INSTITUTO POTOSINO DE INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA, A.C.

POSGRADO EN CIENCIAS APLICADAS

Grazing effects on fungal root symbionts and C and N storage in a shortgrass prairie in Central Mexico

Tesis que presenta

Eduardo Medina Roldán

Para obtener el grado de

Maestro en Ciencias Aplicadas

En la opción de

Ciencias Ambientales

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San Luis Potosí, S. L. P. a 24 de noviembre de 2005

IPICYT

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La tesis "**Grazing effects on fungal root symbionts and soil C and N storage in a shortgrass prairie in Central Mexico**" presentada para obtener el Grado de Maestro(a) en Ciencias Aplicadas en la especialidad de Ciencias Ambientales fue elaborada por **Eduardo Medina Roldán** y aprobada el **24 de noviembre de 2005** por los suscritos, designados por el Colegio de Profesores de la División de Ingeniería Ambiental y Manejo de Recursos Naturales del Instituto Potosino de Investigación Científica y Tecnológica, A.C.

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Durante la realización del trabajo el autor recibió una beca académica del Consejo Nacional de Ciencia y Tecnología (No. de registro **182504**) y del Instituto Potosino de Investigación Científica y Tecnológica, A. C.

La investigación en esa tesis fue financiada como parte del proyecto de ciencia básica SEP-CONACYT num. ref. **42628**

El posgrado en Ciencias Aplicadas con especialización en Ciencias Ambientales del Instituto Potosino de Investigación Científica y Tecnológica se encuentra inscrito dentro del Programa Institucional de Fomento al Posgrado del CONACYT con el número de registro _____

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que se desarrolló bajo la dirección de

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"Reality is infinitely diverse, compared with even the subtlest conclusions of abstract thought, and it does not allow of clear-cut and sweeping distinctions. Reality resists classification".

"La realidad es infinitamente diversa, escapa a las deducciones ingeniosas del pensamiento abstracto, no soporta la clasificación estrecha y exacta, la realidad tiende al fraccionamiento perpetuo, a la variedad infinita"

Fyodor Dostoevsky

Acknowledgments

I'd like to acknowledge Gabriela who helps me in all the moments during our stay in San Luis Potosí, including the sampling period, three hard weeks within many sweet years.

I am thankful to Dr. Tulio and Dr. Elisabeth for sharing with me their experience and knowledge.

I am thankful to Dr. Ángel Alpuche and Dr. Víctor Olalde for their time and comments.

I am grateful for all people who support me and share tow years of their life with me. The time we coexist with each other is one of the most remarkable periods during my life. We engaged in many discussions about science, philosophy and other, more mundane, stuffs. It was very nice to have known you. I hope I did not forget anybody, Andrés, Felipe, Leonardo, Angélica, Margarita, Sosa, Rafa, Pepe, Gris, Pedro, Sandra, Gabriel, Dr. David, Dr. Ricardo, Dr. José Luis.

I thank the Laboratory of Molecular Biology of Plants as well as Laboratory of Medical Biotechnology in IPICYT for the facilities given for the present work.

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Resumen

Efectos del pastoreo sobre hongos simbiontes de la raíz y almacenamiento de C y N en el pastizal mediano abierto del Altiplano Mexicano

Palabras claves: *micorrizas arbusculares, endófitos septados obscuros, herbivoría, funcionamiento del ecosistema, exclusiones de pastoreo de largo plazo*

La ganadería extensiva es una de las principales formas de uso de suelo en los pastizales semiáridos del centro de México. A pesar de la vasta extensión y de su alta degradación la cual está asociada al sobrepastoreo, la comprensión de los efectos del sobrepastoreo sobre aspectos ecológicos cruciales de estos pastizales, como; patrones de colonización de hongos simbióticos de raíces y aspectos de biogeoquímica de elementos como la cantidad de almacén de carbono (C) y nitrógeno (N) es limitada. En este trabajo se determinaron los efectos del apacentamiento sobre la colonización de raíces por hongos simbióticos [micorrizas arbusculares (AMF)] y endófitos septados obscuros (DSE)) asociados con *Bouteloua gracilis* (navajita azul), la especie clave del pastizal mediano abierto, en un gradiente de apacentamiento. Este gradiente incluyó una exclusión de herbívoros de 30 años, un rancho privado con buenas prácticas de manejo y otros dos sitios con diferentes niveles de sobrepastoreo, en un pastizal mediano abierto de la región de los Llanos de Ojuelos, Jalisco. Además, se examinó el efecto del apacentamiento sobre el almacenamiento de C y N y disponibilidad de NH_4 en suelo en respuesta a este gradiente de pastoreo. Se muestrearon plantas de navajita azul y se determinó la colonización de hongos simbióticos durante la estación húmeda. También se colectó suelo a lo largo del gradiente de pastoreo y se analizó la comunidad de esporas de hongos micorrícicos, el carbono (C) total, N total y NH_4 extraíble. Bajo apacentamiento moderado, la concentración de C, N, y de NH_4 fue mayor (alrededor de 20%) en comparación con la exclusión de herbívoros y sobrepastoreo (F = 5.22, P< 0.01; F = 5.92, P < 0.01; F = 5.68, P < 0.001 para C, N y NH_4, respectivamente). Además, el pastoreo afectó la composición (medida como la abundancia de diferentes morfotipos) y diversidad (medida con el índice de diversidad de Simpson) de esporas de AMF (F = 4.48, P = 0.0001; F = 4.08, P = 0.01 para composición y diversidad respectivamente), aunque no se observaron tendencias claras para estas variables en relación con el gradiente de apacentamiento. No se observó efecto del pastoreo sobre la colonización de hongos de las raíces ni sobre la razón C:N (F = 0.660, P > 0.50; F = 1.017, P > 0.35; F = 1.75, P > 0.10 para DSE, AMF y la razón C:N, respectivamente), aunque la colonización en navajita azul fue relativamente alta (colonización media de DSE = 37.24% y de AMF = 10.18%) lo que indica un importante papel de estos organismos como sumideros potenciales de C. Estos resultados sugieren que bajo apacentamiento moderado el almacenamiento de C y N y disponibilidad de N son superiores en el pastizal mediano abierto del centro de México, y que los hongos simbiontes de la raíz juegan un papel importante en la ecología de *B. gracilis* bajo las condiciones de sequía, pastoreo y niveles bajos de nutrientes característicos de este pastizal semiárido.

Abstract

Grazing effects on fungal root symbionts and C and N storage in a shortgrass prairie in Central Mexico

Key words: *arbuscular mycorrhizae, dark septate endophytes, herbivory, ecosystem functioning, long-term grazing exclosures*

Grazing by domestic herbivores is one of the principal drivers of land use change in semiarid grasslands of Central Mexico such as the shortgrass prairie. Despite the vast expansion of these grassland ecosystems and the extent of land degradation associated with overgrazing, we have limited understanding on the effects of overgrazing on root fungal symbionts and ecosystem processes such as nutrient biogeochemistry and soil stocks of carbon (C) and nitrogen (N).

This research focused on studying the grazing effects on root colonization patterns by fungal symbionts [arbuscular mycorrhizae fungi (AMF) and dark septate endophytes (DSE)] associated with *Bouteloua gracilis* (blue grama), a keystone species of the shortgrass prairie, along a grazing gradient comprised of a 30-year old exclosure, a well-managed private ranch and two sites with overgrazing and extreme overgrazing in the subprovince Los Llanos de Ojuelos, Jalisco. Soil nutrient storage and plant nitrogen availability were also examined along the grazing gradient. Roots of blue grama plants were sampled and root fungal colonization (AMF and DSE) determined at the end of the rainy season. Soil samples from all grazing conditions were analyzed for mycorrhizal spore communities, total C and N and extractable ammonium.

Moderate grazing exhibited higher C, N and extractable NH_4 concentration (approximately 20%), while no-grazing and extreme overgrazing tended to have smaller concentrations ($F = 5.22$, $P < 0.01$; $F = 5.92$, $P < 0.01$; $F = 5.68$, $P < 0.001$ for C, N and NH_4, respectively). Furthermore, grazing impacted mycorrhizal spore composition (measured as the abundance of different mycorrhizal spore morphotypes) and diversity as measured by Simpson's diversity index ($F = 4.48$, $P = 0.0001$; $F = 4.08$, $P = 0.01$ for mycorrhizal spore composition and diversity, respectively), although no clear tendencies were observed for these variables along the grazing gradient. No relationship was observed between root fungal colonization and the grazing gradient nor between the C:N ratio and grazing ($F = 0.660$, $P > 0.50$; $F = 1.017$, $P > 0.35$; $F = 1.75$, $P > 0.10$ for DSE, AMF, and C:N ratio, respectively), however root fungal colonization was overall relatively high (average root colonization by DSE = 37.24% and by AMF = 10.18%) suggesting an important C pool.

These results suggest that long-term overgrazing has reduced the storage capacity of important soil nutrients of these grassland ecosystems. The results also suggest that the fungal symbiosis plays an important ecological role in *Bouteloua gracilis* under the interacting influence of low nutrient availability, herbivory and drought characterizing the dominant environmental conditions of the grasslands of central Mexico.

1. Introduction

Overexploitation through grazing by domestic herbivores constitutes one of the principal drivers of land use change in arid and semiarid grasslands throughout the World (WRI, 2001; Ash *et al.,* 2002). Large herbivores impact grassland ecosystems by altering abiotic and biotic properties such as soil physical characteristics, plant resource allocation, plant colonization-extinction patterns, vegetation structure, and by modifying key ecological interactions among organisms (Dysterhuis, 1949; 1958; Coughenour, 1991; Briske and Richards, 1994; 1995; Briske, 1996; McNaughton *et al.,* 1997; Hamilton *et al.,* 1998; Olff and Ritchie, 1998; Wardle *et al.,* 2002, Bardgett and Wardle, 2003; Patra *et al.,* 2005). While much of our knowledge on the effects of grazing is based on aboveground ecosystem components and processes, there is increasing evidence that grazing may indirectly alter soil functions by inducing changes in vegetation, litter quality and consequently in interaction patterns between plants and soil organisms (Ritchie *et al.,* 1998; Bardgett and Wardle, 2003). The plant-fungus symbiosis with arbuscular mycorrhizae (AMF) and dark septate endophytes (DSE) is among the most important and earliest established (at least considering AMF) - from a co-evolutionary perspective - plant-microorganism associations (Taylor 1995; Brundrett, 2002). While acknowledging the increasing importance of these types of aboveground-belowground interactions both for understanding key ecological processes and for implementing natural resource management policies, great uncertainties still remain associated with the occurrence of such interactions in natural ecosystems and their responses to human induced disturbances (Wardle *et al.,* 2004).

The AMF symbiosis is formed between fungi of the phylum Glomeromycota and fine roots of vascular plants of almost all plant families, (Malloch *et al.,* 1980; Brundrett, 2002). Arbuscular mycorrhizal fungi are obligate plant symbionts that receive carbohydrates from the host and in return improve the supply of phosphorous, ammonium or water for the plants through the proliferation of extensive hyphal networks which explore soil pores inaccessible to plants roots (Brundrett, 2002; Finlay, 2004). Another benefit of AMF for plants is enhanced plant resistance against pathogens (Chiariello *et al.,*

1982; Augé, 2001). Dark septate endophytes are a group of melanized fungi of the subphylum Ascomycotina which colonize plant roots growing in nutrient-poor, semiarid-arid and temperate ecosystems (Barrow *et al.*, 1997; Barrow, 2003). While DSE are frequently plant symbionts in grassland ecosystems and often more abundant than the mycorrhizal symbiosis, their ecological role is basically unknown (see Jumpponen, 2001 for a review) (Barrow *et al.*, 1997). Waller *et al.* (2005) found that symbiotic associations with root endophytes promote barley production, enhance resistance to pathogens and decrease osmotic stress, suggesting that DSE act similar to mycorrhizae. Barrow *et al*. (1997) studied the intensity of DSE and AMF colonization in plants of semiarid rangelands in New Mexico and Colorado and found that DSE colonization was more common than AMF colonization (60 to 90 percent DSE colonization versus 3 to 70 percent AMF colonization).

