UNIVERSITY OF
GLOUCESTERSHIRE

This is a peer-reviewed, post-print (final draft post-refereeing) version of the following published document, This is a post-peer-review, pre-copyedit version of an article published in Mycorrhiza. The final authenticated version is available online at: http://dx.doi.org/10.1007/s00572-020-00974-8. and is licensed under All Rights Reserved license:

Faghihinia, Maede, Zou, Yi, Bai, Yongfei, Marrs, Rob and Staddon, Philip L. ORCID logoORCID: https://orcid.org/0000-0002-7968-3179 (2020) Seasonal variation in the response of arbuscular mycorrhizal fungi to grazing intensity. Mycorrhiza, 30 (5). pp. 635-646. doi:10.1007/s00572-020-00974-8

Official URL: http://dx.doi.org/10.1007/s00572-020-00974-8
DOI: http://dx.doi.org/10.1007/s00572-020-00974-8
EPrint URI: https://eprints.glos.ac.uk/id/eprint/8559

Disclaimer

The University of Gloucestershire has obtained warranties from all depositors as to their title in the material deposited and as to their right to deposit such material.

The University of Gloucestershire makes no representation or warranties of commercial utility, title, or fitness for a particular purpose or any other warranty, express or implied in respect of any material deposited.

The University of Gloucestershire makes no representation that the use of the materials will not infringe any patent, copyright, trademark or other property or proprietary rights.

The University of Gloucestershire accepts no liability for any infringement of intellectual property rights in any material deposited but will remove such material from public view pending investigation in the event of an allegation of any such infringement.

PLEASE SCROLL DOWN FOR TEXT.

Seasonal variation in the response of arbuscular mycorrhizal fungi to grazing intensity

Maede Faghihinia [1,2], Yi Zou [2], Yongfei Bai [3], Rob Marrs [1], Philip L. Staddon [2,4,5]

[1] School of Environmental Sciences, University of Liverpool, Liverpool, L69 3GP, UK;

[2] Department of Health and Environmental Sciences, Xi'an Jiaotong-Liverpool University, Suzhou, Jiangsu, 215123, China;

[3] State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing, 100093, China;

[4] Countryside and Community Research Institute, University of Gloucestershire, Cheltenham, GL50 4AZ, UK;

[5] School for Agriculture, Food and the Environment, Royal Agricultural University, Cirencester, GL7 6JS, UK.

*Correspondence author. E-mail: yi.zou@xjtlu.edu.cn (Y. Zou)

Abstract

Despite existing evidence of pronounced seasonality in arbuscular mycorrhizal (AM) fungal communities, little is known about the ecology of AM fungi in response to grazing intensity in different seasons. Here, we assessed AM fungal abundance, represented by soil hyphal length density (HLD), mycorrhizal root colonisation intensity (MI) and arbuscule intensity (AI) throughout three seasons (spring, summer, autumn) in a farm-scale field experiment in typical, grazed steppe vegetation in northern China. Seven levels of field-manipulated, grazing intensities had been maintained for over 13 years within two topographies, flat and slope. We also measured soil nutrients and carbon content throughout the growing season to investigate whether seasonal variation in AM fungal abundance was related to seasonal shifts in soil resource availability along the grazing gradient. We further examined the association between AM fungal metrics in the different grazing treatments through the growing season. Our results showed a pronounced seasonal shift in HLD but there was no clear seasonality in MI and AI. HLD was significantly negatively related to grazing intensity over the course of the growing season from spring to autumn. However, MI and AI were related negatively to grazing intensity only in spring. In addition, differential responses of AM fungal abundance to grazing intensity at the two topographical sites were detected. No strong evidence was found for associations between AM fungal abundance and soil resource availability. Moreover, AM fungal internal and external abundance were correlated positively under the different grazing intensities throughout the growing season. Overall, our study suggests that external AM fungal structures in soil were more responsive to seasonal variation and grazing than internal structures in roots. The findings also suggest that early grazing may be detrimental to AM fungal root colonization of newly-emerged plants.

Keywords: spring grazing, topography, soil resource availability, grazing management, external hyphae, seasonal variation

Introduction

Grasslands play a crucial role in global ecosystem functioning and human well-being (O'Mara 2012; Steinfeld et al. 2006). However, many grasslands are currently facing many pressures, of which over-grazing is one of the key drivers reducing grassland productivity and sustainability (Conant 2010; O'Mara 2012). Pervasive excessive grazing has altered above-ground plant communities, soil water and nutrient availability in grasslands (Conant 2010; McSherry and Ritchie 2013). This could translate to changes in the intimately-connected below-ground microbial community including the most common symbionts in grasslands, arbuscular mycorrhizal (AM) fungi (Birgander et al. 2014; Regan et al. 2014).

AM fungi are keystone soil micro-organisms that play a vital role in maintaining grassland ecosystem productivity and stability (Asmelash et al. 2016; Moora and Zobel 2010). Root symbiotic mycorrhizal fungi establish these mutualistic symbioses with a large proportion of terrestrial plant taxa (over 80%) (Brundrett and Tedersoo 2018). This association is fundamentally a nutritional symbiosis: AM fungi rely on photosynthetic carbon received from the plant in exchange for transfer of nutrients, in particular phosphorus (Doubková et al. 2013; Zavalloni et al. 2012). As such, AM fungi can enhance plant grazing-tolerance by improving nutritional status, and thereby improving plant productivity (Moora and Zobel 2010; Walling and Zabinski 2006).

On the other hand, long-term grazing can alter AM fungal function and communities (Ba et al. 2012; Guo et al. 2016). The effect of grazing on mycorrhizal fungi can be explained in part by the carbon limitation hypothesis; clipping and removing plant photosynthetic tissues through long-term grazing may cause a decrease in carbon allocation to roots and mycorrhizal fungi as a result of competition between the plant and AM fungi for limited carbon resources (Gehring and Whitham 2003). Therefore, a negative response of AM fungi to long-term herbivory is expected. However, contradictory results have been reported (Barto and Rillig 2010; Faghihinia et al. 2020), so research is needed to improve our predictions of grazing effects on ubiquitous symbiotic AM fungi.

The extent of the grazing impact on AM fungal function and community structure depends largely on grazing intensity (Ba et al. 2012; Yang et al. 2020) as it has disparate impacts on above- and below-ground productivity and biodiversity (Yan et al. 2013). Whilst overgrazing has destructive and irreversible negative impacts on plant community and soil properties, under-grazing can be just as harmful as overgrazing to grassland biodiversity and functioning through less stimulation of plant growth and loss of grazing-dependent legumes and grasses (Metera et al. 2010). However, under-grazing is not a common practice worldwide at the moment. On the other hand, moderate grazing has been indicated as a benefit to grassland plant and soil conditions through natural fertilization, seed dispersal, making room for annual and bi-annual plant species growth and expansion, and periodic above-ground defoliation which regulate succession in plant communities (Metera et al. 2010). However, the effects of different grazing intensities on AM fungi have not been sufficiently addressed (van der Heyde et al. 2019).

In addition, the impact of grazing intensity on AM fungal structures may not be significant at particular time points throughout the growing season (Faghihinia et al. 2020). Many studies address the response of AM fungi to grazing at a single seasonal time point (Bai et al. 2013; van der Heyde et al. 2017), with few assessing the seasonal shift in AM fungal responses to herbivory, particularly in temperate systems (Cavagnaro et al. 2019; Staddon et al. 2003b; Wang et al. 2014). Cavagnaro et al. (2019) showed that AM fungal root colonization was significantly greater in summer compared with autumn in both sheep-preferred and non-preferred plant species in a steppe grassland, Argentina. Similarly, Staddon et al. (2003b) and Wang et al. (2014) reported greater mycorrhizal root colonization in the summer but lower values during the autumn in temperate ecosystems. The same seasonal pattern has been reported for fungal hyphal length density in soil (Staddon et al. 2003b). Summer peaks in AM fungal abundance are expected due to greater plant mineral nutrient demand, rapid vegetative growth and root production as a result of high light availability for photosynthesis. In addition, plants may need additional mineral nutrients to fund shoot regrowth, thus allocating more carbon to AM fungi during summer when grazing is most intense (Cavagnaro et al. 2019).

