Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

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

Soil disturbance changes arbuscular mycorrhizal fungi richness and composition in a fescue grassland in Alberta Canada

Holly J. Stover[a,*], M. Anne Naeth[a], Katja Boldt-Burisch[b,c]

[a] Department of Renewable Resources, University of Alberta, Edmonton, Alberta T6G 2H1, Canada
[b] GFZ German Research Centre for Geosciences, Section 5.3 Geomicrobiology, Telegrafenberg, 14473 Potsdam, Germany
[c] Chair of Soil Protection and Recultivation, Brandenburg University of Technology Cottbus-Senftenberg, 03046 Cottbus, Germany

ARTICLE INFO

Keywords:
Arbuscular mycorrhizal fungi
Soil disturbance
Non-native plant invasion
Native grassland
454 pyrosequencing

ABSTRACT

Native grasslands are endangered by non-native plant invasion worldwide, including foothills fescue grasslands in North America. Large populations of non-native plant species have established in these disturbed fescue grasslands, forming dense monocultures and spreading into undisturbed areas. Soil disturbance and plant invasion can alter the arbuscular mycorrhizal fungi (AMF) community, an important symbiotic partner of most land plants, which could negatively affect native plant reestablishment. The objective of this study was to assess whether AMF communities on a fescue grassland shifted in response to disturbances by landfill storage and gravel quarrying and with invasion of non-native plant species relative to undisturbed grassland.

Soil and root-AMF samples were procured from disturbed and undisturbed areas at three sites. Plant canopy cover and species richness were assessed. Soils were analyzed for pH; electrical conductivity; total nitrogen, carbon and phosphorus; and available nutrients. For relative AMF taxa abundance assessment, NS31 and AMF specific primer AML2 were used to amplify a central fragment of the V3 and V4 region of the 18S rRNA gene. AMF were characterized using 454 pyrosequencing and multiplexed barcoded samples amplified from genomic DNA isolated from roots.

There were 92 AMF, including 15 potentially novel taxa detected. AMF communities in disturbed and undisturbed sampling locations were distinct except for one site, and indicator AMF virtual taxa (VT) for undisturbed grassland and disturbed sites were identified. AMF richness was higher in undisturbed (72 VT) than disturbed (64 VT) sites and AMF richness was positively correlated with plant species richness, diversity and native plant cover, and negatively correlated with non-native plant cover. There were 43 AMF VT on undisturbed and disturbed sites, 62% with higher relative abundance on disturbed sites. Site disturbance shifted AMF communities relative to undisturbed native fescue grassland; thus restoration success with native plants might be highly dependent on reintroducing native AMF.

1. Introduction

Temperate natural grasslands, known as the prairie in North America, the pampas in South America, the steppes in eastern Europe and northern Eurasia and the grass veld in South Africa, formed over millions of years and are among the most diverse, fertile and productive terrestrial biomes (Mlot, 1990; Henwood, 1998). Disturbance of grasslands is increasing dramatically, making their conservation and restoration of increasing global concern. Increasing domination of natural ecosystems by humans is transforming them to species poor systems (Loreau et al., 2001) and their restoration is challenged by low native plant reestablishment and competition with non-native plant species (Pyšek et al., 2012). One of the largest natural grasslands in North America is the foothills fescue grasslands of southern Alberta in Canada and Montana in the United States. These grasslands have been reduced to 17% of their former extent (Adams et al., 2009), due to agriculture, industry, fire suppression and invasion by non-native species (Adams et al., 2009; Alberta Government, 2010). Important protected remnants of these grasslands remain in Waterton Lakes National Park, a United Nations Educational, Scientific and Cultural Organization (UNESCO) world heritage site. However, former activities, such as gravel extraction and landfills, have strongly influenced the native plant community. Large populations of non-native plant species, including invasive species such as *Elymus repens* L. Gould (Quack grass), *Centaurea maculosa* Lam. (Spotted knapweed), *Bromus inermis* Leyss. (Smooth brome) and *Poa pratensis* L. (Kentucky bluegrass), have formed