The intensity of AMF colonization of roots is affected by several abiotic and biotic factors such as soil substrate, pH, plant nutrient status, plant density, successional stage and disturbance regime (Johnson *et al.,* 1991; 1992; Genney *et al.,* 2001; Burrows and Pfleger; 2002; Johnson *et al.,* 2003; Lovelock *et al.,* 2003; Rillig *et al.,* 2003), however information on the effects of grazing on fungal symbionts in field conditions are rather scarce (Bethlenfalvay and Dakessian, 1984; Eom *et al.,* 2001; Grigera and Oesterheld, 2004). In a review, Gehring and Whitham (2002) reported for several ecosystems, that herbivory has a negative effect on mycorrhizal colonization of roots; they propose that future studies should take into account the simultaneous effects of herbivory and soil nutrient limitation to address the following three hypotheses 1) root colonization of AMF is greater in plants growing under resource limitation, 2) AMF colonization declines in plants exposed to herbivory irrespective of the soil nutrient conditions and 3) AMF colonization declines more in response to herbivory under nutrient limitation. However, grassland plants have exhibited contrasting responses with respect to AMF colonization to grazing or clipping; they were either positive (Eom *et al.*, 2001; Grigera and Oesterheld, 2004); negative (Bethlenfalvay and Dakessian, 1984; Hetrick *et al.* 1990; Allsopp; 1998) or neutral (Busso *et al.,* 2001) suggesting that responses may be idiosyncratic. However, since AMF (and presumably DSE) depend entirely on the carbon provided by the plant, the underlying mechanisms associated with

grazing effects on root symbionts must be related to the carbon budget of plants and their capacity to maintain fungal colonization. Estimates of carbon allocation to AMF in ungrazed plants range between 10-20% of the total photosynthates (Jakobsen *et al.*, 2002). Hence grazing may suppress fungal associations because plants preferably allocate C to the recovery of leaf tissue rather than to fungal symbionts (Sartor, 2005). However, any reduction in mycorrhizal colonization entails a decrease in mineral nutrient supply for the plant and therefore slows down the process of plant recovery after defoliation. Grazing has also been shown to modify the diversity of mycorrhizal spore communities, although responses range from being negative, neutral to positive (Bethlenfalvay and Dakessian, 1984; Wallace, 1987; Eom *et al.,* 2001; Klironomos *et al.*, 2004), which has been interpreted as interspecific differences in fungal sensitivity to plant carbon shortage imposed by herbivores.

Soil biogeochemical processes in semiarid grasslands are tightly coupled to vegetation structure and traits of blue grama so that higher rates of decomposition, soil organic matter formation and nutrient mineralization are associated with plant cover and the concomitant input of plant litter to the soil (Derner *et al.,* 1997; Vinton and Burke, 1997; Burke *et al.,* 1998; Eipstein *et al.,* 1998; Burke, 1999). Moreover, a large body of literature has addressed how grazing produces predictive changes in plant community composition. Thus, grazing causes the replacement of plants susceptible to herbivory by those exhibiting either traits which reduce plant consumption by herbivores (avoidance through chemical or structural deterrents such as secondary compounds or lignified tissues) or traits that promote plant growth under grazing environments (tolerance through mechanisms such as compensatory photosynthesis) (Briske and Richards, 1994; 1995; Briske, 1996; Strauss and Agrawal, 1999; Rebollo *et al*., 2002). Until recently, it has been suggested that inherent plant traits related to grazing resistance trigger contrasting effects in terms of nutrient cycling, net primary productivity and other ecological processes in that traits related to grazing tolerance enhance soil nutrient cycling as a result of high quality litter while traits related to grazing avoidance slow down biogeochemical processes (Ritchie *et al.,* 1998; Wardle *et al.,* 2002; Bardgett and Wardle, 2003). Tracking the changes in plant functional composition in the grasslands of the Los Llanos de Ojuelos, Arredondo (2002)

found that two populations of *B. gracilis* subjected to contrasting long-term grazing regimes differed in the amount of structural carbon in aboveground tissue with the population from the overgrazed ejido land exhibiting lower lignin content than plants from a moderately grazed site of a local experimental station. Furthermore, Arredondo *et al.* (2005) observed that the population from the overgrazed ejido land had a higher leaf area and specific leaf area than the one from the moderately grazed site. This suggests - when considering leaf traits alone – that grazing tolerant phenotypes of *B. gracilis* could potentially enhance nutrient cycling in heavily overgrazed conditions. However this also depends on the soil biotic and abiotic conditions.

These considerations apply to the grass-fungal symbiosis in the shortgrass prairie where plant carbon gain is simultaneously limited by water and nitrogen availability and continuous herbivory (Sims *et al.,* 1973; Lauenroth and Dodd, 1979; Lauenroth *et al.,* 1980; Austin and Sala, 2002). The shortgrass prairie in Central Mexico is dominated by *Bouteloua gracilis* H.B.K. Lag ex Steud (blue grama) a long-lived caespitose species that makes up 80% of the total aboveground and belowground plant biomass and thus plays a key role in most ecological processes (Coffin *et al.,* 1996; Burke *et al.,* 1999). In many locations of Mexico, overexploitation of grasslands through long-term heavy grazing is related to land tenure. Communal or *ejido* land is usually poorly managed resulting in significant changes in plant community structure and plant species composition, reduction in primary productivity and finally in loss of vegetation cover (Aguado, 1994). Privately owned land, on the other hand, is characterized by better grassland conditions due to proper management practices with moderate stocking rates (*personal observation*). Grazing regimes observed at Los LLanos de Ojuelos have induced changes in species composition and consequently in their functional attributes. Hence, it is expected that grazing induced degradation in Los Llanos de Ojuelos may have modified ecosystem level properties such as soil nutrient storage. Overall, our knowledge on root fungal symbiosis (e.g., percent of colonization, composition of mycorrhizal spore communities) and their response to grazing is very scarce for this region. Reece and Bonham (1978) studied the effects of grazing on AMF colonization in blue grama in a shortgrass prairie in Colorado, however they did not find evidence that grazing affected AMF symbiosis. While there is

evidence that blue grama exhibits root symbiosis with AMF to improve its water relations and enhance its assimilation rates under drought conditions (Allen *et al.,* 1981), we lack knowledge as to the importance and frequency of this symbiosis under natural conditions. This is especially pertinent since *B, gracilis* is the most abundant and hence from an ecosystem functioning perspective important species of the Mexican grassland biome which is currently undergoing substantial changes due to increasing land use change and extreme climate events such as droughts.

In this study, I examined the effects of different grazing regimes on the fungal symbiosis between blue grama and both AMF and DSE,and on ecosystem processes particularly on mineral nitrogen availability and soil nutrient storage in association with *B. gracilis* plants and in open interspaces by comparing soils along a grazing gradient in the shortgrass prairie in Los Llanos de Ojuelos, Jalisco, Mexico. My objectives were: 1) to examine root colonization of blue grama by AMF and DSE along a grazing gradient at the end of the growing season, 2) to characterize the structure of AMF spores (composition and abundance) in response to the grazing gradient, and 3) to determine the effects of different grazing regimes on soil nutrient storage and soil nitrogen availability. Besides focusing on grazing effects on the above mentioned variables, this study was also intended to explore basic knowledge about patterns of AMF and DSE colonization in the shortgrass prairie and on the diversity of AMF spores and the co-occurrence of the two symbionts. I hypothesize that the fungal symbiosis with AMF and DSE differs under contrasting grazing regimes with greater root colonization in ungrazed sites or subjected to moderate grazing compared to sites exposed to heavy grazing. I also expect that the long-term differences in the grazing regime will be reflected in distinct communities of mycorrhizal spores with grazing reducing overall mycorrhizal spore diversity. Finally, I hypothesize that soil nutrient content is low in sites exposed to heavy grazing.

2. Material and methods

2.1. Study area

The study area is located in the physiographic sub-province “Los Llanos de Ojuelos” (21° 49’ N, 101° 37’ W, 2200 m a.s.l). The climate is semiarid and characterized by a rainy season between July and September with 450 mm average annual precipitation and a mean annual temperature of 17-18 ºC (COTECOCA, 1979). The topography is characterized by valleys and gentle rolling hills (0-12% inclination) with soils classified as haplic xerosols associated with lithosols and eutric planosols, and haplic phaeozems associated with lithosols (COTECOCA, 1979). In general, the soils are shallow with silty clay to sandy loam textures and have a cemented layer at roughly 50 cm depth (“tepetate”) (COTECOCA, 197*9*). More soil characteristics are displayed in Table 2.1. The research was carried out along a grazing gradient ranging from sites with extremely heavy grazing (“La Presa” site at the valley bottom, hereafter G^{++}, see Figure 2.1) and heavy grazing (“La Mesa” site at the top of the mesa, hereafter G^{+}; Figure 2.1) over the last 70 years, to sites with moderate grazing (for over 300 yrs) on private land (at the top of the mesa, hereafter G; Figure 2.1) and a 26 year-old 1-ha exclosure in the ejido land (at the valley bottom, hereafter G^{0}, Figure 2.1). The gradient is the product of a series of biophysical (e.g. topography, access to water holes) and socio-economic factors (e.g. land tenure and markedly different grazing histories, closeness of grazing land to the community Vaquerias). Overgrazing has caused sever plant cover loss (> 90% bare soil), and a drastic decline in plant productivity with 80-240 kg dry matter (DM)/ha on the extremely heavy grazing sites to 800-1200 kg DM/ha on the moderately grazed sites (Aguado-Santacruz and García-Moya*,* 1998).