Moreover, the temporal dynamics of AM fungal abundance are confounded by ecosystem complexity, often with no consistent pattern being reported. For instance, no seasonal variation in AM fungal root colonization between summer and winter was reported in a Danish coastal, sandy, temperate grassland (Lekberg et al. 2013). However, another study of seasonal dynamics of AM fungal abundance in five Mediterranean plant species found that the percentage mycorrhizal root colonization and density of external hyphae was greater in autumn than in spring (Varela-Cervero et al. 2016). Further research is, therefore, required to unravel the underlying mechanisms of seasonality impact on AM fungal function and community which has prominent implications for grassland ecosystem management and stability.

Pronounced seasonality in AM fungal abundance also is likely to be attributed to soil resource availability which changes seasonally (Hewins et al. 2015; Wang et al. 2014). Hewins et al. (2015) showed that plant nitrogen and phosphorus content increased from late summer to early spring and the observed trend was associated with a decline in mycorrhizal root colonization in a forest herb in northeastern Ohio, USA. Wang et al. (2014) found a positive correlation between temporal changes of AM fungal root colonization and spore richness as well as soil acid phosphatase activity and available phosphorous in temperate grasslands in the north of China. Seasonal shift in below-ground carbon allocation to AM fungal storage lipids (16:1ω5 NLFA) was observed in a coastal grassland in Denmark (Lekberg et al. 2013). In addition, soil resource availability alters along topographic gradients through topographical-induced changes in soil moisture and nutrient availability and solar exposure (Faghihinia et al. 2020; Murray et al. 2010; Schowalter 2016). Topography also affects animal behavior and distribution via greater livestock density and larger loads of dung and urine in low-lying areas compared with areas at higher elevation (Johnson et al. 2016). Topographic gradients of moisture and nutrient availability may interact with grazing to influence AM fungal variables, but the interaction under natural environments has yet to be discerned. How seasonal variation in AM fungal abundance relates to topographic-induced change in soil resource availability also requires further exploration.

An additional consideration is that AM fungi inhabit two different environments, inside host plant roots and in the surrounding soil. Given that these two media differ in terms of AM fungal community structure (Li et al. 2018; Stevens et al. 2020) and are exposed to disparate biotic interactions (Jansa et al. 2013), various responses of AM fungal internal and external structures to environmental disturbance is highly likely. The external hyphal network in soil has a shorter life-span and higher turnover than internal hyphae within the roots (Varma and Hock 2013), thus, external hyphae respond very quickly to seasonal environmental variations such as pulses in soil moisture and nutrient availability (Treseder et al. 2010). Yet, whether any association exists between AM fungal root colonization and external hyphae in response to grazing intensity over growing seasons is unclear. Examining the linkage between AM fungal abundance in roots and soil is fundamental for some of the crucial functional features of plant-fungal symbiosis including plant nutrient acquisition from the soil and host plant productivity (Jansa et al. 2013).

As far as we are aware, no information is available concerning the interaction of grazing intensity, season and topography on AM fungal abundance (represented by soil hyphal length density (HLD), mycorrhizal root colonization intensity (MI) and arbuscule intensity (AI)). We aimed, therefore, to answer the following three questions (1) Is there a temporal change in mycorrhizal abundance in response to grazing intensity?, (2) Does seasonal variation in AM fungal abundance relate to seasonal shifts in soil resource availability along a grazing gradient in two topographic locations? (3) Is there any association between AM fungal abundance in soil and roots throughout the growing season?

Methods

Study Site

This study was conducted at the Sino-German grazing experimental site in the Xilin River Basin of Inner Mongolia, China (116° 42′ E; 43° 38′ N), a steppe grassland ecosystem with a semi-arid, continental climate. We set up our experiment in 14 plots located in two topographic blocks, flat and slope. The “slope block” had a topographical slope of about 8 degrees, and the “flat block” had no noticeable slope. The two topographic blocks, were significantly distinct in terms of soil moisture, soil bulk density and soil nutrient availability as well as plant community structure and species aggregation (Li et al. 2017; Li et al. 2015; Ren et al. 2018). Each experimental plot, encompassing an area of 2 ha, was subjected to one of seven levels of grazing intensity (GI), from 0 to 9 ewes per ha with an interval increase of 1.5 ewes (35 kg live-weight female sheep). Hereafter, we represent GI by the number of grazers per hectare as 0 (no grazing), 1.5 (very light), 3 (light), 4.5 (light-moderate), 6 (moderate), 7.5 (heavy) and 9 (overgrazing). Ewes were put in plots for 90 days during the grazing season from June to September each year. Until we took samples in 2018, the grazing experiment had been run continuously for 13 years. Plant communities of both topographies are dominated by two perennial C3 grasses, *Leymus chinensis* (Trin.) Tzvel. a rhizomatous grass, and *Stipa grandis* P. Smirn. a bunchgrass, which together account for more than 75% of the total above-ground biomass (Li et al. 2017). A detailed description of the climate, vegetation cover, soil characteristics and the design of the experimental site can be found in previously published papers (Schönbach et al. 2011; Wan et al. 2011) and in the supplementary information (SI-1).

Soil sampling

Five evenly-distributed double soil core samples (2 × 20 cm) were collected from each plot over the growing season at three sampling times in 2018 (2 topographical locations × 7 levels of grazing intensity × 3 seasons × 5 samples). In total 210 soil core samples for mycorrhizal measurement and 210 for soil properties analyses were collected. In the study area, the growing season begins in May, peaks in July and ends in September while the grazing season starts from June continuously to the end of September (Wan et al. 2011). We took samples in early–May, when grazing had not been started yet, mid-July, in the middle of the grazing season and late-September, at the end of the grazing season, representing spring, summer and autumn collections, respectively. Soil samples were kept in an ice box with a temperature of around 0 °C until being placed in storage at -20°C. A schematic illustration of the experimental design is presented in Appendix SI-3 Figure S1.

Soil hyphal length density (HLD) measurement

Soil hyphae were extracted from two sub-samples of 5 g soil from each soil core (420 samples in total) in 500 ml of deionized water (dH_2O) following a modified membrane filter technique from Jakobsen et al. (1992) and Boddington et al. (1999). The hyphae of AM fungi were identified based on microscopic features; angular, aseptate, and 1.0–13.4 µm in diameter (Boddington et al. 1999; Shen et al. 2016). The total length of hyphae (mm) was measured for a minimum 60 fields of view for each filter at × 100 magnification. A modified GIM (Gridline Intersect Method) equation based on (Tennant 1975) was used for calculating the total length of hyphae (mm) per gram of soil (m g^{-1}) (Shen et al. 2016) (SI-2).

