Fungal Ecology 33 (2018) 80–91

Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco

Promotion of *Lotus tenuis* in the Flooding Pampa (Argentina) increases the soil fungal diversity

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ARTICLE INFO

Article history:
Received 21 May 2017
Received in revised form
21 November 2017
Accepted 8 January 2018

Corresponding Editor: Gareth Wyn Griffith

Keywords:
Lotus tenuis
Fusarium
Salado River Basin
Forage herbicide promotion
Glyphosate

ABSTRACT

Forage promotion is an increasingly used agricultural practice that requires herbicide application to remove competing plant species. This work examined the effect of the forage legume *Lotus tenuis* on the fungal community composition at three sites of the Flooding Pampa, Argentina. Replicate paddocks, managed either by herbicide-mediated promotion of *L. tenuis* or unmanaged (dominated by grasses) were compared using 454 pyrosequencing, targeting the fungal internal transcribed spacer (ITS) gene.

Our results suggest that fungal diversity in the studied area varied according to the site and the land use (natural grasses vs. *L. tenuis* promotion). Several factors [pH, Ca^{2+}, P, and HCO_3^-] were the main soil environmental drivers of distribution of fungal classes. Herbicide-mediated *L. tenuis* promotion led to increased fungal diversity, with dominance of *Fusarium* species.

1. Introduction

Pasture promotion is defined as an agricultural practice that uses herbicides to remove competition from weeds and resident species, so that the pasture of interest grows and fully develops. Pasture improvement using promotion is a common practice worldwide (Kehui and Kun, 2007; Lamoureaux and Bourdôt, 2007), and has increased during the last 25 yr (McCormick et al., 2014). A major focus of scientific concern is the use of herbicides in agricultural management, which could have an impact on soil bacterial and fungal communities, as well as on soil chemistry (Jacobsen and Hjelmsø, 2014; Turrini et al., 2015).

The soil biota is directly involved in organic matter decomposition, in carbon, nitrogen, and phosphorus turnover and translocation, also performing important functions on soil structure (Frey et al., 2000, 2003; Ritz and Young, 2004; Kibblewhite et al., 2008; Rachid et al., 2015). Besides, fungi play ecological roles as decomposers, pathogens, and mutualistic symbionts (Newsham et al., 1995; Kibblewhite et al., 2008). For these reasons, it is important to assess the suite of members that best represent the fungal community before and after herbicide-mediated promotion, and to detect those species that reveal a change due to the land use.

The "core microbiome" is defined as the suite of members shared among microbial consortia from similar habitats (Shade and Handelsman, 2012), and it is represented by organisms present in at least 90% of the individual samples (Kellogg et al., 2017; Pose-Juan et al., 2017). Identifying a soil "core mycobiome" is crucial to appreciate the established consortium of species, which are not usually subjected to change; hence, they might possibly be resistant/resilient to disturbances and a varying soil context (Orgiazzi et al., 2013).

Lotus tenuis is a naturalized species with high forage value

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https://doi.org/10.1016/j.funeco.2018.01.001
1754-5048/

reaching its maximum biomass in summer (Colabelli and Miñón, 1994). Its promotion has been conceived as alternative to overcome forage deficiency in this season (Nieva et al., 2016). Although *L. tenuis* has high capacity for natural reseeding (Entio, 2011) and for withstanding water deficit (García and Mendoza, 2014), it is a poor competitor for nutrients during the first stages of seedling establishment (Llobet et al., 2012). Therefore, the application of post-emergence herbicides to remove broad leaf weeds in winter is expected to improve *L. tenuis* establishment, and increase its dominance.

The Flooding Pampa (Buenos Aires Province, Argentina) is a vast region (90,000 km^2; Escaray et al., 2012) characterized by restrictive soil conditions, which limits the land use for cattle production. Under such restrictive soil conditions, *L. tenuis* is the only legume that grows and fully develops here. In a previous study carried out in this region, it was shown that 5–6 yr of *L. tenuis* promotion with herbicides caused no significant effect on the bacterial community structure (expressed as alpha and beta diversity indices), although differences among sampling sites were detected (Nieva et al., 2016). However, no information is available about the effect of *L. tenuis* promotion on the structure of the fungal community in the Flooding Pampa, nor on the possible correlations, between fungal taxa or communities and soil chemical variables.

High-throughput pyrosequencing has become a powerful method to examine highly diverse fungal communities in different soils (Wang et al., 2015; Zhou et al., 2016).

In the present work, we aim at gaining insight on the effect of *L. tenuis* promotion on fungal diversity, core mycobiome, and indicator species, regardless of whether the effect is due to the dominance of the legume induced by the herbicides, or to the herbicides themselves. We used 454 pyrosequencing, targeting the ITS1 and ITS2 regions of the rRNA operon. Information about the composition of fungal populations could help evaluation of the sustainability of this agricultural management, which is becoming a common agronomical practice in the Flooding Pampa in Argentina.