The dominant vegetation is a shortgrass prairie characterized by xeromorphic perennial grasses. *Bouteloua gracilis* (blue grama), *B. scorpioides* Lag (“scorpion grass”), *B. hirsuta* Lag (“hairy grama”), *B. simplex* Lag (“matted grama”), *Aristida divaricata* Humb. & Bompl. (“poverty threeawn”), *Microchloa kuntii* Desv. (Kunth's smallgrass), *Lycurus phleoides* H.B.K. (“common wolfstail”), *Buchloë dactyloides* (Nutt.) Engelm. (“buffalo grass”), and *Muhlen-*

Table 2.1 Soil properties in the study areas (modified from Aguado, 1993)

Site	La Presa		La Mesa		
Depth (cm)	0-25	25-40	0-15	15-30	30-38
pH value	6.5	7.8	6.6	7.1	7.7
Electric conductivity (dS/m)	0.08	0.13	0.14	0.08	0.10
Organic matter (%)	0.6	0.6	2.0	1.2	0.8
Total nitrogen (%)	0.03	0.03	0.10	0.06	0.04
Phosphorous (ppm)	2	2	1	1	1
Interchangeable K cmol/kg	1.20	2.78	1.71	2.01	3.84
Sand (%)	46	54	32	30	32
Silt (%)	29	29	38	35	23
Clay (%)	24	16	30	36	46
Soil texture	loam	sandy loam	clay loam	clay loam	clay
Soil type	Haplic xerosol		Haplic phaeozem		

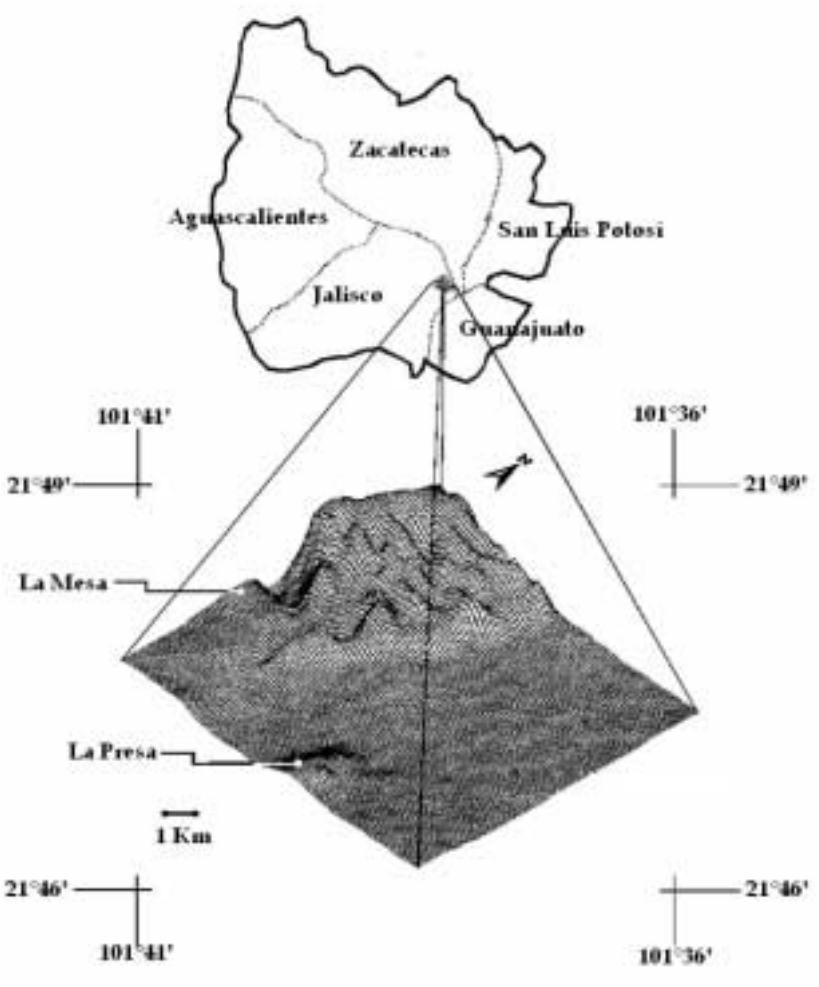

Figure 2.1 Study area (modified from Aguado-Santacruz *et al.*, 2004).

bergia rigida (Kunth) Trin. (“purple muhly”) are the most common grasses in the area. *Mimosa biuncifera* Benth (“catclaw mimosa”), *Dalea bicolor* Humb. & Bompl. in Willd. ("silver prairie clover "), *Brickellia spinulosa* A. Gray, *Opuntia robusta* Wendl., *O. leucotricha* D. C. (arborescent pricklypear), *O. imbricata* Haw., and *O. streptacantha* Lem. are the most common shrubs. Lastly, there is a tree stratum (less than 1 % plant cover) composed of *Acacia schaffneri* (S. Watson) F. J. Herm and *Yucca decipiens* Trel. (for a detailed site description, see Aguado, 1993).

2.2. Experimental design and sampling schemes

The grazing gradient consists of sites with four different levels of stocking density (see above) $G^{++} > G^{+} > G > G^{0}$ with decreasing grazing impacts. In August 2004, a total of sixty soil cores (auger diameter of six cm) were excavated within one-ha plots for all grazing conditions to examine the following variables: communities of mycorrhizal spores, soil nitrogen availability (NH_4^+) and total N and C storage in soil. The sixty cores were sampled along five (replicates) randomly chosen 100 m-transects (twelve cores per transect, taken every eight meters). To examine microsite effects soil cores were taken at two locations, in plant interspaces (gaps greater than 10 cm diameter, after Hook *et al.,* 1991; six cores per transect) and beneath *B. gracilis* plants (six cores per transect). Each soil core was divided into two depths (0-15 and 15-30 cm) to characterize the vertical distribution of the variables. The six soil samples for each microsite by depth combination were composited into a single sample per transect.

To examine root colonization by fungal symbionts in blue grama, three plants were randomly sampled every 33 m along four additional randomly chosen 100 m transects for each grazing condition (N= 48 plants). Plant roots were separated into two depths (0-10 and 10-20 cm) to determine the vertical distribution of fungal symbionts in roots. After sampling, the plant material was stored at 4 °C until further analysis.

2.3. Sample analysis

The composite soil samples were first stored in coolers in the field and then stored at 4 °C until analysis. In the case of mycorrhizal spores, the analysis was carried out during January 2005. The AMF and DSE frequencies of colonization were evaluated between July and August 2005 on samples prepared in February and March 2005. The availability inorganic N in form of ammonium was determined in April 2005. Total soil carbon and nitrogen were analyzed in August 2005.

The percentage of root fungal colonization was determined with a staining method of roots following the protocol from Kormanik and McGrawll (1982). Soil samples were passed though a sieve to recover plant roots smaller than 2 mm diameter. The roots were cut in 1 cm fragments, washed and bleached in a 10% KOH solution and then autoclaved for 20 minutes at 120 °C. Thereafter, roots were washed in H_2O_2 and distilled water, immersed for 3 minutes in 10% HCl and stained with trypan blue in glycerol-acetic acid for 5 minutes at 120 °C. Percent colonization of both DSE and AMF was determined with the method described in McGonigle *et al.* (1990) using the mean value of two slides per soil sample and 150 intersections per slide. Discrimination between the two fungi was based on hyphal color and characteristic structures such as vesicles and microsclerotia for AMF and DSE, respectively (Barrow, 2003).

Mycorrhizal spores were extracted with the sieving and decanting technique described by Daniels and Skipper (1982). Briefly, 100 g of air dried soil were amended with 300 mL of tap water and sieved through three mesh sizes (0.420, 0.149 and 0.038 mm). The recovered soil was transferred into Falcon tubes and mixed with 40 mL water and centrifuged at 2000 rpm for five minutes. Thereafter, the supernatant was eliminated and each tube filled with 40 mL of a sucrose-Tween solution and centrifuged at 3000 rpm for two minutes. The supernatant containing the fungal spores was recovered, rinsed with the thinnest sieve under tap water until the detergent was eliminated and decanted into Petri dishes. The spores in the Petri dishes were counted under a stereomicroscope with 4-fold magnification. Twenty randomly chosen ocular fields were photographed and the images were digitalized for later spore

identification and counts. Spores were grouped based on morphological traits and the average abundance of each morphotype was determined in each soil sample (INVAM, 2004a).

Soil extractable ammonium (NH_4) was determined for one single time only. Soil NH_4 was extracted with 0.5M KSO_4 from triplicates for each sample without incubation. NH_4 was determined with the Berthelot reaction (Bremner, 1965) using sodium salicylate and sodium nitroprusside. One mL of EDTA solution was aggregated to each soil extract (3 mL). Thereafter, 4 mL of sodium salicylate and sodium nitroprusside solution were mixed with the sample and with 2 mL of sodium hypochlorite buffer. The final mixture was kept at 40 °C for 30 minutes and read in the spectrophotometer at 660 nm with NH_4 as the standard. Total soil carbon and nitrogen were determined with a CHNO elemental analyzer (Elemental Combustion System 4010, Costech Analytical Tecnologies Inc).

2.4. Statistical analysis

Fungal colonization (hyphae plus vesicles for AMF, melanized hyphae plus microesclerotia for DSE, as well as total colonization for AMF and DSE) were analyzed with a general linear model with four levels of grazing, two levels of soil depth (0-10 and 10-20 cm) and 12 plant replicates (N = 4 x 2 x 12 = 96) In this case, grazing condition and soil depth were crossed factors. Differences in percent of colonization between AMF and DSE were examined using a t-test. Correlation between DSE and AMF colonization was examined with the simple Pearson correlation analysis to explore co-occurrence patterns in both fungi. Mycorrhizal spore abundances of the different morphotypes were analyzed with a multivariate analysis of variance (MANOVA) following procedures by Bever *et al.* (1996) using ranks of spore abundances in ascending order (i.e., the least spore abundance value within a single morphotype corresponds to rank 1 and so on until rank 80 or less if ties are present) for each spore morphotype. The analysis of ranks of spore abundance was implemented because it improves the highly non-normal distribution of abundances for each morphotype. Ranks were analyzed with a generalized linear model with the three factors grazing (four levels G^{++}, G^{+}, G and G^{0}), microsite (interspace, plant) and soil depths

nested within microsite (0-15 and 15-30 cm for soil samples) with five composite soil samples as replicates (N=4 x 2x 2x 5 = 80). When a statistically significant effect (i.e., grazing, microsite, depth nested within microsite or their interactions) was detected with the MANOVA as shown by the Wilk´s lambda statistic, significant differences in the given effect for each single morphotype were tested with a factorial analysis of variance (ANOVA) and *post hoc* multiple comparisons were done with the Least squares means (LS-means) and the *P*-values were adjusted after the Bonferroni method. Moreover, Simpson's diversity index (Magurran, 1988) as well as total spore densities were calculated for each composite sample and they were analyzed with an ANOVA including the same terms as described previously for ranks of spore abundance. Simpson's diversity index was calculated as

$$1 - D = 1 - \frac{1}{\sum_{i=1}^{n} p_i^{\,2}}$$

where *pi* is the relative abundance of the *i*th species in the sample. The value of this index ranges between one and zero with higher values representing more diverse samples. Data for the biogeochemical variables (NH_4, C, N, C: N ratio) were analyzed with ANOVA with grazing and microsite as crossed factors and depth as a nested factor within microsite. All data except ranks of spore abundance were transformed prior to analysis (arcsine transformation for percentage data of root fungal colonization, percent of carbon, percent of nitrogen and transformation of natural logarithm for ammonium concentration) to meet the assumptions of normality and homogeneity of variance. All statistical tests were run with the SAS system v. 6.12 for windows with a type I error of 0.05 (SAS System, Cary, NC 1997). Back-transformed data are reported in text and figures unless otherwise stated; back-transformed data of percent of carbon and nitrogen were transformed afterwards analysis to a mass basis and together with ammonia are reported on an area basis based on previous knowledge of bulk density (unpublished data)

3. Results

3.1. Root fungal colonization

Root colonization by fungal symbionts differed between the two symbionts (t150=13.64, P<0.001, data not shown) with DSE exhibiting higher root colonization than AMF for all grazing conditions (37.24% versus 10.18% mean root colonization for DSE and AMF, respectively). Grazing did not differently affect root colonization by the symbionts (grazing main effect, F = 0.660, df = 3, P > 0.5 and F = 1.017, df = 3, P > 0.35 for DSE and AMF, respectively; view top panels in Figure 3.1 and Figure 3.2, and Tables A and B in Appendix), although there was high variability in the case of AMF in each grazing condition.