Mycorrhizal root colonization assessment

Roots, comprising multiple plant species, were collected from five soil cores from each plot. The roots were rinsed carefully with distilled water and a sonicator was used to remove soil particles adhering to the root surface. Roots were cut into pieces *ca*. 1 cm long and then approximately 5 g of fine roots of each sample were cleared in 2% KOH (w/v) at 90°C for 60 min and then rinsed thoroughly on a fine sieve before being acidified in 2% HCl (v/v) for 30 min and stained in 0.05% (w/v) trypan blue: glycerol: lactic acid (1:2:1) for 30 min at 90 °C. Root segments of each sub-sample were rinsed with lactic acid: glycerol: dH2O (1:2:1), selected randomly and mounted on slides in 50% glycerol. Thirty pieces of roots from each root sub-sample were observed under the compound microscope (Nikon eclipse Ci-L) at ×200 and ×400 magnification, and mycorrhizal colonization intensity in the root system (MI%) (Percentage of total segment length colonized) and arbuscule intensity (AI%) (arbuscular abundance in the root system) were assessed according to the five-class system of Trouvelot (1986). Although assessed, mycorrhizal frequency was uniformly high and was not informative (data not shown).

Soil resource availability determination

Fresh soil samples were air-dried and sieved through a 2-mm sieve. Soil organic carbon was determined by the acid-potassium dichromate oxidation method (Walkley and Black 1934). Soil available phosphorus (Olsen-P) was extracted with $NaHCO_3$ and determined by spectrophotometry following (Olsen 1954) and soil available nitrogen was measured by the alkali-hydrolyzed diffusion method according to Bao (2000).

Data analysis

We used a three-way nested design to test the interactive effects of grazing intensity, topography and season on AM fungal measures. The data are nested in the sense that sampling was conducted at two sites with contrasting topography; flat and slope. At each topographic location, samples were collected from seven plots, each with different levels of grazing intensity, and sampling was repeated in three seasons (Appendix SI-3, Figure SI1).

We conducted three analyses on our nested hierarchical data. First, we assessed grazing, topography and season effects and their interaction on AM fungal variables by linear mixed effect models (LMEs). Response variables included (i) soil hyphal length density (ii) mycorrhizal root colonization intensity and (iii) arbuscule intensity. Explanatory variables were grazing intensity with interaction with season, and study plot (nested by topography and grazing intensity) was a random variable. LME models fitted by maximum likelihood were applied separately for each AM fungal response variable. Due to the design of this large scale long-term field experiment, we treated grazing intensity as a continuous variable. We first fitted a model with all terms as well as all their interactions. Then, automated model selection using Akaike's information criterion (AIC) were carried out to find the best-fit model.

Second, we assessed the relationship between AM fungal hyphal length density, mycorrhizal root intensity and arbuscule intensity and soil resource variability including (i) available nitrogen, (ii) organic carbon and (iii) available phosphorus in topographic sites using linear regression models. As the effect of environmental conditions on AM fungal responses might not be independent within our soil cores, but could be homogeneous within each plot, we pooled data from the same plot, and analyzed the relationship between AM fungal measures and the means of environmental variables for plots. According to Crawley (2012) and Zuur et al. (2009) statistical analysis on nested data with hierarchical structure should be carried out on means rather than on individual observations so as to provide a conservative estimate of significance and to reduce the likelihood of Type I errors.

Third, we examined the responses of soil resource variables to different grazing intensities by linear mixed effect models. Linear mixed effect models were applied separately to soil available nitrogen, phosphorus and organic carbon, and study plot (nested by topography and grazing intensity) was a random variable. The best-fit models were then selected based on AIC. Finally, to assess relationships between HLD and the other AM fungal variables, Pearson correlation coefficients (r) among the means per plot were calculated.

All data analyses were conducted with R, version 3.6.2 (R Core Team 2018). Linear mixed effect models were applied using the lme() function from the “nlme” package (Pinheiro et al. 2018). Automated model selections were carried out with the package “MuMIn” using the ‘dredge’ function (Barton 2018) to find the best-fit models and statistical inference. All models were validated by checking the distribution of residuals following Zuur et al. (2009). Visual inspection of residual plots did not reveal any noticeable deviations from normality or homoscedasticity.

Results

Seasonality and grazing intensity effects on AM fungal abundance at two topographies

A strong negative relationship between grazing intensity and soil hyphal length density (m/g) (HLD) was detected in all three seasons. HLD decreased significantly with increasing grazing intensity, and this was evident in all seasons: spring (β=-0.23±0.06, P=0.002), summer (β=-0.27±0.06, P=0.001) and autumn (β=-0.43±0.06, $P < 0.001$) in the flat area, as well as in spring (β=-0.48±0.06, $P < 0.001$), summer (β=-0.45±0.06, $P < 0.001$) and autumn (β=-0.54±0.06, $P < 0.001$) in the slope area (Figure1, Table 1).

HLD did vary during the growing seasons but the trends were different in the two topographic locations. HLD increased in the flat area, but decreased in the slope area over the course of the growing seasons. HLD significantly increased from spring to summer (β=1.70±0.45, $P < 0.001$) and from summer to autumn (β=2.13±0.45, $P < 0.001$) in the flat area. In contrast, HLD decreased from spring to summer (β=-1.89±0.45, $P < 0.001$) with no significant difference between summer and autumn in the slope area (β=-0.03±0.45, P=0.952) (Figure 2). Significantly higher HLDs were observed in the slope site in spring (β=-4.09±0.45, $P < 0.001$) and autumn (β=1.66±0.45, $P < 0.001$) compared with the flat site (Table 1).

There was a negative relationship between mycorrhizal root intensity (MI) and grazing intensity in spring but no such relationship in summer and autumn. As grazing intensity increased, MI decreased marginally in spring (β=-0.91±0.43, P=0.057) in the flat area, and significantly in spring (β=-2.02±0.43, $P < 0.001$) in the slope area (Figure1, Table 1).

MI variation along the growing season differed at the two topographic locations: MI increased in the flat area while it decreased in slope area during the growing seasons (Figure 2). No significant difference was observed between spring and summer (β=-6.43±3.29, P=0.052) but MI significantly increased from summer to autumn (β=9.16±3.29, P=0.006) in the flat area. In contrast, MI significantly decreased from spring to summer (β=-11.21±3.29, P=0.001) and remained unchanged from summer to autumn (β=-1.60±3.29, P=0.627) in the slope area (Figure 2, Table 1). Topography significantly impacted MI with higher abundance in slope site in spring (β=-9.57±3.29, P= 0.004) but not in summer (β=-4.79±3.29, P=0.1473) and autumn (5.97±3.29, P=0.072) (Table 1).

Arbuscule intensity (AI) showed the same pattern as MI to grazing intensity with a negative relationship with grazing intensity in spring in the flat (β=-0.42± 0.15, P=0.017) and slope sites (β=-0.56±0.15, P=0.003) (Figure 1, Table 1). No grazing intensity effect on AI was found in summer and autumn. No seasonal shifts were found in AI in the flat area, while AI significantly decreased from summer to autumn (β=-3.88±1.14, P=0.001) in the slope area (Figure 2, Table 1). AI was significantly higher in the slope site throughout the growing season in spring (β=0.42±0.15, P=0.017), summer (β=-2.62±1.14, P=0.023) and autumn (β=2.48±1.14, P=0.031) compared with the flat site (Table 1).

The interaction of grazing intensity and topography was only significant for HLD but not for MI and AI. Model fitting and selection revealed non-significant effects of three way interactions between grazing intensity, topography and season on HLD, MI and AI (Table 1).

Seasonality and grazing intensity effects on soil resource availability at two topographies

Soil available nitrogen (AN) (mg/kg) was not related to grazing intensity at the two topographic locations but it did change over the growing season in the flat area; AN increased from spring to summer (β=10.28±3.38, P=0.003) and then decreased from summer to autumn (β=-8.21±3.38, P=0.016). AN marginally increased from spring to summer (β=5.99±3.38, P=0.079) while it remained unchanged from summer to autumn in the slope site (Table 1, Figure S3). The flat site exhibited significantly higher availability of soil nitrogen in summer (β=-10.52±3.38, P=0.002) and autumn (β=-7.63±3.38, P=0.025) compared with the slope site (Table 1, Figure S3).

