intensity of the mycorrhizal symbiotic relationship are greatly affected by morphological and ecological characteristics of plant species, 6 different plant species were selected in this study. General information on their growth form, root system, reproduction method and phenology are summarised in Table 1.

Table 1.Characteristics of 6 plant species studied in this research (Kanppa and Seastedt, 1986; Blair, 1997; Kitchen *et al*., 2009; Dhillion and Anderson, 1993)

Species	Growth form	Root system	Reproduction organ	Phenology
Agropyron trichophorum*Richter*	Grass	Deep and strong	Rhizomes and seeds	April -August
Artemisia aucheri Boiss	Shrub	Extensive and deep roots	seed	May-September
Poa bulbosa L.	Geophytes	Superficial roots	Seeds and bulbs	April-May
Stipa barbata Desf.	Bunch	Extensive roots system	Seeds and stolon	April - August
*Astragalus gossypinus F*isch	Cushion plant	Deep roots	seed	May - August
Pimpinella tragium Vill.	Perennial forb	Deep roots	Rhizomes and seeds	April- May

Sampling method and laboratory measurements

In each plot, 3 individual plants were randomly selected for each species. About 1 kg soil and some roots (100-200 g) were taken from root media of the selected plants. Soil samples were air-dried. Roots were transferred to the laboratory and thoroughly washed and placed in a solution of 50% ethanol at 4 °C. To determine the colonization rate, roots were stained by trepan blue, and then, were washed with KOH 10% and HCL 1% (Phillips and Hayman, 1970). Colonization rate was determined by a method suggested by Glovannetti and Mosse (1980). For counting mycorrhiza spores, soil samples were grinded and sieved with mesh numbers 18, 35, 120 and 230. The contents of mesh No. 230 were transferred to centrifuged pipes using sucrose 60%, and samples were put on a centrifuge for 5 minutes.

Data Analysis

Data were recorded and sorted in Microsoft Excel and analysed in Minitab 17. Data were analysed by the analysis of variance (ANOVA) or t-test. Means were compared using Tukey tests.

Results and Discussion

Results of a two-way ANOVA for the effects of prescribed fire and plant species on number of mycorrhiza spores in root media and their inoculation rate with rangeland species are shown in Table 1. For both variables, burning had significant effects at $p \leq 0.01$, which means different amounts of spore density and mycorrhiza colonization rates in the roots of plant species sampled from burnt plots as compared to those plants growing in control plots. Effects of species were also significant on both dependent variables, which indicate the differences in spore density and colonization rates of different range plant species of this study. Interaction between the effects of mycorrhiza and species was not significant regarding both spore density and inoculation rate.

Table 2. Result of a two-way ANOVA for the effects of prescribed fire and plant species, and their interaction, on number of mycorrhiza spores in root media and inoculation rate with rangeland plants.

SOV	df	MS	
		Colonization	Spore
Treatment	1	0.227**	2570.2**
Species	5	0.158**	168.40
Treatment × species	5	0.034	23.6
Error	24	0.031	21.78

**: significant at 1% probability level

Comparing the prescribed burning vs. control

Effects of prescribed fire on number of mycorrhiza spores and colonization rate are presented in Fig. 2. In our study, a prescribed burning had increased both spore density and mycorrhiza inoculation for the range plants of Dehbar. Such positive effects might be due to the favorable conditions after the burning event. Most of the wildfire burnings in the rangelands occur during the dry seasons (early summer), and hence, they increase drought stress on the existing plant species. However, in our study, burning was conducted at early spring when the available soil moisture and moderate air temperature could provide favorable conditions for plants and mycorrhiza to repair the fire injuries; it was also regarded as an opportunity for plants to use high amounts of nutrient released in soil after the fire event. Eom *et al*. (1991) also found the increases in the spore number in Rhizosphere of range plants after the spring fire. However, as it has been reported by Bentivenga and Hetrick (1992), the effects of burning on mycorrhiza spore and colonization may be temporal and can be vanished by time.

In this research, we found some increases in the number of spores and colonization rate in the burning site as compared to control. Fire can temporarily (during the fire event) increase temperature in the soil surface (upper 2 cm) up to 700°C (Jankju, 2009). This may lead to destructive effects of fire on the symbiotic relationship between plant and mycorrhiza (McGuire, 2007; Cline *et al*., 2005; Schroeder *et al*., 2012). A long-term increase (5-10 °C) in soil temperature also happens because of lower soil vegetation and litter cover in the burnt than control plots. Higher soil temperature if coincident with high soil moisture can increase the decomposition and nutrient release and hereby growth of external hypha (Wilson *et al*., 2009; Rillig, 2004; Schreiner *et al*., 1997). Therefore, in our research, increases in mycorrhiza symbiosis with range plants may be due to favorable conditions provided by long-term temperature rather than its sudden increase and destructive effects.