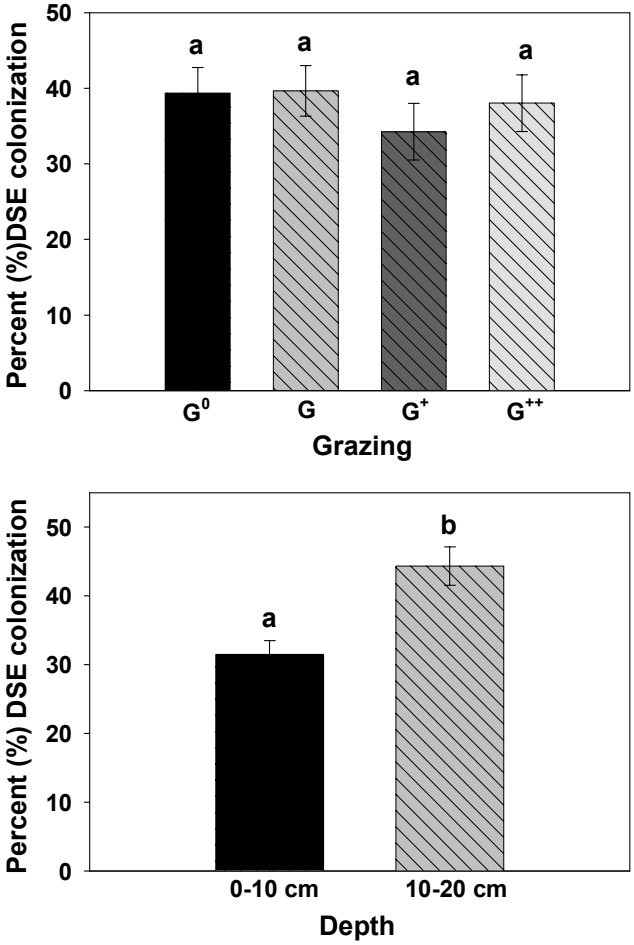

Figure 3.1 LS-means (±standard error) of percent root colonization by dark septate endophytes (DSE) of *Bouteloua gracilis* plants sampled along a grazing gradient of Los Llanos de Ojuelos, Jalisco at the end of the rainy season in 2004 (top) and at two soil depths (bottom). Different letters indicate statistical differences among treatments with $P \leq 0.05$. G^0 = no grazing, G = moderate grazing G^+ = heavy grazing and G^{++} = extremely heavy grazing.

Regarding the vertical distribution of root colonization, only DSE showed significant difference between sampling depths with greater colonization at 10-20 cm depth than at 0-10 cm (soil depth main effect, $F = 12.796$, $df = 1$, $P < 0.001$; see bottom panels of Figure 3.1 and Figure 3.2). Overall, no arbuscules were found in the soil samples. Percent root colonization of AMF and DSE was positively correlated only for the heavy grazing (G^+) condition ($r = 0.59$, $t_{16} = 2.92$, $P < 0.01$; Figure 3.3 bottom-left panel, Table C in Appendix) while for the other grazing conditions the correlation between the colonization of the two fungal symbionts was variable yet not significantly different (G^0 condition, $r = 0.24$, $t_{17} = 1.02$, $P = 0.33$; G condition, $r = -0.05$, $t_{18} = -0.21$, $P = 0.80$, G^{++} condition, $r = 0.29$, $t_{17} = 1.25$, $P = 0.22$; Figure 3.3).

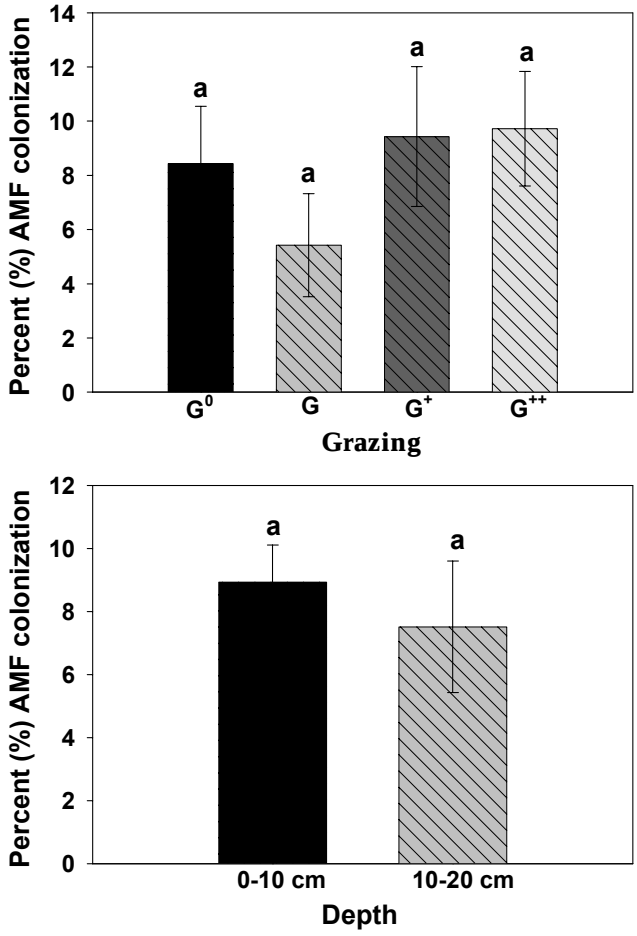

Figure 3.2 LS-means (±standard error) of percent root colonization of arbuscular mycorrhizal fungi (AMF) of *Bouteloua gracilis* plants sampled along a grazing gradient of Los Llanos de Ojuelos, Jalisco at the end of the rainy season in 2004 (top) and at two sampling depths (bottom). Different letters indicate statistical differences among treatments with $P \leq 0.05$. G^0 = no grazing, G = moderate grazing G^+ = heavy grazing and G^{++} = extremely heavy grazing.

When the data of root colonization were pooled for the two symbionts, there was no significant difference along the grazing gradient for total fungal colonization (grazing main effect, F = 0.42, df = 3, P = 0.73; Table D in Appendix).

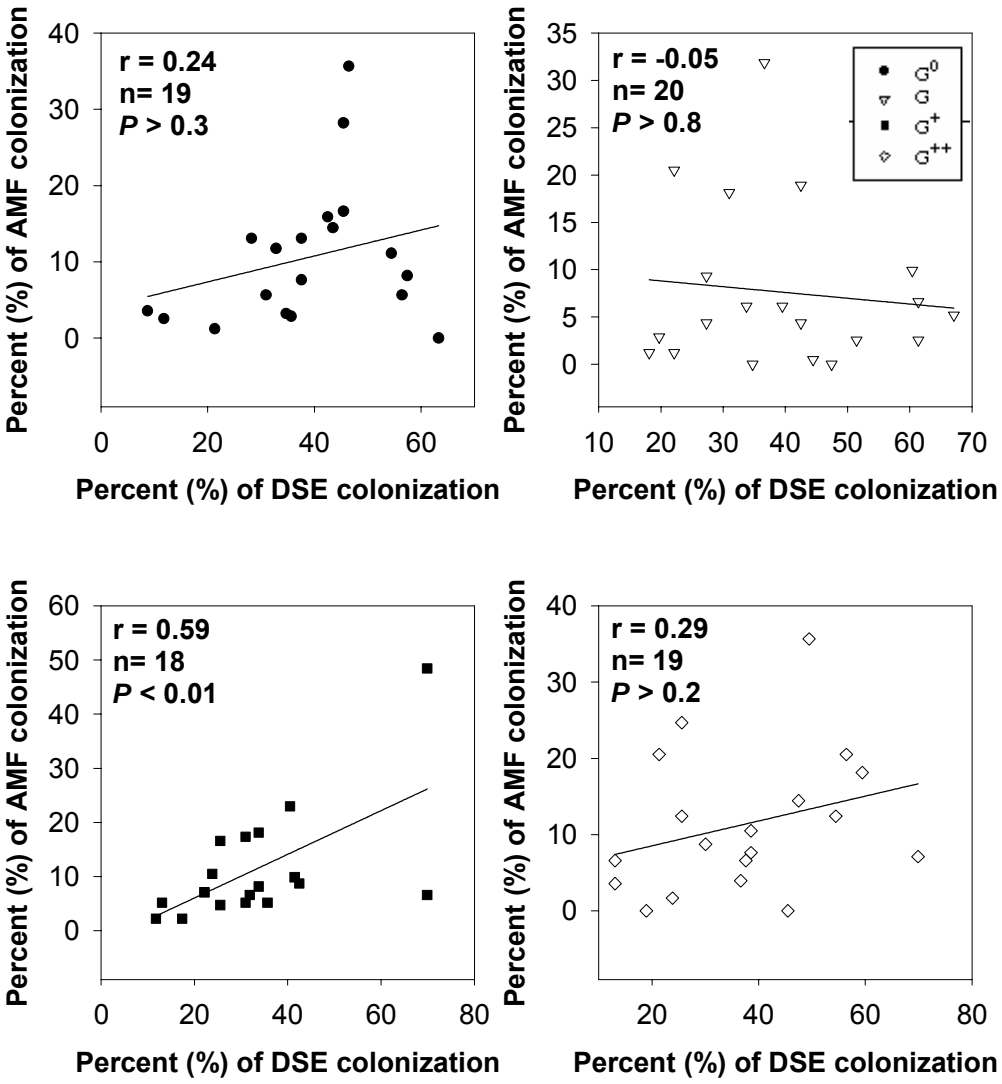

Figure 3.3 Correlation between percent of dark septate endophytes (DSE) colonization (x axis) and percent of arbuscular mycorrhizae fungi (AMF) colonization (y axis) of *Bouteloua gracilis* plants sampled along a grazing gradient in Los Llanos de Ojuelos, Jalisco at the end of the rainy season in 2004. Correlation analyses are based on arcsin transformed data of both colonization types but graphs show non-transformed values. Lines are linear least-square fits to depict the tendencies of associations. r= Pearson correlation coefficient, n= number of observations and *P*= probability associated with single r-value.

3.2. Morphotypes of arbuscular mycorrhizae spores

Four distinct morphotypes of AMF spores, named black, amber, yellow and brown morphotypes, were identified based on the external characteristics of color and shape. Overall, the black morphotype constituted the most abundant spore morphotype followed by the amber, brown and yellow morphotypes (Table 3.1). Ranks of morphotype abundance differed along the grazing gradient as shown by the MANOVA (grazing main effect; Wilk's lambda (Λ) = 0.468, F = 4.48, P = 0.0001; see Table E in Appendix). The results of single ANOVAs showed that grazing effects were significant only for the amber and yellow morphotypes (grazing main effect, F = 4.262, P = 0.008 and F = 9.856, P < 0.0001, respectively; Figure 3.4). Mean discrimination analysis revealed that ranks of abundance for the amber morphotype were lowest in the G^{+} condition, while they did not differ in the other grazing sites (grazing main effect, F = 9.856, P < 0.0001; Figure 3.4). On the other hand, the yellow morphotype had the highest abundance in the G^{+} grazing intensity, intermediate abundance in the G^{++} and G grazing conditions and the least abundance in the ungrazed area (Figure 3.4). The black morphotype was similarly abundant with respect to different grazing intensities (grazing main effect, F = 1.023, P = 0.38) and the brown morphotype was only marginally affected (grazing main effect, F = 2.62, P = 0.0579). When considering ranks of spore abundance, morphotypes differed significantly between the two sampling depths within microsite with higher ranks of spore abundances in the shallow soil layer [depth (microsite) main effect, Λ = 0.423, F = 8.216, P = 0.0001; Figure 3.5, Table E in Appendix]. There was also a marginal significant interaction between grazing and soil depth nested within microsite [grazing X depth (microsite) interaction, Λ= 0.572, F = 1.55, P = 0.055] as a result of different responses of ranks spore abundance in the yellow and brown morphotypes to the grazing gradient (Figure 3.6).

When abundance data for all four morphotypes were pooled, there were no differences in total spore abundance among grazing conditions (grazing main effect, F = 1.1, P = 0.35), nor between plant interspaces and plant microsites (microsite main effect, F = 1.33, P = 0.25). For this analysis, total spore abundances differed remarkably between the two sampling depths

(depth(microsite) effect, F = 27.65, P < 0.001) with a near three-fold higher spore density in 0-15 cm depth (80 spores/g soil) than in 15-30 cm (30 spores/g soil).