Soil available phosphorus (AP) (mg/kg) was related positively to grazing intensity in spring in the flat area (β=0.31±0.08, P=0.004) and in autumn in the slope area (β=0.43±0.08, P < 0.001) (Table 1, Figure S2). AP did not change along the growing season in the flat area but it significantly, though marginally, decreased from summer to autumn in the slope area (β=-1.32±0.64, P=0.042) (Table 1, Figure S3). There was significantly greater phosphorus availability in summer (β=-1.79±0.64, P=0.006) and autumn (β=-3.43±0.64, P < 0.001) in the flat site compared to the slope site (Table 1, Figure S3).

Soil organic carbon (SOC) (%) was related negatively to grazing in spring (β=-0.06±0.03, P=0.052) and autumn (β=-0.07±0.03, P=0.033) in the flat area and in summer (β=-0.1±0.03, P=0.005) in the slope area (Table 1, Figure S2). Pronounced seasonality was observed for SOC but the pattern differed between the topographic locations. SOC decreased from spring to summer (β=-0.69±0.22, P=0.002) and then increased in autumn (β=0.49±0.22, P=0.027) in the flat area. In contrast, SOC significantly increased from spring to summer (β=0.60±0.22, P=0.007) and decreased from summer to autumn (β=-0.78±0.22, P=0.001) in the slope area (Table 1, Figure S3).

Relationship between AM fungal abundance and soil resource availability

HLD was not related to any measured variables (Table S1, Figure S4 and S5). MI was related negatively to soil available nitrogen in the flat site (β=-0.36±0.15, P=0.029) and AI was significantly negatively related to soil available phosphorus (β=-0.84±0.29, P=0.01) in the flat site (Table S1, Figure S4 and S5).

Relationship between AM fungal abundance in soil and roots

There was a significant positive association between HLD and MI in both the flat (Pearson r =0.49, P= 0.024) and the slope sites (Pearson r =0.61, P=0.003) throughout the growing season (Figure 3). HLD was significantly correlated with AI in flat site (Pearson r =0.54, P=0.011), but no significant association was detected for the slope site (Pearson r =0.37, P=0.103).

Discussion

Climatic seasonality and inter-annual variations in temperature and precipitation are expected to moderate the effects of grazing on plant and soil related factors, and thereafter on below-ground biota including mycorrhizal fungi. Nevertheless, the interaction of seasonality and grazing effects on AM fungal abundance has not been investigated fully, particularly for HLD (Faghihinia et al. 2020). Our findings demonstrated significant negative relationships between HLD and grazing intensity and this trend persisted in all three seasons (Figure 1). The negative response of HLD to grazing has been reported previously in several studies in grassland ecosystems (Ren et al. 2018; van der Heyde et al. 2017; Vowles et al. 2018). Grazing-induced reduction in above-ground vegetation cover and below-ground root biomass

(Hao and He 2019) would reduce the range of plants root types and the range of root exudates (Wilson et al. 2018) which would consequently impact soil microbes including AM fungi (Wang et al. 2014). Given that hyphal extension and germination of AM fungal spores is known to take place preferentially in the presence of roots and root exudates (Smith and Read 2008; Tahat et al. 2010), reduction in HLD with increasing grazing intensity is expected. Noteworthy, however, is the consistent trend in the response of HLD to long-term grazing intensity from early in the season to the end of the growing season supporting the hypothesis that the effects of grazing intensity on external hyphal abundance is moderated by seasonality.

Seasonal dynamics were not pronounced in mycorrhizal root colonization variables. MI and AI were significantly negatively related to grazing intensity only in spring but not in the summer and autumn (Figure 1). One possible explanation is that plants allocate less carbon to below-ground root colonizers in spring due to lack of mature leaf tissues and thus lower total photosynthetic activity (Hewins et al. 2015). Plants generally allocate more carbon to leaf elongation rather than root growth at the early stages of their growth (Waterton and Cleland 2016), suggesting that mycorrhizal root colonization is most likely governed by plant physiological status. Moreover, herbaceous vegetation is susceptible to herbivory during the early stage of the growing season due to small plant sizes, undeveloped physical (e.g., hard shells, thorns or spines) and low chemical defense mechanisms (e.g., producing secondary metabolites such as alkaloids, terpenoids, phenolics) as well as high palatability and nutritional quality (Quintero et al. 2014). This finding suggests that the potential impact of early grazing would not only be detrimental to newly emerged plants, as reported in previous studies (Quintero et al. 2014; Waterton and Cleland 2016), but also to AM fungal root colonizers. This impact on the mycorrhizal symbiosis has large implications for grassland management in term of the timing of grazing within the growing season. These insights can help with management decisions aimed at maintaining sustainable grassland productivity and soil biodiversity.

Clear differential responses of AM fungal abundance to grazing intensity were observed between the two topographic locations. Overall, we observed higher HLD, MI and AI in the slope site compared with the flat site, particularly in the spring (Figure 2). Previous studies in the same site have shown that the two topographies are distinct in terms of soil properties and plant communities. The flat site has a greater plant richness, above-ground biomass, soil nitrogen and phosphorus availability, soil moisture and pH compared with the slope site (Li et al. 2017; Ren et al. 2018; Schönbach et al. 2011; Wan et al. 2011). The slope area is therefore more nutrient-limited than the flat area. Given that the arbuscular mycorrhizal symbiosis involves a carbon and nutrient trade-off between the plant and fungal partners (Hodge et al. 2010), it is likely that plants are more dependent on mycorrhizal fungi for obtaining nutrients in the slope area and allocate more carbon below-ground in the search for additional nutrients (Johnson 2010). Plant demand for nutrients is greater in spring when they are in their rapid vegetative growth stage and leaf elongation takes place. There might not be as high a demand in the flat site at early stage of the growing season when soil mineral nutrients are abundantly available to plants compared with the more nutrient-limited slope area.

Furthermore, previous studies reported greater plant species richness (41 vs. 20 plant species) and above-ground biomass (129.02 vs. 77.06 g m^{-2}) in the flat area compared to the slope area (Li et al. 2017; Wan et al. 2011). The heterogeneity of the plant community has resulted in a higher ecological threshold of community structure and ecosystem functioning to grazing intensity in the flat (3.75 sheep ha^{-1}) compared with the slope site (3 sheep ha^{-1}). As a result, it has been suggested that the plant community composition in the flat site is more resistant and resilient to grazing disturbance than that in the slope site (Li et al. 2017). Accordingly, AM fungi appeared more tolerant to some perturbations associated with grazing intensity in the flat site compared with the slope site because the corresponding plant community is itself more resilient to grazing impacts. This may in part explain the increasing HLD and MI from spring, when no grazing happens, to the end of growing season when grazing intensity is becoming intense. In contrast, reduction in HLD and MI throughout the growing season in the slope site was linked to lower nutrient availability and concomitant lower capability of plant species to respond to grazing pressure and defoliation.

The differential responses of AM fungal abundance at the two topographies can also be explained by differences among plant community composition and grazers' diet preferences. The vegetation at our experimental site is dominated by *L.chinensis* and *S.grandis*. The above-ground biomass and richness of *L. chinensis* is greater than that of *S. grandis* in the flat site (Schönbach et al., 2011; Wan et al., 2011). It has been shown that the above-ground biomass of palatable and highly mycorrhizal *L. chinensis* decreased substantially with increasing grazing intensity, whereas the biomass of relatively unpalatable, less mycorrhizal *S. grandis* remained unchanged along the grazing gradient (Wan et al. 2011). Thus, lower AM fungal abundance in the flat site could have been caused by the strong negative effects of grazing on dominant, palatable *L. chinensis*.