* Corresponding author at: Department of Biology, Biological and Geological Sciences Building, Western University, London, Ontario N6A 5B7, Canada.
E-mail addresses: hstover@uwo.ca (H.J. Stover), anne.naeth@ualberta.ca (M.A. Naeth), katja.boldt@b-tu.de (K. Boldt-Burisch).

https://doi.org/10.1016/j.apsoil.2018.07.008
Received 9 April 2018; Received in revised form 20 July 2018; Accepted 23 July 2018

dense monocultures and have spread into undisturbed areas. Seeding disturbed sites with native cultivars resulted in limited success in reducing non-native species invasion in foothills fescue grassland and methods to restore native fescue communities are ongoing (Alberta Government, 2010).

Worldwide invasion of terrestrial ecosystems by non-native species has considerable associated ecological and economic costs, which has stimulated interest in better understanding biotic and abiotic interactions associated with invasive plant species (Mummey and Rillig, 2006). One such interaction is mycorrhizal symbiosis (Menzel et al., 2017). Over 80% of terrestrial plants (Smith and Read, 2010) are estimated to live in a mutualistic interaction with arbuscular mycorrhizal fungi (AMF). AMF are important, often obligate, symbiotic partners for plants, known to improve nutrition, carbon economy and plant health (Smith and Read, 2010) and strongly influence plant competition and community diversity (Bever et al., 1997; van der Heijden et al., 1998). AMF community specificity may occur with ecological plant groups (generalists vs specialists) (Davison et al., 2011) and with ecosystems (Veresoglou and Rillig, 2014). AMF is the dominant type of mycorrhizae in grasslands (Read, 1991) with roots harbouring unique AMF communities (Vályi et al., 2015). Grassland specific AMF communities could be directly related to plant community stability. A lack or alteration of fungal community composition, quantity and spatial distribution by soil disturbance (Klironomos et al., 1993; Hart and Reader, 2004; Voříšková et al., 2016) could significantly affect reestablishment of native plant communities on disturbed sites. The extensive AMF hyphal networks, which usually range across multiple host plants, could be damaged by soil disturbance, decreasing the proportion of infectious propagules for new plants and interrupting service of the AMF network that some plant species depend on. Reestablishing native plants in ecological restoration might be strongly influenced by these factors.

Koziol and Bever (2017) suggest that later successional grassland species are more affected by native AMF than early successional species. They found plots inoculated with specific AMF isolated from the prairie were dominated by native prairie plants, whereas plots inoculated with other AMF species or not inoculated were dominated by weeds and non-native species. The role of AMF for invasive plant success may be determined by mycorrhizal dependency of the invader relative to native plants, and the ability of the invasive plant to change AMF abundance and composition in the community being invaded (Pringle et al., 2009; Lekberg et al., 2013).

Restoration activities are often unsuccessful because of abiotic constraints and unfavourable biotic conditions since the soil community is an important driver of plant community development (Wubs et al., 2016). Thus successful plant community restoration may require restoration of beneficial soil microorganisms such as AMF and therefore more knowledge on alteration of AMF communities due to soil disturbance is needed. The objective of this study was to analyze whether AMF communities on a fescue grassland shifted in response to past disturbances by landfill and gravel quarry activities and invasion by non-native plant species. We hypothesized that disturbed sites with non-native (invasive) plant species would have a different AMF taxa composition and lower AMF richness than natural undisturbed fescue grassland, and that relative abundance of AMF taxa on disturbed and undisturbed sites would be higher on undisturbed. Such information will contribute to a better understanding of changes within a fescue grassland AMF community from disturbance, to improve restoration strategies for consistent and reliable reestablishment of native plants in fescue grasslands.