Mycorrhizal spore diversity was significantly higher in ungrazed conditions than in overgrazed (G^+) conditions (grazing main effect, F = 4.08, P = 0.01; Figure 3.7, see Table F in Appendix) while the levels of diversity were intermediate in the heavy overgrazing and moderate grazing conditions. There occurred a significant grazing X depth within microsite interaction indicating that in the G^0 condition spore diversity was higher in the shallow soil layer while in the other grazing conditions spore diversity was higher at greater soil depth (Figure 3.8).

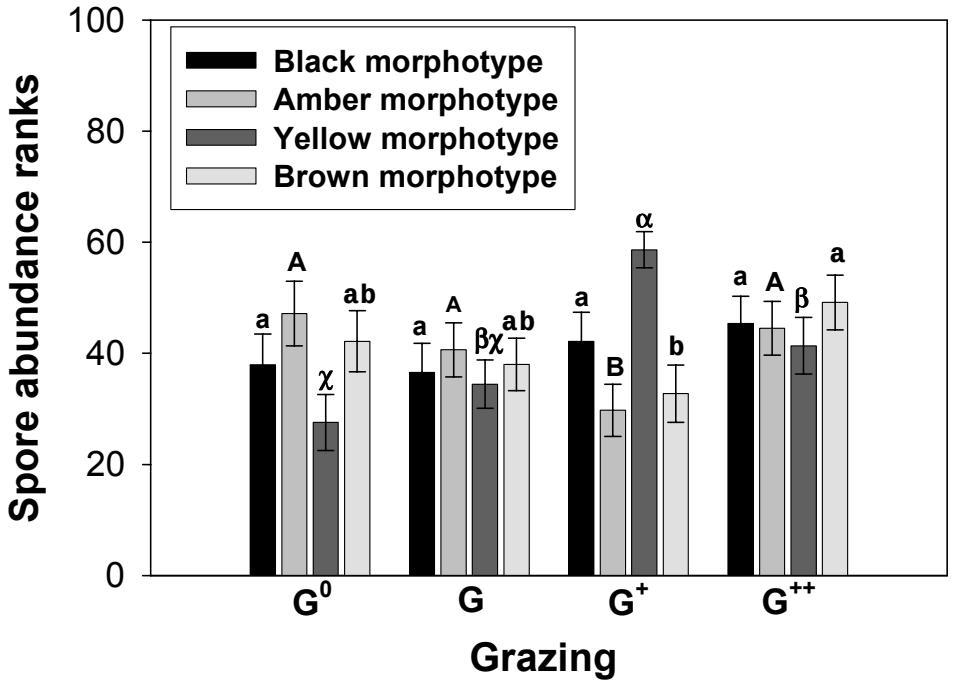

Figure 3.4 LS-means (± standard error) of ranks of mycorrhizal spore abundances along a grazing gradient in Los Llanos de Ojuelos, Jalisco at the end of the rainy season in 2004. The least rank spore abundance value within a single morphotype corresponds to rank 1 and so on until the rank 80 or less if ties were present. G^0 = no grazing, G = moderate grazing G^+ = heavy grazing and G^{++} = extremely heavy grazing. MANOVA results are presented in Table E in Appendix I. Different symbols by morphotype indicate significant differences at P < 0.05.

Table 3.1 Mean abundances (± se) of arbuscular mycorrhizal (AMF) spores morphotypes along a grazing gradient in Los Llanos de Ojuelos, Jalisco at the end of the rainy season in 2004. Values are spores number/soil g.

AMF morphotypes	Grazing gradient			
	G^0	G	G^+	G^{++}
Black	41.17± 7.86	39.72 ± 8.32	49.25 ± 8.99	52.02 ± 8.15
Amber	20.40 ± 3.85	13.723 ± 2.23	9.66 ± 1.80	15.59 ± 2.25
Yellow	0.49 ± 0.09	0.85 ± 0.20	3.43 ± 0.63	1.19 ± 0.29
Brown	3.11 ± 0.71	2.19 ± 0.33	2.00 ± 0.36	4.80 ± 1.06

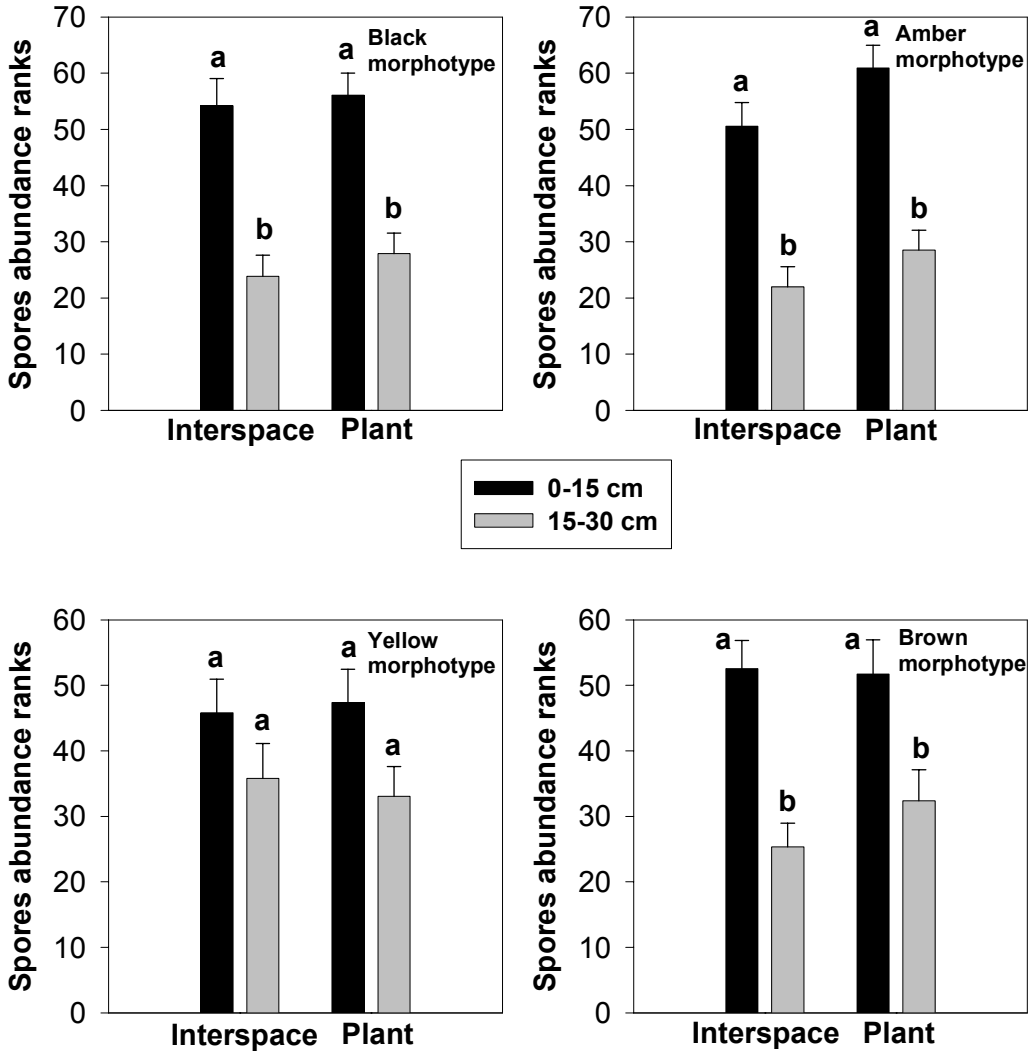

Figure 3.5 Means (± standard error) of ranks of mycorrhizal spore abundance between the two sampling depths within microsite for each mycorrhizae morphotype found along a grazing gradient in Los Llanos de Ojuelos, Jalisco at the end of the rainy season in 2004. The least rank spore abundance value within a single morphotype corresponds to rank 1 and so on until the rank 80 or less if ties were present. MANOVA results are presented in Table E in Appendix.

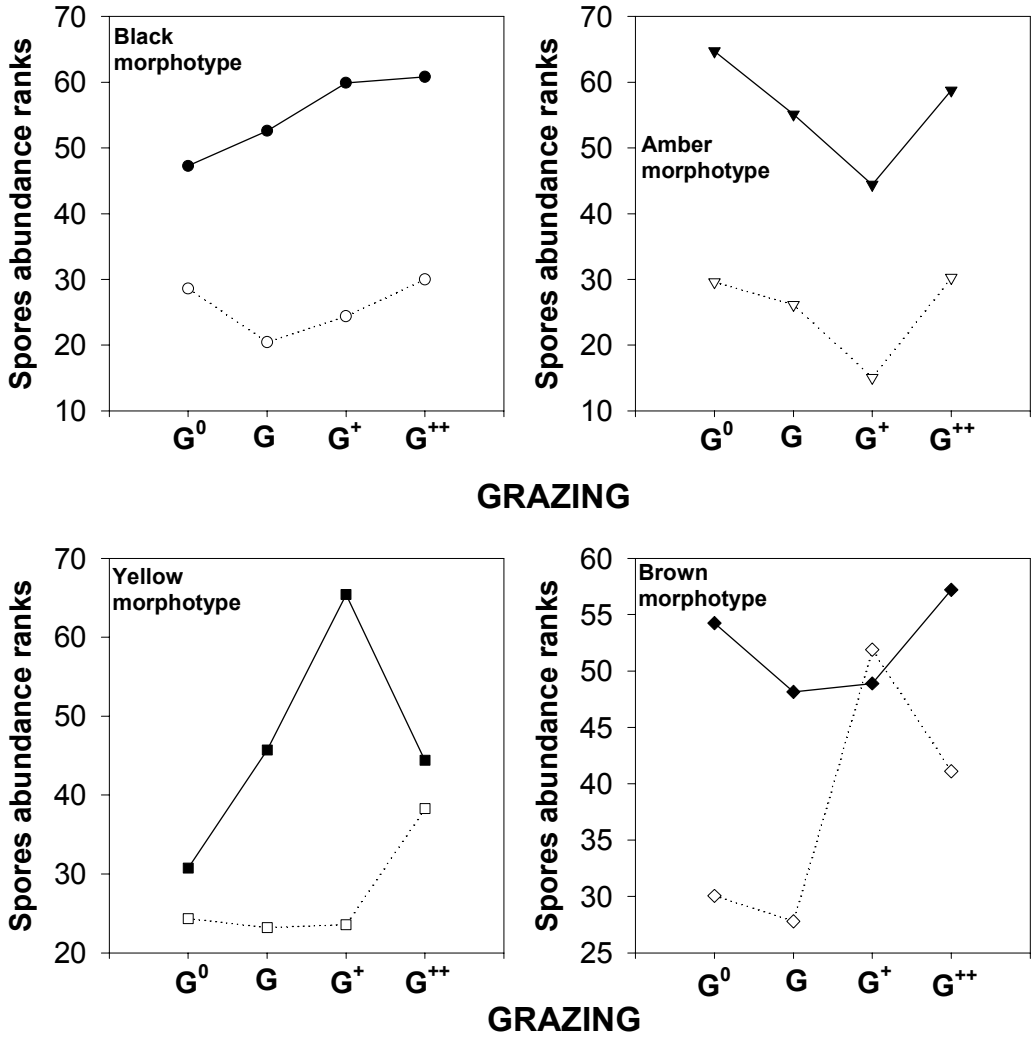

Figure 3.6 Means of spores ranks abundance of mycorrhizal morphotypes found along a grazing gradient in Los Llanos de Ojuelos, Jalisco at the end of the rainy season in 2004. The least rank spore abundance value within a single morphotype corresponds to rank 1 and so on until rank 80 or less if ties were present. G^0 = no grazing, G = moderate grazing G^+ = heavy grazing and G^{++} = extremely heavy grazing. Filled symbols correspond to 0-15 cm sampling depth and open symbols correspond to 15-30 cm depth. For more details, see text.