Seasonal differences in AM fungal abundance have been shown to be driven by shifts in relative abundance of soil resource availability (Hewins et al. 2015; Lekberg et al. 2013). However, we did not find similar seasonal trends in AM fungi and soil resources, and the relationships are not particularly strong for these variables. We found a marginally-significant negative relationship between (1) MI and soil available nitrogen, and (2) AI and soil available phosphorus in the flat site. Whether the seasonal shifts in AM fungal abundance are directly associated with soil resource availability cannot be confirmed in this current study and requires further investigation.

We found a strong positive association between hyphal length density in soil and the intensity of root colonization (Figure 3) suggesting that changes in AM fungal internal abundance in roots are positively associated with those of external abundance in soil over the growing season. The positive correlation between HLD and MI in our grazed study sites is not surprising because thin, fragile runner hyphae can be easily disrupted by the activities of large herbivores leading to lower nutrient uptake by the associated plants and lower redistribution of recently fixed carbon through the soil, thereby reduced colonization capacity of the fungi (Gui et al. 2018; van der Heyde et al. 2017). Such a relationship between the various metrics of AM fungal dynamics rarely has been reported in previous studies, particularly in grassland ecosystems. Considering that nutrient uptake and carbon use differ among and within AM fungal structures (Smith and Read 2008) and that AM fungal isolates differ in their rates of colonization and hyphal extension (Hart and Reader 2005), this relationship between AM fungal structures may change across different ecosystems. Studies are needed to unravel underlying mechanisms.

Conclusions

In summary, we showed that the effects of grazing intensity on AM fungal abundance is mediated by both topography and seasonality in this Inner Mongolia grassland. While we acknowledge that true replicates of each individual plot at a given grazing intensity and topography would have added increased robustness to our conclusions, to repeat such a large scale experiment with multiple large plots, in this case a total of 14 plots of 2 hectares each, is extremely expensive and unrealistic. By careful application of appropriate statistical analyses, our results clearly showed that, in the study site, HLD was negatively related to grazing intensity over the course of the growing season and MI and AI were significantly negatively related with grazing intensity only at the early stage of the growing season at both topographic locations.

That seasonal shifts in mycorrhizal abundance were more pronounced in HLD, but not so marked in MI and AI, suggest that external AM fungal structures in soil are more responsive to seasonal variation than internal mycorrhizal structures in roots. This can be explained by the mycorrhizal hyphae in the soil experiencing a much broader range of environmental conditions than those within the relatively stable conditions within plant roots. Indeed, soil HLD, containing a large proportion of AM fungal hyphae with short longevity and high turnover rate (Staddon et al. 2003a) is more susceptible to environmental disturbance compared with AM fungal hyphae inside roots (Varma and Hock 2013). MI and AI were significantly negatively related to grazing intensity only in spring which suggests that mycorrhizal root colonization is driven by plant physiology rather than by sampling time *per se*. Furthermore, our data suggest that early grazing can be detrimental to AM fungal root colonization of newly emerged plants. Overall, early-spring grazing should be avoided in Inner Mongolia grazed steppe to prevent damage of plant growth and thereby their root-associated symbiotic partners. This will lead to maintaining healthy plant communities and soil biota with sustainable function of the grassland ecosystem.

Acknowledgements:

This study was finically supported by XJTLU Research Development Fund (RDF-15-02-13). We appreciate the kind cooperation of the Inner Mongolia Grassland Ecosystem Research Station of the Chinese Academy of Sciences for kindly providing research station facilities and a plant dataset. The authors acknowledge the kind help of Lijia Dong, Zirui Hu, Yuwei He, Ye Wang, Chuanyu Zhou, Hanyu Jian, Fanjing Liu and Shasha Zou for collecting data in the lab.

Supplementary data

References

Asmelash F, Bekele T, Birhane E (2016) The Potential Role of Arbuscular Mycorrhizal Fungi in the Restoration of Degraded Lands Front Microbiol 7:1095 doi:10.3389/fmicb.2016.01095

Ba L, Ning J, Wang D, Facelli E, Facelli JM, Yang Y, Zhang L (2012) The relationship between the diversity of arbuscular mycorrhizal fungi and grazing in a meadow steppe Plant and Soil 352:143-156

Bai G, Bao Y, Du G, Qi Y (2013) Arbuscular mycorrhizal fungi associated with vegetation and soil parameters under rest grazing management in a desert steppe ecosystem Mycorrhiza 23:289-301

Bao S-D (2000) Analysis of soil and agricultural chemistry Analysis of organic matter contents in soil:30-34

Barto EK, Rillig MC (2010) Does herbivory really suppress mycorrhiza? A meta - analysis Journal of Ecology 98:745-753

Barton K (2018) Package 'MuMIn'. R package version 1.40. 4.

Birgander J, Rousk J, Olsson PA (2014) Comparison of fertility and seasonal effects on grassland microbial communities Soil Biology and Biochemistry 76:80-89

Boddington CL, Bassett EE, Jakobsen I, Dodd JC (1999) Comparison of techniques for the extraction and quantification of extra-radical mycelium of arbuscular mycorrhizal fungi in soils Soil Biology and Biochemistry 31:479-482

Brundrett MC, Tedersoo L (2018) Evolutionary history of mycorrhizal symbioses and global host plant diversity New Phytol 220:1108-1115 doi:10.1111/nph.14976

Cavagnaro RA, Pero E, Dudinszky N, Golluscio RA, Grimoldi AA (2019) Under pressure from above: Overgrazing decreases mycorrhizal colonization of both preferred and unpreferred grasses in the Patagonian steppe Fungal Ecology 40:92-97 doi:https://doi.org/10.1016/j.funeco.2018.09.003

Conant RT (2010) Challenges and Opportunities for Carbon Sequestration in Grassland Systems: A Technical Report on Grassland Management and Climate Change Mitigation Integrated Crop Management, Rome: FAO 9

Crawley MJ (2012) The R book. John Wiley & Sons,

Doubková P, Vlasáková E, Sudová R (2013) Arbuscular mycorrhizal symbiosis alleviates drought stress imposed on Knautia arvensis plants in serpentine soil Plant and soil 370:149-161

Faghihinia M, Zou Y, Chen Z, Bai Y, Li W, Marrs R, Staddon PL (2020) Environmental drivers of grazing effects on arbuscular mycorrhizal fungi in grasslands Applied Soil Ecology 153:103591 doi:https://doi.org/10.1016/j.apsoil.2020.103591

Gehring CA, Whitham TG (2003) Mycorrhizae-Herbivore Interactions: Population and Community Consequences. In: van der Heijden MGA, Sanders IR (eds) Mycorrhizal Ecology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 295-320. doi:10.1007/978-3-540-38364-2_12

Gui W et al. (2018) Plant functional group influences arbuscular mycorrhizal fungal abundance and hyphal contribution to soil CO2 efflux in temperate grasslands Plant and Soil 432:157-170 doi:10.1007/s11104-018-3789-0

Guo Y, Du Q, Li G, Ni Y, Zhang Z, Ren W, Hou X (2016) Soil phosphorus fractions and arbuscular mycorrhizal fungi diversity following long-term grazing exclusion on semi-arid steppes in Inner Mongolia Geoderma 269:79-90

Hao Y, He Z (2019) Effects of grazing patterns on grassland biomass and soil environments in China: A meta-analysis PloS one 14:e0215223