Burning can release high amount of nutrients in the soil which can help plants and mycorrhiza to repair the injuries from fire and start a quick regrowth (Carleton and Loftin, 2000; Dale *et al*., 2002; Cassie *et al*., 2009). The nutrients first help plants to recover and then extend their roots towards mycorrhiza (Purdy *et al*., 2002). Therefore, in our research, the effects of fire on mycorrhiza have been more related to its effects on soil fertility rather than temperature increase.

Comparing between species for spore density and colonization rate

Total number of mycorrhiza spores in the root media was varied based on plant species. Accordingly, the highest spore density (42.22) was found around the root media of *Pimpinella tragium* whereas all other species showed similarly low spore density. *P. tragium* is a rhizomatous species from Apiaceae (Fig. 3). Having the secondary compounds and low growth rate in Apiaceae family may have increased their dependency on the symbiotic relationships with the mycorrhiza fungus as it was already reported by Kapoor *et al.* (2004).

Two shrub species (*Artemisia aucheri* and *Astragalus gossypinus*) showed significantly higher colonization rate than three perennial grass species i.e. *Agropyron trichophorum*, *Poa bulbosa* and *Stipa barbata*. Shrubs usually provide a mild and fertile microclimate with higher soil moisture under their canopy as compared to the adjacent open areas (Jankju *et al.,* 2008). Such favorable conditions may provide a suitable environment for the growth and activity of microorganisms (Bailey, 1970; Moro *et al*., 1997). Especially for *Astragalus gossypinus* as a legume species, possible nitrogen fixation capability may enhance its mycorrhiza symbiosis as it was reported earlier by Jones *et al*. (2003).

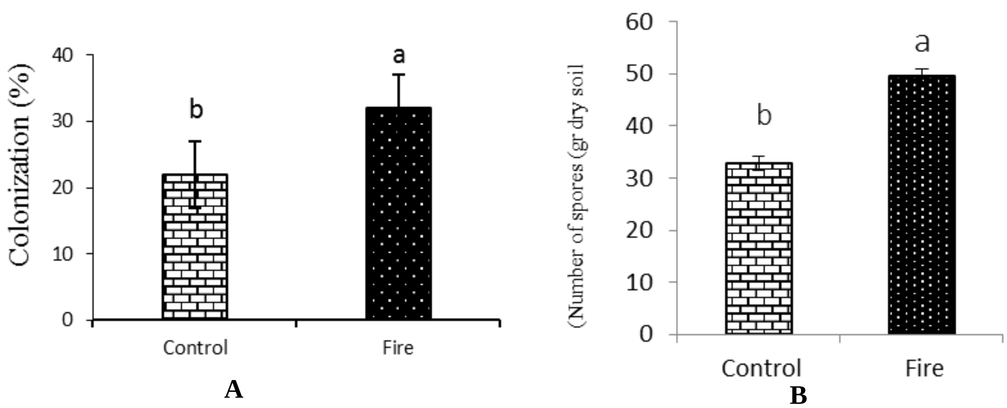

Fig. 2. Effects of prescribed spring fire on number of mycorrhiza spores in root media (A) and on root colonization rate (B). Column with different alphabetic letters indicate significant difference at p≤ 0.01.

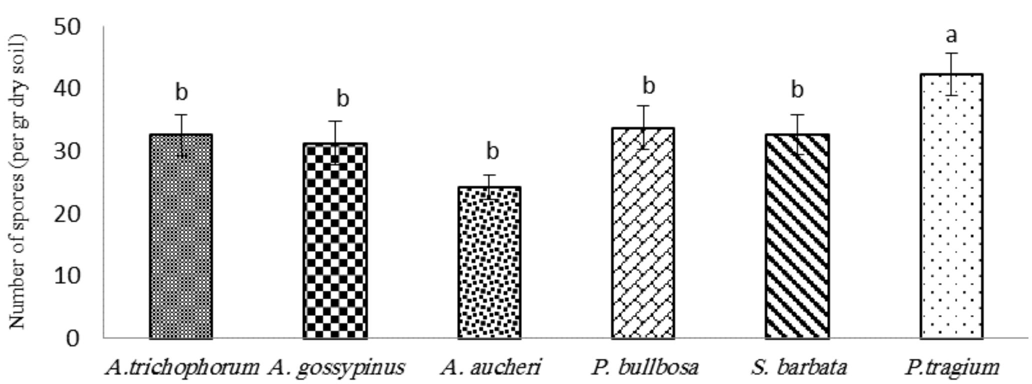

Fig. 3. Comparing number of spores in rhizospher of different plant species.
Column with different alphabetic letters indicate significant difference at p≤ 0.01.