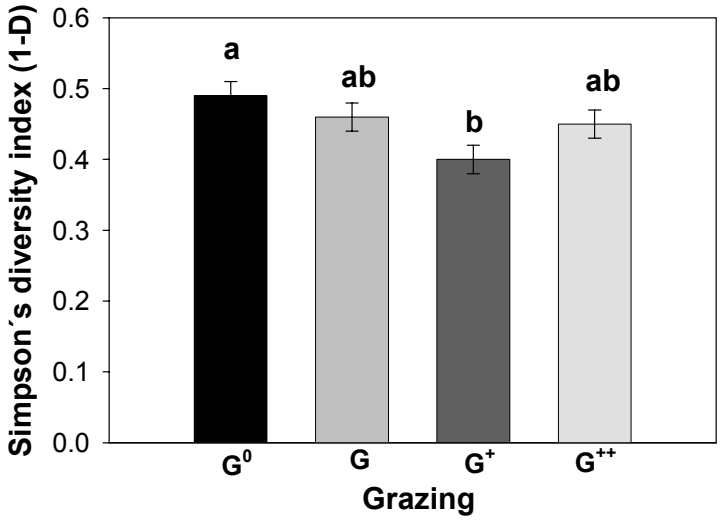

Figure 3.7 LS-means (± standard error) of Simpson's diversity index (1-D where D is Simpson's dominance) based on abundances of mycorrhizal spores found along a grazing gradient in Los Llanos de Ojuelos, Jalisco at the end of the rainy season in 2004. Different letters indicate statistical differences among treatments with $P \leq 0.05$. G^0 = no grazing, G = moderate grazing G^+ = heavy grazing and G^{++} = extremely heavy grazing.

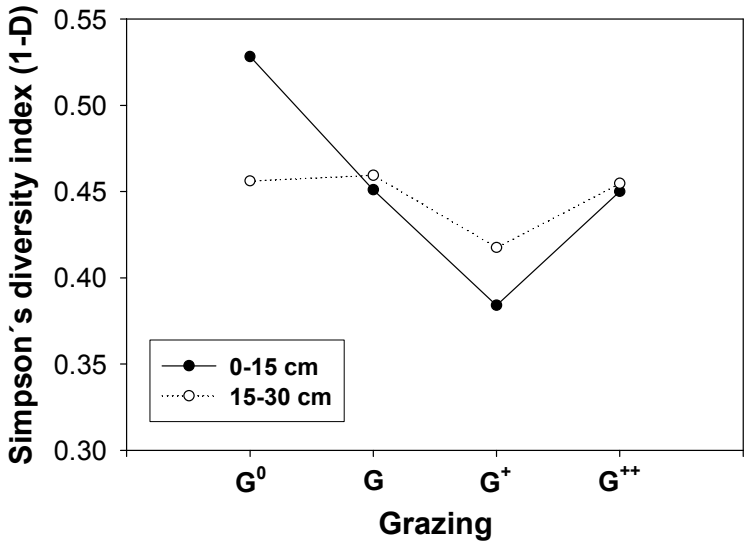

Figure 3.8 Means for two sampling depths of Simpson's diversity index (1-D where D is Simpson's dominance) based on abundances of mycorrhizal spores found along a grazing gradient in Los Llanos de Ojuelos, Jalisco at the end of the rainy season in 2004. G^0 = no grazing, G = moderate grazing G^+ = heavy grazing and G^{++} = extremely heavy grazing. For more details see the text.

3.3. Biogeochemical variables

Grazing had a significant effect on total soil carbon (C) (grazing main effect, F = 5.22, df = 3, P < 0.01, Figure 3.9 top panel, Table H in Appendix) with C

differing by almost 30 % between the moderate grazing treatment (5043 gC/m^2, G condition) and extremely heavy grazing (3901 gC/m^2, G^{++} condition). Furthermore, the C content differed significantly between microsites with 4790 g C/m^2 beneath plants vs. 4139 gC/m^2 for the interspaces (microsite main effect, F = 8.68, df = 1, P < 0.01; Figure 3.9 top panel). Significant differences in C content between soil depths within microsites were also observed with greater C in the top 15 cm compared to the 15-30 cm beneath plants, while in the open interspaces the C content did not differ between the two depths (depth (microsite) main effect; F = 21.63, df = 2, P < 0.001; Figure 3.9 bottom panel).

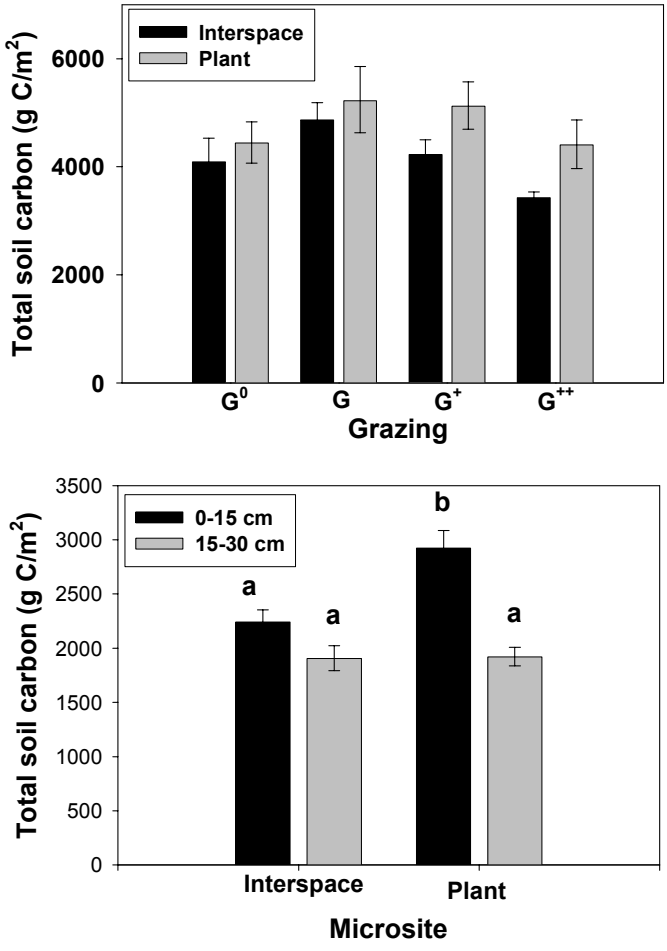

Figure 3.9 LS-means (± standard error) of total soil carbon (including the total sampling depth of 30 cm) for grazing and microsite effects (top) and for the two depths nested within microsites (bottom). For explanation see text. Different letters indicate statistical differences among treatments with $P \leq 0.05$. G^0= no grazing, G= moderate grazing G^+= heavy grazing and G^{++}= extremely heavy grazing.

Total soil nitrogen (N) differed significantly for the different grazing conditions along the grazing gradient (grazing main effect, $F = 5.92$, $df = 3$, $P < 0.01$, top, Table I in Appendix) with N content being highest under G and G^{+} and lowest under G^{0} and G^{++} (Figure 3.10, top panel). Total soil N differed by almost 20% with respect to grazing (462 gN/m^2 under G and 373 g N/m^2 under G^{++}, respectively.) Total N was significantly higher beneath plants (447 g N/m^2) than in the interspaces (404 gN/m^2) (microsite main effect, $F = 6.05$, $df = 1$, $P < 0.05$; Figure 3.10 top panel). Soil N content was higher in 0-15 cm beneath plants in comparison to the interspaces and to the 15-30 cm in both microsites (depth (microsite) main effect; $F = 18.27$, $df = 2$, $P < 0.001$) (Figure 3.10, bottom panel).

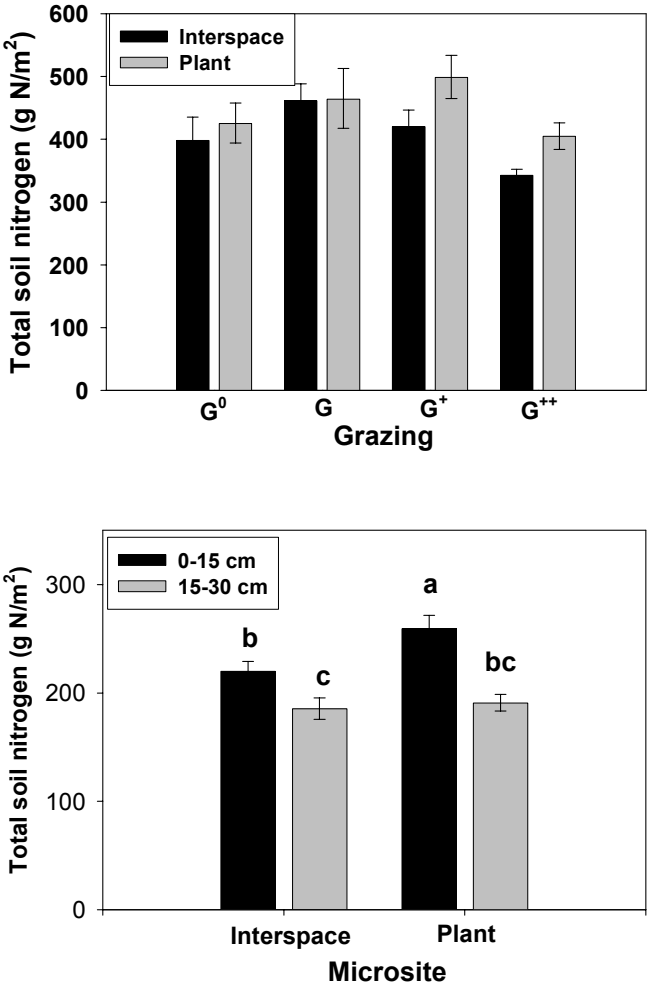

Figure 3.10 LS-means (± standard error) of total soil nitrogen along a grazing gradient by interspace and plant microsites (top, it includes the 30 cm depth of sampling) and for the two depths within microsites (bottom). Different letters indicate statistical differences among treatments with $P \leq 0.05$. G^{0}= no grazing, G= moderate grazing G^{+}= heavy grazing and G^{++}= extremely heavy grazing.

The C:N ratio differed significantly between sampling depths nested within microsites (depth(microsite) main effect, F = 6.285, df = 2, P = 0.003; Figure 3.11, bottom panel) in that beneath plants it was higher in 0-15 cm (11.27) than at 15-30 cm (10.06) where it was similar to the C:N ratios at both soil depths in the interspaces (10.17). Grazing (grazing main effect, F = 1.75, df = 3, P > 0.15, Table I in Appendix) did not influence the C:N ratio, which was about 10.4 for the four grazing conditions (Figure 3.11 top panel).