Hart MM, Reader RJ (2005) The role of the external mycelium in early colonization for three arbuscular mycorrhizal fungal species with different colonization strategies Pedobiologia 49:269-279 doi:https://doi.org/10.1016/j.pedobi.2004.12.001

Hewins CR, Carrino-Kyker SR, Burke DJ (2015) Seasonal variation in mycorrhizal fungi colonizing roots of Allium tricoccum (wild leek) in a mature mixed hardwood forest Mycorrhiza 25:469-483

Hodge A, Helgason T, Fitter A (2010) Nutritional ecology of arbuscular mycorrhizal fungi Fungal ecology 3:267-273

Jakobsen I, Abbott L, Robson A (1992) External hyphae of vesicular ‐ arbuscular mycorrhizal fungi associated with Trifolium subterraneum L New Phytologist 120:371-380

Jansa J, Bukovská P, Gryndler M (2013) Mycorrhizal hyphae as ecological niche for highly specialized hypersymbionts – or just soil free-riders? Frontiers in Plant Science 4 doi:10.3389/fpls.2013.00134

Johnson NC (2010) Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales New Phytologist 185:631-647

Johnson NC, Gehring C, Jansa J (2016) Mycorrhizal mediation of soil: Fertility, structure, and carbon storage. Elsevier,

Lekberg Y, Rosendahl S, Michelsen A, Olsson PA (2013) Seasonal carbon allocation to arbuscular mycorrhizal fungi assessed by microscopic examination, stable isotope probing and fatty acid analysis Plant and soil 368:547-555

Li S, Shakoor A, Wubet T, Zhang N, Liang Y, Ma K (2018) Fine-scale variations of fungal community in a heterogeneous grassland in Inner Mongolia: Effects of the plant community and edaphic parameters Soil Biology and Biochemistry 122:104-110

Li W, Xu F, Zheng S, Taube F, Bai Y (2017) Patterns and thresholds of grazing‐induced changes in community structure and ecosystem functioning: Species‐level responses and the critical role of species traits Journal of Applied Ecology 54:963-975

Li W, Zhan S, Lan Z, Wu XB, Bai Y (2015) Scale-dependent patterns and mechanisms of grazing-induced biodiversity loss: evidence from a field manipulation experiment in semiarid steppe Landscape ecology 30:1751-1765

McSherry ME, Ritchie ME (2013) Effects of grazing on grassland soil carbon: a global review Glob Chang Biol 19:1347-1357 doi:10.1111/gcb.12144

Metera E, Sakowski T, Słoniewski K, Romanowicz B (2010) Grazing as a tool to maintain biodiversity of grassland-a review Animal Science Papers and Reports 28:315-334

Moora M, Zobel M (2010) Arbuscular mycorrhizae and plant-plant interactions Positive plant interactions and community dynamics:79-98

Murray TR, Frank DA, Gehring CA (2010) Ungulate and topographic control of arbuscular mycorrhizal fungal spore community composition in a temperate grassland Ecology 91:815-827

O'Mara FP (2012) The role of grasslands in food security and climate change Ann Bot 110:1263-1270 doi:10.1093/aob/mcs209

Olsen SR (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. vol 939. US Department of Agriculture,

Pinheiro J, Bates D, DebRoy S, Sarkar D, Team RC (2018) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-137. https://CRAN.R-project.org/package=nlme

Quintero C, Lampert EC, Bowers MD (2014) Time is of the essence: direct and indirect effects of plant ontogenetic trajectories on higher trophic levels Ecology 95:2589-2602

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.

Regan KM et al. (2014) Seasonal controls on grassland microbial biogeography: are they governed by plants, abiotic properties or both? Soil Biology and Biochemistry 71:21-30

Ren H et al. (2018) Long-term effects of grazing and topography on extra-radical hyphae of arbuscular mycorrhizal fungi in semi-arid grasslands Mycorrhiza 28:117-127 doi:10.1007/s00572-017-0812-x

Schönbach P et al. (2011) Grassland responses to grazing: effects of grazing intensity and management system in an Inner Mongolian steppe ecosystem Plant and Soil 340:103-115

Schowalter TD (2016) Insect ecology: an ecosystem approach. Academic Press,

Shen Q, Kirschbaum MU, Hedley MJ, Arbestain MC (2016) Testing an alternative method for estimating the length of fungal hyphae using photomicrography and image processing PloS one 11:e0157017

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic press, sandiego. USA,

Staddon PL, Ramsey CB, Ostle N, Ineson P, Fitter AH (2003a) Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of 14C Science 300:1138-1140

Staddon PL, Thompson K, Jakobsen I, Grime JP, Askew AP, Fitter AH (2003b) Mycorrhizal fungal abundance is affected by long‐term climatic manipulations in the field Global Change Biology 9:186-194

Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M, De Haan C (2006) Livestock's long shadow FAO, Rome 2006

Stevens BM, Propster JR, Öpik M, Wilson GW, Alloway SL, Mayemba E, Johnson NC (2020) Arbuscular mycorrhizal fungi in roots and soil respond differently to biotic and abiotic factors in the Serengeti Mycorrhiza:1-17

Tahat M, Sijam K, Othman R (2010) Spores Germination and Ralstonia solanacearum Growth in vitro International Journal of Plant Pathology 1:1-12

Tennant D (1975) A test of a modified line intersect method of estimating root length The Journal of Ecology:995-1001

Treseder KK, Schimel JP, Garcia MO, Whiteside MD (2010) Slow turnover and production of fungal hyphae during a Californian dry season Soil Biology and Biochemistry 42:1657-1660 doi:https://doi.org/10.1016/j.soilbio.2010.06.005

Trouvelot A (1986) Mesure du taux de mycorhization VA d'un systeme radiculaire. Recherche de methodes d'estimation ayant une significantion fonctionnelle Mycorrhizae: physiology and genetics:217-221

van der Heyde M, Abbott LK, Gehring C, Kokkoris V, Hart MM (2019) Reconciling disparate responses to grazing in the arbuscular mycorrhizal symbiosis Rhizosphere:100167

van der Heyde M, Bennett JA, Pither J, Hart M (2017) Longterm effects of grazing on arbuscular mycorrhizal fungi Agriculture, Ecosystems & Environment 243:27-33

Varela-Cervero S, López-García Á, Barea JM, Azcón-Aguilar C (2016) Spring to autumn changes in the arbuscular mycorrhizal fungal community composition in the different propagule types associated to a Mediterranean shrubland Plant and soil 408:107-120

Varma A, Hock B (2013) Mycorrhiza: structure, function, molecular biology and biotechnology. Springer Science & Business Media,

Vowles T, Lindwall F, Ekblad A, Bahram M, Furneaux BR, Ryberg M, Björk RG (2018) Complex effects of mammalian grazing on extramatrical mycelial biomass in the Scandes forest ‐ tundra ecotone Ecology and Evolution 8:1019-1030

Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method Soil science 37:29-38

Walling SZ, Zabinski CA (2006) Defoliation effects on arbuscular mycorrhizae and plant growth of two native bunchgrasses and an invasive forb Applied Soil Ecology 32:111-117

Wan H, Bai Y, Schönbach P, Gierus M, Taube F (2011) Effects of grazing management system on plant community structure and functioning in a semiarid steppe: scaling from species to community Plant and Soil 340:215-226

Wang Q, Bao Y, Liu X, Du G (2014) Spatio-temporal dynamics of arbuscular mycorrhizal fungi associated with glomalin-related soil protein and soil enzymes in different managed semiarid steppes Mycorrhiza 24:525-538 doi:10.1007/s00572-014-0572-9