Our objectives in this study were to: (1) examine the effect of 6–7 yr of continuous *L. tenuis* promotion on fungal taxonomic composition and diversity; (2) determine the core mycobiome under two land uses; and (3) detect relationships between specific fungal taxa and physicochemical soil variables. As well as determining the occurrence of particular fungal species (Lauber et al., 2008; Rousk et al., 2010), we aimed to evaluate the relationship between fungal taxa and major soil properties. To achieve these objectives, fungal diversity, taxonomic composition, and soil properties were analyzed and compared among three sites, each one having two different land uses: *L. tenuis* promotion with herbicides and natural grassland.

2. Materials and methods

2.1. Study sites characteristics

Soil samples were taken from three representative sites of the North of the Flooding Pampa, in the areas of Chascomús (Buenos Aires, Argentina: Manantiales 1 [(M) S 35°45′01″, W 58°02′22″]; La Bellaca [(L) S 35°35′55″, W 57°56′44″]; Manantiales 2 [(T) S 35°45′39″, W 58°03′38″]. Manantiales 1 (M) is 20 km apart from La Bellaca (L), whereas the two sites in the Manantiales area (M and T) are at a 1 km of distance one from each other. These sites are covered by different kinds of sodic soils (i.e. Solonetzes) with prevalence of Typic Natraquolls from the General Guido Series, which covers about 200,000 ha in the region (Lavado and Taboada, 1988). The soil has a loamy and organic matter-rich A horizon (0–0.14 m), overlying a clayey and impervious natric horizon (0.14–0.68 m) (INTA, 1980, 2002). All paddocks were grazed by cattle at similar stocking rates (about 1 cow ha^{-1} yr^{-1}). Topography was similar among sites, with plain or concave relief.

2.2. Herbicide treatment

L. tenuis promotion was achieved by the application of glyphosate (*N*-(phosphonomethyl) glycine; 3.5 l/ha), followed by two applications of 2,4 DB (4-(2,4-dichlorophenoxy) butyric acid, 1 l/ha), and a single dose of Quizalofop-p-ethyl (Ethyl (R)-2-[4-(6-chloro-2-quinoxalyloxy) phenoxy]propionate; 1.2 l/ha), in 6 (M and T) or 7 (L) annual cycles from June to August. Control paddocks received no management or agrochemicals since 1970. No fertilizer was applied in any case. Herbicides treatment led to *L. tenuis* monoculture, whereas in paddocks where no herbicide was ever applied, plant community was represented by native species and naturalized forages such as *Festuca arundinacea, Lolium multiflorum, Distichlis spicata, Ambrosia tenuifolia, Esporobolus indicus, Paspalum vaginatum,* and *L. tenuis.*

2.3. Experimental design

Sampling followed a randomized block design with two factors: site, with 3 levels, and land use, with 2 levels, natural cover (nat) or *L. tenuis* promotion with herbicides (lot). There were three replicates of each site per land use combination. Each replicate consisted of a mixture of 20 pooled and thoroughly mixed soil subsamples (Fig. 1). Soil subsamples were collected in November 2015 from neighboring paddocks (100 × 50 m, approximately) subjected to one of the types of land use. Soil cores (subsamples, 20 per paddock) were taken with a borer (20 cm length x 7 cm diameter) every 10 m. Replicates were identified by the initial of each site followed by the land use (e.g.: Mnat; Mlot, Tlot, etc.). Soil replicates were introduced into individual plastic bags and immediately transported on ice to the laboratory, where they were sieved through a 2 mm mesh and used for nucleic acid extraction.

2.4. Soil DNA extraction, PCR, and pyrosequencing

Ground soil was thoroughly mixed before subsampling for DNA extraction. Total DNA for amplicon sequence libraries was obtained from a 0.25 g aliquot of each soil replicate using PowerSoil DNA isolation kit (MO BIO Laboratories, Inc, CA, USA) following manufacturer's instructions. DNA quality and concentration in each sample was evaluated by spectrophotometer (Synergy™ H1, BIOTEK) and 1.5% agarose gel electrophoresis. Genomic DNAs for PCR and pyrosequencing procedures were stored at −20 °C. Amplicon libraries were prepared on the base of the ITS1- ITS2 region of the 18s rDNA gene, considered as the universal DNA barcode marker for Fungi. (Schoch et al., 2012). Universal primers ITS1F: 5′ CTTGGTCATTTAGAGGAAGTAA 3′(Gardes and Bruns, 1993) and ITS4: 5′TCCTCCGCTTATTGATATGC 3′(White et al., 1990) were used and a re-amplification was performed to include the Roche 454 sequencing A and B adaptors, and a nucleotide "multiple identifier" (MID) to sort samples (Supplementary Table 1). The PCR mixture (final volume 25 μl) contained 2.5 μl FastStart High Fidelity 10 × reaction Buffer (Roche Applied Science, Mennheim, Germany), 20 ng of template DNA, 0.4 mM of each primer, and 1.25 U FastStart High Fidelity Enzyme Blend (Roche Applied Science) and 0.2 mM dNTPs. The PCR conditions were 95 °C, 5 min for initial denaturalization, followed by 95 °C 45 s, 57 °C 45 s, 72 °C 60 s in 30 cycles, and a final elongation step at 72 °C 4 min. Two negative control reactions, containing all components excepting the template were performed. Libraries were purified using Agencourt AMPure XP.