Conclusions

This study aimed to investigate the effect of spring prescribed fire on symbiotic relationships between 6 range plant species and mycorrhiza. Overall, a prescribed spring fire increased spore density in soil and mycorrhiza colonization with plants. Here, in this experiment, we did not measure the soil temperature and fertility within the burnt and control sites. However, based on the previous literature, an increase in mycorrhiza symbiosis with range plants after spring time burning might be related to a sudden increase in soil nutrients that provided favorable growth conditions for both mycorrhiza and range plants. A perennial forb from Apiaceae family showed the highest colonization rate which was referred to its slow growth rate and production of secondary compounds. Perennial shrubs also showed higher symbiosis with mycorrhiza which was related to favorable growth conditions under their canopy.

Finally, this research led to two important findings: 1) a spring time prescribed burning may increase plant-mycorrhiza relationships in a short time after the burning, and 2) range plants with different growth forms (shrubs vs. grasses) or ecological traits (secondary compounds) may respond differently to mycorrhiza symbiosis. Further researches are needed in future for a more detailed understanding on the mechanisms of fire effects on plant-mycorrhiza relationship in which soil microclimate, fire intensity, and fuel content (above ground biomass) are measured.

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بررسی اثر آتش‌سوزی تجویزشده بهاره بر همزیستی قارچ میکوریزا و برخی گیاهان مرتعی

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چکیده. آتش یکی از شیوه‌های مدیریت پوشش گیاهی برای علفزارها و مراتع نیمه خشک است. آتش-سوزی ممکن است بر رابطه همزیستی گیاهان مرتعی و قارچ‌های میکوریز تأثیرگذار باشد. از این‌رو، این پژوهش با هدف بررسی اثرات احتمالی آتش‌سوزی بهاره‌ی تجویزشده (به عنوان یک روش اصلاح مرتع) بر روابط همزیستی بین قارچ میکوریز و شش گونه‌ی گیاه مرتعی انجام شد. آتش‌سوزی در مرتع نیمه استپی واقع در دهبار، طرقبه، خراسان رضوی ایران انجام شد. آتش‌سوزی تجویز شده در ۲ اردیبهشت ۱۳۹۴ صورت گرفت. نمونه‌برداری خاک و گیاه یک ماه بعد از آتش‌سوزی انجام شد. درصد کلونیزاسیون و تراکم اسپور در ریشه و ریزوسفر شش گونه مختلف مرتعی اندازه‌گیری شد. آتش‌سوزی بهاره میزان اسپور ریزوسفر را در همه گونه‌های گیاهی به میزان قابل‌توجهی افزایش داد. بیشترین فراوانی اسپور (۴۲ در هر گرم خاک خشک) مربوط به گونه‌ی *Pimpinella tragium* و کمترین مقدار (۲۴ در هر گرم خاک خشک) مربوط به *Artemisia aucheri* بود. اثر آتش‌سوزی بر کلونیزاسیون (همزیستی) متنوع بود؛ در برخی باعث افزایش، در بعضی کاهش و در بعضی موارد بی‌تأثیر بود. افزایش همزیستی میکوریزا پس از آتش‌سوزی تجویز شده در طول فصل رویش ممکن است به خاطر افزایش ناگهانی مواد مغذی حاصل از خاکستر گیاه باشد. اگرچه گونه‌های مورد مطالعه ازنظر مورفولوژی (تاج پوشش و سیستم ریشه‌ای)، فنولوژی و فرم زیستی (ژئوفیت، گراس‌های چندساله و بوته‌ها) متفاوت بودند، آتش‌سوزی بهاره میزان کلونیزاسیون را برای گونه‌هایی که رشد رویشی را شروع کرده بودند (*Stipa barbata* *Artemisia aucheri* و*Pimpinella tragium*) افزایش داد اما هیچ اثری برگونه‌هایی که فصل رشد خود را در زمان سوختن کامل کرده بودند (*Poa bulbosa*، *Agropyron trichophorum* و *Astragalus gossypinus*) نداشت. بنابراین از منظر رابطه گیاه و میکوریز، آتش‌سوزی تجویزشده بهاره ممکن است از طریق تأثیر بر روابط بین گیاه و میکوریز باعث افزایش توان رقابتی گیاهان چندساله مرتعی در برابر گیاهان مهاجم یک‌ساله و تروفیت مرتع شود.

کلمات کلیدی: مراتع، احیای بوم‌شناختی، برهم‌کنش‌های گیاهی، بیولوژی خاک