2. Materials and methods

2.1. Study area

Waterton Lakes National Park is located in the Rocky Mountains of southwestern Alberta, Canada. Grasslands are characterized by Chernozem soils with predominantly calcareous soil parent materials, good drainage and dark colored mineral surface horizons high in organic matter (Coen and Holland, 1976). Mean annual precipitation is 808 mm; mean minimum and maximum temperatures are −1.3 and 10.6 °C, respectively (Environment Canada, 2012).

Three disturbed grassland sites were located within a 3 km radius with similar topography and soils, surrounded by undisturbed native grassland. Site 1 (49°09′N, 113°88′W, WGS 84/NAD 83), approximately 5.8 ha in size, was used for disposal of various waste materials from 1952 to 1999 (Naeth and Jobson, 2007). Soil and ground water had elevated strontium, nickel, silver, aluminum, copper, zinc and iron, reflecting natural background concentrations. Thus the site was classified as very low risk with no required remediation (Canadian Council of Ministers of the Environment, 2008). Restoration activities in 2006 and 2007 included recontouring and application of wood chips and topsoil to rebuild the soil. Native grasses, forbs and shrubs from wild collected seed were seeded and transplanted (Naeth and Wilkinson, 2008). Non-native plant species remained dominant at the site and few native species established despite revegetation efforts.

Site 2 (49°11′N, 113°83′W), 1.8 ha in size, was used for gravel excavation during the 1960s and decommissioned in the late 1970s. It became heavily infested with *Centaurea maculosa*, which was herbicided and hand pulled in the 1980s, then plowed and revegetated with native plant species. Site 3 (49°13′N, 113°85′W), 2.2 ha in size, was also used for gravel excavation until park staff recently seeded native plant species and tried to control target weeds.

The disturbed areas contained a population of non-native species including *Centaurea maculosa*, *Cirsium arvense* L. (Canada thistle), *Bromus inermis*, *Elymus repens*, *Poa pratensis*, *Poa compressa* L. (Canada bluegrass) and *Agropyron cristatum* L. (Crested wheatgrass). Non-native plant species were most abundant at Site 1, intermediate at Site 2 and least at Site 3, with the opposite trend for native species. The undisturbed native grasslands are dominated by *Festuca campestris* Rybd. (Foothills rough fescue), *Danthonia parryi* Scribn. (Parry oatgrass), *Festuca idahoensis* Elmer (Idaho fescue) and numerous forb and shrub species. Non-native species were absent from undisturbed native grassland.

2.2. Experimental design and sampling

At each of the three disturbed sites, 0.3 ha with uniform topography were selected for study, and three 200 m^2 sampling plots were established. Sites were surrounded by undisturbed native grassland, within which one 200 m^2 plot was located approximately 400 m from the disturbed plots. On each 200 m^2 plot, 0.1 m^2 quadrats were randomly located for vegetation, soil and root-AMF sampling.

In each quadrat, plant species present and their percent canopy cover were assessed in the first week of August 2011. Botanical nomenclature followed Kuijt (1982) and Tannas (2003). Soil and roots were sampled in the third week of September 2011. A 15 × 15 × 15 cm hole was dug in the center of selected sampling quadrats with trowels and 500 g of soil and 100 g of roots were removed as individual samples. Samples were placed in labeled plastic bags and stored at 4 °C until processing.

2.3. Soil analyses

Soils were analyzed for total nitrogen and carbon by dry combustion (Bremner, 1996). Inorganic carbon was determined through carbon dioxide release; organic carbon by subtracting inorganic carbon from total carbon (Loeppert and Suarez, 1996). Total phosphorus was determined with strong acid extraction and inductively coupled plasma mass spectrometry (US Environmental Protection Agency, 1996). Sand, silt and clay were determined by hydrometer (Kroetsch and Wang, 2008); electrical conductivity, pH, sodium adsorption ratio and available soil calcium, magnesium, sodium and potassium in saturated paste