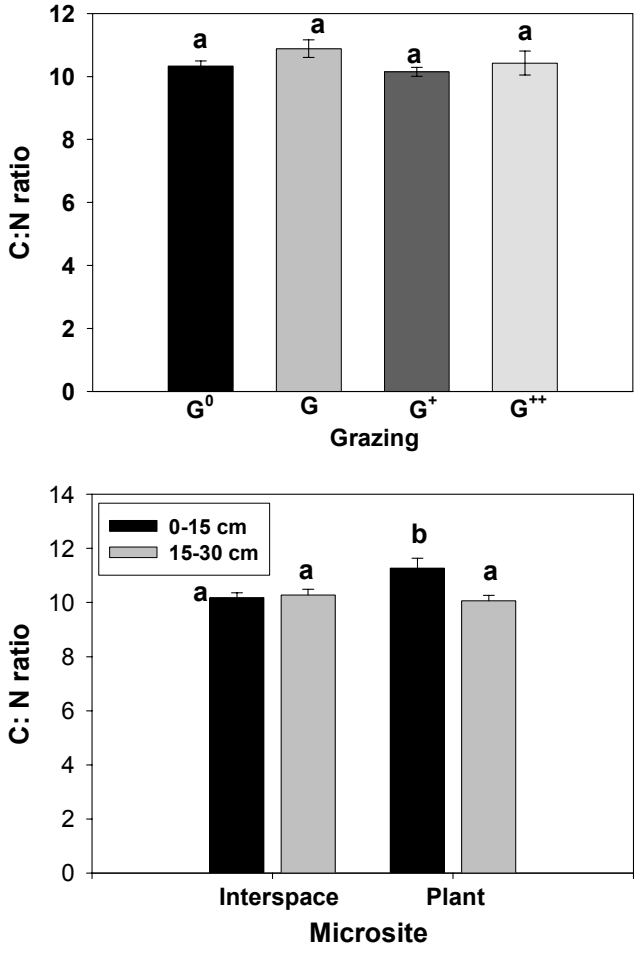

Figure 3.11 LS-means (± standard error) of soil C:N ratio along a grazing gradient (top, it includes the 30 cm depth of sampling) and at two soil depths within microsites (bottom). Different letters indicate statistical differences among treatments with $P \leq 0.05$. G^0= no grazing, G= moderate grazing G^+= heavy grazing and G^{++}= extremely heavy grazing.

Soil ammonium content was highest in the G grazing condition (0.571 g NH_4/m^2) and lowest under G^{++} and G^{+} (0.217 gNH_4/m^2 and 0.244 gNH_4/m^2, respectively) (Figure 3.12, top panel) representing a 62 % difference (grazing main effect, F = 5.687, df = 3, P < 0.01, See Table K in Appendix). The microsites beneath plants had higher NH_4 content than the ones in interspaces (microsite main effect, F = 12.12, df = 1, P = 0.001, Figure 3.12, top panel). There was twice as much ammonium in the top 15 cm (0.417 gNH_4/m^2) beneath plants compared to the 15-30 cm depth and in the interspaces (depth nested within microsite main effect, F = 22 df = 2, P < 0.001, Figure 3.12 bottom).

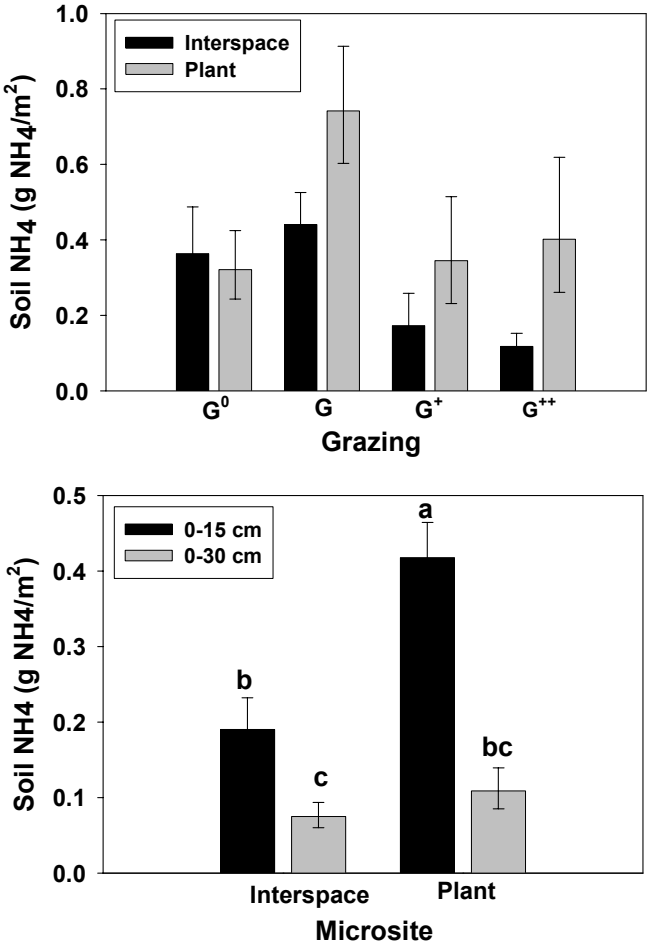

Figure 3.12 LS-means (± standard error) of soil extractable ammonium content along a grazing gradient for different microsites (top, includes the 0-30 cm depth of sampling) and for the two depths within microsites (bottom). Different letters indicate statistical differences among treatments with $P \leq 0.05$. G^0= no grazing, G= moderate grazing G^+= heavy grazing and G^{++}= extremely heavy grazing.

4. Discussion

4.1. The fungal symbiosis in *Bouteloua gracilis* and mycorrhizal community along a grazing gradient

My results showed that fungal associations between AMF and DSF and roots of Bouteloua gracilis are important symbioses in the shortgrass prairie and that different grazing regimes had no contrasting effects on the frequency these symbioses. Since AMF are obligatory symbionts and plants may encounter potential trade-offs in carbon allocation in response to grazing, I hypothesized that the frequency of the fungal symbiosis would decrease with increasing grazing intensity. Our data did not support this hypothesis, root colonization by AMF and DSE did not seem to have been differently affected by the contrasting grazing intensities along the grazing gradient (Figure 3.1 and Figure 3.2). Overall, the symbiosis with DSE was more common than with AMF. To our knowledge this is one of the first reports to show that B. gracilis invests into DSE symbiosis. Although, the functional role of the DSE is still unclear this symbiosis was more pronounced at the 10-20 cm (Figure 3.1 and Figure 3.2) while mycorrhizal colonization was similar for the two soil depths (Figure 3.2). Blue grama exhibits morphological and physiological traits typical for grazing tolerant species; e.g. the position of meristems near the soil surface (Branson; 1953; Sims, 1973), compensatory photosynthesis (Detling *et al.,* 1980), greater allocation of photosynthates to aboveground production at the expense of belowground production (Bekele *et al.,*1974; Detling *et al.,* 1980), and increased relative tillering (Reece *et al.,* 1998), which all explain the dominance of blue grama in the shortgrass prairie where grazing pressure is extremely high, especially under current land use conditions. Maintenance of fungal symbiosis has been considered a trait of grazing tolerant grasses (Allsopp; 1998; but see Busso *et al.,* 2001). Since the fungal symbioses in this study did not change in response to the grazing gradient, this suggests that the fungal symbiosis could be part of the mechanisms conferring grazing tolerance to blue grama. However, the present study does not provide evidences to probe the above argument. Semiarid grasslands are notoriously nutrient poor environments and herbivory has been one of the most important selective forces during the co-

evolutionary development of these ecosystems (Miclchunas *et al.,* 1988). The absence of a response of the fungal symbiosis to grazing in grasslands studied in the Llanos de Ojuelos coincides with the findings of a previous work by Reece and Bonham (1978) in which blue grama plants also showed no difference in AMF colonization between grazed and ungrazed shortgrass prairie in Colorado. Other factor such as high levels of NH_4 and phosphorous (20 to 40-fold higher NH_4 concentrations than those obtained in this study) (Hays *et al.,* 1982) may suppress AMF infection of blue grama roots. Although, my study reported differences in NH4 there was not associated changes in AMF colonization in *B. gracilis*.

A full account of the mycorrhizal responses to disturbance always requires a concomitant assessment of the effects on root length (Kliromonos *et al.,* 1994). I did not measure fungal symbiosis in relation to root variables due to limitations in the sampling scheme. Nevertheless, there is good evidence that grazing does curtail root growth in blue grama both in clipping experiments and in field studies along grazing gradients (Bekele *et al.,* 1974; Medina-Roldán, 2003) While different grazing intensities did not affect the percentage of fungal colonization in my study, on heavily grazed sites it may have reduced total root colonization due to reductions in root biomass.

Although descriptive, the results in this study permited us to get insight on the ecology of plant - fungal symbiosis in the semiarid grasslands. For instance, it was shown that the DSE symbiosis was more frequent than the AMF symbiosis at least for the sampling period at peak standing crop biomass. This pattern has been observed in other arid and semiarid ecosystems suggesting that the DSE symbiosis may play a complementary functional role to AMF (Barrow *et al.*, 1997; Jumponen, 2001; Barrow, 2003). AMF and DSE showed a clear positive correlation only under heavy grazing conditions (G+), whereas in the other grazing conditions the two fungal symbionts simply co-colonized the roots. Additional studies are necessary to explore the functional role of these coexisting symbionts and overall the ecological roles of these fungi in the shortgrass prairie.

In spite of the four observed morphotypes are rough taxonomic estimates of AMF spore identity, grazing modified the composition (measured through ranks of abundance) and diversity of the AMF spore communities at this

taxonomic resolution (Figure 3.4 and Figure 3.7). The morphotypes responses to grazing was variable and not always significant (Figure 3.4 and Figure 3.6), suggesting that mycorrhizal species differ in susceptibility to grazing. However, unlike my expectations of observing a reduced diversity with increasing grazing intensity, a significant reduction in spore diversity was only noticed for the heavily grazed site (Figure 3.7) but not for the extremely overgrazed site. The mechanism for the lowest level of mycorrhizal spore diversity at a grazing level other than the extreme overgrazing is not clear at the moment although I observed a trend to have less spores diversity in grazed conditions compared to the ungrazed area. Since the same four AMF spores morphotypes were registered through the grazing gradient, the changes in diversity observed correspond to changes in evenness with a trend of black morphotype to dominate in grazed environments (Table 3.1). Other studies have documented reduced AMF spores diversity in response to grazing (Bethlenfalvay and Dakessian, 1984; Eom *et al.,* 2001) but given the level of knowledge on AMF diversity in this region, additional work is necessary addressing seasonal variation in spore composition with a more rigorous treatment of taxonomic units. Here a molecular biology approach such as ITS-PCR assay can be used together with sequencing analysis in order to estimate AMF spores identity and diversity (Redecker *et al.,* 1997). Grazing, on the other hand, did not affect total mycorrhizal spore abundance so that changes in AMF diversity were produced by reductions in evenness with increasing levels of herbivory. Together, these results may imply the occurrence of grazing "tolerant" species of mycorrhizal fungi which compensate at least in term of sporulation.

4.2. The soil C and N pools and plant available N along a grazing gradient

The response of biogeochemical variables to grazing regimes addresses the more general notion of grazing effects on ecosystem functioning (McNaughton *et al.,* 1997; Ritchie *et al.,* 1998; Wardle *et al.,* 2002, Bardgett and Wardle, 2003; Patra *et al.,* 2005). Grazing intensity did influence total soil C, total soil N and extractable NH_4 with higher concentrations at sites exhibiting intermediate stocking rates (Figure 3.9, Figure 3.10 and Figure 3.12 top panels). Previous

studies (Burke *et al*., 1998; Vinton and Burke, 1997) proposed that in grasslands receiving < 700 mm of annual precipitation such as the shortgrass prairie, it is the belowground processes that control ecosystem functioning. Hence water availability and the spatial structure of vegetation (plant cover versus bare ground openings) control the biogeochemical processes rather than different grazing histories that may act on plant traits and thereby influence litter quality. In contrast, Reeder and Shuman (2002, and multiple references therein) reported that heavy grazing increased soil carbon in the 15-30 cm layer in comparison to light or moderate grazing in the shortgrass prairie. They concluded that increased carbon is likely a result of changes in physiological traits of blue grama, particularly the allocation of carbon to crowns and root exudates. Plant cover on the other hand, is positively correlated with belowground biomass in shortgrass prairie (Medina-Roldán, 2003) therefore greater plant cover (and therefore biomass inputs to the soil) observed in the exclosure and moderate grazing may cause the higher levels of total soil C and N in these grazing conditions in comparison to the extremely heavy grazing area.