Waterton J, Cleland EE (2016) Trade ‐ off between early emergence and herbivore susceptibility mediates exotic success in an experimental California plant community Ecology and evolution 6:8942-8953

Wilson CH, Strickland MS, Hutchings JA, Bianchi TS, Flory SL (2018) Grazing enhances belowground carbon allocation, microbial biomass, and soil carbon in a subtropical grassland Global change biology 24:2997-3009

Yan L, Zhou G, Zhang F (2013) Effects of different grazing intensities on grassland production in China: a meta-analysis PLoS One 8:e81466 doi:10.1371/journal.pone.0081466

Yang X, Chen J, Shen Y, Dong F, Chen J (2020) Global negative effects of livestock grazing on arbuscular mycorrhizas: A meta-analysis Science of The Total Environment 708:134553 doi:https://doi.org/10.1016/j.scitotenv.2019.134553

Zavalloni C et al. (2012) Exposure to warming and CO2 enrichment promotes greater above-ground biomass, nitrogen, phosphorus and arbuscular mycorrhizal colonization in newly established grasslands Plant and soil 359:121-136

Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer Science & Business Media,

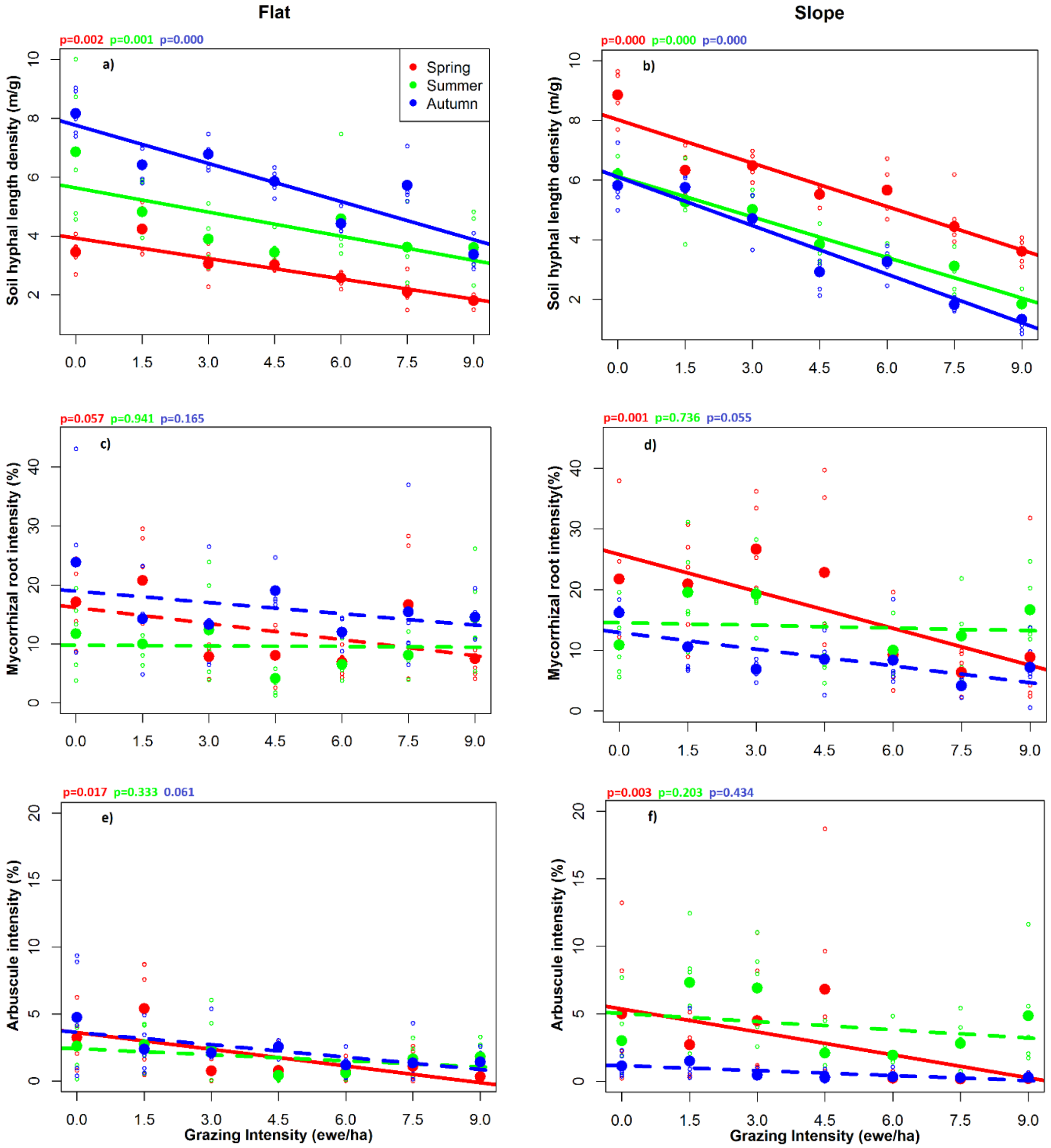

Figure 1. Soil hyphal length density in flat (a) and slope sites (b), mycorrhizal root intensity in flat (c) and slope sites (d) and arbuscule intensity in flat (e) and slope sites (f) in response to grazing intensities at three seasonal time points. Solid and hollow circles indicate mean and individual observations at each grazing intensity, respectively. Lines represent regressions from linear mixed-effects models, with solid and dashed lines indicating significant (P<0.05) and non-significant (P>0.05) relationships, respectively.

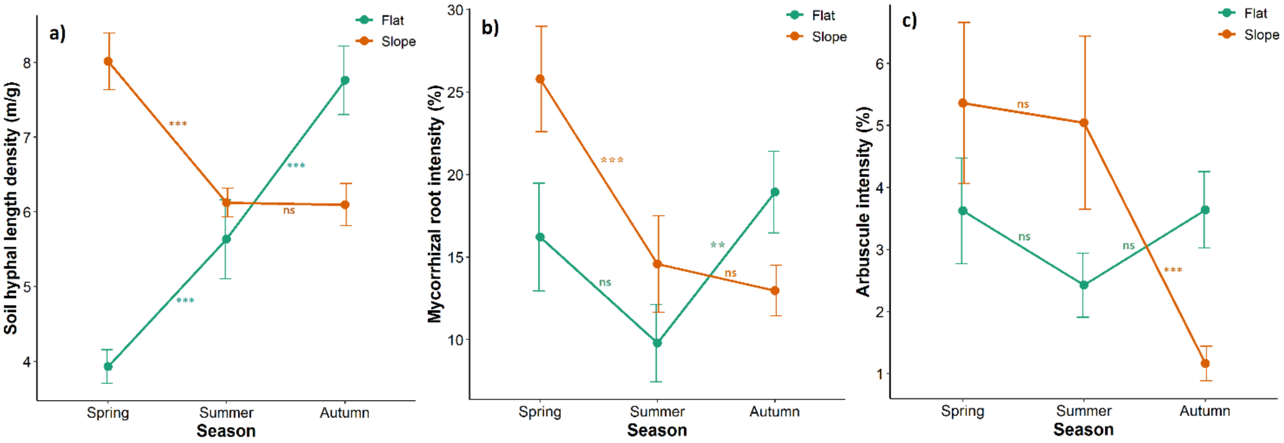

Figure 2. Estimated coefficients from general linear mixed models applied to (a) soil hyphal length density, (b) mycorrhizal root intensity and (c) arbuscule intensity in response to season in the flat and slope sites. Asterisks represent significance levels obtained from the model results, p < .001, "***", p < .01, "**", p < .05, "*", NS: non-significant.