Interestingly, the heavy grazing treatment despite of exhibiting important reductions in plant cover, maintained C and N levels higher than those observed on the exclosure beneath the plant microsite, although both grazing conditions had similar C and N contents under bare ground openings. Other mechanisms which enhance nutrient retention and nutrient cycling in grassland ecosystems in response to grazing include greater root: shoot ratio, greater root and tissue turnover as well as livestock inputs of organic nitrogen (McNaughton, 1979; Hillbert *et al*., 1981; McNaughton *et al*., 1997; Conant and Paustian, 2002; Hawkes and Sullivan, 2001) so that higher C and N content under heavy grazing conditions in comparison with the exclosure could result from direct N inputs through the excreta of herbivores.

Results from this study together with those from Arredondo (2002), Arredondo *et al.* (2005), Aguado (1994), Medina-Roldán (2003) suggest that grazing may impact important soil processes such as, soil water dynamics in the shortgrass prairie of Los Llanos de Ojuelos by changing vegetation structure and composition. In his work, Medina-Roldán (2003) found that soil water dynamics are related to plant cover in such a way that greater plant cover enhances soil

water inputs and soil water usage. Since biological processes in semiarid grasslands are mainly water-limited, variation in soil nutrient contents may reflect both direct grazing effects on soil litter inputs and indirect grazing effects on soil water dynamics.

Results in this study support the argument that *B. gracilis* grasslands require the influence of disturbance factors (grazing) to maintain a successful community. Management practices recommended based on the above arguments should prevent plant cover loss by maintaining moderate stocking rates to avoid nutrient loss form the system. Soil resource distribution between plant and interspace microsites differed by 14% for C, 10% for N, and more than 40% for ammonium with greatest concentrations under the plant in the upper soil layer (0-15 cm). This pattern has been described for the shortgrass prairie of Colorado in other studies (Derner *et al.,* 1997; Vinton and Burke, 1997; Burke *et al.,* 1998; Eipstein *et al.,* 1998; Burke, 1999; Hook and Burke, 2000) and indicates the vulnerability of shortgrass prairie to management practices that disturb shallow soil layer such as ploughing.

5. Conclusions

Approaches linking the above- and belowground components are necessary in order to understand ecosystems in a more integrative way. In this work, grazing by domestic herbivores constituted the link between these ecosystem compartments and it was an important influence on soil functioning in terms of carbon and nitrogen storage. These trends could be explained by both direct modifications of grazing on vegetation structure which cause plant biomass loss and by indirect effects of grazing on other ecosystem processes such as soil water dynamics. Furthermore, this work indicated that colonization of arbuscular mycorrhizal fungi as well as dark septate endophytes in *Bouteloua gracilis* plants did not fit to the view of carbon allocation tradeoffs in response to grazing which supposedly limit fungal symbioses frequency. This result suggests that fungal symbiosis can be a mechanism of grazing tolerance in this poor-nutrient adapted species. On the other hand, the composition and diversity of arbuscular mycorrhizal spores responded to grazing intensity in rather idiosyncratic ways which deserves more studies that explore in depth the ecological significance of arbuscular mycorrhizal species in shortgrass prairie. Overall, land use change in the form of overgrazing which negatively impact plant cover and the top soil layer may cause nutrient redistribution and degradation of these semiarid grasslands.

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Appendix of statistical tables

Table A. ANOVA table of percent of colonization (%) in *Bouteloua gracilis* (blue grama) roots by dark septate endophytes for different grazing conditions (no grazing, moderate grazing, heavy grazing and extremely heavy grazing) and two sampling depths (0-10 and 10-20 cm) in a shortgrass prairie in Central Mexico.

Source of variation	*df*	Sums of Squares	Mean square	*F*	*P*
Grazing	3	0.048	0.0162	0.660	0.56
Depth	1	0.314	0.3139	12.796	0.0006
Grazing X Depth	3	0.038	0.0128	0.522	0.67
Error	68	1.6679	0.025		

Table B. ANOVA table of percent of colonization (%) in blue grama roots by arbuscular mycorrhizae for different grazing conditions (no grazing, moderate grazing, heavy grazing and extremely heavy grazing) and two sampling depths (0-10 and 10-20 cm) in a shortgrass prairie in Central Mexico.

Source of variation	*df*	Sums of Squares	Mean square	*F*	*P*
Grazing	3	0.078	0.026	1.017	0.39
Depth	1	0.013	0.013	0.523	0.47
Grazing X Depth	3	0.032	0.011	0.421	0.73
Error	68	1.731	0.025		

Table C. Statistical summary of correlation analysis between root colonization of dark septate endophytes and arbuscular mycorrhizal fungi in *Bouteloua gracilis* plants sampled along a grazing gradient in Los Llanos de Ojuelos, Jalisco during the ending of the rainy season at 2004. G^0 = no grazing, G = moderate grazing, G+ = heavy grazing and G++ = extremely heavy grazing. *r* = Pearson correlation coefficient, n = sample size, *sr* = standard error of correlation coefficient.

Grazing condition	*r*	n	s_r	*t*	*df*	*P*
G^0	0.24	19	0.235	1.02	17	0.33
G	-0.05	20	0.235	-0.21	18	0.81
G^+	0.59	18	0.201	2.92	16	0.001
G^{++}	0.29	19	0.232	1.25	17	0.22

Table D. ANOVA table of total fungal colonization (%) in blue grama roots for different grazing conditions (no grazing, moderate grazing, heavy

grazing and extremely heavy grazing) and two sampling depths (0-10 and 10-20 cm) in a shortgrass prairie in Central Mexico.

Source of variation	*df*	Sums of Squares	Mean square	*F*	*P*
Grazing	3	0.032	0.010	0.42	0.73
Depth	1	0.215	0.215	8.65	0.004
Grazing X Depth	3	0.029	0.009	0.39	0.75
Error	68	1.694	0.025		

Table E. MANOVA results of ranks of mycorrhizal spores abundance of four mycorrhizae morphotypes found under different grazing conditions (no grazing, moderate grazing, heavy grazing, extremely heavy grazing), microsites (under plant and under bare ground openings) and sampling depths (0-15 and 15-30 cm) in a shortgrass prairie in Central Mexico.

Source of variation	Num *df*	Den *df*	Wilks' Lambda	*F*	*P*
Grazing	12	161.68	0.468	4.481	0.0001
Microsite	4	61	0.898	1.72	0.15
Depth within Microsite	8	122	0.4228	8.216	0.0001
Grazing X Microsite	12	161.68	0.790	1.25	0.25
Grazing X Depth within Microsite	24	214.01	0.572	1.55	0.055

Table F. ANOVA table of mycorrhizal spores diversity (Simpson's diversity index 1-D where D is Simpson's dominance) based on abundances of mycorrhizal morphotypes found along a grazing intensity gradient (no grazing, moderate grazing, heavy grazing, extremely heavy grazing), under different microsites (under plant and under bare ground openings) and sampling depths (0-15 and 15-30 cm) in a shortgrass prairie in Central Mexico.

Source of variation	*df*	Sums of Squares	Mean square	*F*	*P*
Grazing	3	0.085	0.028	4.08	0.01
Microsite	1	0.003	0.003	0.53	0.47
Depth within Microsite	2	0.023	0.011	1.67	0.19
Grazing X Microsite	3	0.038	0.012	1.83	0.15
Grazing X Depth within Microsite	6	0.097	0.0161	2.32	0.04
Error	64	0.443	0.007		

Table G. ANOVA table of total mycorrhizal spores abundance (spores number/ g soil) along a grazing intensity gradient (no grazing, moderate

grazing, heavy grazing, extremely heavy grazing), under different microsites (under plant and under bare ground openings) and sampling depths (0-15 and 15-30 cm) in a shortgrass prairie in Central Mexico.

Source of variation	*df*	Sums of Squares	Mean square	*F*	*P*
Grazing	3	1.21	0.403	1.10	0.35
Microsite	1	0.48	0.485	1.33	0.25
Depth within Microsite	2	20.2	10.1	27.65	< 0.001
Grazing X Microsite	3	1.77	0.6	1.61	0.19
Grazing X Depth within Microsite	6	0.55	0.092	0.25	0.35
Error	64	23.37	0.36		

Table H. ANOVA table of total soil carbon (g C/m^2 soil) along a grazing intensity gradient (no grazing, moderate grazing, heavy grazing, extremely heavy grazing), under different microsites (under plant and under bare ground openings) and sampling depths (0-15 and 15-30 cm) in a shortgrass prairie in Central Mexico.

Source of variation	*df*	Sums of Squares	Mean square	*F*	*P*
Grazing	3	0.002	0.0006	5.222	0.002
Microsite	1	0.001	0.001	8.677	0.004
Depth within Microsite	2	0.005	0.002	21.631	0.0001
Grazing X Microsite	3	0.0003	0.0001	0.730	0.53
Grazing X Depth within Microsite	6	0.0006	0.0001	0.839	0.54
Error	62	0.008	0.0001		

Table I. ANOVA table of total soil nitrogen (g N/m^2 soil) along a grazing intensity gradient (no grazing, moderate grazing, heavy grazing, extremely heavy grazing), under different microsites (under plant and under bare ground openings) and sampling depths (0-15 and 15-30 cm) in a shortgrass prairie in Central Mexico.

Source of variation	*df*	Sums of Squares	Mean square	*F*	*P*
Grazing	3	0.0001	0.00003	5.917	0.001
Microsite	1	0.000	0.000	6.053	0.016
Depth within Microsite	2	0.0003	0.00015	18.272	0.0001
Grazing X Microsite	3	0.000	0.000	0.994	0.401
Grazing X Depth within Microsite	6	0.000	0.000	1.150	0.344
Error	62	0.0005	0.00001		

Table J. ANOVA table of soil C: N ratio along a grazing intensity gradient (no grazing, moderate grazing, heavy grazing, extremely heavy grazing),

under different microsites (under plant and under bare ground openings) and sampling depths (0-15 and 15-30 cm) in a shortgrass prairie in Central Mexico.

Source of variation	*df*	Sums of Squares	Mean square	*F*	*P*
Grazing	3	0.0016	0.0005	1.751	0.16
Microsite	1	0.001	0.001	3.092	0.083
Depth within Microsite	2	0.004	0.002	6.285	0.003
Grazing X Microsite	3	0.0004	0.0001	0.440	0.72
Grazing X Depth within Microsite	6	0.00242	0.0003	1.178	0.32
Error	62	0.012	0.0003		

Table K. ANOVA table of soil extractable soil ammonium (g NH_4/m^2 soil) along a grazing intensity gradient (no grazing, moderate grazing, heavy grazing, extremely heavy grazing), under different microsites (under plant and under bare ground openings) and sampling depths (0-15 and 15-30 cm) in a shortgrass prairie in Central Mexico.

Source of variation	*df*	Sums of Squares	Mean square	*F*	*P*
Grazing	3	9.839	3.28	5.687	0.0017
Microsite	1	6.99	6.99	12.12	0.001
Depth within Microsite	2	25.38	12.7	22.005	0.0001
Grazing X Microsite	3	3.91	1.3	2.264	0.09
Grazing X Depth within Microsite	6	4.08	0.68	1.18	0.33
Error	58	33.44	0.57		