Variable	Model No	GI	Tp Slope	Season Summer	Season Autumn	GI: Tp Slope	GI: Season Summer	GI: Season Autumn	Tp Slope: Season Summer	Tp Slope: Season Autumn	GI: Tp Slope: Season Summer	GI: Tp Slope: Season Autumn	AICc
Soil hyphal length density (m/g)	1	-0.23±0.06 **(0.005)**	4.09±0.51 **(0.000)**	1.70±0.45 **(0.004)**	3.83± 0.36 **(0.000)**	-0.25±0.08 **(0.003)**	-0.04±0.08 (0.613)	-0.20±0.08 **(0.003)**	-3.59±0.75 **(0.000)**	-5.749±0.54 **(0.000)**	0.07± 0.14 (0.594)	0.14± 0.09 (0.257)	555.93
	2	-0.27±0.05 **(0.000)**	3.76±0.31 **(0.000)**	1.54±0.33 **(0.000)**	3.51±0.29 **(0.000)**	-0.18±0.04 **(0.000)**	-0.01±0.05 (0.906)	-0.13±0.05 **(0.007)**	-3.26±0.41 **(0.000)**	-5.11±0.29 **(0.000)**	-	-	553.75
Mcorrhizal root intensity	1	-0.91±0.43 (0.057)	9.56±3.29 **(0.004)**	-6.43±3.76 (0.052)	2.72±3.17 (0.391)	-1.12±0.61 (0.069)	0.87±0.61 (0.225)	0.27±0.59 (0.703)	-4.77±4.83 (0.324)	-15.53±5.37 **(0.001)**	0.99±0.89 (0.264)	0.84±0.83 (0.406)	1454.76
	2	-1.46±0.31 **(0.001)**	4.55±1.85 **(0.015)**	-8.68±2.63 **(0.001)**	0.84±2.56 (0.743)	-	1.37±0.41 **(0.001)**	0.68±0.41 (0.098)	-0.28±2.75 (0.920)	-11.77±2.48 **(0.000)**	-	-	1451.67
Arbuscule intensity	1	-0.42±0.15 **(0.028)**	1.74±1.14 (0.130)	-1.19±1.14 (0.359)	0.02±1.13 (0.989)	-0.15±0.21 (0.482)	0.27±0.21 (0.210)	0.11±0.21 (0.609)	0.88±1.63 (0.588)	-4.212±1.60 **(0.009)**	0.09±0.30 (0.745)	0.34±0.33 (0.257)	1019.72
	2	-0.49± 0.11 **(0.001)**	1.06± 0.63 (0.092)	-1.42± 0.91 (0.122)	-0.74± 0.91 (0.416)	-	0.31± 0.15 **(0.034)**	0.28± 0.15 (0.063)	1.32± 0.90 (0.143)	-2.70± 0.88 **(0.003)**	-	-	1014.33
Available phosphorus (mg/kg)	1	0.31± 0.08 **(0.004)**	-0.12± 0.64 (0.854)	0.63± 0.64 (0.327)	0.95± 0.55 (0.085)	-0.36± 0.12 **(0.003)**	-0.25± 0.12 **(0.037)**	-0.23± 0.10 **(0.026)**	-1.67± 1.03 (0.105)	-3.31± 0.77 **(0.000)**	0.28± 0.19 (0.140)	0.71± 0.14 **(0.000)**	725.43
Available nitrogen (mg/kg)	1	0.92±0.44 (0.059)	-6.23±3.38 (0.067)	10.28±3.38 **(0.003)**	2.06±2.82 (0.465)	-0.60±0.63 (0.341)	-1.48±0.63 **(0.019)**	-1.65±0.52 **(0.002)**	-4.29±5.46 (0.433)	-1.40±3.99 (0.726)	1.16±1.01 (0.253)	1.35±0.74 **(0.070)**	1415.57
	2	0.63±0.31 (0.068)	-8.91±1.88 **(0.000)**	7.67±2.51 **(0.003)**	-0.96±2.29 (0.676)	-	-0.90±0.37 **(0.016)**	-0.98±0.37 **(0.009)**	0.92±3.02 (0.761)	4.66±2.23 **(0.038)**	-	-	1413.36
Organic carbon (%)	1	-0.06± 0.03 **(0.052)**	-0.57± 0.22 **(0.011)**	-0.69± 0.22 **(0.002)**	-0.20± 0.14 (0.161)	0.049± 0.04 (0.236)	0.07± 0.04 (0.108)	-0.01± 0.03 (0.783)	1.3± 0.39 **(0.001)**	0.03± 0.20 (0.904)	-0.15± 0.07 **(0.038)**	0.04± 0.04 (0.279)	175.81
	2	-0.04±0.02 (0.094)	-0.35±0.14 **(0.012)**	-0.40±0.14 **(0.004)**	-0.24±0.08 **(0.004)**	-	-	-	0.62±0.25 **(0.016)**	0.21±0.11 (0.066)	-	-	173.25

Table-1 Linear mixed-effects models of the effects of grazing intensity (GI), topography (Tp) and season on AM fungi and soil variables. Mixed-effects models were applied to nested (multi levels) data. The data are nested in the sense that samples were taken from two topographic locations (flat and slope) and in each topography from seven sites (called "plot" hereafter) representing seven levels of grazing intensity. In each plot, sampling was conducted at three seasons (spring, summer and autumn). The full model (model No. 1) and the best model selected according to Akaike's information criteria (AIC) (model No. 2) are presented. Topography flat and season spring are reference groups in data presented here. Bold numbers represent the significant relationships ($p<0.05$).

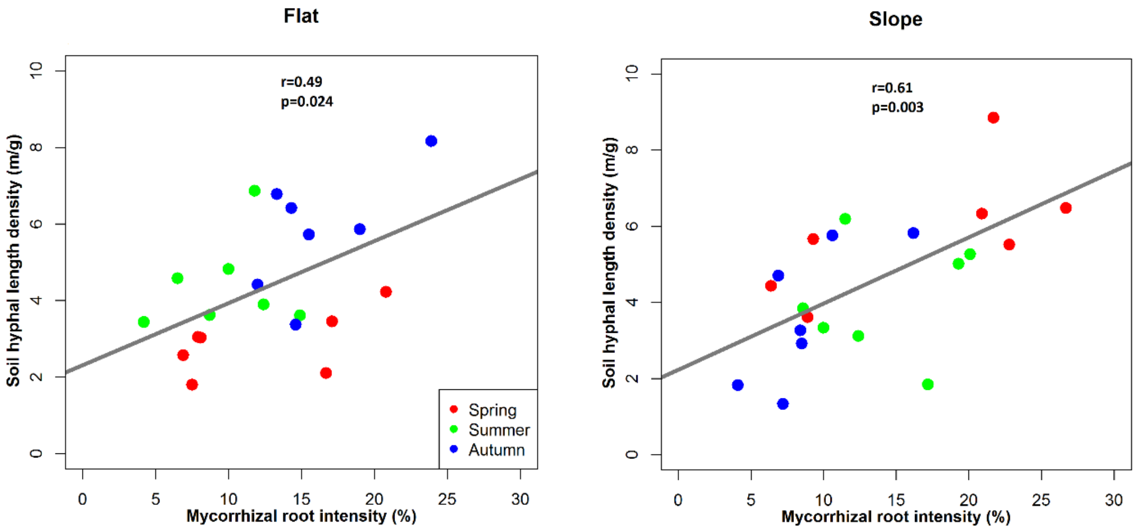

Figure 3. Pearson correlation coefficients (r) between soil hyphal length density and mycorrhizal root colonization.